Homocysteine and methylmalonic acid in diagnosis and risk assessment from infancy to adolescence\textsuperscript{1-3}

Anne Lise Bjørke Monsen and Per Magne Ueland

ABSTRACT

The concentration of total homocysteine (tHcy) in serum and plasma is elevated in both folate and cobalamin deficiencies, whereas methylmalonic acid (MMA) in serum, plasma, or urine is a specific marker of cobalamin function. The combined measurement of both metabolites is useful for the diagnosis and follow-up of these deficiency states. In addition, tHcy is elevated under various pathologic states (eg, renal failure), and hyperhomocysteinemia is associated with an increased risk of cardiovascular disease, cognitive dysfunction, and adverse pregnancy outcomes. The diagnostic utility of tHcy and MMA concentrations as markers of folate and cobalamin deficiencies in healthy and diseased children has been documented. This article briefly summarizes the biochemical background of tHcy and MMA and the associations of tHcy and MMA with various disease states and focuses on novel data obtained in infants, children, and adolescents, with emphasis on cobalamin status in infants. The utility of tHcy and MMA as indicators of cobalamin and folate deficiencies in adults can be extended to infants and older children. Furthermore, as in adults, tHcy is related to unhealthy lifestyle factors and is a risk factor for vascular disease. High MMA concentrations in newborns, occasionally denoted as benign methylmalonic aciduria, may reflect impaired cobalamin function. Am J Clin Nutr 2003;78:7–21.

KEY WORDS  Lifestyle, cardiovascular disease, neurologic disorders, folate deficiency, cobalamin deficiency

INTRODUCTION

In adults, total homocysteine (tHcy) and methylmalonic acid (MMA) in serum or plasma are sensitive markers of cobalamin status and are used for the diagnosis and follow-up of cobalamin deficiency (1–3). tHcy is also elevated in folate deficiency and is used as an indicator of this deficiency state (2, 4).

MMA is a sensitive but specific marker of cobalamin function, because—apart from cobalamin deficiency—renal impairment and rare inborn errors affecting methylmalonate-CoA mutase activity are the only known conditions causing markedly elevated concentrations of MMA (5). In contrast, tHcy is elevated in both folate and cobalamin deficiencies and also in pathologic states such as renal failure, thyroid dysfunction, heart transplantation, and the acute phase after a cardiovascular event (6). In addition, the tHcy concentration is influenced by a diversity of genetic and acquired factors and by interactions between such factors (6). The most prevalent genetic cause of hyperhomocysteinemia is the 677C–&gt;T polymorphism of the methylenetetrahydrofolate reductase (MTHFR) gene, which predisposes to hyperhomocysteinemia under conditions of impaired folate status. Several drugs may influence the tHcy concentration by acting as vitamin antagonists (7). Of the physiologic and lifestyle determinants, increasing age, male sex, poor nutrition with low vitamin intake, smoking, and heavy coffee consumption cause high tHcy concentrations, whereas young age, premenopausal state, pregnancy, B vitamin intake, and exercise are associated with low tHcy concentrations (6). Thus, tHcy shows a remarkable association with numerous physiologic and lifestyle factors, which often parallel their negative effect on health.

Whereas MMA is regarded as a sole marker of cobalamin deficiency, there is an increasing body of evidence that a high tHcy concentration may itself be hazardous by predisposing to occlusive vascular disease, cognitive dysfunction, adverse pregnancy outcomes, and malformations (8–11). Therefore, strategies to reduce modifiable factors predisposing to hyperhomocysteinemia may have a health-promoting effect.

Most data on the diagnostic utility of tHcy and MMA and the health effects of hyperhomocysteinemia are based on clinical and epidemiologic studies in adult, middle-aged, and elderly populations (9). However, in past years, several studies on the clinical utility of tHcy and MMA measurements in newborns and children have been published. These studies are largely motivated by the recognized importance of cobalamin and folate status for growth and development and by the growing evidence that cobalamin deficiency in infancy and childhood may cause irreversible neurologic damage (12–15). Furthermore, it is conceivable that elevated homocysteine (Hcy) in blood and tissues is associated with organ dysfunction in infants that may lead to disease later in life.

This article reviews pediatric literature on the normal concentrations and determinants of tHcy, the diagnostic utility of tHcy and MMA, and the possible pathogenic and predictive role of hyperhomocysteinemia in relation to disease. The role of tHcy and MMA as markers of the inborn errors of Hcy (16) or cobalamin metabolism (17, 18) and methylmalonic acidurias (19) will only briefly be mentioned, because these topics were comprehensively reviewed elsewhere.

\textsuperscript{1}From the Department of Pediatrics (ALBM) and the LOCUS for Homocysteine and Related Vitamins (PMU), University of Bergen, Norway.

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\textsuperscript{3}Address reprint requests to PM Ueland, Clinical Pharmacology Unit, Central Laboratory, 5021 Haukeland Hospital, Bergen, Norway. E-mail: per.ueland@ikk.uib.no.

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AdoMet Met
N,N-dimethylglycine
Ado
glycine
FIGURE 1. Homocysteine (Hcy) formation, remethylation, and transsulfuration and the enzymes and B vitamins involved in these processes. Hcy is formed from S-adenosylhomocysteine (AdoHcy). Remethylation to methionine (Met), in most tissues, is catalyzed by the ubiquitous methionine synthase (MS), which requires cobalamin (B-12) as cofactor and 5-methyltetrahydrofolate (CH₃THF) as substrate. CH₃THF is formed by the action of the flavin adenine dinucleotide-dependent enzyme methylenetetrahydrofolate reductase (MTHFR), which thus resides at a critical metabolic locus directing the folate pool to Hcy remethylation at the expense of folate used for DNA and RNA biosynthesis.

