The nutritional burden of methylation reactions

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Purpose of review
Methyl group metabolism is a metabolically demanding process that has significant nutritional implications. Methionine is required not only for protein synthesis but also as the primary source of methyl groups. However, demethylated methionine can be remethylated by methyl groups from methylneogenesis (via folate) and betaine (synthesized from choline). This review discusses the impact of methylation precursors and products on the methionine requirement.

Recent findings
Recent evidence has clearly demonstrated that transmethylation reactions can consume a significant proportion of the flux of methionine. In particular, synthesis of creatine and phosphatidylcholine consume most methyl groups and their dietary provision could spare methionine. Importantly, methionine can become limiting for protein and phosphatidylcholine synthesis when creatine synthesis is upregulated. Other research has shown that betaine and choline seem to be more effective than folate at reducing hyperhomocysteinemia and impacting cardiovascular outcomes suggesting they may be limiting.

Summary
It appears that methyl groups can become limiting when dietary supply is inadequate or if transmethylation reactions are upregulated. These situations can impact methionine availability for protein synthesis, which can reduce growth. The methionine requirement can likely be spared by methyl donor and methylated product supplementation.

Keywords
infant nutrition, methionine requirement, methyl groups, remethylation, transmethylation

INTRODUCTION

Many amino acids are important not only for the synthesis of proteins but also as metabolites for critical metabolic reactions. Some of these 'non-protein' roles of amino acids can utilize a significant proportion of the total amino acid pool. For indispensable amino acids, these alternative metabolic pathways must be carefully considered when establishing the dietary requirement of that amino acid. The upregulation or downregulation of these pathways can tax or spare the amino acid requirement, respectively, depending on the pathway in question and the availability of alternate metabolites. For example, methionine is an essential amino acid that is necessary for the synthesis of cysteine and taurine as well as for the supply of methyl groups. These methyl groups are needed, *inter alia*, for the synthesis of creatine, phosphatidylcholine and carnitine and for regulating gene expression via cytosine and histone methylation. Methyl groups for remethylation to methionine can be provided by methyl donors such as choline and betaine and by methylneogenesis (via folate). In adult humans, the major dietary sources of labile methyl groups are methionine (~10 mmol methyl per day) [1], choline (~30 mmol methyl per day) [1], betaine (~26–75 mmol methyl per day) [2], and methylneogenesis via folate (~5–10 mmol methyl per day) [1]. Because folate, choline and methionine are all essential nutrients, the dietary availability of folate, choline and betaine can obviously influence the amount of methionine needed. Moreover, dietary supply of methylated products such as creatine, carnitine and phosphatidylcholine could reduce the need for methyl groups and further spare methionine requirement. In growing animals, this balance becomes more precarious as growth requires even more amino acids for expansion of body protein mass as well as for the increased need for methyl groups for critical reactions.

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KEY POINTS

- Methylation reactions can demand a significant proportion of methionine flux and limit the availability of methyl groups.
- Methyl donors such as betaine and choline can likely spare the methionine requirement.
- The effects of dietary methyl donors and methylated products must be considered when determining the methionine requirement, especially for growing neonates.

One key question remains unanswered: What is the impact of the supply of methyl groups on methylation reactions? The metabolic deficiency of methyl groups must have profound effects throughout the body given the scope of methylation reactions. So if folate, betaine and/or choline are deficient in the diet, then presumably more methionine is required to maintain the critical methylation reactions and, consequently, less methionine is available for protein synthesis and growth. Conversely, if these methyl nutrients are in excess, then less methionine is required and folate, betaine and/or choline can spare methionine and perhaps enhance growth. With respect to DNA methylation, methyl group supply can have permanent effects on gene expression, which can have broad effects on whole body metabolism and even lead to chronic diseases in adulthood [3]. Because most infants are exposed to dietary extremes of methyl-related nutrients, elucidation of the transmethylation pathways and the effects of dietary methyl supply in neonates will not only optimize infant health, but also adult health.

METHIONINE METABOLISM

In Fig. 1, the pathways for sulfur amino acid metabolism are presented and can be summarized by transmethylation (methionine to homocysteine), transsulfuration (homocysteine to cysteine) and remethylation (homocysteine to methionine). Briefly, methionine is adenylated to *S*-adenosylmethionine (SAM), which is the methyl donor in over 50 different reactions including DNA methylation, creatine synthesis and phosphatidylcholine synthesis. The end-products are the methylated compound and *S*-adenosylhomocysteine (SAH), which is converted to homocysteine. Homocysteine can either be irreversibly catabolized to cysteine via the transsulfuration pathway or remethylated to methionine.

