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Review

Homocysteine, folic acid and vitamin B_{12} in relation to pre- and postnatal health aspects

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Abstract

Studies linking hyperhomocysteinemia (HHCY) and B-vitamin deficiency to some health aspects in children have been accumulating. Low B-vitamin status in early life, even as early as the time of conception, may endanger the potential for new life and may negatively influence the health of the offspring. Early abortion, pregnancy complications and poor pregnancy outcomes have been linked to elevated concentrations of total plasma homocysteine (tHcy) and low folate or vitamin B_{12}. Maternal vitamin status predicts that of fetuses and neonates. Lactating women are likely to experience low micronutrient status, which might affect breast-milk composition and hence the nutritional status of their breast-fed infants. Elevated concentrations of methylmalonic acid (MMA) is common in infants (age <6 months), which may indicate a transient inadequate vitamin B_{12} status. Deficiency of B-vitamins might confer deleterious effects on the physical and mental health of the child, such as impaired growth, gross motor function, poor school performance and other adaptive skills. The importance of maintaining adequate B-vitamin status during periods of progressive growth and development should be emphasized because symptoms related to folate and vitamin B_{12} deficiency are difficult to detect. Serum levels of tHcy and MMA should be estimated in several target groups of children, pregnant and lactating women and those planning for pregnancy. Concentrations of tHcy and MMA are useful indicators of B-vitamin status in the pediatric laboratory. Using these functional markers may facilitate detecting suboptimal B-vitamin status in children.

Keywords: folate; homocysteine; pediatrics; vitamin B_{12}.

Homocysteine: a new marker in pediatric settings

Numerous studies have investigated concentrations of plasma total homocysteine (tHcy) in children during the last few years. Concentrations of tHcy and methylmalonic acid (MMA) are sensitive and specific metabolic markers for B-vitamin status (1, 2). The classic hematological manifestations associated with folate or vitamin B_{12} deficiency are not uniquely expressed (3). Moreover, serum concentrations of these vitamins have poor specificity and sensitivity (2). Therefore, the assessment of tHcy and MMA offers a useful diagnostic tool in the pediatric laboratory.

Concentrations of tHcy in infants (range 4–9 μmol/L) are relatively higher than in older children (1). Vitamin B_{12} is the major modulator of tHcy in infants younger than 1 year and folate becomes a main determinant of tHcy at older age (1). In children over 1 year, as well as in adults, age becomes a significant factor that modulates tHcy levels (1). In general, children (>1 year) have lower tHcy concentrations than adolescents (4). Boys have slightly higher tHcy than girls and this sexual dimorphism of tHcy concentrations occurs as early as 10 years of age (5). Elevated tHcy concentration in children may be related to inherited or acquired factors (6) (Table 1).

Homocysteine, a risk factor for pregnancy complication and outcome

The importance of B-vitamins in human reproduction has been repeatedly addressed. Low maternal status of B-vitamins or hyperhomocysteinemia (HHCY) has been linked to pregnancy complications and poor birth outcomes (7–9).

Concentrations of tHcy decrease during pregnancy and are usually lower in pregnant women supplemented with folic acid compared to unsupplemented women (10). An elevated concentration of tHcy during pregnancy was independently associated with the risk of recurrent pregnancy loss (7), preeclampsia, prematurity and low birth weight (8–10). The association between maternal HHCY and intrauterine growth retardation has not been confirmed by all investigators (11). Different findings from studies in Europe and Canada could be related to different study designs or to improved folate status in Canada since the fortification of food with folic acid in 1998. In line with this suggestion, Canadian women homozygous for the mutation in the methylenetetrahydrofolate reductase gene (*MTHFR* C677T) who were not taking multivitamins during pregnancy were at increased risk of having a child with intrauterine growth restriction (12). The importance of vitamin B_6 during pregnancy has also been outlined in several studies (13).

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Table 1 Common causes of hyperhomocysteinemia in newborns and children.

