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ARTICLE

Common Metabolic Profile in Infants Indicating Impaired Cobalamin Status Responds to Cobalamin Supplementation

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What's Known on This Subject

Studies suggest that cobalamin deficiency, particularly in breastfed infants, may be prevalent. The prevailing opinion is that the metabolic profile encountered in these infants reflects immature organ systems, rather than impaired cobalamin status.

What This Study Adds

This intervention trial shows that cobalamin supplementation in 6-week-old infants changes all markers toward the profile observed in cobalamin-replete older children and adults, contradicting the assumption that low cobalamin status in infants is attributable to organ immaturity.

ABSTRACT

OBJECTIVE. A metabolic profile consistent with impaired cobalamin status is prevalent in breastfed infants. We investigated whether this profile reflects immature organ systems or impaired cobalamin status.

METHODS. In a single-center, randomized, placebo-controlled trial, we studied 107 six-week-old infants. The infants were randomly assigned to receive either an intramuscular injection of 400 μg of cobalamin or no intervention. Concentrations of cobalamin and folate in serum and total homocysteine, methylmalonic acid, and cystathionine in plasma were determined at enrollment and at the age of 4 months.

RESULTS. There were no significant differences between the intervention group (n = 54) and the control group (n = 53) in the concentrations of any vitamin marker at baseline (6 weeks). At 4 months, the supplement-treated infants had a 75% higher median serum cobalamin level and remarkable reductions in median plasma total homocysteine (from 7.46 to 4.57 μmol/L) and methylmalonic acid (from 0.58 to 0.20 μmol/L) levels, whereas levels of both metabolites were essentially unchanged during the follow-up period in the control group.

CONCLUSIONS. Cobalamin supplementation changed all markers of impaired cobalamin status (low cobalamin, high total homocysteine, and high methylmalonic acid levels) toward a profile observed in cobalamin-replete older children and adults. Therefore, the high total homocysteine and methylmalonic acid levels reported for a large fraction of infants reflect not immature metabolism but rather insufficient cobalamin levels to fully sustain cobalamin-dependent reactions fully. *Pediatrics* 2008;122:83–91

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This trial has been registered at www.clinicaltrials.gov (identifier NCT00479479).

Key Words
infant, breastfeeding, cobalamin deficiency, cobalamin supplementation

Abbreviations
tHcy—total homocysteine
MMA—methylmalonic acid

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THERE HAS BEEN increasing awareness of cobalamin deficiency in breastfed infants.[1–3] In exclusively breastfed infants, the cobalamin content in the milk, and therefore the maternal cobalamin status during lactation, has a profound impact on the infant cobalamin status.[4] Infant cobalamin deficiency is considered secondary to maternal deficiency, which may be attributable to vegetarianism, malabsorption, or unrecognized early pernicious anemia.[5]

High rates of cobalamin deficiency in pregnant and breastfeeding women and their infants have repeatedly been demonstrated in developing countries.[1,6–9] In developed countries, exclusive breastfeeding for the first 6 months is encouraged,[10] and this places great nutritional demands on the mother.[11]

During fetal life and infancy, adequate cobalamin status is important for normal growth and central nervous system development.[2] In infants, cobalamin deficiency may present as failure to thrive, developmental delays or regression, progressive or persistent neurologic disorders, or hematologic changes. The symptoms may be evident as early as 3 weeks of age but often are subtle and difficult to detect, partly because of the large variation in normal development in this age group.[12–16] Long-term neurologic consequences depend on the severity and duration of cobalamin deficiency, but deficiency during infancy, even when treated successfully, may result in permanent developmental disabilities.[14,17–19]

Cobalamin is a coenzyme in a folate-dependent methyl transfer reaction that converts homocysteine to methionine and in a separate reaction that converts L-methylmalonyl-CoA to succinyl-CoA. Consequently, elevated levels of total homocysteine (tHcy) and/or methylmalonic acid (MMA) in the blood are measures of impaired cobalamin status.[20] Homocysteine also can be condensed with serine to form cystathionine,[21] and elevated levels of cystathionine are found in the serum of most patients with cobalamin and folate deficiencies.[22]

There have been reports of low cobalamin concentrations combined with elevated concentrations of the metabolic markers tHcy and MMA in apparently healthy, breastfed infants born to mothers on a westernized diet.[23–27] This metabolic profile may be an innocuous phenomenon caused by immaturity of liver or kidney function[28,29] or may reflect common occurrence of impaired cobalamin function in infants.[2,30,31]

We conducted a randomized, controlled, cobalamin intervention trial with infants 6 weeks of age. The purpose of the study was to determine whether cobalamin supplementation influenced the metabolic profile related to cobalamin status in infants.