REMETHYLATION

Remethylation of homocysteine to methionine occurs either via cobalamin-dependent methionine synthase and folate or via an equally important route, betaine–homocysteine methyltransferase (BHMT), which uses betaine, a product of choline oxidation, as the methyl donor (Fig. 1). Importantly, during choline or betaine deprivation, more folate is required to facilitate remethylation, thereby increasing folate requirements and, conversely, folate deficiency leads to increased use of betaine for methyl groups, thereby increasing choline requirements [1]. *N*,*N*-Dimethylglycine (DMG) is formed from betaine (*N*,*N*,*N*-trimethylglycine) by demethylation via BHMT. Betaine demethylation is the only known source of DMG in mammals, so the presence of DMG is a direct result of methylation of homocysteine to methionine [4]. DMG is further demethylated to sarcosine (*N*-methylglycine) via the flavoprotein dimethylglycine dehydrogenase, which converts the methyl group into 5,10-methylene tetrahydrofolate [4]. Sarcosine can also be demethylated to glycine by mitochondrial sarcosine dehydrogenase. Because sarcosine is also a product of transmethylation via glycine *N*-methyltransferase (GNMT), it is not an unambiguous marker of its demethylation pathway. Folate-dependent remethylation via methionine synthase derives its methyl group primarily via the glycine cleavage system and serine hydroxymethyltransferase (SHMT), both of which require vitamin B6 as coenzyme, to generate 5,10-methylene tetrahydrofolate [5▪,6]. However, it should be noted that 5,10-methylene tetrahydrofolate is also derived from the catabolism of DMG and sarcosine [7] (Fig. 1).

The regulation of the methionine pathways is complex. When cysteine is unavailable, transmethylation and transsulfuration are increased to oxidize methionine and synthesize cysteine. Similarly, when methionine is in excess, transmethylation and transsulfuration are upregulated to eliminate methionine, which is toxic at relatively low concentrations. When transmethylation is upregulated, all extra methyl groups are eliminated by GNMT (Fig. 1) [8]. Indeed, elevated methionine leads to increased expression of GNMT [9], which consumes excess SAM and still conserves the carbon skeleton in homocysteine, which can be remethylated. When methionine is undersupplied, then transsulfuration is downregulated and remethylation is upregulated to regenerate methionine. The rate-limiting enzymes for transmethylation, remethylation and transsulfuration are primarily regulated by SAM [8,10]. The partitioning of transmethylation, remethylation and transsulfuration during various conditions has been studied in various in-vitro

