

Cobalamin Status from Pregnancy to Early Childhood: Lessons from Global Experience

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ABSTRACT

Low cobalamin intake and status during pregnancy or lactation have been linked to adverse maternal and perinatal health outcomes, whereas low cobalamin status during early childhood is associated with impaired development in children. Women who begin pregnancy with depleted stores (low or very low plasma cobalamin) will give birth to depleted infants who are likely to develop deficiency symptoms during the first few weeks or months postpartum. Newly ingested cobalamin during pregnancy and lactation (from diet or supplements) is transferred to the child and is not likely to correct cobalamin status in depleted women. The prevalence of low cobalamin status is high especially in low-income settings or in populations with a low intake of animal products. Folate and cobalamin play interdependent roles in one-carbon metabolism. Although folic acid supplementation during early pregnancy is widely recommended and practiced, cobalamin supplementation during pregnancy and lactation has received little attention. Furthermore, the intake recommendations for pregnant and lactating women and in early life need reevaluation in the light of newly available evidence in the field. *Adv Nutr* 2017;8:971–9.

Keywords: cobalamin, infants, pregnancy, lactation, cord blood, child development, supplementation, recommended intake

Cobalamin in Pregnancy and Health Outcomes

Cobalamin requirements increase during pregnancy and lactation to meet the demands of the mother, the fetus, and the infant. Women of childbearing age from low-income settings and those with low intake of animal products are at risk of cobalamin deficiency (1). The deficiency also is common in the same at-risk groups during pregnancy (2, 3).

Maternal cobalamin deficiency has been related to an increased risk of early and recurrent miscarriage (4, 5), preterm birth (6), and low birth weight (7, 8). Preconception cobalamin concentrations ≥ 258 pmol/L were associated with a 60% reduced risk of preterm birth, but they did not affect pregnancy loss or conception in Chinese women (6). Plasma methylmalonic acid (MMA), a functional marker of low cobalamin status, was positively associated with the probability of spontaneous miscarriage in Brazilian women (OR 3.8, 95% CI: 1.4, 10.6 per quartile increase in MMA) (9).

Serious developmental defects of the central nervous system [e.g., neural tube defects (NTDs)] are associated with maternal cobalamin deficiency, although the evidence for a causal role of low cobalamin status in NTDs is moderate compared with that of low folate (10). The incidence of NTDs is 6.7–8.2/1000 live births in some parts of India (11) where cobalamin status is low (12). NTD risk was associated with low maternal holotranscobalamin (holoTC) levels and maternal transcobalamin 2 genotypes that influence cobalamin levels (13). Researchers in Ireland reported that plasma cobalamin < 185 pmol/L increased the risk of NTD by 2.5- to 3-fold, after adjusting for folate status (14). According to that study, preconception cobalamin concentrations of > 221 pmol/L are likely to be associated with a low risk of NTDs (14).

Anemia is a common complication of pregnancy in tropical countries, where multiple nutrients deficiency is common and evidence for an association between low cobalamin and maternal anemia has been regarded as limited (15). Even when cobalamin deficiency was not the main cause of anemia, cobalamin administration (i.e., 200–250 $\mu\text{g}/\text{wk}$ initially, followed by 50 $\mu\text{g}/\text{wk}$) alleviated the symptoms in many cases (16), suggesting that screening anemic women for cobalamin deficiency may be

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Abbreviations used: AI, adequate intake; EAR, estimated average requirement; HC, haptocorrin; Hcy, homocysteine; holoTC, holotranscobalamin; MMA, methylmalonic acid; NTD, neural tube defect; TC, transcobalamin; tHcy, total homocysteine.

useful in detecting undiagnosed cases that would benefit from supplementation.

The increased risk of disease was observed at serum cobalamin concentrations within the reference range. There could therefore be a threshold for serum cobalamin above which the risk of adverse health outcomes is low.

Cobalamin Requirements during Pregnancy and Lactation

The RDAs for cobalamin were set at 2.6 $\mu\text{g}/\text{d}$ for pregnant women and 2.8 $\mu\text{g}/\text{d}$ for lactating women (17). The National Academies of Sciences, Engineering, and Medicine, Institute of Medicine, Food and Nutrition Board, defined the RDAs based on the following assumptions: 1) half of total body cobalamin is stored in the liver (18), 2) cobalamin absorption increases during pregnancy to cover the requirements of the fetus and mother (19, 20), and 3) recently absorbed cobalamin is transferred to the fetus (17). It has been estimated that 0.1–0.2 μg cobalamin are stored in the fetal liver per day of pregnancy. Thus, the estimated average requirement (EAR) of nonpregnant women (2.2 $\mu\text{g}/\text{d}$) should be increased by 0.2 $\mu\text{g}/\text{d}$ to satisfy fetal needs. The EAR for lactating women was set by increasing the EAR for nonpregnant women by 0.33 $\mu\text{g}/\text{d}$ cobalamin (i.e., the amount of cobalamin excreted in milk during the first 6 mo of lactation) (17).

The assumptions used to estimate the RDAs were not corroborated by other studies. For example, increased cobalamin absorption following oral cobalamin administration was not confirmed based on the CobaSorb test at any time during pregnancy (21). Moreover, the enhanced transport of recently ingested cobalamin from the mother to the fetus or the infant theoretically can exacerbate cobalamin depletion in women with low serum cobalamin. To estimate cobalamin intake recommendations for pregnant and lactating women, more research is needed to address the gaps in knowledge in this field (Text Box 1).

Changes in Cobalamin Markers during Pregnancy

Cobalamin acutely administered to pregnant animals or humans is actively transported and accumulates in the placenta

and fetal organs (20, 22–24). Cobalamin supplementation (50 $\mu\text{g}/\text{d}$) to deficient pregnant Indian women from 14 gestational weeks to 6 wk postpartum led to higher maternal plasma cobalamin in the second and third trimesters, but it did not lead to differences in metabolic markers of deficiency [e.g., MMA and total homocysteine (tHcy)] compared with the placebo group (3). The decline in plasma cobalamin between the first and second trimesters was attenuated in the cobalamin-treated group (3). In the same study, 6-wk-old newborns from women receiving supplements had higher cobalamin and lower MMA and tHcy compared with newborns from placebo-treated women (3). As such, there seems to be a prioritization of available cobalamin to the placental-fetal unit at the expense of maternal needs, because maternal cobalamin metabolic markers were not corrected in the study from India (3). It remains unclear how maternal cobalamin deficiency may affect homeostasis of the vitamin and its fetal transport during pregnancy.