Ado, adenosine; AdoMet, S-adenosylmethionine; BHMT, betaine homocysteine methyltransferase; CBS, cystathionine β-synthase; CH₂THF, 5,10-methylenetetrahydrofolate; CHOTHF, formyltetrahydrofolate; CHTHF, methylenetetrahydrofolate; CL, cystathionine lyase; Cys, cysteine; DHF, dihydrofolate; DHFR, dihydrofolate reductase; dTMP, deoxythymidine monophosphate; dUMP, deoxyuridine monophosphate; MT, methyltransferase; R, methyl acceptor; SAHH, S-adenosylhomocysteine hydrolase; THF, tetrahydrofolate; TS, thymidylate synthase. Modified from reference 7.

BASIC AND CLINICAL BIOCHEMISTRY

Homocysteine

Hcy is formed from methionine during S-adenosylmethionine-dependent methylation reactions (Figure 1). Its further metabolism is dependent on several B vitamins. Hcy may be salvaged to methionine, and this reaction is catalyzed in most tissues by the ubiquitous enzyme methionine synthase (5-methyltetrahydrofolate homocysteine methyltransferase; EC 2.1.1.13), which requires 5-methyltetrahydrofolate as cosubstrate and cobalamin as cofactor (Figure 2). Alternatively, during methionine excess, superfluous Hcy is directed into the transsulfuration pathway, which converts Hcy to cysteine, catalyzed by 2 sequential vitamin B-6-dependent reactions. The cystathionine β-synthase reaction is the rate-limiting step in this sequence (20). These biochemical pathways are depicted in Figure 1.

Folate or cobalamin deficiency impairs remethylation of Hcy, which is exported into the extracellular compartment, including plasma (21, 22). This explains why fasting tHcy becomes a sensitive marker of intracellular folate or cobalamin status (1). The tHcy response obtained post methionine load (PML tHcy) is also influenced by folate status (23), but vitamin B-6 status is a major determinant of PML tHcy (23–25). The latter variable is used mostly for research purposes (25) and is particularly impractical in infants.

Blood sampling and processing are important in obtaining reliable tHcy values (4). The release of Hcy from the blood cells is time- and temperature-dependent, which in adults causes an increase in serum or plasma tHcy by ≈10%/h at room temperature. Therefore, it is recommended that blood samples are put on ice before centrifugation or that the plasma or serum fraction is separated from the blood cells within 30 min (4). Alternative strategies to avoid the artificial increase in tHcy have been reported, including blood sampling into acidic citrate (26), sodium fluoride (27), or 3-deazaadenosine (28). Plasma tHcy in placental and neonatal blood samples was recently shown to be stable for 24 h at 4°C in acidic citrate (29).

Prevention of an artificial increase in plasma tHcy is paramount in research settings that establish reference values or investigate associations between tHcy and disease. The release of Hcy from blood cells has a minor influence on the high tHcy concentrations observed in inborn errors of Hcy metabolism (16) or cobalamin deficiency (18).

In serum or plasma, Hcy exists in several interchangeable forms, among which protein-bound Hcy is the predominant species, followed by low-molecular-weight mixed disulfides, whereas only trace amount of the reduced thiol has been detected.
These Hcy forms are collectively measured as tHcy after quantitative reduction (30). The quantitative relation between different Hcy species in normal plasma and recommended nomenclature (31) are summarized in Figure 3.

A plethora of methods for tHcy has been published (4, 32). A key feature in the pediatric setting is the volume requirement, which should be < 100 μL. Assay specifications and performance of various assays are compared in review articles (4, 32).

**Methylmalonic acid**

MMA in serum, plasma, and urine is derived from the hydrolysis of o-methylmalonyl-CoA (MMA-CoA), which is a metabolic intermediate in the conversion of propionic acid to succinic acid (1). This hydrolytic reaction is catalyzed by o-methylmalonyl-CoA hydrolase (EC 3.1.2.17). The conversion to succinyl-CoA is an alternative metabolic route of MMA-CoA, catalyzed by the sequential actions of D,L-methylmalonyl-CoA racemase (EC 5.1.99.1) and L-methylmalonyl-CoA mutase (EC 5.4.99.2). The latter enzyme requires adenosylcobalamin as cofactor (Figure 4). Thus, impaired cobalamin function causes an increased concentration of extracellular MMA (33), which thereby becomes a sensitive and specific marker of cobalamin status (1).

The development of specific assays for MMA in urine, serum, or plasma has made MMA available for the diagnosis of cobalamin deficiency and its differentiation from folate deficiency. In adults, serum and urinary MMA concentrations > 0.4 μmol/L (1) and 3.2 mmol/mol creatinine (34), respectively, are early indicators of cobalamin deficiency. In infants, a cutoff for MMA excretion of 20–23 mmol/mol creatinine is recommended for indicating cobalamin deficiency (35, 36), which is a higher cutoff than used.
for adults and older children. In addition, the mean (± SD) plasma MMA concentration in newborns (0.36 ± 0.26 μmol/L) (37) is higher than that measured in adults. The measurement of MMA has been advocated when serum or plasma samples are required for the determination of complementary variables such as cobalamin and tHcy (34). However, serum MMA is not a specific marker of cobalamin status because the concentration is elevated in renal failure (38), in thyroid disease, in small-bowel bacterial overgrowth, and in conditions of hemococoncentration (39). If serum MMA is not falsely high, there is a high correlation with urinary MMA (r = 0.98) (40). Urinary MMA, however, is normalized to creatinine concentrations and thereby corrected for renal insufficiency and dehydration. Furthermore, the measurement of urinary MMA is noninvasive and requires only a random urine specimen, which may be advantageous in the routine pediatric setting (39).