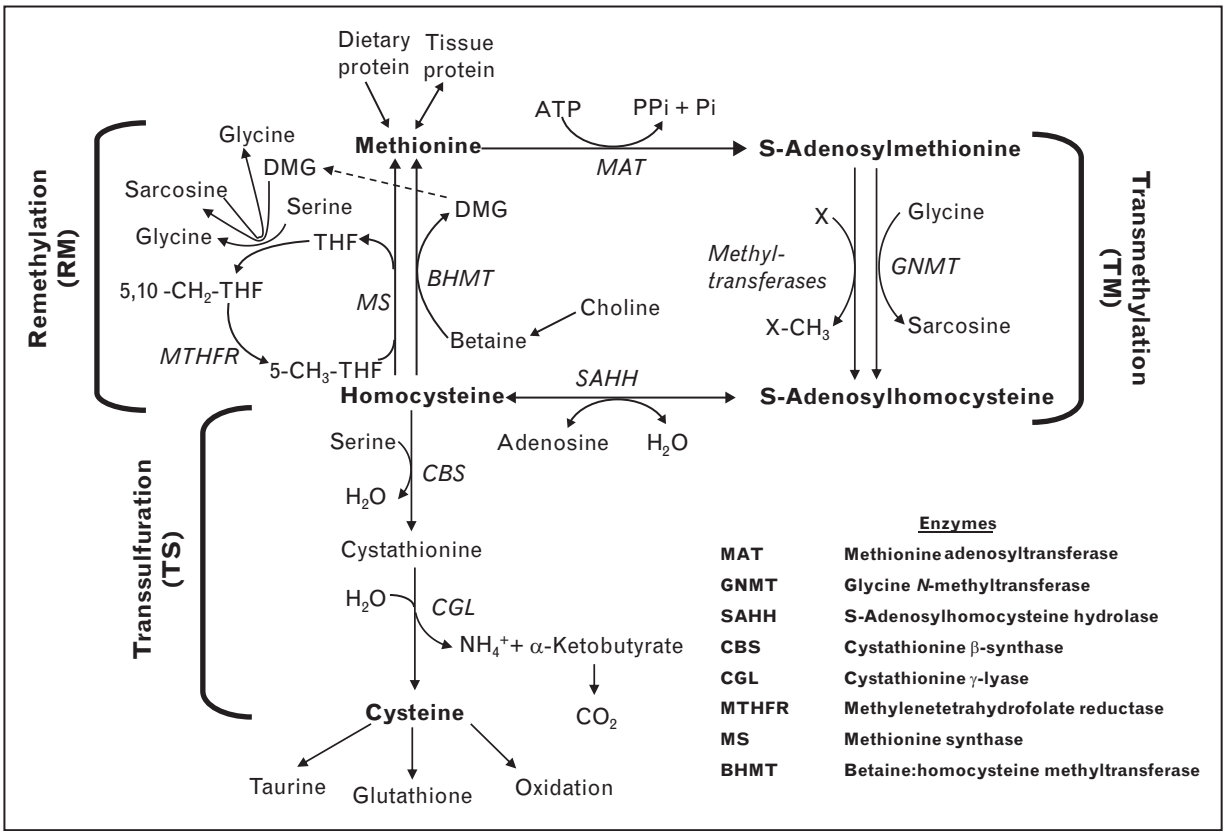

FIGURE 1. Schematic of the methionine cycle including transmethylation, remethylation and transsulfuration pathways. BHMT, betaine–homocysteine methyltransferase; CBS, cystathionine β-synthase; CGL, cystathionine γ-lyase; DMG, dimethylglycine; GNMT, glycine *N*-methyltransferase; MAT, methionine adenosyltransferase; MS, methionine synthase; MTHFR, methylene tetrahydrofolate reductase; SAHH, *S*-adenosylhomocysteine hydrolase; THF, tetrahydrofolate.

systems and less so, *in vivo* [11], but the partitioning of methyl groups within transmethylation has not been studied extensively. An unanswered question is which methylation reactions have priority when methyl groups are scarce?

TRANSMETHYLATION REQUIREMENTS

The nonprotein requirement for methionine can be viewed as the transmethylation requirement of the body; if cysteine intake is adequate, then the requirement for methyl groups will drive the nonprotein requirement for methionine. There are more than 50 methylation reactions but quantitatively, the most important enzymes are phosphatidylethanolamine methyltransferase (PEMT), which synthesizes about one-third [12] of endogenous phosphatidylcholine; guanidinoacetate methyltransferase (GAMT), which synthesizes creatine; and GNMT, which serves as an overflow pathway for methionine catabolism. GAMT has a very significant methyl requirement, especially in neonates who are rapidly expanding their lean tissue mass and associated creatine pool. Indeed, we have recently estimated that the suckling piglet accrues creatine at a rate three times higher than sow milk can provide and this endogenous creatine synthesis rate would consume up to one-third of dietary methionine [13]. Obviously, creatine synthesis alone requires either a large proportion of dietary methionine or, considering sow milk contains negligible betaine (unpublished data), a very high rate of methylneogenesis and/or choline oxidation. Put in perspective, creatine synthesis alone obliges a transmethylation rate of 0.64 mmol/kg per day in the piglet [13] compared with the total transmethylation rate (for all transmethylation reactions) of approximately 0.85 mmol/kg per day in postweaned piglets [14] and 0.17 mmol/kg per day in adult humans [11]. Moreover, methyl demand for phosphatidylcholine synthesis via PEMT (which uses three methyl groups per phosphatidylcholine synthesized) could be even more quantitatively important than that for creatine synthesis [15], especially when one considers the extremely high phosphatidylcholine needs of a growing neonate [16]. With respect to infant nutrition, if an infant is fed cow milk or soy formula, choline or creatine,

respectively, can be inadequate [17]. And although the quantitative importance of DNA methylation has not been estimated, its developmental importance and sensitivity to dietary manipulation [18] makes this methylation pathway perhaps the most important of all, with respect to the long-term health of the organism.

These pathways all share the same need for a common substrate: methyl groups. During growth and development, all of these pathways are highly active not only for maintaining basic functions but also for expanding the pools of their respective products to meet the demands for growth. We recently demonstrated competition among these pathways for a limited methyl supply [19]. We infused guanidinoacetate (GAA) intraportally in 15–18-day-old piglets to drive creatine synthesis and consume hepatic methyl groups (unpublished). Compared with a saline infusion, GAA infusion doubled the methyl incorporation rate into creatine, but decreased methyl incorporation into phosphatidylcholine by 85% and reduced protein synthesis by 40% (Fig. 2). These data suggest that methyl groups were limited for phosphatidylcholine synthesis and that methionine was diverted from protein synthesis. GAA infusion did not affect methyl incorporation into DNA. The partitioning of available methionine between its primary maintenance role as a methyl donor and its role in growth via protein synthesis has important implications when determining amino acid recommendations for neonates. Moreover, this study demonstrated that methyl supply can be limited in the neonate and that this leads to a repartitioning of methyl groups among methylation reactions.