Hemodilution, decline in albumin, and increased glomerular filtration rate during pregnancy affect blood concentrations of metabolites and nutrients. Serum cobalamin declines during pregnancy (25, 26) but returns to preconception concentrations postpartum (27). The decline in serum cobalamin has raised concerns about the depletion of maternal cobalamin during pregnancy. In nondeficient women not receiving nutritional supplements, serum holoTC falls between preconception and the first trimester but then remains stable throughout the remainder of a pregnancy (21, 28); this could indicate a mechanism that preserves the biologically available form of cobalamin throughout pregnancy. In support of a decline in cobalamin status at late pregnancy, serum MMA initially falls during early and mid-normal pregnancy but gradually increases by late pregnancy to higher concentrations than at preconception or early pregnancy (26). The increase in MMA occurs in the face of the physiological forces leading to reduced concentrations of other plasma metabolites or nutrients and the increase is greater in women beginning their pregnancy with a lower plasma holoTC (<67 pmol/L), suggesting that it could

TEXT BOX 1 GAPS IN KNOWLEDGE REGARDING COBALAMIN REQUIREMENTS DURING PREGNANCY AND LACTATION

How is cobalamin distribution regulated between maternal and fetal tissues, particularly in cobalamin-depleted women?

Is cobalamin absorption from food (protein bound) and from supplements (free) higher during pregnancy compared with preconceptional absorption?

Is there a clearance of newly absorbed cobalamin to fetal tissues (during pregnancy) or to the infant (through human milk during lactation)?

Can cobalamin stored in the kidney be mobilized more efficiently in pregnant women with low status than in those with high status?

Should the estimated average requirement calculations consider preservation of sufficient maternal cobalamin stores (i.e., based on functional markers such as methylmalonic acid, and total homocysteine) during pregnancy and lactation instead of being based solely on the amount assumed to be transported to the fetus or infant?

be related to impaired maternal cobalamin status in late pregnancy (26). Similar results regarding increases in plasma tHcy concentrations (29, 30) during late pregnancy were reported, despite the presence of physiological factors expected to produce the opposite effect. The decline in serum total cobalamin between 13 and 36 wk gestation was associated with an increase in tHcy (3.8–5.0 $\mu\text{mol/L}$) and MMA (0.10–0.16 $\mu\text{mol/L}$) (21). The contribution of impaired cobalamin status to the increase in tHcy in late pregnancy has not been quantified; the increase in both MMA and tHcy in late pregnancy suggests a decline in maternal cobalamin stores, possibly because of preferential transfer to the fetus.

Cobalamin-binding proteins and their saturation also change during pregnancy (21, 25, 28, 31). Total TC concentrations increase and TC saturation decreases during pregnancy (25, 31). The decline in serum total cobalamin has been explained by a reduction in total haptocorrin (HC) and holohaptocorrin (21, 28).

Balanced Cobalamin and Folate

Nutrients such as cobalamin, folate, vitamin B-6, methionine, choline, betaine, riboflavin, and cysteine participate in the one-carbon metabolic network. Cobalamin is a cofactor for methionine synthase, the enzyme that converts homocysteine (Hcy) to methionine, through use of the methyl groups provided by the folate cycle. Cobalamin is a rate-limiting factor for Hcy methylation when folate status is replete (32). Cobalamin deficiency impairs the folate and methionine cycles, thus reducing purine and pyrimidine synthesis and affecting DNA replication and epigenetic regulation in growing cells. The conversion of 5,10-methylenetetrahydrofolate to 5-methyltetrahydrofolate by methylenetetrahydrofolate reductase is irreversible. As such, in cobalamin-deficient subjects folate remains trapped as 5-methyltetrahydrofolate and cannot be used in the remethylation of Hcy to methionine. Alternative sources of methyl groups can change the balance in the folate cycle (33–36) in cobalamin-deficient women or those with an imbalanced intake of the nutrients (37).

Preconceptional folic acid (400–800 $\mu\text{g/d}$) is recommended to reduce the risk of NTDs. Mandatory fortification with folic acid (200 $\mu\text{g/d}$) has been implemented in >70 countries and voluntary fortification is in place in some countries. Targeted supplementation of high doses of folic acid (1–5 mg/d) during early pregnancy is a common practice in countries such as India and Sri Lanka (38). Neonates born to Indian women with low cobalamin intake who consume >1000 $\mu\text{g/d}$ of folic acid and those with a low cobalamin:total folate intake ratio are at increased risk of being born small for gestational age (39). High red blood cell folate in pregnant Indian women has been associated with adiposity (40) and an increased risk of insulin resistance in their children if maternal plasma cobalamin concentrations also were low (40, 41). The risk of gestational diabetes was higher in cobalamin-deficient Indian women, and risk of permanent diabetes in the cobalamin-deficient women with

gestational diabetes was higher in those with higher folate status (41). In pregnant women from the United Kingdom, BMI was negatively associated with both folate and cobalamin statuses, and cobalamin deficiency also was associated with insulin resistance in these women (42). Women with a combination of low plasma cobalamin (<170 pmol/L) and folate (<10.3 nmol/L) statuses had the highest BMIs, whereas those with high cobalamin (>238 pmol/L) and folate (>18.3 nmol/L) statuses had the lowest BMIs (42). Taken together, women with imbalanced folate-cobalamin intakes (e.g., vegans) may require additional sources of cobalamin before and during pregnancy to reduce the risk of NTDs or other adverse health outcomes in the mother or child.

Determinants of Cobalamin in Breast Milk

Recent maternal cobalamin intake is the main source and determinant of breast milk cobalamin. Milk cobalamin is a major determinant of cobalamin status in exclusively breastfed infants and is correlated strongly with maternal blood cobalamin (43, 44) and maternal intake. A single injection of 50 μg cobalamin caused a 150-fold increase in milk cobalamin concentrations, but the effect lasted for only 7 d in a study of Indian women (43). Oral supplementation of cobalamin until 6 wk postpartum led to a 2-fold increase in mean breast milk cobalamin (3) and markers of cobalamin status in the infants compared with the placebo group. Infant cobalamin status declined when maternal supplementation was stopped (3). Breast milk cobalamin may be depleted in lactating women consuming a strict vegetarian diet or in women from low-income settings (45, 46). A 10-wk-long diet providing $\sim 8.6 \mu\text{g/d}$ cobalamin beginning at 5 wk postpartum did not increase cobalamin concentrations in breast milk (44). Therefore, in women with low to moderate cobalamin intake (<10 $\mu\text{g/d}$), the amount of cobalamin available for transport to the child during lactation may be limited.