There is one preliminary report suggesting that MMA excretion into urine increases in infants (aged 5 d to 16 mo) during periods of apnea (36). Elevated MMA excretion in these infants may be related to inhibition of MMA-CoA catabolism under conditions of tissue hypoxia and respiratory acidosis (36).

No special precautions are required for blood sampling and storage of MMA, which is stable in blood, serum, and plasma at room temperature. MMA is measured with laborious chromatographic techniques (34, 41–46), which require expensive equipment and trained personnel.

**FIGURE 4.** The role of the cobalamin-dependent enzyme L-methylmalonyl-CoA mutase in the metabolism of methylmalonic acid. Adenosyl-Cbl, adenosylcobalamin; MCH, D-methylmalonyl-CoA hydratase; MCM, L-methylmalonyl-CoA mutase; MCR, D,L-methylmalonyl-CoA racemase.

**PHYSIOLOGIC, GENETIC, AND LIFESTYLE DETERMINANTS OF HOMOCYSTEINE**

tHcy concentrations in children from birth to adolescence have been published, and most tHcy determinants established in adults (6) have been investigated in children.

**Physiologic and genetic factors**

The mean tHcy concentration is ~4–8 μmol/L in children aged <15 y, which is ~60% of the values detected in adults (47–50). tHcy increases as a function of age (50, 51). Some (52–54) but not all (49, 50, 55–58) studies have shown a slightly higher tHcy concentration in boys than in girls, and this sex effect is enhanced during and after puberty (>15 y) (56, 59) (Table 1). Conceivably, inconsistency regarding a sex effect could be related to differences in vitamin and nutritional status and ethnicity between the study populations. The small sex effect might become more apparent in large B vitamin-replete populations (52, 53).

As in adults, a higher tHcy concentration in boys than in girls, but also the age effect, could be explained by increases in muscle mass according to age and male sex. This contention is supported by a positive relation between tHcy and creatinine in healthy children without renal dysfunction (49, 58).

tHcy concentrations vary according to ethnicity and are higher in black than in white (67) or Hispanic (52) children. Notably, the effect of the MTHFR 677C→T polymorphism on tHcy and folate status reported in adults, characterized by a 25% higher tHcy and lower serum folate concentration in homozygous TT than in CC subjects (7), was shown in 92 children with familial hypercholesterolemia aged 6–11 y (71), in 64 healthy children aged ≥10 y, but not in 63 younger children (51). Likewise, there are consistent reports of no effects from the MTHFR 677C→T and 1298A→C polymorphisms on plasma tHcy in newborns (61, 64).

**B vitamin status according to age**

The effects of B vitamins on plasma tHcy in children are similar to those previously shown in adults. In children aged ≥24 mo, plasma tHcy correlated strongly with serum folate and less so with serum cobalamin (49, 51, 52, 56, 59); of the B vitamins, vitamin B-6 had the weakest effect (50, 52) or no effect (72). Accordingly, tHcy was lower in multivitamin users than in nonusers (52). As in adults, smoking was associated with elevated tHcy (50, 52, 56), which may partly be attributable to impaired vitamin status (52) and poor nutrition (73) in smokers.

The associations between tHcy and B vitamin concentrations are different in newborns and infants as compared with children aged >1 y (Table 1). In the first year of life, plasma tHcy shows a remarkable strong correlation with serum cobalamin (29, 37, 60, 61), whereas the relation to circulating folate is weaker (29, 60) or absent (37, 61). Furthermore, there are consistent reports of mean tHcy concentrations in newborns and infants of 6–9 μmol/L (29, 37, 60, 61), which is higher than the concentrations of 5–6 μmol/L (50, 51, 56, 57, 68) often seen in children 1–10 y of age. tHcy concentrations according to age and B vitamin status are summarized in Table 1.
HOMOCYSTEINE AND METHYLMALONIC ACID

HOMOCYSTEINE IN DISEASES OTHER THAN VITAMIN DEFICIENCIES

Cardiovascular disease

Episodes of cardiovascular disease in children are rare, ≈2/100 000 persons annually. In ≈30% of the cases, no causative factor or underlying disease was identified (88). A few reports published during the past 5 y suggest that an elevated tHcy concentration is a risk factor of vascular disease and stroke in children.

Elevated plasma tHcy in children or young adults is associated with premature (<55 y of age) cardiovascular disease in male relatives (49) or parents (68, 89) and was detected in children with parents or grandparents with occlusive artery disease (90, 91). In children with familial hypercholesterolemia, tHcy was significantly higher in those with than in those without parental cardiovascular disease (59, 92). These observations indicate that the familial risk of cardiovascular disease is partly explained by elevated tHcy concentrations or may reflect an environmental effect on both cardiovascular disease risk and tHcy concentration. Furthermore, in hypercholesterolemic children with a Familial burden of cardiovascular disease, further risk enhancement is of particular concern, and a reduction in tHcy concentrations by vitamin supplementation should be considered.

tHcy has been associated with stroke (69, 93–95) and also with venous thrombosis (96) in newborns and children. Because of the low incidence of these events, the study populations are small (≈400 cases have been studied to date), but the relations in most studies are remarkably strong, corresponding to odds ratios > 4 (69, 94, 96). In one study of 118 children, the tHcy concentration was not related to cerebrovascular disease or stroke, but children with the homozygous MTHFR TT genotype had elevated tHcy concentrations and a significantly enhanced risk of a primary and recurrent episode (97). Likewise, 2 studies including > 140 cases each showed that the TT and CT genotypes are associated with an increased risk of stroke (93) or venous thrombosis (96), whereas smaller studies (74, 98–101) provided inconsistent results. Thus, the published data suggest that the tHcy concentration is a strong risk factor for occlusive vascular disease in children. A possible significant relation between the MTHFR 677C>T polymorphism and cardiovascular disease risk in children, as opposed to adults (102) requires substantiation.

