Biomarkers of Nutrient Exposure and Status in One-Carbon (Methyl) Metabolism

Joel B. Mason

Tufts University, Boston, MA 02111

ABSTRACT One-carbon metabolism is a network of interrelated biochemical reactions that involve the transfer of one-carbon groups from one compound to another. The coenzymes necessary for several of these reactions include the B-vitamins, folate, vitamin B-12, vitamin B-6 and riboflavin (vitamin B-2), whereas important intermediary compounds in this schema include methionine and choline. There has been renewed interest in one-carbon metabolism during the past several years, engendered by recent insights that indicate that modest dietary inadequacies of the abovementioned nutrients, of a degree insufficient to cause classical deficiency syndromes, can still contribute to important diseases such as neural tube defects, cardiovascular disease and cancer. Traditional means of assessing nutrient exposure with food frequency questionnaires, and nutrient status with plasma and urine vitamin assays, has some genuine validity and utility. Assessing the concentration of appropriate intermediary compounds, such as plasma homocysteine for folate and methylmalonic acid for vitamin B-12, provides further insights because they appear to add a degree of sensitivity that does not exist with the more traditional assays. There may also be value in developing measures that integrate the status of all these nutrients and express it as a functional “methylation capacity” of the individual. Plasma or tissue concentrations of S-adenosylmethionine and S-adenosylhomocysteine, and genomic DNA methylation are two potential candidates in this regard although much work is yet to be done to define the nature of these relationships. J. Nutr. 133: 941S–947S, 2003.

KEY WORDS: biomarkers • diet assessment • epidemiology • folate • nutrition • one-carbon metabolism

One-carbon metabolism is a network of interrelated biochemical reactions that involve the transfer of one-carbon groups from one site to another. It is more accurate to refer to this network of reactions as one-carbon metabolism than methyl metabolism because the one-carbon moiety, depending on the particular reaction, can be donated in the form of a methenyl, formyl or methyl group. The past decade witnessed a renewed interest in dietary components that mediate, or in some manner facilitate, one-carbon metabolism. The dietary components of interest are the B-vitamins, folate and vitamin B-12, as well as choline and methionine. Collectively, these nutrients are sometimes called “dietary lipotropes,” a term that arose from the observation that a diet deficient in choline alone, or choline in combination with other lipotropes, leads to an excessive accumulation of triglyceride in the liver of laboratory rodents.

Strictly speaking vitamin B-6 is not a lipotrope. Nevertheless it is integrally involved in one-carbon metabolism, and deficiency of the vitamin may interfere with methylation reactions in certain settings because a deficiency of vitamin B-6 leads to a rise in blood levels of homocysteine. Similarly, riboflavin (vitamin B-2) is a cofactor for the critical folate-dependent enzyme, methylenetetrahydrofolate reductase, and studies in laboratory animals suggest that vitamin B-2 status can impair activity of this enzyme and therefore interconversion of the different forms of folate. Not surprisingly, at least one study in humans suggests that one-carbon metabolism can be interrupted by diminished riboflavin status.

A renewed interest in these compounds was largely engendered by recent indications that modest dietary inadequacies of certain lipotropes can cause several disease conditions of considerable clinical impact. Inadequate folate intake early in pregnancy now unequivocally is shown to increase the risk of births complicated by a neural tube defect (NTD). Inadequate intake of folate, vitamin B-12 or vitamin B-6 raises serum levels of a one-carbon intermediary product, homocysteine, which is highly associated with an increased risk of occlusive vascular disease. Inadequate intake of folate and methionine (and more recently, vitamins B-6 and B-12) is increasingly implicated in carcinogenesis in certain tissues. Our concepts about what constitutes
a “deficiency state” for each of these nutrients is in evolution because the abovementioned diseases seem to result from degrees of nutrient depletion that fall far short of the magnitude of depletion that is classically defined as a deficiency state. For example women who consume lesser amounts of folate are at increased risk of delivering an infant with a neural tube defect even though their mean intake is well within a range that prevents the classical deficiency syndrome, megaloblastic anemia, from occurring. Thus there is considerable interest in either redefining normative ranges for desirable blood levels for some of the lipotropes or developing novel, and more revealing, biomarkers, but each biomarker measures a different aspect of nutrient status.