In a Mexican study, 15% of the women had plasma cobalamin concentrations of <74 pmol/L during pregnancy, and levels <103 pmol/L were found in 30% of them during lactation (45), suggesting that depleted cobalamin reserves persist in the postpartum period. Milk cobalamin concentrations <362 pmol/L [cutoff suggested by Specker and colleagues (47, 48)] were found in 31 of 50 Mexican women (45). The presence of anemia was associated with significantly lower breast milk cobalamin concentrations (mean 285 pmol/L in anemic women compared with 418 pmol/L in nonanemic women) (45). The association between plasma cobalamin and breast milk cobalamin was smaller than expected, possibly because cobalamin status was low in all of the Mexican women studied (45).

In a study of lactating Danish women, milk cobalamin concentrations declined between 2 wk and 4 mo and remained at this low concentration at 9 mo (49). At 4 mo low milk cobalamin concentration was associated with the lowest serum holoTC and the highest MMA in breastfed children (49), suggesting that the breast milk cobalamin

concentration at 4 mo was not sufficient to maintain a child's metabolic functions on the same level as before that age. Another study on lactating Brazilian women confirmed the decline in milk cobalamin concentrations during the first 3 mo of lactation (from 0.35 to 0.25 nmol/L) (50).

Mean milk cobalamin concentrations were compared between mother-infant cohorts of high (50) to low cobalamin status (47, 51). The highest milk cobalamin content (mean 0.91 $\mu\text{g/L}$) was observed in women receiving supplements (51), whereas the lowest content (0.31 $\mu\text{g/L}$) was detected in vegans (47). Milk cobalamin concentrations $<0.49 \mu\text{g/L}$ from vegan women not receiving supplements were associated with elevated urinary MMA in their infants (52).

Large variations exist between studies of the association between serum and milk cobalamin, making interstudy comparisons difficult (44, 45). Timing of sample collection and methodological inaccuracy in human milk cobalamin determination are important sources of variation (53). Milk HC content increases during lactation, and unbound HC ($>10 \text{ pmol/L}$) interferes with assay methods (54) and should be removed before cobalamin is measured (49, 54). Differences between studies also are explained by intervention regimes (timing, duration, and dose) and baseline cobalamin status of the participants.

The associations between markers of cobalamin status in lactating mothers, breast milk, and their breastfed offspring have not been studied adequately (3, 55), especially in women who are depleted of the vitamin (56). In general, there is evidence that milk cobalamin content declines after 6 mo of lactation and this decline could be critical for fulfilling a child's requirements, especially when the mother is depleted.

Cobalamin Intake Recommendations in Infants

The adequate intake (AI) for cobalamin in infants aged 0–6 mo was estimated to be $\sim 0.40 \mu\text{g/d}$. The AI for children from 6 mo to $\leq 1 \text{ y}$ was extrapolated from this estimate and adjusted to body weight (57). This AI for infants was estimated from human milk cobalamin concentrations and the amount of milk consumed per day in asymptomatic infants (57), in addition to the association between maternal milk cobalamin concentration and urinary MMA in the offspring of vegan mothers (52).

The approach to defining the AI of cobalamin in infants has been questioned, mainly as a result of analytical limitations in studies of milk cobalamin content, including timing of sample acquisition, because milk cobalamin content declines in the postpartum period (49, 54, 58). Moreover, maternal or child plasma cobalamin status markers were not considered when defining the AI of cobalamin in infants. Fluctuations in cobalamin concentrations during the first years of life also appear to be a critical factor. Cobalamin status decreases in exclusively breastfed infants between 4 and 6 mo after birth, even in those born to nondeficient mothers (49); this has been shown by high urinary MMA and plasma homocysteine (59) and low plasma holoTC and cobalamin. Urinary MMA in exclusively breastfed infants of vegetarian

mothers was studied in infants with a mean age of 7 mo (range: 2–14 mo) (47, 52) who showed a wide overlap with results from omnivorous infants. Large interindividual variation in serum MMA have been reported in the first year of life and may be only partly explained by cobalamin status (60). **Text Box 2** shows the gaps in knowledge related to cobalamin intake recommendations in infants.

Cobalamin Status in the Baby at Birth and during Lactation and Prolonged Lactation

Cobalamin status (total cobalamin and holoTC in cord serum) at birth is positively related to maternal cobalamin status (26, 61, 62). Low maternal status during pregnancy remains a relevant predictor of low child cobalamin in preschool-age children; this is likely to be explained by low child vitamin stores at birth (61) and continued insufficient dietary intake after weaning (63). The strong association between maternal and child cobalamin status beyond the age of lactation is explained by socioeconomic status and diet. Predictors of cobalamin status in preschool-age and school-age children include maternal supplement use; cobalamin status at birth; feeding patterns (breast milk and formula) (64, 65); and introduction, quality, and frequency of animal-based complementary foods after weaning (66). Infants born to well-nourished women have high plasma cobalamin concentrations at birth (31, 67), whereas those born to vegetarian mothers not taking supplements are depleted at birth (47, 52). The effect of depleted maternal cobalamin status (based on cobalamin markers) during lactation was evident in Guatemalan infants at 12 mo postpartum (55).

Cobalamin markers in blood (total cobalamin, MMA, and tHcy) continue to change postpartum $\leq 19 \text{ y}$ (60, 68). Age-related changes are not always associated with symptoms of deficiency, but they are more pronounced in exclusively breastfed or depleted infants. The association between cobalamin depletion and subtle signs of deficiency or developmental delay is not well studied because there are no established reference ranges for cobalamin markers at or after birth.

Cobalamin deficiency after birth is a public health problem in countries with endemic deficiency and prevalent or prolonged breastfeeding practices associated with widespread food insecurity. For example, breastfeeding was associated with low plasma cobalamin and elevated MMA in Nepali children aged 6–11, 12–23, and 24–35 mo (65). The differences in cobalamin markers between breastfed and formula-fed infants were greatest in the first year of life, in which complementary foods from animal sources are not likely to be consumed. In a study of Guatemalan infant-mother pairs, infant urinary MMA at age 3 mo was related to maternal serum cobalamin and was partly explained by breast milk cobalamin (69). Maternal intake of cobalamin during lactation (median: 1.47 $\mu\text{g/d}$) was unlikely to be sufficient, thus explaining the high prevalence of very low ($<148 \text{ pmol/L}$, in 13%) and marginally low serum cobalamin levels (148–221 pmol/L , in 33%) in the infants (69).

TEXT BOX 2 GAPS IN KNOWLEDGE REGARDING COBALAMIN INTAKE RECOMMENDATIONS IN INFANTS

Analytical laboratory methods should be standardized for the purposes of valid cross-comparison or pooled analyses of different populations and studies.

The choice of cobalamin marker in women and infants is essential. A combination of several markers is recommended. Urinary methylmalonic acid alone lacks specificity and shows interstudy variability. It is not clear how urinary methylmalonic acid can be affected by biological factors that are unrelated to cobalamin status.