Renal disease

Hyperhomocysteinemia is common in adult renal transplant recipients, in patients with chronic renal failure, and occurs almost uniformly in patients with end-stage renal failure. The tHcy concentration can be reduced but is usually not normalized by high doses of folic acid. This may be clinically important because tHcy
TABLE 1

Total homocysteine (tHcy) in plasma or serum of healthy children according to sex, age, and B vitamin status

<table>
<thead>
<tr>
<th>Reference and year</th>
<th>Sex</th>
<th>Age</th>
<th>No. of subjects</th>
<th>tHcy</th>
<th>Folate</th>
<th>Cobalamin</th>
<th>Comments</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td></td>
<td></td>
<td>( \mu \text{mol/L} )</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Infants aged &lt; 1 y</td>
<td></td>
<td></td>
<td></td>
<td>( r_p )</td>
<td>( r_s )</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Minet et al, 2000 (60)</td>
<td>M+F</td>
<td>5 (1-28) d</td>
<td>123</td>
<td>7.8 ± 3.1</td>
<td>( r_p = -0.30 )</td>
<td>( r_p = -0.64 )</td>
<td>Breastfed</td>
</tr>
<tr>
<td></td>
<td>M+F</td>
<td>12 (4-20) wk</td>
<td>30</td>
<td>10.4 ± 3.4</td>
<td>( r_p = 0.04 )</td>
<td>( r_p = 0.24 )</td>
<td>Increase with age</td>
</tr>
<tr>
<td>Bjarke-Monsen et al, 2001 (37)</td>
<td>M+F</td>
<td>4 d</td>
<td>173</td>
<td>6.2 (5.0-7.5)</td>
<td>( r_p = -0.06 )</td>
<td>( r_p = -0.52 )</td>
<td>Formula-fed</td>
</tr>
<tr>
<td>Guerra-Shinohara et al, 2002 (29)</td>
<td>M+F</td>
<td>6 wk</td>
<td>45</td>
<td>7.4 (6.5-8.9)</td>
<td>( r_p = -0.24 )</td>
<td>( r_p = -0.53 )</td>
<td></td>
</tr>
<tr>
<td>Molloy et al, 2002 (61)</td>
<td>M+F</td>
<td>0 d</td>
<td>69</td>
<td>6.6 ± 2.8</td>
<td>( r_p = -0.22 )</td>
<td>( r_p = -0.29 )</td>
<td></td>
</tr>
<tr>
<td>Bartesaghi et al, 2001 (62)</td>
<td>M+F</td>
<td>4 d</td>
<td>1400</td>
<td>4.9 ± 2.7</td>
<td>( r_p = 0.36 )</td>
<td>( r_p = -0.21 )</td>
<td>Formula-fed</td>
</tr>
<tr>
<td>Fokkema et al, 2002 (63)</td>
<td>M+F</td>
<td>40 d</td>
<td>53</td>
<td>7.4 ± 1.6</td>
<td>( r_p = 0.36 )</td>
<td>( r_p = -0.21 )</td>
<td></td>
</tr>
<tr>
<td></td>
<td>M+F</td>
<td>40 d</td>
<td>15</td>
<td>9.1 ± 2.4</td>
<td>( r_p = 0.36 )</td>
<td>( r_p = -0.21 )</td>
<td></td>
</tr>
<tr>
<td>Infante-Rivard et al, 2002 (64)</td>
<td>M+F</td>
<td>0 d</td>
<td>438</td>
<td>5.1 (4.9-5.2)</td>
<td>( r_p = 0.36 )</td>
<td>( r_p = -0.21 )</td>
<td></td>
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</tbody>
</table>

Children aged > 1 y

<table>
<thead>
<tr>
<th>Reference and year</th>
<th>Sex</th>
<th>Age</th>
<th>No. of subjects</th>
<th>tHcy</th>
<th>Folate</th>
<th>Cobalamin</th>
<th>Comments</th>
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<tr>
<td></td>
<td></td>
<td></td>
<td></td>
<td>( \mu \text{mol/L} )</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Rogers et al, 1999 (53)</td>
<td>M+F</td>
<td>15-19 Y</td>
<td>87</td>
<td>9.8 (6.7-14.3)</td>
<td>( r_p = 0.20 )</td>
<td>( r_p = 0.30 )</td>
<td>Increase with age</td>
</tr>
<tr>
<td>Jacques et al, 1999 (53)</td>
<td>M+F</td>
<td>12-15 Y</td>
<td>347</td>
<td>6.6 (5.5-8.0)</td>
<td>( r_p = 0.20 )</td>
<td>( r_p = 0.30 )</td>
<td>Increase with age</td>
</tr>
<tr>
<td>van Beynum et al, 1999 (69)</td>
<td>M+F</td>
<td>8.6 (0-19.3)</td>
<td>234</td>
<td>9.1 (4.3-20.0)</td>
<td>( r_p = 0.20 )</td>
<td>( r_p = 0.30 )</td>
<td>Adequate cobalamin status</td>
</tr>
<tr>
<td>Rogers et al, 2002 (70)</td>
<td>M+F</td>
<td>8-12 Y</td>
<td>371</td>
<td>8.8 ± 1.9</td>
<td>( r_p = 0.20 )</td>
<td>( r_p = 0.30 )</td>
<td></td>
</tr>
</tbody>
</table>

(Continued)
concentrations predict both cardiovascular disease morbidity and mortality in renal patients (103).