The interest in the dietary determinants of methyl metabolism as it relates to chronic disease largely pertains to various degrees of deficiency rather than toxicity. Although it is an oversimplification, one-carbon metabolism can be perceived as serving two critically important functions: biological methylation and the synthesis of nucleotides (Fig. 1). The enzymatic addition of a methyl group to certain compounds is a critical synthetic step in many biochemical schemes and, in more than 80 such reactions, the proximate methyl group donor is an intermediary product of one-carbon metabolism, S-adenosylmethionine (SAdoMet). Dietary deficiencies of the lipotropes often (but not invariably) lead to a decrease in cellular SAdoMet levels and therefore diminish the methylation capability of the tissue. By donating its labile methyl group, SAdoMet becomes S-adenosylhomocysteine (SAH), and an accumulation of the latter compound inhibits methylation reactions from occurring. This constitutes the basis for why the “SAdoMet:SAH” ratio is often used as an indicator of the methylation capability of a particular tissue (7). It is also important to recognize that SAH is hydrolyzed to homocysteine via a reversible reaction that favors synthesis of SAH. Therefore, any accumulation of homocysteine usually translates into increases in SAH, thereby impeding methylation reactions.

It is nevertheless important not to focus solely on biological methylation when one is concerned with lipotrope nutriture because existing hypotheses that attempt to explain how lipotrope deficiency produces chronic disease such as cancer implicate aberrations in nucleotide synthesis as well. In this case the intracellular pool of folate coenzymes can alternatively be routed into a biochemical avenue that leads to the de novo synthesis of thymidine or the purines, adenine and guanine. Interestingly, these two major synthetic pathways appear to compete for folate coenzymes. Under normal circumstances SAdoMet serves as a major regulator in determining whether folate will be routed into one path versus the other, largely through its role as an allosteric inhibitor of methyltetrahydrofolate reductase (MTHFR) (8).

Polymorphisms in several enzymes, such as methionine synthase and MTHFR, were recently described, and they determine in part how folate coenzymes are distributed between methylation and nucleotide synthesis. For instance, the common 677 mutation in the MTHFR gene inhibits the conversion of 5,10-methylenetetrahydrofolate into 5-methyltetrahydrofolate, and therefore appears to inhibit methylation reactions and instead favor nucleotide synthesis. This is particularly evident when folate availability is limited. Thus compared to wild-type subjects, under low folate conditions subjects with the homozygous TT (mutant) genotype possess higher blood concentrations of homocysteine owing to impaired remethylation of the compound to methionine (9) as well as lower levels of genomic DNA methylation in their peripheral blood cells (10, 11).

**A brief biochemical overview**

Before embarking on a study, considerable thought should go into precisely what the investigator wants to know because the selection of a biomarker should serve the needs of the particular research questions at hand. This is entirely true of lipotropes because each nutrient can be assessed by a variety of direct measures of nutrient exposure or status.

**Biomarkers of nutrients involved in methyl metabolism: direct measures of nutrient exposure or status**

Before embarking on a study, considerable thought should go into precisely what the investigator wants to know because the selection of a biomarker should serve the needs of the particular research questions at hand. This is entirely true of lipotropes because each nutrient can be assessed by a variety of biomarkers, but each biomarker measures a different aspect of the metabolism of that nutrient.