Inherited
• Hetero- or homozygosity for mutations in genes encoding enzymes (e.g., CBS or MS)
• *MTHFR* mutation
• Cbl E/G and F defects
• Inherited transcobalamin deficiency
Acquired
Primary
• Folate, vitamin B_{12} or B_6 deficiency
• Vegetarian, vegan or macrobiotic diet
• Poverty or malnutrition
• Newborns of vitamin-deficient mothers
Secondary
• HIV-infected children
• Malabsorption, bacterial overgrowth
• Medications (anti-epileptics, theophylline)
• Renal dysfunction

CBS, cystathione-β-synthase; MS, methionine synthase; Cbl, cobalamin.

Poor maternal vitamin B_6 status during pregnancy has been related to low birth weight of the offspring (13).

Taken together, it is recommended that women of reproductive age should increase their dietary intake of folate, vitamin B_{12} and B_6. Deficiency of one of these vitamins should be suspected in women with a current or a previous unfavorable event during pregnancy.

Homocysteine in diseased children

Homocysteinuria, the disease firstly linked to disturbed tHcy metabolism, is a rare genetic defect that causes a marked reduction in the activity of cystathionine-β-synthetase and therefore severe HHCY (tHcy >100 μmol/L). HHCY is a risk factor for ocular, cerebral, skeletal and clotting pathologies in homocysteinuric children (14). Large-scale screening programs of newborns are not currently recommended (15). However, the importance of selective screening for HHCY in pediatric patients has recently been highlighted (16). In contrast to severe HHCY, modest elevation of tHcy (10–30 μmol/L) is frequently encountered. Nevertheless, the implications of HHCY in children have not been fully explored.

Children treated with certain medications, such as anti-epileptic drugs, had elevated concentrations of tHcy (17). Mild to moderate HHCY is a risk factor for occlusive vascular disease, cerebrovascular lesions and thrombosis in children (17–19). HHCY is also very common in pediatric renal patients, but can be lowered by therapeutic doses of folic acid (20). Evidence available from vitamin intervention studies in adult renal patients is promising and suggests that similar studies should be implemented in children as well.

Higher concentrations of tHcy and lower concentrations of folate and vitamin B_{12} were found in HIV-infected children (21). Malabsorption, low dietary intake and increased demands may contribute to micronutrient deficiency in this case. Neurological and neurodevelopmental disorders are common in such children (21). B-vitamins are required for the production of S-adenosylmethionine, the methyl donor for numerous biochemical reactions, including the formation of neurotransmitters. Therefore, B-vitamin deficiency may cause deprivation of the methyl groups in the central nervous system.

A notable exception of elevated tHcy concentrations in the context of childhood disease is the case of Down syndrome (trisomy 21). The gene encoding cystathionine-β-synthetase enzyme located on chromosome 21 is overexpressed in children with Down syndrome. Accordingly, concentrations of tHcy, methionine and S-adenosylmethionine were lower in children with Down syndrome compared to normal siblings (22).

Vitamin treatment lowered tHcy concentrations and improved some measures of endothelial function in pediatric patients (23). Prospective studies may clarify whether tHcy-lowering therapy may improve the progression and outcome of tHcy-related morbidities in children.

Periods of high demands for B-vitamins

Folate and vitamin B_{12} play a fundamental role in the biosynthesis of purine and pyrimidine and in maintaining DNA methylation. A high rate of DNA synthesis and gene expression characterizes all rapidly dividing cells. Therefore, increased demands for B-vitamins in pregnant women and during early life are expected.

Body stores of folates are limited and depletion occurs rapidly. A marginal, usually asymptomatic, low vitamin status during the periconception period may traverse into a deficiency state during pregnancy or lactation. Prolonged lactation may deplete serum folate and cause tHcy elevation in mothers with a low daily intake ($\leq$380 μg) (24). Nevertheless, prolonged lactation did not cause folate depletion in mothers taking 1 mg of folic acid daily (24). Other target groups for which adequate intake and status of the B-vitamins should be stressed are pre-school and school-age children (25). Long-term deficiency may have deleterious neurological and developmental consequences (discussed below) (25, 26).

Predictors of tHcy and B-vitamin status in early life

Maternal concentrations of vitamin B_{12} and folate predict those in the neonate at birth (27, 28). The placenta sequesters vitamin B_{12}, B_6 and folate. Moreover, the concentrations of these vitamins are two- to six-fold higher in cord blood than in maternal serum (27, 28). This may support the high metabolic rate in the developing fetus.