Diet composition will also affect the relative requirements of these pathways, especially early in life. For example, a creatine-free diet (i.e. soy-based formula or vegan diet) will require the synthesis of the entire requirement for creatine, both for maintenance and for growth. Similarly, some bovine milk-based and soy-based formulas have little choline compared with breast-milk and plasma choline concentrations in formula-fed neonates are half that of breast-fed infants [17]. Moreover, maternal choline status modifies choline in milk, which in turn reflects choline status of infants [16]. Diets low in choline will determine the activity of PEMT. Moreover, a casein-based formula has much more methionine (and low cysteine) than a whey-based formula or breast milk. But ultimately, these pathways depend on the

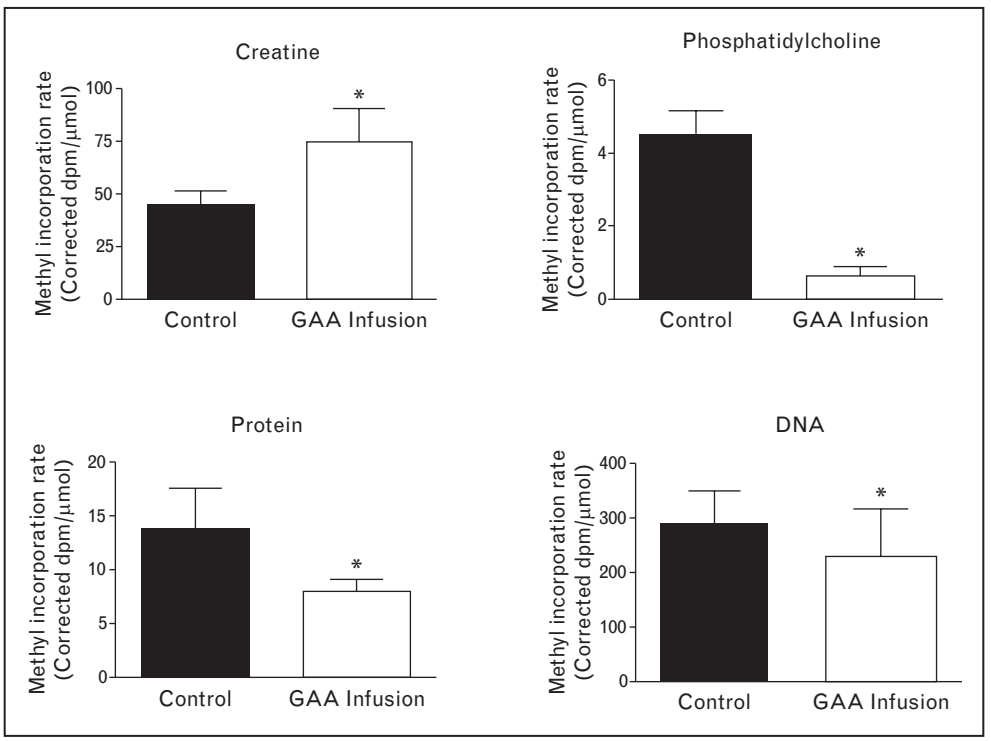

FIGURE 2. Methyl incorporation rate into hepatic creatine, phosphatidylcholine, protein and DNA following a 2-h infusion of saline (closed bars) or guanidinoacetate (GAA) (open bars) in 15–18-day-old piglets. Methyl incorporation rate was determined following a 1-h bolus infusion of [methyl-^{3}H]methionine and data were corrected for specific radioactivity of SAM (for creatine, PC, DNA) or methionine (for protein). Each bar represents the mean + SD of $n = 5$ piglets. Asterisk (*) indicates data are significantly different ($P < 0.05$). PC, phosphatidylcholine; SAM, *S*-adenosylmethionine.

methyl supply, both from the diet and from de-novo synthesis. Choline, betaine, methionine and folate-dependent methylneogenesis are responsible for supplying methyl groups and their interdependency requires further research. If one or two of these nutrients are limiting, then the other(s) must compensate to maintain supply. It is imperative that the requirement for methionine accommodates its flux not only to protein and cysteine but also to transmethylation pathways, fully considering its remethylation back to methionine.