Cobalamin adequate intake for infants should be determined in age groups (0–4, 4–6, and 6–12 mo) because cobalamin status markers fluctuate in early life and cobalamin status declines between 4 and 6 mo. Thus, extrapolation of adequate intake to other age groups is not straightforward.

What are the short- and long-term consequences (e.g., anemia, growth, weight gain) of marginally inadequate cobalamin intake in the first year of life? Which intake levels are associated with normal biomarkers and low disease risks? Should the adequate intake recommendations be adjusted to ensure covering the requirements for vulnerable populations who are born with low cobalamin stores?

Low levels of food security in India are associated with cobalamin deficiency in children as a result of prolonged breastfeeding (66). Prolonged exclusive breastfeeding is a strong predictor of low serum cobalamin concentrations in children from families with low socioeconomic status (64, 70). Moreover, prolonged breastfeeding in Indian children (mean age: 16 mo) from families of low to middle socioeconomic status was associated with stunting, anemia, low weight, or wasting (low weight-for-length) (66, 71).

Cobalamin Deficiency and Its Manifestations in Infants and Children

Cobalamin deficiency in children is common in several African (72, 73), Southeast Asian (74–76), and South American (45, 69) populations. The risk of low serum cobalamin (<148 pmol/L) was 2-fold higher in Kenyan schoolchildren whose diet contained no meat than it was in schoolchildren whose diet contained some meat, 5-fold higher in the lowest compared with the highest tertile of milk consumption, and almost 7-fold in the lowest compared with the highest tertile of any animal food source (73). Prevalent cobalamin deficiency (indicated by plasma cobalamin, MMA, and tHcy) in other populations such as Guatemalan schoolchildren (mean age: 10 y, range: 8–12 y) (77), however, was not explained by cobalamin intake (mean: 5.5 µg/d) that was expected to cover daily requirements for this age group (1.8 µg/d) (78). Infection with *Helicobacter pylori* or bacterial overgrowth could interfere with cobalamin absorption (79), but further investigation is needed.

In affluent countries cobalamin deficiency can affect people with alternative dietary practices, such as strict vegetarians or individuals consuming a macrobiotic diet (80, 81). A macrobiotic diet contains minimal amounts of meat and focuses on the consumption of local and seasonal products. Feeding a macrobiotic diet during the first 6 y of life had long-term effects on biochemical markers of cobalamin in adolescents (elevated MMA or lowered cobalamin) who consumed a lacto-vegetarian or lacto-ovo-vegetarian or omnivorous diet from the age of 6 y onward (82).

Nutritional cobalamin deficiency in infancy is manifested between ages 3 and 6 mo in parallel with the decline in cobalamin supply in breastfed infants. Symptoms differ in severity and include physical, hematological, and neurological signs (83). The majority of symptoms are reversible if treated during the early stages of the disease, but long-term neurological complications have been reported (83). Screening for cobalamin deficiency is warranted in children with low birth weight, unexplained anemia, poor physical growth, or neurodevelopmental delays. Moreover, cobalamin deficiency should be explored in children with diseases that increase the risk of deficiency such as malabsorption disorders. Subtle cobalamin deficiency is common in school-age children and it may not lead to acute illness, but it can affect child physical growth, cognitive development, and behavior (84).

The physical growth of children and adolescents who consume macrobiotic diets, vegan diets, or diets poor in cobalamin has been studied extensively. In a study of Dutch infants born to women consuming a macrobiotic diet, the mean birth weight of the newborns was 200 g ($P < 0.05$) lower than that of a Dutch reference population. In a study of a macrobiotic community, age-specific anthropometric measurements (length, weight, sitting height, and arm circumference) in infants aged <6 mo were found to be similar to the Dutch standards; however, markedly lower height, weight, and other physical measures were observed in the macrobiotic group compared with the reference group after age 6 mo (81). Cobalamin status in macrobiotic children was positively associated with bone mineral density and content even after adjusting for potential confounders (85). A causal role for cobalamin deficiency in childhood diseases could not be established because of possible confounding by factors such as infections or multiple nutrients deficiency, which also can affect disease outcomes (72, 73, 77).

Indian infants (aged 12–18 mo) with biochemical signs of cobalamin deficiency had lower psychomotor and mental development scores compared with infants with higher cobalamin status (86). In line with this, children consuming a macrobiotic diet performed worse in psychomotor development

(gross motor and speech-language) than their omnivorous counterparts (81). Differences in cognitive abilities such as fluid intelligence test scores measuring reasoning, problem solving, learning, and abstract thinking were associated with elevated MMA in adolescents who were formerly children who consumed a macrobiotic diet (87).

Prevention and Treatment of Cobalamin Deficiency in Children

Cobalamin supplementation during pregnancy and lactation can prevent maternal depletion and deficiency in infants (3, 64). For example, cyanocobalamin supplementation (250 µg/d) during pregnancy corrected biochemical signs of cobalamin deficiency and increased breast milk concentrations in women from Bangladesh, reflecting increased cobalamin status in infants at birth and thereafter (88).

Formula milk is enriched in cyanocobalamin, which prevents depletion after birth; however, the amounts of cobalamin added to the milk are not based on strong scientific evidence. Furthermore, the effect of using such formula milk on clinical endpoints is unknown and may be confounded by critical differences in other components in the milk.

The treatment of clinically manifested deficiency in children is empirical and influenced by the causes and severity of the manifestations. Treatment may extend from a few months up to 2 y. In 6- to 12-mo-old infants with cobalamin deficiency caused by nutritional factors (e.g., vegan mother), cobalamin injections can be initiated as a first treatment step (1 mg intramuscularly, any cobalamin form). Injections often were continued up to several years, depending on the clinical response (89). Switching to oral cobalamin after several injections is common practice. The improvement of symptoms varies. Hematological signs (e.g., megaloblastic red blood cells) improve within 1 wk of treatment initiation. Recovery from anemia is faster when treatment with folic acid and iron supplements also includes cobalamin. Muscle hypotonia, communication signs, apathy, vomiting, and irritation improve within a few days. Some neurological damage may persist for a longer time.

Clinical trials assessing the effect of cobalamin on child neurodevelopment are scarce. In Norwegian infants <8 mo old with mildly elevated plasma tHcy (>6.5 µmol/L), a single intramuscular cobalamin injection of 400 µg hydroxycobalamin improved motor function and regurgitations and enhanced cobalamin status markers (90).