As in adults with renal disease, tHcy concentrations are markedly elevated in pediatric patients with chronic renal failure, including patients receiving hemo- or peritoneal dialysis, and in pediatric renal transplant recipients (104, 105). The tHcy concentration was closely related to measures of impaired renal function (high serum creatinine or low glomerular filtration rate), increased with age (104, 105), and was slightly higher in renal patients with the MTHFR T allele than in those with the CC genotype (65, 106). In children with chronic renal failure who were not routinely supplemented with folic acid, folate deficiency was common (=60%). The main determinants of hyperhomocysteinemia, in addition to renal function, were folate and cobalamin status (65). Hyperhomocysteinemia was responsive to high-dose folic acid (104, 106-108). The additional tHcy-lowering effect resulting from high-dose cobalamin supplementation in adult renal patients (109) has not been documented in children.

High-dose folic acid supplementation and the accompanying reduction in tHcy concentrations is associated with improved flow-mediated dilatation in children with chronic renal failure, which contrasts with the lack of such an effect in adult renal patients (110). This finding suggests that folic acid therapy may decrease cardiovascular disease morbidity and mortality in children with renal failure.

**Diabetes**

In adults with diabetes, tHcy concentrations are moderately elevated in those with microangiopathy, and the observed hyperhomocysteinemia is assumed to result from the diabetic nephroplesis (111). Likewise, in children who have had type 1 diabetes for >10 y and who have diabetic complications, tHcy is moderately elevated relative to concentrations in control subjects (112).

Insulin by itself actually reduces tHcy concentrations, and tHcy is below normal in early diabetes, probably because of glomerular hyperfiltration (111). In diabetic children, vascular sequelae and nephropathy may not have developed, and one may expect normal or low tHcy concentrations in these patients. This is actually what is observed in children (and adolescents) with type 1 diabetes (66, 112-115). Both fasting and PML tHcy concentrations are significantly lower and serum folate and cobalamin concentrations moderately higher than in aged-matched control subjects; however, B vitamin status may only partly account for the low tHcy concentration (66).

Low tHcy concentrations in children with early type I diabetes suggest that the diabetic angiopathy is not attributable to hyperhomocysteinemia. However, endothelial dysfunction in such patients, measured both as flow-mediated vasodilation and by markers of endothelial activation, is closely related to the concentrations of circulating folate (116). This observation motivates intervention trials to assess whether folate improves endothelial function in children with diabetes.

**Neural tube defects**

Folate deficiency is an established risk factor for neural tube defects (NTDs), and periconceptional folate supplementation reduces the recurrence and occurrence of NTDs. This is consistent with the observation of mildly elevated tHcy concentrations in mothers of children with NTDs. The search for genetic defects explaining impaired folate metabolism has mainly focused on the MTHFR 677C→T polymorphism, and most studies have shown that the TT genotype in mothers (but not fathers) and their infants confers an increased risk of NTDs. Maternal folate status and NTDs is the subject of several comprehensive review articles (10, 117-119).

Assessment of Hcy and folate status in children with NTDs has been carried out mainly to explore the possible involvement of fetal folate metabolism in the development of NTDs. Some (120-122), but not all (123, 124), studies have shown elevated tHcy concentrations in children with NTDs. In one study, elevated tHcy concentrations were confined to children with the MTHFR T allele (120). One limitation of most studies on Hcy in children with NTDs is the lack of data on kidney function, which is occasionally compromised in these patients (125).

**Down syndrome**

Folate and Hcy status and related polymorphisms have been considered as maternal risk factors for Down syndrome (126). One study showed elevated tHcy concentrations and an increased prevalence of the MTHFR T allele in mothers of children with Down syndrome (127). This observation could not be confirmed in other studies (128), including one from Italy (129). Furthermore, the prevalence of the T allele but not Down syndrome is higher in Italy than in other European countries (129). However, ethnicity, variations in folate status, and interaction with other genetic polymorphisms related to folate and Hcy status may explain the observed differences. An example is the 66A→G
polymorphism of the methionine synthase reductase (MTRR) gene. The GG MTRR variant alone or in combination with the MTHFR TT/CT genotypes confers substantial risk enhancement (128, 130). These preliminary findings should motivate larger studies of the interactions between nutrition and genetics affecting folate and homocysteine metabolism as risk factors for Down syndrome.

Low fasting and PML tHcy concentrations in children with Down syndrome were shown 15 y ago by Chadeaux et al (131) and was attributed to the increased gene dosage and activity of cystathionine-β-synthase, which is located on chromosome 21. Low fasting tHcy concentrations were recently confirmed in 42 patients with a mean age of 7.4 y, and the metabolic profile (low methionine, S-adenosylhomocysteine, and S-adenosylmethionine concentrations and elevated cystathionine and cysteine concentrations) detected in the patients with Down syndrome is in line with increased Hcy degradation through the transsulfuration pathway (132). It has been speculated whether increased Hcy degradation explains the low frequency of atherosclerosis in patients with Down syndrome (133). Furthermore, a low Hcy concentration may also decrease the methionine synthase reaction, which in turn may trap 5-methyltetrahydrofolate and thereby impair folate function. Some clinical features of patients with Down syndrome could be related to functional folate deficiency. These features include enhanced methotrexate sensitivity (134), elevated mean corpuscular volume, and gastrointestinal malabsorption (132). Impaired folate function may explain deoxynucleotide-pool imbalance and elevations in folate-sensitive fragile sites and DNA strand breaks. These lesions may be related to the high incidence of leukemia observed in patients with Down syndrome (132). Conceivably, an impaired S-adenosylmethionine–dependent transmethylation reaction may have a diversity of effects, including dysfunction of the central nervous system.