In particular, little attention is paid to methylation products and their influence on methionine requirements. Indeed, studies to determine methionine requirements in adult humans typically control for methylation precursors (e.g. B vitamins, choline) [20], but do not consider the dietary content of methylation products (e.g. creatine). Because such studies almost invariably employ protein-free, crystalline amino acid based diets, it is likely these diets are very low in creatine, which would require more methionine. Ball *et al*. [20] have defined the minimum obligatory methionine requirement as 'the dietary intake of methionine that cannot be replaced by cysteine and that will not be reduced by addition of any methyl donor, cofactor or any other metabolite'. Thus, if the inclusion of creatine in the diet spares methionine, then current methionine requirement estimates that were determined using creatine-poor test diets are likely to be overestimated.

CAN METHYL DONORS SPARE METHIONINE?

There has been significant research exploring the interdependency between methionine and cysteine, both in adults and during growth [20]. If cysteine is not supplied in the diet, then one needs twice as much methionine; in other words, dietary cysteine can 'spare' about half of the methionine requirement. If cysteine is supplied in excess, then the methionine requirement can be reduced to that needed for protein synthesis and for methylation reactions. The concept of alternate dietary methyl 'donors' (i.e. betaine, choline, methylneogenesis precursors) sparing the methionine requirement has recently been discussed [21], but few studies have addressed this concept directly.

Because folic acid [22], betaine [23–26] and choline [27] can effectively lower plasma homocysteine in humans by increasing remethylation to methionine, these nutrients are very likely to be capable of sparing the methionine requirement. Indeed, in a recent, elegant isotope study, folic acid and choline doubled remethylation to methionine when methionine was limiting [28▪▪]. However, it should be noted that supplemental folic acid, in addition to vitamin B_6 and cyanocobalamin, are not providing methyl groups *per se*, but rather the cofactors for remethylation. In this way, the requirement for these vitamins must be met, but beyond that, these vitamins cannot spare the methionine requirement. Indeed, it is well described that the lowering effect of B vitamin supplementation on plasma homocysteine is not graded and a maximum reduction is reached, beyond which additional supplementation is not effective [29]. Furthermore, it has recently been shown that in spite of folic acid's homocysteine-lowering effects, there appears to be no beneficial effect on adverse cardiovascular outcomes [30,31▪], unlike for betaine [32▪]. Although the inadequacy of these vitamins in some populations can affect the ability to process methyl supply [22], these B vitamins are generally adequate in Western diets [33] and likely do not affect the supply of methyl groups. However, caution should be used when associating low vitamin status and plasma homocysteine, as Lamers *et al*. [34▪] have recently shown that rates of remethylation, transsulfuration, transmethylation and DNA methylation are unaffected by dietary vitamin B_6 restriction in healthy adults, in spite of an accentuated postprandial rise of plasma homocysteine. However, estimates of remethylation, transsulfuration and transmethylation using isotopes also need to be interpreted with caution [11]. Given the growing interest in the role of these vitamins in methyl metabolism, future experiments will need to address the potential sparing effects of these cofactors on methionine requirements, especially in populations with vitamin inadequacy.

It has been suggested that perhaps choline, betaine or other one-carbon donors such as DMG, sarcosine, serine or glycine, which are precursors of methyl groups via 5-methyl-tetrahydrofolate, should be considered in addition to vitamin B supplementation [35]. Most foods contain some choline, choline esters and betaine [36] and with a healthy diet, most of the body's betaine needs can likely be met by choline oxidation [4]. However, most people do not consume adequate choline and the mean intake is 40% below the recommended adequate intake [37]. Moreover, intakes of betaine and choline from food in adults can predict both fasting and postmethionine-load homocysteine concentrations, especially in individuals with low folate and cobalamin status [38,39]. This association is supported by other trials that have shown that supplementation with choline or betaine can decrease plasma homocysteine concentrations in humans [23–26]. It is perhaps surprising then that

most published reviews on homocysteine lowering in humans focused more on vitamin B supplements rather than methyl supply. It is likely that a metabolic deficiency of methyl groups might be responsible for some hyperhomocysteinemia [35].

But the question still remains as to whether methyl donors can effectively 'spare' the methionine requirement. In food animals, this concept has been explored by animal scientists primarily interested in how methionine requirement can be reduced by less costly supplements of methyl-containing choline and/or betaine. Numerous studies have demonstrated that choline and/or betaine can spare the methionine requirement in growing chickens [40,41] and young pigs [42,43], not only for the synthesis of protein but also for creatine and carnitine synthesis [41]. The fact that these studies were able to show a sparing effect of methyl groups on the methionine requirement demonstrates the enormous demands of these pathways. Although data in humans are not available, it is likely a similar effect occurs in infants and children as well as adults. So, the actual choline and betaine content of the diet will have a bearing on the methionine requirement for growth and nongrowth functions.