**Intakes of nutrients**

If a biomarker is being sought that merely reflects the exposure of an individual or population to that nutrient, then measuring dietary intake is highly appropriate. Dietary recall or dietary records have some validity in this case but they are expensive, require trained personnel to administer and are prone to sampling biases because they only assess intake over a limited time period (12). The last point is a particularly important one because diet-associated risks in chronic degenerative diseases such as cardiovascular disease and cancer are largely related to habitual dietary patterns. Food frequency

![FIGURE 1 A simplified version of one-carbon metabolism, emphasizing the interconversions of the different coenzymatic forms of folate. Enzyme 1 is methionine synthase and enzyme 2 is methyltetrahydrofolate reductase (MTHFR). THF refers to tetrahydrofolate. Methylene tetrahydrofolate is underlined to emphasize that it sits at the metabolic intersection whereby folate is either directed toward nucleotide synthesis (i.e., purines and thymidylate) or toward biological methylation reactions. Modified with permission from Choi and Mason (1).](https://academic.oup.com/jn/article-abstract/133/3/941S/4688062)
questionnaires (FFQs) have therefore assumed an important role in assessing habitual intake. They have been successfully cross-validated by various means and shown to be a suitably accurate means of assessing exposure to some of the nutrients involved in methyl metabolism. For example, the correlations between the dietary intakes of vitamins B-2, B-6, folate and B-12, as assessed by the Willett semiquantitative FFQ, and the mean of two, 1-wk dietary records is shown in Table 1.

Correlations are invariably stronger when vitamin intakes include vitamin sources that are contained in supplements. This is because 1) the absolute amount of intake is larger when the content of supplements is taken into consideration and therefore the range over which a correlation is created is wider and the issue of FFQ sensitivity is attenuated; and 2) the precision of the amount of intake can be more accurately determined in a commercially formulated mixture than in a natural foodstuff. This also is generally true of the regression function between nutrient intake as assessed by FFQ and dietary records.

Habitual dietary intake of a particular nutrient is a major (although not the sole) determinant of nutrient status. Therefore, another means of validating the FFQs is by comparing values obtained from the FFQ with a biochemical indicator of nutrient status. An example of this is shown in Table 2.

The negative correlations observed with vitamins B-2 and B-6 merely reflect the fact that the biochemical assays for riboflavin and vitamin B-6 are “activity coefficients” of vitamin-dependent enzymes, the values of which are inversely related to vitamin status (see below).

The relationships between vitamin intake, as assessed by FFQ, and biochemical status are not as robust as those observed when FFQ intakes are compared to diet records. In large part this is because nutrient status is related to many factors, only one of which is dietary intake. A prime example is vitamin B-12. Intake of the vitamin is frequently shown to be only a rough predictor of vitamin B-12 status because common pathophysiological factors, such as the presence of atrophic gastritis, have a very large impact on the bioavailability of food-borne vitamin B-12. The issue is of lesser magnitude with vitamin B-12 supplements but there is a large impact on the bioavailability of food-borne vitamin B-12. Because the latter is also largely determined by habitual intake (16).

Habitual intake of methionine is estimated by the Willett FFQ by relying on the methionine content listed in the National Reference Food Tables published by the U.S. Department of Agriculture (www.nalusda.gov/fnic/cgi-bin/nut-search).

### Table 1

<table>
<thead>
<tr>
<th></th>
<th>Pearson correlation coefficient</th>
<th>Regression coefficient</th>
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<tbody>
<tr>
<td>Vitamin B-2</td>
<td>0.88</td>
<td>1.03</td>
</tr>
<tr>
<td>w/o supplements</td>
<td>0.53</td>
<td>0.46</td>
</tr>
<tr>
<td>Vitamin B-6</td>
<td>0.85</td>
<td>1.02</td>
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<tr>
<td>w/o supplements</td>
<td>0.73</td>
<td>0.85</td>
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<tr>
<td>Vitamin B-12</td>
<td>0.56</td>
<td>0.63</td>
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<tr>
<td>w/o supplements</td>
<td>0.52</td>
<td>0.32</td>
</tr>
<tr>
<td>Folate</td>
<td>0.77</td>
<td>0.78</td>
</tr>
<tr>
<td>w/o supplements</td>
<td>0.70</td>
<td>0.84</td>
</tr>
</tbody>
</table>

1 Adapted from Rimm et al. (13). Values based on energy-adjusted intakes that are deattenuated for within-person variability. All correlation coefficients significant, \( p < 0.05 \).