FOLIC ACID FORTIFICATION

In the United States, fortification of grain products was issued in March 1996, with an effective date of 1 January 1998 (135). An investigation of a US population showed a reduction in tHcy concentrations and an elevation in serum folate in blood collected after (September 1997 to March 1998) compared with before fortification (136). In Canada, folic acid fortification was fully implemented in January 1998, and a mandatory program was introduced in November 1998 (137). An increase in blood cell folate in the period from mid-1997 to mid-1999 was reported (137). No European country has enacted regulations concerning folate fortification (135).

Conceivably, implementation of folic acid fortification in the United States and Canada will probably affect the relation between tHcy and its lifestyle determinants and disease risk, and such relations may be different from those in Northern European countries. However, only a few publications on plasma tHcy concentrations in children are based on US (52, 55, 68, 79, 132) or Canadian (64, 65, 127, 130) populations. The sample collection for the 2 large US population-based studies—the Bogalusa Heart Study (68) and the CATCH study (52)—were completed in 1994, and 2 US studies (55, 79) were published before 1998. One recent US study reported low plasma tHcy concentrations and associated metabolic changes in serum samples (collected after fortification; J James, personal communication, 2002) from patients with Down syndrome (132). This study extends and essentially confirms the findings of the article on Down syndrome published by Chadeaux et al (131) in 1988. Canadian studies of Down syndrome (127, 130) and renal patients (65) were based on samples collected before fortification (J James, A Merouani, personal communication, 2002). Notably, a recent Canadian investigation of newborns showed lower mean tHcy concentrations (5.06 μmol/L) than reported previously, and the tHcy concentrations were independent of MTHFR genotype, features attributable to fortification and supplementation with folic acid (64). Except for that study, essentially all of the publications on tHcy concentrations in children quoted in this review are based on samples taken before folic acid fortification was implemented in the United States and Canada.

FOLATE DEFICIENCY

In countries that have not implemented the folic acid fortification of flour, the main dietary sources of folate are fruit and vegetables (138). In the United Kingdom and other countries, breakfast cereals and snack foods are fortified with a range of vitamins, including folate, on a voluntary basis. These food items are popular among children and adolescents. Breakfast cereals are eaten by a high portion of schoolchildren and contribute significantly to their total folate intake (139).

There is a transfer of folate from the maternal to the fetal circulation (18), and the folate concentration in the cord blood, erythrocytes, and plasma of newborns is 2–3 times higher than in the erythrocytes and plasma of their mothers (18, 37). Folate deficiency is seldom detected in newborns (18); moderate deficiency is found in <10% of preschool children, but may be more common in schoolchildren (140). This finding agrees with the observation of low dietary folate in a substantial portion of UK schoolchildren (139). However, data on folate status in children are sparse, and only a few studies have evaluated plasma tHcy as a folate indicator. The strong relation (coefficients ranging from 0.2 to 0.6; Table 1) between serum folate and tHcy in children aged 2–19 y indicates that tHcy is a sensitive marker of folate status in this age group.

A few studies have investigated the usefulness of tHcy in assessing folate status in children and adolescents assumed to be at increased risk of negative folate balance. In 40 female adolescents (aged 11–18 y) with anorexia nervosa, tHcy was moderately elevated, normalized after nutritional rehabilitation, and was weakly associated with serum folate but not with serum cobalamin. Both folate and cobalamin were normal in these patients, but serum creatinine was higher than in control subjects (141). Elevated tHcy concentrations may reflect impaired folate status in the anorectic patients, but the elevated creatinine concentrations point to the possibility of enhanced tHcy production or a renal mechanism.

Of 69 HIV-infected children aged 1–15 y, serum cobalamin concentrations were normal, but elevated tHcy or low serum folate concentrations were detected in about one-half of the patients. tHcy was strongly correlated with serum folate (Spearman’s r = −0.60) but not with serum cobalamin, which indicates that hyperhomocysteinemia reflects impaired folate status in these patients (142).

The occurrence of subclinical folate deficiency and the justification of routine folic acid supplementation in children with sickle cell disease (SCD) have been debated (143) and have been addressed by measuring plasma tHcy concentrations in these patients. Such studies have also been motivated by the finding of higher median tHcy in
young SCD patients with than without an episode of stroke (144). A study of children with SCD from the Netherlands Antilles showed that these patients had hyperhomocysteinemia, which was responsive to folic acid but not to vitamin B-6 and cobalamin (145). These findings were not supported by an investigation of SCD patients from the United States who were not supplemented with folic acid, but who had normal tHcy concentrations (146). Another study of SCD patients from the United States, of whom only 30% were supplemented with folic acid, found elevated fasting tHcy concentrations only in patients older than 10 y, elevated PML tHcy irrespectively of age, and normal circulating folate concentrations (147). Taken together, these findings indicate that children with SCD have a greater B vitamin requirement than do children without SCD and should motivate further studies of plasma tHcy as a measure of B vitamin status in children with SCD.