An interesting aspect of betaine metabolism is that it can potentially provide a methyl group for both remethylation pathways. Demethylation of betaine via BHMT results in DMG, which in turn is sequentially demethylated to sarcosine and then to glycine (Fig. 1). Both DMG and sarcosine can be used to synthesize methylene THF, thereby providing a methyl group for methylneogenesis via the methionine synthase pathway [4,7]. To my knowledge, the extent to which each of these three precursors is used for methylneogenesis is unknown. Interestingly, DMG has recently been approved for use in agriculture as a feed supplement and has been shown to improve feed digestibility and utilization in poultry [44,45] and swine [46]. The role of methyl metabolism in these outcomes has not been investigated, but considering DMG is also known to inhibit BHMT activity in rats [4,47], its supplementation would have significant effects on methyl metabolism. In chickens, betaine or choline supplementation increases folate-dependent remethylation, and not BHMT remethylation, suggesting a role for DMG in methionine synthase remethylation [40]. Clearly, the role of betaine as a precursor for the two remethylation pathways warrants further research.

CONCLUSION

With respect to human health and development, we need to understand more than just the effects of methyl donors on growth; we need a more detailed exploration of the nongrowth requirements for optimal health. For example, if methyl groups are limiting for DNA methylation, this can have implications for chronic disease development in adult life. In addition, the variable diet of human neonates, most of whom are not breast-fed exclusively (unlike other mammals), necessitates a clear understanding of the roles of excesses and deficiencies of methionine, choline, betaine and creatine. Furthermore, how the body partitions methionine when limiting amounts are available needs to be elucidated. Is protein synthesis and growth sacrificed to maintain essential methylation reactions? Which of these methylation reactions are most important? It seems inconceivable that DNA methylation, a presumably minor pathway from a quantitative point of view, would be changed by dietary methyl group availability; yet, recent studies have demonstrated exactly this in several species and conditions [48,49,50▪]. The impact of methylation reactions on the methionine requirement during neonatal development is important not only to simply define nutritional requirements in infants but also in light of the emerging evidence of a link between methyl metabolism and the developmental origins of adult disease.

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Conflicts of interest

There are no conflicts of interest.

REFERENCES AND RECOMMENDED READING

Papers of particular interest, published within the annual period of review, have been highlighted as:

- ▪ of special interest
- ▪▪ of outstanding interest

Additional references related to this topic can also be found in the Current World Literature section in this issue (p. 117).

1. Niculescu MD, Zeisel SH. Diet, methyl donors and DNA methylation: interactions between dietary folate, methionine and choline. J Nutr 2002; 132 (8 Suppl):2333S–2335S.
2. Craig SAS. Betaine in human nutrition. Am J Clin Nutr 2004; 80:539–549.
3. McMillen IC, Robinson JS. Developmental origins of the metabolic syndrome: prediction, plasticity, and programming. Physiol Rev 2005; 85:571–633.
4. Lever M, Slow S. The clinical significance of betaine, an osmolyte with a key role in methyl group metabolism. Clin Biochem 2010; 43:732–744.
5. Stover PJ, Field MS. Trafficking of intracellular folates. Adv Nutr 2011; ▪ 2:325–331.

An excellent review of one-carbon flux among the various folate-dependent pathways.

6. Davis SR, Stacpoole PW, Williamson J, *et al.* Tracer-derived total and folate-dependent homocysteine remethylation and synthesis rates in humans indicate that serine is the main one-carbon donor. Am J Physiol Endocrinol Metab 2004; 286:E272–E279.