Intervention studies in subtle cobalamin deficiency have been conducted in infants, preschool-age children, or school-age children from low-income settings through the use of dietary modifications or low doses of cobalamin (close to the RDA) alone or in combination with other micronutrients. Recovery from anemia or other health outcomes (e.g., weight and stunting) have been reported. In school-age children from rural Kenya, serum cobalamin increased with increasing intake of either milk or meat in 1 school meal/d for 1 school year compared with the control group (72); however, no effects were observed in blood hemoglobin, ferritin, or iron. This lack of effect was explained by the high prevalence of

infections and the unchanged low status of several nutrients such as retinol, iron, zinc, riboflavin, and folate. Similarly, providing a snack containing ~9 µg cobalamin/d for 3 mo to Colombian schoolchildren led to improved cobalamin status as well as increased height-for-age z scores and fewer sick days and doctor visits (91). Indian children aged 6–30 mo treated with cyanocobalamin (0.9 µg/d for infants ≤1 y of age and 1.8 µg/d for those >1 y), folic acid (75 or 150 µg/d), or a combination of those vitamins for 6 mo showed an increase in cobalamin status markers and a reduction in the risk of low neurodevelopmental Ages & Stages Questionnaire-3 scores in children whose growth is stunted (71). Indian children who are stunted, wasted, or underweight showed increases in weight-for-age and height-for-age z scores after supplementation with cobalamin or cobalamin plus folic acid (92). In a recent study of 5-y-old Nepalese infants, cobalamin status markers (MMA, tHcy, cobalamin, or their combinations) that were measured at the age of 2–12 mo showed positive associations with several domains of social perception and visuospatial processing scores, suggesting that cobalamin deficiency is one potential cause of adverse developmental outcomes in this population (93).

Summary and Future Directions

Cobalamin deficiency during pregnancy, lactation, and early life is a public health problem in populations with low intake of animal products such as vegetarians and vegans. Intake recommendations for pregnant and lactating women and infants need to be revised in line with recent developments, with special attention paid to women who begin pregnancy with depleted stores of cobalamin. Moreover, a balanced cobalamin-folate intake during pregnancy is necessary for the prevention of poor health outcomes. Cobalamin deficiency during pregnancy can be detected optimally by measuring holoTC as a first-line marker, followed by MMA when necessary. Total plasma cobalamin declines as a result of physiological factors during pregnancy. Cobalamin is important for fetal growth and development. Children with cobalamin deficiency showed improvement in physical and cognitive outcomes after cobalamin supplementation.

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References

1. Rosenthal J, Lopez-Pazos E, Dowling NF, Pfeiffer CM, Mulinare J, Vellozzi C, Zhang M, Lavoie DJ, Molina R, Ramirez N, et al. Folate and vitamin B12 deficiency among non-pregnant women of childbearing-age in Guatemala 2009-2010: prevalence and identification of vulnerable populations. *Matern Child Health J* 2015;19:2272–85.
2. Samuel TM, Duggan C, Thomas T, Bosch R, Rajendran R, Virtanen SM, Srinivasan K, Kurpad AV. Vitamin B(12) intake and status in early pregnancy among urban South Indian women. *Ann Nutr Metab* 2013;62:113–22.
3. Duggan C, Srinivasan K, Thomas T, Samuel T, Rajendran R, Muthayya S, Finkelstein JL, Lukose A, Fawzi W, Allen LH, et al. Vitamin B-12 supplementation during pregnancy and early lactation increases maternal, breast milk, and infant measures of vitamin B-12 status. *J Nutr* 2014;144:758–64.