COBALAMIN DEFICIENCY

Occurrence and children at risk

Cobalamin deficiency typically occurs in middle-aged and elderly persons (148) and is considered to be rare in infants. In infancy, cobalamin deficiency is usually secondary to maternal deficiency. Cobalamin deficiency in breast-feeding women may be due to malabsorption (including gastric surgery and short-gut syndrome) or unrecognized early pernicious anemia, but the most common cause is vegetarianism (149). Most of the infants found to be cobalamin deficient were breastfed or born to mothers adhering to a strict vegetarian diet. Under these conditions, there is probably insufficient cobalamin transfer across the placenta, which leads to low cobalamin stores in newborns (150). The inadequate cobalamin status in newborns is further deteriorated by insufficient cobalamin in the breast milk, the cobalamin content of which is closely correlated with low serum cobalamin in vegetarian mothers (151). These infants may develop deficiency symptoms as early as 4–6 mo after birth (150).

Lower cobalamin and folate concentrations in low-birth-weight infants than in normal-birth-weight infants was shown 25 y ago (152). Cobalamin supplementation was found to reduce the severity of anemia in premature infants (153).

In developing countries such as India, Mexico, and Guatemala, a high prevalence of cobalamin deficiency in pregnant and breastfeeding women and their babies has consistently been found. Folate deficiency is less frequent. Common causes of cobalamin deficiency are a poor diet low in animal products and intestinal parasite infection, eg, Giardia lamblia infection (18, 150, 154, 155). A high prevalence of cobalamin deficiency has also been found in older children. Studies in Mexico (155), Venezuela (156), and Kenya (157) show low plasma cobalamin in 33–52% of the children. Animal foods—including meat, milk, and poultry—are the main sources of dietary cobalamin (158). Breakfast cereals and snack foods have been fortified with micronutrients, including cobalamin, for many years, and cereals contribute significantly to daily cobalamin intakes in UK schoolchildren (139). The view prevails that cobalamin deficiency is rare in children and adolescents consuming a typical Western diet. Data from the third National Health and Nutrition Examination Survey (1988–1994) show that >95% of children in the United States consume more than the daily cobalamin requirement of 0.4–2.4 µg (159), and the frequency of children with serum cobalamin <200 pg/mL was <1% at that time (160). However, the pediatric reference ranges for cobalamin are poorly defined (161), and for newborns the concentrations decrease during the first 6–8 wk (37, 162–164), which complicates the assessment of cobalamin status from serum cobalamin.

In Western countries, dietary guidelines to reduce cholesterol concentrations in children may decrease cobalamin intake (165), and assessment of cobalamin status in children consuming a low-cholesterol diet is mandatory (18). Furthermore, the fortification of grain-cereal products with folic acid was put into effect in the United States in 1998 (136). A high folic acid intake in children may possibly delay the diagnosis or accelerate the development of neuropsychiatric complications of cobalamin deficiency, as has been the concern in adults (166).

Inborn errors of cobalamin absorption and transport

Rare forms of hereditary cobalamin deficiency are related to various defects of cobalamin absorption or cellular uptake, including low or no secretion of intrinsic factor (MIM 261000), low or no synthesis of functional transcobalamin II (MIM 275350), or impaired uptake of the intrinsic factor–cobalamin complex by the intestinal epithelium (MIM 2611000, Imerslund-Gräsbeck disease) (167). These inborn errors present with typical symptoms and signs of cobalamin deficiency in childhood, but the age of onset is different. Most transcobalamin II–deficient patients develop severe megaloblastic anemia as early as 1–3 mo after birth, whereas in infants with congenital malabsorption, symptoms develop later, between 12 and 18 mo, by the time cobalamin stores during pregnancy are exhausted (18, 167).

Symptoms, signs, and consequences

Cobalamin deficiency usually occurs in middle-aged and elderly persons (148), and the cardinal signs are megaloblastic bone marrow and myelopathy. However, the introduction of the cobalamin markers tHcy and MMA since the mid-1980s has shown that tissue cobalamin deficiency commonly occurs without the involvement of bone marrow or nervous system (1, 2).

The typical symptoms from overt cobalamin deficiency in infancy are failure to thrive, movement disorders, developmental delay and regression, and megaloblastosis, but neurologic symptoms and signs can develop even without hematologic abnormalities (149). In infants with cobalamin deficiency who were treated, the long-term consequences have been poor intellectual performance (12). Notably, in apparently healthy children from macrobiotic families, metabolic signs of persistent cobalamin deficiency (14) and impaired cognitive performance (15) were observed in adolescence even after consumption of animal products since the age of 6 y. This finding emphasizes the need for prevention, early recognition, and treatment of cobalamin deficiency in infants.

Assessment of cobalamin status by metabolic markers

Since the late 1970s, there have been sporadic reports on methylmalonic aciduria in breastfed infants born to vegetarian mothers (168–172). The potential use of urinary MMA measurement in cobalamin diagnostics in vegetarians was shown by Speckter et al (173) as early as 1988. They reported that the concentration and range of urinary MMA excretion in infants born to cobalamin-deficient vegetarian mothers (2.6–790.9 µmol/mmol creatinine) were higher than in control infants (1.7–21.4 µmol/mmol.
creatinine) and correlated strongly with maternal serum cobalamin \( r = -0.68 \) and maternal urinary MMA \( r = 0.69 \). Schneede et al (48) made complementary observations in infants aged 10–20 mo who were consuming a macrobiotic diet. Plasma MMA [geometric \( \bar{x} \pm 1.96 SD: 1.44 (0.19–15.0) \mu \text{mol/L} \)] was 7.5-fold higher in the macrobiotic infants than in the control subjects \( 0.18 (0.06–0.51) \mu \text{mol/L} \), and tHcy was 2-fold higher. Plasma MMA (compared with tHcy and hematologic variables) afforded the highest sensitivity and specificity to discriminate between the macrobiotic and control infants (48). These studies highlight the usefulness of urinary and plasma or serum MMA concentrations as markers of impaired cobalamin status in children.