7. Pike ST, Rajendra R, Artzt K, Appling DR. Mitochondrial C1-tetrahydrofolate synthase (MTHFD1L) supports the flow of mitochondrial one-carbon units into the methyl cycle in embryos. J Biol Chem 2010; 285:4612–4620.
8. Stipanuk MH. Sulfur amino acid metabolism: pathways for production and removal of homocysteine and cysteine. Annu Rev Nutr 2004; 24:539–577.
9. Rowling MJ, McMullen MH, Chipman DC, Schalinske KL. Hepatic glycine *N*-methyltransferase is up-regulated by excess dietary methionine in rats. J Nutr 2002; 132:2545–2550.
10. Hoffer LJ. Homocysteine remethylation and trans-sulfuration. Metabolism 2004; 53:1480–1483.
11. Hoffer LJ. Methods for measuring sulfur amino acid metabolism. Curr Opin Clin Nutr Metab Care 2002; 5:511–517.
12. Williams KT, Schalinske KL. New insights into the regulation of methyl group and homocysteine metabolism. J Nutr 2007; 137:311–314.
13. Brosnan JT, Wijekoon EP, Warford-Woolgar L, *et al.* Creatine synthesis is a major metabolic process in neonatal piglets and has important implications for amino acid metabolism and methyl balance. J Nutr 2009; 139:1292–1297.
14. Riedijk MA, Stoll B, Chacko S, *et al.* Methionine transmethylation and transsulfuration in the piglet gastrointestinal tract. Proc Natl Acad Sci U S A 2007; 104:3408–3413.
15. Stead LM, Brosnan JT, Brosnan ME, *et al.* Is it time to reevaluate methyl balance in humans? Am J Clin Nutr 2006; 83:5–10.
16. Caudill MA. Pre and postnatal health: evidence of increased choline needs. J Am Diet Assoc 2010; 110:1198–1206.
17. Ilcol YO, Ozbek R, Hamurtekin E, Ulus IH. Choline status in newborns, infants, children, breast-feeding women, breast-fed infants and human breast milk. J Nutr Biochem 2005; 16:489–499.
18. Waterland RA. Does nutrition during infancy and early childhood contribute to later obesity via metabolic imprinting of epigenetic gene regulatory mechanisms? Nestle Nutr Workshop Ser Pediatr Program 2005; 56:157–171.
19. McBreairty LE, Lamarre SG, McGowan RA, *et al.* Increased methyl demand for creatine synthesis changes the partitioning of methyl groups in runt and large Yucatan miniature piglets. Appl Physiol Nutr Metab 2011; 36:468–469.
20. Ball RO, Courtney-Martin G, Pencharz PB. The in vivo sparing of methionine by cysteine in sulfur amino acid requirements in animal models and adult humans. J Nutr 2006; 136 (6 Suppl):1682S–1693S.
21. Fukagawa NK. Sparing of methionine requirements: evaluation of human data takes sulfur amino acids beyond protein. J Nutr 2006; 136 (6 Suppl):1676S–1681S.
22. Homocysteine Lowering Trialists' Collaboration. Dose-dependent effects 326 of folic acid on blood concentrations of homocysteine: a meta-analysis of the randomized trials. Am J Clin Nutr 2005; 82:806–812.
23. Schwab U, Törrönen A, Toppinen L, *et al.* Betaine supplementation decreases plasma homocysteine concentrations but does not affect body weight, body composition, or resting energy expenditure in human subjects. Am J Clin Nutr 2002; 76:961–967.
24. Olthof MR, van Vliet T, Boelsma E, Verhoef P. Low dose betaine supplementation leads to immediate and long term lowering of plasma homocysteine in healthy men and women. J Nutr 2003; 133:4135–4138.
25. Steenge GR, Verhoef P, Katan MB. Betaine supplementation lowers plasma homocysteine in healthy men and women. J Nutr 2003; 133:1291–1295.
26. Ueland PM, Holm PI, Hustad S. Betaine: a key modulator of one-carbon metabolism and homocysteine status. Clin Chem Lab Med 2005; 43:1069–1075.
27. Olthof MR, Brink EJ, Katan MB, Verhoef P. Choline supplemented as phosphatidylcholine decreases fasting and postmethionine-loading plasma homocysteine concentrations in healthy men. Am J Clin Nutr 2005; 82:111–117.
28. ■■ Wilson FA, Holtrop G, Calder AG, *et al.* Effects of methyl-deficient diets on methionine and homocysteine metabolism in the pregnant rat. Am J Physiol Endocrinol Metab 2012; 302:E1531–E1540.

This elegant isotope study quantifies methionine cycle fluxes in the pregnant rat and fetus. It clearly demonstrated the role of methyl precursors on remethylation flux when methionine is limiting.

29. Bailey LB. Do low doses of folic acid result in maximum lowering of homocysteine? Am J Clin Nutr 2005; 82:717–718.
30. Clarke R, Halsey J, Lewington S, *et al.* B-Vitamin Treatment Trialists' Collaboration. Effects of lowering homocysteine levels with B vitamins on cardiovascular disease, cancer, and cause-specific mortality: Meta-analysis of 8 randomized trials involving 37 485 individuals. Arch Intern Med 2010; 170:1622–1631.
31. ■ Zhou YH, Tang JY, Wu MJ, *et al.* Effect of folic acid supplementation on cardiovascular outcomes: a systematic review and meta-analysis. PLoS One 2011; 6:e25142.

A thorough review of the literature demonstrating that folic acid supplementation is not effective at attenuating cardiovascular outcomes, in spite of effects on hyperhomocysteinemia.