4. Hübner U, Alwan A, Jouma M, Tabbaa M, Schorr H, Herrmann W. Low serum vitamin B12 is associated with recurrent pregnancy loss in Syrian women. *Clin Chem Lab Med* 2008;46:1265–9.
5. Candito M, Magnaldo S, Bayle J, Dor JF, Gillet Y, Bongain A, Van Obberghen E. Clinical B12 deficiency in one case of recurrent spontaneous pregnancy loss. *Clin Chem Lab Med* 2003;41:1026–7.
6. Ronnenberg AG, Goldman MB, Chen D, Aitken IW, Willett WC, Selhub J, Xu X. Preconception homocysteine and B vitamin status and birth outcomes in Chinese women. *Am J Clin Nutr* 2002;76:1385–91.
7. Fréry N, Huel G, Leroy M, Moreau T, Savard R, Blot P, Lellouch J. Vitamin B12 among parturients and their newborns and its relationship with birthweight. *Eur J Obstet Gynecol Reprod Biol* 1992;45:155–63.
8. Relton CL, Pearce MS, Parker L. The influence of erythrocyte folate and serum vitamin B12 status on birth weight. *Br J Nutr* 2005;93:593–9.
9. Guerra-Shinohara EM, Pereira PM, Kubota AM, Silva TA, Reis JL, Miyashita GS, D'Almeida V, Allen RH, Stabler SP. Increased MMA concentration and body mass index are associated with spontaneous abortion in Brazilian women: a pilot study. *Clin Chim Acta* 2010;411:423–7.
10. Ray JG. Folic acid food fortification in Canada. *Nutr Rev* 2004;62:S35–9.
11. Cherian A, Seená S, Bullock RK, Antony AC. Incidence of neural tube defects in the least-developed area of India: a population-based study. *Lancet* 2005;366:930–1.
12. Antony AC. Vegetarianism and vitamin B-12 (cobalamin) deficiency. *Am J Clin Nutr* 2003;78:3–6.
13. Godbole K, Gayathri P, Ghule S, Sasirekha BV, Kanitkar-Damle A, Memane N, Suresh S, Sheth J, Chandak GR, Yajnik CS. Maternal one-carbon metabolism, MTHFR and TCN2 genotypes and neural tube defects in India. *Birth Defects Res A Clin Mol Teratol* 2011;91:848–56.
14. Molloy AM, Kirke PN, Troendle JF, Burke H, Sutton M, Brody LC, Scott JM, Mills JL. Maternal vitamin B12 status and risk of neural tube defects in a population with high neural tube defect prevalence and no folic acid fortification. *Pediatrics* 2009;123:917–23.
15. Finkelstein JL, Layden AJ, Stover PJ. Vitamin B-12 and perinatal health. *Adv Nutr* 2015;6:552–63.
16. Berry CG. Anaemia of pregnancy in Africans of Lagos. *BMJ* 1955;2:819–23.
17. Institute of Medicine. Dietary reference intakes for thiamin, riboflavin, niacin, vitamin B6, folate, vitamin B12, pantothenic acid, biotin, and choline. Washington (DC): National Academy Press; 1998. p. 390–422.
18. Vaz Pinto A, Torras V, Sandoval JF, Dillman E, Mateos CR, Cordova MS. Folic acid and vitamin B12 determination in fetal liver. *Am J Clin Nutr* 1975;28:1085–6.
19. Robertson JA, Gallagher ND. Increased intestinal uptake of cobalamin in pregnancy does not require synthesis of new receptors. *Biochim Biophys Acta* 1983;757:145–50.
20. Hellegers A, Okuda K, Nesbitt RE Jr., Smith DW, Chow BF. Vitamin B12 absorption in pregnancy and in the newborn. *Am J Clin Nutr* 1957;5:327–31.
21. Greibe E, Andreasen BH, Lildballe DL, Morkbak AL, Hvas AM, Nexø E. Uptake of cobalamin and markers of cobalamin status: a longitudinal study of healthy pregnant women. *Clin Chem Lab Med* 2011;49:1877–82.
22. Graber SE, Scheffel U, Hodkinson B, McIntyre PA. Placental transport of vitamin B12 in the pregnant rat. *J Clin Invest* 1971;50:1000–4.
23. Ullberg S, Kristofferson H, Flodh H, Hanngren A. Placental passage and fetal accumulation of labelled vitamin B12 in the mouse. *Arch Int Pharmacodyn Ther* 1967;167:431–49.
24. Giugliani ER, Jorge SM, Goncalves AL. Serum vitamin B12 levels in parturients, in the intervillous space of the placenta and in full-term newborns and their interrelationships with folate levels. *Am J Clin Nutr* 1985;41:330–5.
25. Koebnick C, Heins UA, Dagnelie PC, Wickramasinghe SN, Ratnayaka ID, Hothorn T, Pfahlberg AB, Hoffmann I, Lindemans J, Leitzmann C. Longitudinal concentrations of vitamin B(12) and vitamin B(12)-binding proteins during uncomplicated pregnancy. *Clin Chem* 2002;48:928–33.
26. Murphy MM, Molloy AM, Ueland PM, Fernandez-Ballart JD, Schneede J, Arija V, Scott JM. Longitudinal study of the effect of pregnancy on maternal and fetal cobalamin status in healthy women and their offspring. *J Nutr* 2007;137:1863–7.
27. Milman N, Byg KE, Bergholt T, Eriksen L, Hvas AM. Cobalamin status during normal pregnancy and postpartum: a longitudinal study comprising 406 Danish women. *Eur J Haematol* 2006;76:521–5.
28. Morkbak AL, Hvas AM, Milman N, Nexø E. Holotranscobalamin remains unchanged during pregnancy. Longitudinal changes of cobalamins and their binding proteins during pregnancy and postpartum. *Haematologica* 2007;92:1711–2.
29. Murphy MM, Scott JM, Arija V, Molloy AM, Fernandez-Ballart JD. Maternal homocysteine before conception and throughout pregnancy predicts fetal homocysteine and birth weight. *Clin Chem* 2004;50:1406–12.
30. Murphy MM, Scott JM, McPartlin JM, Fernandez-Ballart JD. The pregnancy-related decrease in fasting plasma homocysteine is not explained by folic acid supplementation, hemodilution, or a decrease in albumin in a longitudinal study. *Am J Clin Nutr* 2002;76:614–9.
31. Obeid R, Morkbak AL, Munz W, Nexø E, Herrmann W. The cobalamin-binding proteins transcobalamin and haptocorrin in maternal and cord blood sera at birth. *Clin Chem* 2006;52:263–9.
32. Green R, Miller JW. Vitamin B12 deficiency is the dominant nutritional cause of hyperhomocysteinemia in a folic acid-fortified population. *Clin Chem Lab Med* 2005;43:1048–51.
33. Fernández-Roig S, Cavalle-Busquets P, Fernandez-Ballart JD, Ballesteros M, Berrocal-Zaragoza MI, Salat-Batlle J, Ueland PM, Murphy MM. Low folate status enhances pregnancy changes in plasma betaine and dimethylglycine concentrations and the association between betaine and homocysteine. *Am J Clin Nutr* 2013;97:1252–9.
34. Colomina JM, Cavallé-Busquets P, Fernández-Roig S, Solé-Navais P, Fernandez-Ballart JD, Ballesteros M, Ueland PM, Meyer K, Murphy MM. Maternal folate status and the BHMT c.716G>A polymorphism affect the betaine dimethylglycine pathway during pregnancy. *Nutrients* 2016;8:pii: E621.
35. García-Minguillán CJ, Fernandez-Ballart JD, Ceruelo S, Ríos L, Bueno O, Berrocal-Zaragoza MI, Molloy AM, Ueland PM, Meyer K, Murphy MM. Riboflavin status modifies the effects of methylenetetrahydrofolate reductase (MTHFR) and methionine synthase reductase (MTRR) polymorphisms on homocysteine. *Genes Nutr* 2014;9:435.
36. Bueno O, Molloy AM, Fernandez-Ballart JD, García-Minguillán CJ, Ceruelo S, Ríos L, Ueland PM, Meyer K, Murphy MM. Common polymorphisms that affect folate transport or metabolism modify the effect of the MTHFR 677C > T polymorphism on folate status. *J Nutr* 2016;146:1–8.
37. Dominguez-Salas P, Moore SE, Cole D, da Costa KA, Cox SE, Dyer RA, Fulford AJ, Innis SM, Waterland RA, Zeisel SH. DNA methylation potential: dietary intake and blood concentrations of one-carbon metabolites and cofactors in rural African women. *Am J Clin Nutr* 2013;97:1217–27.
38. Gomes TS, Lindner U, Tennekoon KH, Karandagoda W, Gortner L, Obeid R. Homocysteine in small-for-gestational age and appropriate-for-gestational age preterm neonates from mothers receiving folic acid supplementation. *Clin Chem Lab Med* 2010;48:1157–61.
39. Dwarkanath P, Barzilay JR, Thomas T, Thomas A, Bhat S, Kurpad AV. High folate and low vitamin B-12 intakes during pregnancy are associated with small-for-gestational age infants in South Indian women: a prospective observational cohort study. *Am J Clin Nutr* 2013;98:1450–8.
40. Yajnik CS, Deshpande SS, Jackson AA, Refsum H, Rao S, Fisher DJ, Bhat DS, Naik SS, Coyaji KJ, Joglekar CV. Vitamin B12 and folate concentrations during pregnancy and insulin resistance in the offspring: the Pune Maternal Nutrition Study. *Diabetologia* 2008;51:29–38.
41. Krishnaveni GV, Veena SR, Karat SC, Yajnik CS, Fall CH. Association between maternal folate concentrations during pregnancy and insulin resistance in Indian children. *Diabetologia* 2014;57:110–21.