Poor nutrition and malabsorption in developing countries predispose to cobalamin deficiency, which has been studied in infants and children with the use of cobalamin markers MMA and tHcy. Elevated urinary MMA concentrations were found in 12% of 113 infants of Guatemalan, breastfeeding women. The elevated urinary MMA concentrations were related to maternal cobalamin concentration in serum and milk (174).

A high mean tHcy concentration of 15.9 \( \mu \text{mol/L} \) was found in Nigerian adolescent girls between 12 and 16 y of age. Most of the girls had a tHcy concentration greater than the upper reference range for their age group. The hyperhomocysteinemia was frequently attributable to cobalamin deficiency and seldom to folate deficiency (175). A similar observation was recently made among 553 Guatemalan schoolchildren. Low or marginal plasma cobalamin concentrations were detected among 33% of these children, and the frequency of elevated tHcy or MMA concentrations was higher than in children with adequate plasma cobalamin (70). Thus, MMA or tHcy may be useful for the detection and follow-up of cobalamin deficiency in populations with an inadequate diet.

Increasing evidence based on measurements of MMA and tHcy supports the notion suggested by Rosenblatt and Whitehead (18) that cobalamin deficiency may be widespread and undetected in babies born to nonvegetarian women consuming Westernized diets. Specker et al (35) noted >10 y ago that the normal range of urinary MMA concentrations in infants \( 0.4–23 \mu \text{mol/mmol creatinine} \) was wider and higher than that of adults \( 0.7–3.2 \mu \text{mol/mmol creatinine} \); and children \( 2.0–5.1 \mu \text{mol/mmol creatinine} \). There was a strong, inverse correlation between serum cobalamin and urinary MMA, and newborns fed formulas, which have a higher cobalamin content than does breast milk, had a low urinary MMA concentration of <5 \( \mu \text{mol/mmol creatinine} \). In a recent study of 123 neonates, tHcy was strongly correlated with serum cobalamin, which was lower in breastfed infants than in formula-fed infants. Fifteen percent of the infants had an elevated tHcy concentration, i.e., tHcy > 11 \( \mu \text{mol/L} \). In a placebo-controlled trial, elevated tHcy concentrations returned to normal in breastfed neonates given cobalamin supplements (60). Similarly, in 68 low-birth-weight infants, tHcy concentrations increased during the first 40 d in those fed breast milk, but not in those fed formula (63). In 173 neonates, MMA and tHcy were strongly related to serum cobalamin (but not to serum folate), and their cobalamin status, measured as plasma MMA and serum cobalamin, was positively related to maternal cobalamin status (37). At 6 wk after birth, serum cobalamin was reduced to one-half, and this reduction was associated with a drastic increase in MMA (from 0.36 ± 0.26 to 1.36 ± 1.7 \( \mu \text{mol/L} \)), particularly in infants of multiparous mothers (37).

The consistent finding of elevated urinary or serum MMA concentrations in apparently healthy infants aged <1 y (35, 37, 60) could be regarded as an innocuous phenomenon possibly related to the immaturity of organ or enzyme systems involved in MMA clearance or in the production of MMA or its precursors by intestinal microorganisms (177). Alternatively, elevated serum and urinary MMA concentrations may reflect impaired cobalamin function. This possibility is supported by some observations, including the concurrent decrease in serum cobalamin concentrations and elevated tHcy concentrations (37), which suggests impaired homocysteine remethylation. Furthermore, the elevation in MMA is provoked by several factors known to cause negative cobalamin balance, such as low maternal cobalamin status (35, 37) and multiparity (37), and the concurrent hyperhomocysteinemia is ameliorated by formula feeding and cobalamin supplementation (60, 63). The possibility that cobalamin deficiency is common in infants born to nonvegetarian mothers deserves further investigation.

Despite that low birth weight and prematurity predispose to cobalamin deficiency, assessment of cobalamin status on the basis of MMA concentrations in these infants has not been investigated. One preliminary report suggests that the tHcy concentration in 9 preterm infants was actually lower \( 3.8 ± 0.3 \mu \text{mol/L} \) than that in term infants \( 6.1 ± 1.3 \mu \text{mol/L} \), and the tHcy concentration was normalized within 1–2 wk (178). The low and variable tHcy concentration during the first weeks of life in premature infants may preclude the use of tHcy as an indicator of vitamin status in these infants.

**CONCLUSION**

The determinants and diagnostic utility of serum or plasma tHcy and MMA concentrations are similar in children, adolescents, and adults, but some important differences have been established. In adults and in the elderly, tHcy is mainly used in cardiovascular disease risk assessment and in the diagnosis and follow-up of folate and cobalamin deficiencies. Deficiencies of these vitamins and renal failure are the most common causes of hyperhomocysteinemia, and MMA is a specific adjunct to serum cobalamin and tHcy for the diagnosis of cobalamin deficiency.

The usefulness of tHcy in evaluating the rare events of pediatric stroke has not been established, and tHcy and MMA measurements in infants with symptoms indicating homocystinuria, methylmalonic aciduria, or both are obligatory but are not common applications of these metabolite assays.

In children, adequate folate and cobalamin status is crucial for optimal organ development, including central nervous system function, and subtle or atypical deficiencies of these vitamins are probably more common than previously recognized. In this respect, the possibility of impaired cobalamin status in a significant fraction of breastfed infants represents a particular challenge in preventive medicine. To meet this challenge, efforts should be made to establish reference ranges of MMA and plasma tHcy in infants with adequate cobalamin and folate status. This could be accomplished by investigating infants of mothers with defined vitamin status or who are being supplemented with cobalamin or folate. Intervention studies with cobalamin supplementation of infants should also be considered.

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