2 The regression coefficient here represents the slope of the relationship between FFQ and dietary record assessment.

### Table 2

<table>
<thead>
<tr>
<th>Nutrient</th>
<th>Pearson correlation coefficient</th>
<th>Biochemical assessment</th>
</tr>
</thead>
<tbody>
<tr>
<td>Vitamin B-2</td>
<td>-0.13</td>
<td>rbc glutathione reductase, activity coefficient</td>
</tr>
<tr>
<td>w/o supplements</td>
<td>0.21^2</td>
<td>rbc aspartic acid transaminase, activity coefficient</td>
</tr>
<tr>
<td>Vitamin B-6</td>
<td>-0.15</td>
<td>rbc aspartic acid transaminase, activity coefficient</td>
</tr>
<tr>
<td>w/o supplements</td>
<td>0.05</td>
<td>plasma B12</td>
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<tr>
<td>Vitamin B-12</td>
<td>0.36^2</td>
<td>serum folate</td>
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<tr>
<td>w/o supplements</td>
<td>0.61^2</td>
<td>folate</td>
</tr>
<tr>
<td>Folate</td>
<td>0.55–0.56^2</td>
<td>folate</td>
</tr>
<tr>
<td>w/o supplements</td>
<td>0.38–0.40^2</td>
<td>folate</td>
</tr>
</tbody>
</table>

1 All values, except those for rbc folate, adapted from Jacques et al. (14). Values based on energy-, age- and sex-adjusted intakes. Coefficients correlated with rbc folate adapted from Reference 15.

2 Significant correlation, \( p < 0.05 \).

However, there has been little or no attempt to cross-validate these estimates with dietary records or biochemical indices. Dietary assessment of choline intake is even more difficult because standards for the choline content of foods are not yet published by the USDA. Nevertheless individual research laboratories have published tables indicating the choline content of common foods (17).

### Conventional biochemical assessment of nutrient status

**Folate.** Historically the serum (or plasma) folate concentration and red blood cell (RBC) concentration of folate were the two most commonly used indicators of folate status. In the human, serum folate tends to be more a reflection of short-term folate balance during the preceding 1–2 d (18). The measurement of serum folate nevertheless has substantial predictive value in defining the risk of megaloblastic anemia as well as other indicators of systemic folate status, such as homocysteine levels (19). In contrast, the folate that is packaged into a developing blood cell (which is primarily a function of folate availability at the time of erythropoiesis) is no longer metabolized after maturation of that red cell occurs. Red cell folate concentration therefore represents an integration of dietary folate intake during the preceding 120 d, the half-life of a red cell. It is therefore not surprising that RBC folate tends to be a more accurate reflection of tissue folate status (18), because the latter is also largely determined by habitual intake over a prolonged period. Although normative values vary somewhat from laboratory to laboratory, two standard deviations below the mean for serum and RBC folate concentrations are typically 3 ng/mL and 160 ng/mL, respectively, and values less than that are associated with a markedly increased risk of developing megaloblastic anemia (18); serum folate concentrations of 3–5 ng/mL are considered to be marginally deficient. Serum and RBC folate continue to be important tools in the clinical evaluation of folate status although it is worth remembering that what we presently consider to be acceptable normative ranges may need to be modified in view of the growing consensus that subtle degrees of folate depletion of insufficient magnitude to cause megaloblastic anemia appear to have pathologic consequences.
Depletion of folate does not occur in a symmetric fashion among the various tissues of the body. Animal models of folate depletion clearly demonstrate that some tissues are more susceptible to depletion than others (20). Therefore, the measurement of the folate content in the tissue of interest may provide additional important information. Because methylated forms of folate generally participate in methylation reactions whereas nonmethylated forms play other physiologic roles, recent work suggests that measurement of the distribution of different coenzymatic forms of folates in a tissue has important meaning in terms of the functional capability of that tissue to carry out methylation reactions (21). Because of recent technological advances, the means of assessing the distribution of folate coenzymes is now feasible in small tissue samples such as biopsies (22).

**Vitamin B-12.** The serum cobalamin assay remains the conventional means of assessing vitamin B-12 status. Two standard deviations below the mean are typically found to be 150 pg/mL, and levels below that are clearly associated with a high risk of developing clinical manifestations of vitamin B-12 deficiency (23).