Fetal and maternal tHcy concentrations are strongly correlated (27–29). Concentrations of tHcy at the time of labor were lower in mothers taking folic acid compared to unsupplemented mothers (10). Low concentration of vitamin B_{12} (or high MMA) in mothers was associated with lower B_{12} and higher MMA in their newborns (30). Moreover, babies who had the highest

MMA (and lowest B_{12}) values at birth also had the highest MMA values (and lowest B_{12}) at 6 weeks (30).

Concentrations of tHcy in neonates are influenced by nutritional factors (folate, B_{12} and B_6) (31–34). Vitamin B_{12} intake may be limited during the first year of life because of a low vitamin content in breast milk and an immature intrinsic factor system. The estimated stores of vitamin concentrations B_{12} in the neonatal liver are expected to cover the requirements for this vitamin during the first year of life. Vitamin stores in newborns of depleted mothers may be readily depleted, especially in breast-fed infants. Previous studies demonstrated that breast-fed infants of vegetarian mothers excrete more MMA in their urine than infants of non-vegetarian women (32, 34). Moreover, lower concentrations of tHcy and higher concentrations of B_{12} were found in formula-fed infants compared to breast-fed infants (35). Several studies reported a positive correlation between maternal serum and milk content of the vitamin (32, 33). Moreover, the occurrence and the reversible nature of methylmalonic aciduria in breast-fed infants from deficient mothers confirm that maternal B_{12} status determines that of the newborns (32, 34, 35). Vitamin B_{12} status cannot be easily restored in newborns of deficient mothers, especially if vitamin intake continues to be in the lower range (36, 37). As a consequence, newborns of deficient mothers may develop a real deficiency syndrome later in life (36–38).

Occurrence of a sub-normal B-vitamin status in early life

A high incidence of B-vitamin deficiency has been documented worldwide. This occurs frequently in the context of poverty or prolonged inadequate intake. Vitamin B_{12} deficiency is highly prevalent in vegetarians, macrobiotic communities and in some developing countries (33, 36).

A transient inadequate status of vitamin B_{12} may be common at age <6 months (1). Concentrations of MMA and tHcy were higher in infants (<6 months) than that in older children (1). Measurement of MMA in conjunction with tHcy improved the specificity of these tests (1). The usefulness of serum levels of holotranscobalamin, the vitamin B_{12} portion bound to transcobalamin, in estimating vitamin B_{12} status in infants should be determined.

Vitamin B_{12} deficiency was very prevalent in Guatemalan lactating mothers and their infants at 3 months postpartum (33). B_{12} content in breast milk was low in 31% of these women and correlated to urinary MMA in their infants (33). An intake below the recommended daily amount (RDA) for B_{12} (0.3 μg/day) was recorded in 16% of the infants (33). In a study of lactating women in Mexico, dietary intake of vitamin B_{12} was only 50% of that recommended and 62% of the mothers had a low content of B_{12} in their breast milk (39). Low vitamin B_{12} status was also common in Guatemalan and Kenyan schoolchildren (40, 41). Furthermore, folate deficiency was very common (43%) and folate intake was also below that recommended during lactation (500 μg/day). In addition, folate content in human milk was not adequate to support the requirements of many infants (39, 42).

A high incidence of B_{12} deficiency was found in infants consuming a macrobiotic diet (36). Ingesting a strict vegetarian diet in early life led to a low vitamin B_{12} status in adolescence, despite increasing B_{12} intake since the age of 6 years (43).

Homocysteine and B-vitamins in relation to birth defects

Several congenital anomalies may be prevented by folic acid supplementation before and during early pregnancy (44). The exact mechanism by which folic acid exerts its protective effect is unclear. However, the role of the vitamin in one-carbon metabolism, nucleic acid synthesis and the integrity of DNA are plausible explanations.