42. Knight BA, Shields BM, Brook A, Hill A, Bhat DS, Hattersley AT, Yajnik CS. Lower circulating B12 is associated with higher obesity and insulin resistance during pregnancy in a non-diabetic white British population. *PLoS One* 2015;10:e0135268.
43. Srikantia SG, Reddy V. Megaloblastic anaemia of infancy and vitamin B12. *Br J Haematol* 1967;13:949–53.
44. Bae S, West AA, Yan J, Jiang X, Perry CA, Malysheva O, Stabler SP, Allen RH, Caudill MA. Vitamin B-12 status differs among pregnant, lactating, and control women with equivalent nutrient intakes. *J Nutr* 2015;145:1507–14.
45. Black AK, Allen LH, Pelto GH, de Mata MP, Chavez A. Iron, vitamin B-12 and folate status in Mexico: associated factors in men and women and during pregnancy and lactation. *J Nutr* 1994;124:1179–88.
46. Shapiro J, Alberts HW, Welch P, Metz J. Folate and vitamin B12 deficiency associated with lactation. *Br J Haematol* 1965;11:498–504.
47. Specker BL, Black A, Allen L, Morrow F. Vitamin B-12: low milk concentrations are related to low serum concentrations in vegetarian women and to methylmalonic aciduria in their infants. *Am J Clin Nutr* 1990;52:1073–6.
48. Specker BL. Nutritional concerns of lactating women consuming vegetarian diets. *Am J Clin Nutr* 1994;59:1182S–6S.
49. Greibe E, Lildballe DL, Streym S, Vestergaard P, Rejnmark L, Mosekilde L, Nexø E. Cobalamin and haptocorrin in human milk and cobalamin-related variables in mother and child: a 9-mo longitudinal study. *Am J Clin Nutr* 2013;98:389–95.
50. Trugo NM, Sardinha F. Cobalamin and cobalamin binding capacity in human milk. *Nutr Res* 1994;14:23–33.
51. Donangelo CM, Trugo NM, Koury JC, Barreto Silva MI, Freitas LA, Feldheim W, Barth C. Iron, zinc, folate and vitamin B12 nutritional status and milk composition of low-income Brazilian mothers. *Eur J Clin Nutr* 1989;43:253–66.
52. Specker BL, Miller D, Norman EJ, Greene H, Hayes KC. Increased urinary methylmalonic acid excretion in breast-fed infants of vegetarian mothers and identification of an acceptable dietary source of vitamin B-12. *Am J Clin Nutr* 1988;47:89–92.
53. Hampel D, Shahab-Ferdows S, Domek JM, Siddiqua T, Raqib R, Allen LH. Competitive chemiluminescent enzyme immunoassay for vitamin B12 analysis in human milk. *Food Chem* 2014;153:60–5.
54. Lildballe DL, Hardlei TF, Allen LH, Nexø E. High concentrations of haptocorrin interfere with routine measurement of cobalamins in human serum and milk. A problem and its solution. *Clin Chem Lab Med* 2009;47:182–7.
55. Deegan KL, Jones KM, Zuleta C, Ramirez-Zea M, Lildballe DL, Nexø E, Allen LH. Breast milk vitamin B-12 concentrations in Guatemalan women are correlated with maternal but not infant vitamin B-12 status at 12 months postpartum. *J Nutr* 2012;142:112–6.
56. Bijur AM, Desai AG. Composition of breast milk with reference to vitamin B12 and folic acid in Indian mothers. *Indian J Pediatr* 1985;52:147–50.
57. Institute of Medicine. Vitamin B12. In: Dietary reference intakes for thiamin, riboflavin, niacin, vitamin B6, folate, vitamin B12, pantothenic acid, biotin, and choline. Washington (DC): National Academy Press; 2000. p. 306–56.
58. Hampel D, Allen LH. Analyzing B-vitamins in human milk: methodological approaches. *Crit Rev Food Sci Nutr* 2016;56:494–511.
59. Karademir F, Suleymanoglu S, Ersen A, Aydinöz S, Gultepe M, Meral C, Ozkaya H, Gocmen I. Vitamin B12, folate, homocysteine and urinary methylmalonic acid levels in infants. *J Int Med Res* 2007;35:384–8.
60. Monsen AL, Refsum H, Markestad T, Ueland PM. Cobalamin status and its biochemical markers methylmalonic acid and homocysteine in different age groups from 4 days to 19 years. *Clin Chem* 2003;49:2067–75.
61. Koc A, Kocyigit A, Soran M, Demir N, Sevinc E, Erel O, Mil Z. High frequency of maternal vitamin B12 deficiency as an important cause of infantile vitamin B12 deficiency in Sanliurfa province of Turkey. *Eur J Nutr* 2006;45:291–7.
62. Guerra-Shinohara EM, Morita OE, Peres S, Pagliusi RA, Sampaio Neto LF, D'Almeida V, Irazusta SP, Allen RH, Stabler SP. Low ratio of S-adenosylmethionine to S-adenosylhomocysteine is associated with vitamin deficiency in Brazilian pregnant women and newborns. *Am J Clin Nutr* 2004;80:1312–21.
63. Bhat V, Deshpande S, Bhat D, Joshi N, Laddkat R, Watve S, Fall C, de Jager CA, Refsum H, Yajnik C. Vitamin B12 status of pregnant Indian women and cognitive function in their 9-year-old children. *Food Nutr Bull* 2008;29:249–54.
64. Hay G, Johnston C, Whitelaw A, Trygg K, Refsum H. Folate and cobalamin status in relation to breastfeeding and weaning in healthy infants. *Am J Clin Nutr* 2008;88:105–14.
65. Ulak M, Chandyo RK, Adhikari RK, Sharma PR, Sommerfelt H, Refsum H, Strand TA. Cobalamin and folate status in 6 to 35 months old children presenting with acute diarrhea in Bhaktapur, Nepal. *PLoS One* 2014;9:e90079.
66. Pasricha SR, Shet AS, Black JE, Sudarshan H, Prashanth NS, Biggs BA. Vitamin B-12, folate, iron, and vitamin A concentrations in rural Indian children are associated with continued breastfeeding, complementary diet, and maternal nutrition. *Am J Clin Nutr* 2011;94:1358–70.
67. Hay G, Clausen T, Whitelaw A, Trygg K, Johnston C, Henriksen T, Refsum H. Maternal folate and cobalamin status predicts vitamin status in newborns and 6-month-old infants. *J Nutr* 2010;140:557–64.
68. Ueland PM, Monsen AL. Hyperhomocysteinemia and B-vitamin deficiencies in infants and children. *Clin Chem Lab Med* 2003;41:1418–26.
69. Casterline JE, Allen LH, Ruel MT. Vitamin B-12 deficiency is very prevalent in lactating Guatemalan women and their infants at three months postpartum. *J Nutr* 1997;127:1966–72.
70. Taneja S, Bhandari N, Strand TA, Sommerfelt H, Refsum H, Ueland PM, Schneede J, Bahl R, Bhan MK. Cobalamin and folate status in infants and young children in a low-to-middle income community in India. *Am J Clin Nutr* 2007;86:1302–9.
71. Kvestad I, Taneja S, Kumar T, Hysing M, Refsum H, Yajnik CS, Bhandari N, Strand TA; Folate and Vitamin B12 Study Group. Vitamin B12 and folic acid improve gross motor and problem-solving skills in young North Indian children: a randomized placebo-controlled trial. *PLoS One* 2015;10:e0129915.
72. Siekmann JH, Allen LH, Bwibo NO, Demment MW, Murphy SP, Neumann CG. Kenyan school children have multiple micronutrient deficiencies, but increased plasma vitamin B-12 is the only detectable micronutrient response to meat or milk supplementation. *J Nutr* 2003;133:3972S–80S.
73. McLean ED, Allen LH, Neumann CG, Pearson JM, Siekmann JH, Murphy SP, Bwibo NO, Demment MW. Low plasma vitamin B-12 in Kenyan school children is highly prevalent and improved by supplemental animal source foods. *J Nutr* 2007;137:676–82.
74. Chanarin I, Malkowska V, O'Hea AM, Rinsler MG, Price AB. Megaloblastic anaemia in a vegetarian Hindu community. *Lancet* 1985;2:1168–72.
75. Bondevik GT, Schneede J, Refsum H, Lie RT, Ulstein M, Kvale G. Homocysteine and methylmalonic acid levels in pregnant Nepali women. Should cobalamin supplementation be considered? *Eur J Clin Nutr* 2001;55:856–64.
76. Gomber S, Kumar S, Rusia U, Gupta P, Agarwal KN, Sharma S. Prevalence & etiology of nutritional anaemias in early childhood in an urban slum. *Indian J Med Res* 1998;107:269–73.
77. Rogers LM, Boy E, Miller JW, Green R, Sabel JC, Allen LH. High prevalence of cobalamin deficiency in Guatemalan schoolchildren: associations with low plasma holotranscobalamin II and elevated serum methylmalonic acid and plasma homocysteine concentrations. *Am J Clin Nutr* 2003;77:433–40.
78. Rogers LM, Boy E, Miller JW, Green R, Rodriguez M, Chew F, Allen LH. Predictors of cobalamin deficiency in Guatemalan school children: diet, *Helicobacter pylori*, or bacterial overgrowth? *J Pediatr Gastroenterol Nutr* 2003;36:27–36.
79. Serin E, Gumurdulu Y, Ozer B, Kayaselcuk F, Yilmaz U, Kocak R. Impact of *Helicobacter pylori* on the development of vitamin B12 deficiency in the absence of gastric atrophy. *Helicobacter* 2002;7:337–41.
80. Schneede J, Dagnelie PC, van Staveren WA, Vollset SE, Refsum H, Ueland PM. Methylmalonic acid and homocysteine in plasma as indicators of functional cobalamin deficiency in infants on macrobiotic diets. *Pediatr Res* 1994;36:194–201.