Some individuals develop clinical manifestations of vitamin B-12 deficiency even though their serum B-12 level is in the low-to-normal range, i.e., 150–400 pg/mL (24). It has been estimated that up to 5–15% of the elderly whose serum B-12 falls in this range are functionally deficient in vitamin B-12 (25). The measurement of serum methylmalonic acid (MMA) and homocysteine were shown to be effective means of detecting such subtle deficiencies although MMA is more specific because it does not also rise in folate deficiency (26). Nevertheless, confounding factors that may artificially alter MMA levels include renal insufficiency or hypervolemia (27) and concurrent use of antibiotics (28). Measuring levels of the serum transport protein, transcobalamin II bound to cobalamin, has been suggested as a sensitive means of detecting vitamin B-12 depletion (29) although it has not been explored as extensively as MMA and homocysteine.

**Vitamin B-6.** The major form of vitamin B-6 in the circulation is pyridoxal-5'-phosphate (PLP), the concentration of which is often used as an indicator of vitamin B-6 status. It is among the most sensitive measures of systemic vitamin B-6 status available although various reports suggest it may be influenced by factors such as physical exercise, pregnancy and the level of plasma alkaline phosphatase (30). Other measures of human vitamin B-6 status include erythrocyte PLP, total plasma B-6, urinary excretion of either total B-6 or a metabolite, 4-pyridoxic acid, or the activation coefficients of either of two erythrocyte enzymes dependent on vitamin B-6: alanine aminotransferase (ALT) and aspartate aminotransferase (AST). Among these, the urinary excretion of 4-pyridoxic acid or total B-6 appear to reflect recent dietary intake of the vitamin whereas the enzyme activity coefficients are reflective of long-term intake. In addition, the urinary excretion of xanthurenic acid after an oral tryptophan load is sometimes used as a functional indicator of vitamin B-6 status. Each of these measures of vitamin B-6 status was repeatedly shown to be a valid measure of B-6 status in closely controlled studies in healthy human volunteers (31, 32, 33). Under these conditions, xanthurenic acid excretion was observed to be the most sensitive indicator (31). However, experience with xanthurenic acid excretion indicates that it is easily affected by numerous confounding factors such as drugs and disease states and therefore its widespread application appears limited outside of carefully controlled studies.

**Vitamin B-2.** Riboflavin status is most commonly assessed by measuring flavin adenine dinucleotide (FAD)-dependent glutathione reductase activity in freshly lysed red blood cells before and after addition of FAD (34). This is another so-called “activation coefficient” assay because it assesses how much additional enzyme activity is gained by adding exogenous cofactor. Studies performed under well-controlled depletion and repletion conditions in healthy subjects (35), as well as those performed in large-scale nutrition surveys (36), indicate this is a sensitive measure of vitamin B-2 status.

One limitation of this assessment tool is that the results appear to be artifically altered in individuals with glucose-6-phosphate dehydrogenase deficiency (37), the most common form of which affects ~10% of the African-American population in the United States.

Total urinary riboflavin excretion is also used (35) as an indication of riboflavin status. It appears to be more indicative of recent riboflavin intake rather than bodily status of the vitamin, is not as sensitive as the EGR assay and the values obtained during depletion are not proportional to the magnitude of deficiency, as is true of the enzymatic EGR assay (35, 36). Plasma and whole blood concentrations of riboflavin are also used but do not appear to be as useful as the EGR assay and urinary riboflavin excretion.

**Selected biochemical parameters that have potential as functional measures of lipotrope status**

A functional measure of nutrient status is defined here as a parameter that reflects the integrity of a biochemical system or physiologic process that is dependent on the nutrient of interest.