Neural tube defects (NTDs) are the most common congenital malformations that have been linked to maternal folate deficiency and HHCY (44–46). In addition, concentrations of tHcy were higher in mothers of children with NTDs compared to mothers of normal children (45). Concentrations of folate were lower in children with spina bifida compared to normal children (46). Low blood folate was associated with an odds ratio of 2.6 for having NTD and of 3.1 for being a mother of a child with an NTD (46). Recent strategies for the primary prevention of NTDs include preconceptional folic acid supplementation (47). In 1998, the US Food and Drug Administration mandated that all grain products be fortified with folic acid (140 μg per 100 g of grain). A similar policy has also been put in place in Canada, Chile and Hungary. This national health policy is proposed to provide a daily intake of at least 400 μg/day folic acid in women of reproductive age. Folate status and concentrations of tHcy have been improved in women of childbearing age after such fortification (48). This metabolic improvement has also been associated with a marked reduction in birth defects (between 15% and 30%) (49).

Down syndrome or trisomy 21 is a common genetic disorder that is due to disturbed segregation of chromosome 21 during meiosis. DNA hypomethylation, due to methyl-group deficiency, influences chromosome segregation. Folate and vitamin B_{12} are required for controlled DNA methylation and genomic stability. The effect of abnormal folate metabolism on the maternal risk of having a child with Down syndrome has been examined (50). A significant increase in tHcy levels was observed in mothers of children with Down syndrome and an approximately three-fold higher risk was found in mothers homozygous for the *MTHFR* C677T mutation compared to mothers without the T substitution (50).

Genes encoding enzymes that participate in the distribution of folate forms or in folate metabolism and transport are important for embryonic development. Several mutations in genes involved in folate and methionine metabolism have been linked to the risk

Table 2 Target groups who should increase their intake of vitamin B_{12} and folic acid.

- Pregnant and lactating women
- Mothers with previous pregnancy loss, complication or poor pregnancy outcome
- Women planning for pregnancy
- Infants of strict vegetarian mothers
- Infants with unexplained neurological syndromes
- Infants with developmental delay
- Infants with siblings affected by homocysteinuria, birth defects or developmental delay
- Breast-fed infants of vitamin-deficient mothers
- Pediatric patients (renal patients, diabetes, epilepsy)

of some congenital anomalies (50–54). A specific pattern of gene-nutrient interaction has been described. Therefore, improvement of the maternal nutritional status may modify the associated risk of certain genetic variants (46, 51). Subjects homozygous for the *MTHFR* C677T mutation have higher tHcy levels when folate and/or B_{12} supply is limited. A positive association has been reported between the *MTHFR* TT genotype and NTDs (55). The risk for NTDs increased in *MTHFR* carriers who had low folate levels (46). Other mutations in genes encoding homocysteine remethylation enzymes may also increase the risk of NTDs (54).

Role of vitamin B_{12} status in postnatal development

Deficiency of one or more of the B-vitamins may lead to irreversible neurological disorders. Folate or vitamin B_{12} deficiencies occur mostly in infants of deficient mothers (33, 34, 36, 38, 56, 57). Symptoms of vitamin B_{12} deficiency in neonates become evident at 4–9 months of age (38). A serious neurological syndrome and developmental disorders have been described in breast-fed infants of strict vegetarian mothers (38, 58). A few studies relating B_{12} status to cognitive development in children are available. A significant association was found between biochemical markers of B_{12} status and performance on tests measuring cognitive function in children (59, 60). School achievement was negatively related to vitamin B_{12} status in Guatemalan children (25, 60). Moreover, lower scores in some measures of cognitive performance were found in adolescents who previously consumed a macrobiotic diet compared to those who consumed an omnivorous diet from birth onwards (37). Vitamin B_{12} deficiency in infants was associated with marked developmental regression, poor brain growth or poor intellectual outcome (25, 60). Other signs of developmental delay include impaired communicative reactions, and fine and gross motor functions.

In conclusion, deficiency of the B-vitamins in infancy leads to long-term metabolic and neurological abnormalities. Table 2 summarizes target groups for which the intake of folate and vitamin B_{12} should be increased. Early recognition and prevention of these deficiencies should be emphasized. Age-specific reference ranges for blood concentrations of folate, B_{12} and their surrogate markers should be established, because adults reference values cannot be extrapolated to infants and children.

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