81. Dagnelie PC, van Staveren WA. Macrobiotic nutrition and child health: results of a population-based, mixed-longitudinal cohort study in The Netherlands. *Am J Clin Nutr* 1994;59:1187S–96S.
82. van Dusseldorp M, Schneede J, Refsum H, Ueland PM, Thomas CM, de Boer E, van Staveren WA. Risk of persistent cobalamin deficiency in adolescents fed a macrobiotic diet in early life. *Am J Clin Nutr* 1999;69:664–71.
83. Graham SM, Arvela OM, Wise GA. Long-term neurologic consequences of nutritional vitamin B12 deficiency in infants. *J Pediatr* 1992;121:710–4.
84. Allen LH. The nutrition CRSP: what is marginal malnutrition, and does it affect human function? *Nutr Rev* 1993;51:255–67.
85. Dhonukshe-Rutten RA, van Dusseldorp M, Schneede J, de Groot LC, van Staveren WA. Low bone mineral density and bone mineral content are associated with low cobalamin status in adolescents. *Eur J Nutr* 2005;44:341–7.
86. Strand TA, Taneja S, Ueland PM, Refsum H, Bahl R, Schneede J, Sommerfelt H, Bhandari N. Cobalamin and folate status predicts mental development scores in North Indian children 12–18 mo of age. *Am J Clin Nutr* 2013;97:310–7.
87. Louwman MW, van Dusseldorp M, van de Vijver FJ, Thomas CM, Schneede J, Ueland PM, Refsum H, van Staveren WA. Signs of impaired cognitive function in adolescents with marginal cobalamin status. *Am J Clin Nutr* 2000;72:762–9.
88. Siddiqua TJ, Ahmad SM, Ahsan KB, Rashid M, Roy A, Rahman SM, Shahab-Ferdows S, Hampel D, Ahmed T, Allen LH, et al. Vitamin B12 supplementation during pregnancy and postpartum improves B12 status of both mothers and infants but vaccine response in mothers only: a randomized clinical trial in Bangladesh. *Eur J Nutr* 2016;55:281–93.
89. Dror DK, Allen LH. Effect of vitamin B12 deficiency on neurodevelopment in infants: current knowledge and possible mechanisms. *Nutr Rev* 2008;66:250–5.
90. Torsvik I, Ueland PM, Markestad T, Bjorke-Monsen AL. Cobalamin supplementation improves motor development and regurgitations in infants: results from a randomized intervention study. *Am J Clin Nutr* 2013;98:1233–40.
91. Arsenault JE, Mora-Plazas M, Forero Y, López-Arana S, Marín C, Baylin A, Villamor E. Provision of a school snack is associated with vitamin B-12 status, linear growth, and morbidity in children from Bogota, Colombia. *J Nutr* 2009;139:1744–50.
92. Strand TA, Taneja S, Kumar T, Manger MS, Refsum H, Yajnik CS, Bhandari N. Vitamin B-12, folic acid, and growth in 6- to 30-month-old children: a randomized controlled trial. *Pediatrics* 2015;135:e918–26.
93. Kvestad I, Hysing M, Shrestha M, Ulak M, Thorne-Lyman AL, Henjum S, Ueland PM, Midttun Ø, Fawzi W, Chandyo RK, et al. Vitamin B-12 status in infancy is positively associated with development and cognitive functioning 5 y later in Nepalese children. *Am J Clin Nutr* 2017;105:1122–31.