**Serum or plasma homocysteine.** An elevation in the serum concentration of homocysteine is found to be a sensitive indicator of both folate and vitamin B-12 depletion because the remethylation of homocysteine to methionine is a B-12- and folate-dependent process (an alternative pathway exists, but only in the liver). Studies conducted under metabolic unit conditions indicate that serum homocysteine begins to rise perceptibly before the level of serum folate falls into a frankly deficient range (38). Similarly elevations in homocysteine are more sensitive in subtle vitamin B-12 deficiency than plasma B-12 alone; in the elderly, 5–10% of true vitamin B-12 deficiencies might be overlooked if plasma B-12 alone is used as a criteria, although in this setting both homocysteine and methylmalonic acid levels should be checked to facilitate the distinction between folate and vitamin B-12 deficiency (26, 28). Specificity is a concern in certain settings because chronic renal insufficiency, advancing age (especially >60 y), and chronic alcohol abuse also predictably cause a elevation, although if these factors are taken into account, serum homocysteine appears to be an excellent means of detecting even modest levels of folate or vitamin B-12 depletion. In a large population-based survey of healthy elderly, mean plasma homocysteine was observed to rise when habitual folate intake was <250 μg per d or when serum folate was <2.2 ng/mL (19). In the same study, a significant rise in plasma homocysteine was observed when plasma B-12 dropped below 190 pg/mL. Other factors that should be taken into account (39) include gender, because before menopause men have higher homocysteine levels than women, hypothyroidism (40), regular use of coffee (41), as well as the use of drugs such as folate antagonists and L-dopa.

In contrast to vitamin B-12 and folate, an elevation in fasting plasma homocysteine is not a reliable indicator of vitamin B-6 depletion because, in the vitamin B-6–deplete fasting state, homocysteine disposal occurs readily through the alternative remethylation pathway (42) (Fig. 1). In a nonfasting state, elevations in homocysteine become more re-
fective of defective vitamin B-6 status because the alternative pathway for homocysteine disposal is inhibited. Thus in large observational studies, nonfasting plasma homocysteine concentrations correlate inversely with dietary vitamin B-6 intake and plasma PLP levels quite well (19). Nevertheless, the accuracy with which a postprandial homocysteine concentration can diagnose vitamin B-6 depletion in an individual subject is not yet well defined. For the purposes of increasing the rigor of scientific studies, postprandial homocysteine concentrations are usually mimicked by instead administering a standardized oral dose of methionine (100 mg/kg) and then measuring the plasma homocysteine concentration 2–4 h after the methionine load (43).

S-adenosylmethionine (S-AdoMet) and S-adenosylhomocysteine (SAH). The concentrations of S-AdoMet and SAH are in large part determined by adequate availability of lipotropes and therefore they constitute functional reflections of lipotrope status. Of additional importance is the fact that S-AdoMet serves as the proximate methyl donor for nearly all the biochemical reactions that result in the methylation of DNA, RNA, phospholipids, proteins and other macromolecules (Fig. 1). Increased tissue concentrations of S-AdoMet, as well as decreased concentrations of SAH generally correlate with the extent to which these macromolecular methyl acceptors becomes methylated (7) although it is important to note that perturbation of the S-AdoMet:SAH ratio is not invariably reflected in altered methylation of macromolecules (44). The measurement of S-AdoMet and SAH therefore has added potential as a functional indicator of methylation capability.

Many studies in animals indicate that folate, methionine, choline or vitamin B-12 deficiencies each promptly lead to diminished levels of hepatic S-AdoMet, often to rises in hepatic SAH, and usually to a significant decrease in the SAM:SAH ratio (44, 45, 46). SAH tends to rise in any situation where homocysteine levels are increased because the hydrolysis of SAH to homocysteine is reversible and thermodynamically favors the reverse reaction (Fig. 1).

Despite the attractive nature of using S-AdoMet and SAH as indicators of lipotrope status and methylation capacity, one must keep in mind that the measurement of these metabolites in a single tissue does not necessarily mean that this measurement is representative of the state of affairs in every tissue. For instance, in one rodent study of folate depletion no concurrent alterations in S-AdoMet and SAH occurred in the colonic mucosa as was observed in the liver (45).