32. ■ Lever M, George PM, Elmslie JL, *et al.* Betaine and secondary events in an acute coronary syndrome cohort. PLoS One 2012; 7:e37883.

This prospective study demonstrated that betaine insufficiency and excess are associated with adverse cardiovascular outcomes.

33. Allen LH. How common is vitamin B-12 deficiency? Am J Clin Nutr 2009; 89:693S–696S.
34. ■ Lamers Y, Coats B, Ralat M, *et al.* Moderate vitamin B-6 restriction does not alter postprandial methionine cycle rates of remethylation, transmethylation, and total transsulfuration but increases the fractional synthesis rate of cystathionine in healthy young men and women. J Nutr 2011; 141:835–842.

This whole-body in-vivo study demonstrated that vitamin B6 is not critical for maintaining the methionine cycle and supports the idea that remethylation via BHMT can compensate for methionine synthase downregulation.

35. Benevenga NJ. Consideration of betaine and one-carbon sources of N5-methyltetrahydrofolate for use in homocystinuria and neural tube defects. Am J Clin Nutr 2007; 85:946–949.
36. Zeisel SH, Mar MH, Howe JC, Holden JM. Concentrations of choline-containing compounds and betaine in common foods. J Nutr 2003; 133:1302–1307.
37. Zeisel SH, da Costa KA. Choline: an essential nutrient for public health. Nutr Rev 2009; 67:615–623.
38. Cho E, Zeisel SH, Jacques P, *et al.* Dietary choline and betaine assessed by food-frequency questionnaire in relation to plasma total homocysteine concentration in the Framingham Offspring Stud. Am J Clin Nutr 2006; 83:905–911.
39. Lee JE, Jacques PF, Dougherty L, *et al.* Are dietary choline and betaine intakes determinants of total homocysteine concentration? Am J Clin Nutr 2010; 91:1303–1310.
40. Pillai PB, Fanatico AC, Blair ME, Emmert JL. Homocysteine remethylation in broilers fed surfeit choline or betaine and varying levels and sources of methionine from eight to twenty-two days of age. Poult Sci 2006; 85:1729–1736.
41. Zhan XA, Li JX, Xu ZR, Zhao RQ. Effects of methionine and betaine supplementation on growth performance, carcass composition and metabolism of lipids in male broilers. Br Poult Sci 2006; 47:576–580.
42. Kroening GH, Pond WG. Methionine, choline and threonine interrelationships for growth and lipotropic action in the baby pig and rat. J Anim Sci 1967; 26:352–357.
43. Fernandez-Figares I, Wray-Cahen D, Steele NC, *et al.* Effect of dietary betaine on nutrient utilization and partitioning in the young growing feed-restricted pig. J Anim Sci 2002; 80:421–428.
44. Kalmar ID, Cools A, Buyse J, *et al.* Dietary N,N-dimethylglycine supplementation improves nutrient digestibility and attenuates pulmonary hypertension syndrome in broilers. J Anim Physiol Anim Nutr (Berl) 2010; 94:e339–e347.
45. Kalmar ID, Verstegen MW, Maenner K, *et al.* Tolerance and safety evaluation of N,N-dimethylglycine, a naturally occurring organic compound, as a feed additive in broiler diets. Br J Nutr 2012; 107:1635–1644.
46. Cools A, Maes D, Buyse J, *et al.* Effect of N,N-dimethylglycine supplementation in parturition feed for sows on metabolism, nutrient digestibility and reproductive performance. Animal 2010; 4:2004–2011.
47. Liu Y, Liu YQ, Morita T, Sugiyama K. Effects of betaine supplementation and choline deficiency on folate deficiency-induced hyperhomocysteinemia in rats. J Nutr Sci Vitaminol (Tokyo) 2012; 58:69–77.
48. Kotsopoulos J, Sohn KJ, Kim YI. Postweaning dietary folate deficiency provided through childhood to puberty permanently increases genomic DNA methylation in adult rat liver. J Nutr 2008; 138:703–709.
49. Burdge GC, Lillycrop KA, Phillips ES, *et al.* Folic acid supplementation during the juvenile-pubertal period in rats modifies the phenotype and epigenotype induced by prenatal nutrition. J Nutr 2009; 139:1054–1060.
50. ■ Cordero P, Gomez-Uriz AM, Campion J, *et al.* Dietary supplementation with methyl donors reduces fatty liver and modifies the fatty acid synthase DNA methylation profile in rats fed an obesogenic diet. Genes Nutr 2012. doi: 10.1007/s12263-012-0300-z [Epub ahead of print].

This study demonstrated that dietary methyl supply can alter gene-specific epigenetic profile and phenotype, even when supplemented in adulthood.