There is only a small amount of data defining how S-AdoMet and SAH concentrations in humans are altered by lipotrope deficiencies, largely because there was no way to reliably measure S-AdoMet and SAH in the plasma until very recently. However, there is now a published HPLC method with coulometric detection for measuring S-AdoMet and SAH in plasma (47). In healthy controls, elevations in plasma SAH are observed to be associated with higher homocysteine concentrations and low plasma pyridoxal-5'-phosphate (47, 48). Nevertheless, no one has yet quantitatively defined the relationships between plasma S-AdoMet, SAH and the lipotrope nutrients in any detail. However, the use of plasma SAH in particular looks promising as a biomarker because the existing data shows excellent correlations between it and other presumptive functional markers of lipotrope status. For example, the correlation between plasma homocysteine and plasma SAH is reported to be quite strong ($r = 0.73, p < 0.001$). Plasma SAH correlates inversely with genomic DNA methylation in blood lymphocytes in an equally strong fashion ($r = -0.74, p < 0.001$), consistent with the concept that SAH is an inhibitor of most methylation reactions. In contrast, plasma S-AdoMet has no significant correlation with either plasma homocysteine or DNA methylation, although the existing data also indicates that plasma S-AdoMet:SAH ratios are diminished in individuals with elevated homocysteine levels (48). These observations are entirely consistent with the known determinants of homocysteine and SAH levels, as discussed earlier in this review. Thus initial data suggests that plasma SAH as well as the S-AdoMet:SAH ratio in humans may provide meaningful data about lipotrope status, although considerable work needs to be performed to fully define the qualitative and quantitative nature of the relationship.

Genomic DNA methylation. Approximately 4% of the cytosine residues in the dinucleotide sequence, CCG, are methylated in the human genome.

The patterns of methylation are highly conserved after each replication of a cell, a function performed by specific DNA methyltransferases that utilize S-AdoMet as a methyl donor. The stringent degree of conservation is important because these patterns serve several important functions, including the control of gene transcription and genetic stability. A detailed discussion of these functions of DNA methylation is beyond the scope of this discussion, but is reviewed elsewhere (49). It is particularly pertinent that aberrations of DNA methylation are essentially a universal phenomenon for most cancers. Genomic DNA hypomethylation is generally observed concurrent with selected loci (often within the promoter regions of genes) of hypermethylation. Mechanistically, it was proposed that hypomethylation within the coding region of a gene might create DNA instability and mutations, that hypomethylation in the promoter region of a protooncogene could cause inappropriate overexpression of that gene whereas hypermethylation in a promoter region could repress the expression of important tumor suppressor genes (49).

A combined deficiency of methionine, choline, folate and vitamin B-12 (50), or a marked deficiency of folate alone (51), are shown to induce genomic DNA hypomethylation in laboratory rodents. However, a more moderate degree of folate depletion, even when maintained for several months does not appear to be sufficient to induce genomic hypomethylation (44). Interestingly, in a rodent experiment, multiple lipotrope deficient diets have been shown initially to create loci-specific hypomethylation, which later evolve into loci of hypermethylation, probably as a compensatory response (52). Thus lipotrope depletion appears to be capable of creating paradoxical hypermethylation at selected loci, at least in animal models.

Genomic DNA methylation is emerging as a sensitive biomarker of dietary folate intake. Two studies in healthy human volunteers independently demonstrated that a folate-deplete diet containing ~50–120 µg of folate per d for 7–9 wk was sufficient to induce genomic DNA hypomethylation of blood mononuclear cells and that folate repletion over several weeks caused the hypomethylation to return toward normal (38, 53). Of considerable import is the observation that DNA hypomethylation evolved in both studies even though mean plasma folate levels never dropped below the normal range and anemia did not emerge, suggesting that DNA methylation is a rather sensitive tool for the identification of folate depletion. Nevertheless as suggested by Figure 2, rises in plasma homocysteine appear a bit earlier than the evolution of DNA hypomethylation. As mentioned earlier in this review, it is now evident that the ability of low folate status to diminish genomic DNA methylation is dependent on interactions with specific genotypic variants, such as the MTHFR 67T (C to T) polymorphism (10, 11). In these studies the combination of the TT genotype and low folate status was the necessary condition to produce genomic hypomethylation; either condition alone was
values are means ± SEM. The mean values for plasma folate and homocysteine at the end of each diet phase were different from those values at day 5 (p < 0.05) or §, compared to day 84. The § in the upper panel indicates the mean methylation at day 90 was different from day 69, p < 0.05). Dashed line indicates lower limit of reference range for plasma folate, 6.8 nmol/L (3.1 ng/mL). From Jacob et al. (38) with permission.

FIGURE 2 DNA methylacceptance, which is an inverse measure of DNA methylation (top panel) and plasma folate and homocysteine (bottom panel) in eight healthy women who were subjected to folate depletion (days 6–69) followed by folate repletion (days 70–91). Plotted values are means ± SEM. The mean values for plasma folate and homocysteine at the end of each diet phase were different from those values at day 5 (* = p < 0.05) or §, compared to day 84. The § in the upper panel indicates the mean methylation at day 90 was different from day 69, p < 0.05). Dashed line indicates lower limit of reference range for plasma folate, 6.8 nmol/L (3.1 ng/mL). From Jacob et al. (38) with permission.

insufficient to induce hypomethylation. Thus a relationship between folate depletion and DNA methylation might only become apparent when genetic or metabolic states impose additional limitations to the methylation system.

In the past genomic methylation was usually measured by enzymatic, semiquantitative methods that were prone to relatively large inter- and intraassay variations. A new liquid chromatography/mass spectrometry method has now been described, which is quantitative in nature (i.e., it measures ng of methylcytosine/ug of DNA). It has a high degree of precision: intra- and interday relative standard deviations vary from 5–7% (11, 54).

The measurement of genomic DNA methylation, like the measurement of SAH, holds promise as a functional biomarker of lipotrope status. Both parameters are particularly attractive because they may be useful as integrated measures of the status of multiple lipotropes. Nevertheless, utilizing either of these parameters must be done with the recognition that they are affected by factors other than lipotrope availability. Furthermore, much work must be done to more fully understand the precise quantitative nature of the relationships between these biomarkers and lipotrope status.

Uracil misincorporation into DNA. Folate is a necessary coenzyme for the de novo synthesis of thymidine from its pre-cursor, uridine. Folate depletion alters the cellular pool of these two nucleosides to favor the latter compound although only the former is normally incorporated into DNA. However, cell culture studies indicate that inhibition of folate metabolism leads to inappropriate insertion of uridine where thymidine should be because of the promiscuous nature of some of the DNA polymerases. A single report in humans (55) suggests that frank folate deficiency leads to excessive uridine (more commonly referred to by its parent base, uracil) incorporation into DNA, a phenomenon that was observed to reverse with vitamin repletion. Although this might prove to be an effective biomarker of folate depletion in the future, there must be some confirmation of the original report for this concept to move forward. Unlike plasma S-AdoMet/SAH levels and DNA methylation, uracil misincorporation should be perceived only as a potential candidate for the functional assessment of folate status.

SUMMARY AND FUTURE DIRECTIONS

Food frequency questionnaires are moderately good tools for assessing chronic exposure to those nutrients integrally involved in one-carbon metabolism. It is worth noting, however, that chronic nutrient exposure does not necessarily predict nutrient status; this seems particularly true for vitamins B-2 (riboflavin), B-6 and B-12. The nutrient status for the B-vitamins associated with this pathway (folate, vitamin B-12, vitamin B-6 and riboflavin) can be assessed by conventional biochemical tests with moderate degrees of accuracy. Unfortunately, comparable assays for methionine and choline status are not yet available.

Given the renewed interest in lipotrope depletion, and particularly the interest focused on subtle degrees of depletion that produce disease states, a great deal of exciting possibilities exist for functional tests of nutrient status that are sensitive to very mild degrees of depletion. Plasma homocysteine, plasma S-AdoMet/SAH and DNA methylation are three such promising candidates, although as “downstream” indicators of nutrient status, they are influenced by factors other than the nutrient status. The data on uracil misincorporation in DNA as a functional indicator of folate status is quite preliminary at this point and must be confirmed before its further development as a biomarker.

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