METHODS

Study Population and Design

Healthy term infants and their mothers were recruited by a local public health nurse during well-infant visits at 5 different health services. Between December 2004 and April 2006, 107 healthy infants, including 3 pairs of twins, and their 104 mothers were enrolled. The infants were 6 ± 2 weeks of age at entry. They were invited back for a second investigation at 4 months of age. At baseline and 4 months, blood samples were obtained from infants and mothers through antecubital venipuncture. A questionnaire on infant and maternal nutrition, vitamin supplementation, growth parameters, parity, and maternal use of tobacco was completed.

Infants were assigned, through block randomization, to receive either cobalamin (n = 54) or no treatment (n = 53; control group). Four infants were lost to follow-up monitoring, 2 from each group. After blood sampling at the first visit, the infants in the intervention group received an intramuscular injection of 400 μg of hydroxycobalamin (vitamin B_{12} depot; Nycomed Pharma, Zürich, Switzerland). Treatment with 400 μg of hydroxycobalamin has been associated with increased hemoglobin levels[32] and reduced tHcy and MMA levels in premature infants (A-L.B-M., T.M., H. Reigstad, P.M.U., unpublished data, 2003).

Because of ethical concerns, no placebo injection was given to the control subjects. The laboratory personnel responsible for blood sampling and analyses but not the rest of the study staff members or the mothers were blinded with respect to group assignment. Ethical approval of the protocol was granted by the local committee on medical research ethics, and the mothers gave written informed consent.

Blood Sample Collection and Biochemical Analyses

Serum was obtained by collecting blood into Vacutainer tubes (Becton Dickinson, Franklin Lakes, NJ) with no additive. Blood was allowed to clot at room temperature for 30 minutes before the serum fraction was transferred to an empty glass vial. The blood samples used for preparation of EDTA-treated plasma were collected into Vacutainer tubes (Becton Dickinson, Franklin Lakes, NJ) and placed in ice water, and plasma was separated within 4 hours. The samples were stored at −80°C until analysis.

Serum cobalamin levels were determined with a *Lactobacillus leichmannii* microbiologic assay[33] and serum folate levels with a *Lactobacillus casei* microbiologic assay.[34] Plasma levels of tHcy, MMA, and cystathionine were determined by using a gas chromatography-mass spectrometry method based on methylchloroformate derivatization.[35]

Statistical Analyses

The calculation of the sample size was based on data from our previous studies[23,26] on cobalamin status in infants, with the assumption that cobalamin supplementation would result in tHcy levels located in the lower quartile (<6.14 μmol/L) for infants at 4 months. A calculated sample size of 65 (ie, 33 in each group) would give the study a statistical power of >90% to detect a 25% relative reduction in tHcy levels. However, on the basis of our experience from earlier studies, a dropout rate of ~40% was expected, and a total of ~100 infants were recruited.

Results are presented as median and interquartile range and mean ± SD. Medians were compared with the Wilcoxon signed-ranks test and Mann-Whitney U test, and means were compared with Student's t test. Differences in categorical variables were tested with the χ^2 test. Multivariate linear regression models were used to assess the relationships of intervention, infant nutrition, and maternal vitamin status to infant serum cobalamin, serum folate, plasma tHcy, MMA, and cystathionine levels at 6 weeks and 4 months. Because the additional solid food (cereals) used at 4 months did not contain cobalamin, infant nutrition was defined as breastfed, combined breastfed and formula-fed, or formula-fed.

We also used quantile regression analysis,[36] which examines the simultaneous influence of the covariates on the entire distribution of metabolic responses of the infants, to determine changes in infant cobalamin, folate, tHcy, or MMA levels as a function of pretreatment concentrations of these variables at 6 weeks, intervention, infant nutrition at 4 months, maternal cobalamin concentrations at 4 months, and, for changes in infant folate and tHcy levels, maternal folate levels at 4 months. For each covariate, the point estimate is the impact of a 1-unit change in the covariate on infant vitamin or metabolite concentrations at 4 months, with the other covariates held fixed. Because the respective infant indices at 6 weeks were included as independent variables,

TABLE 1 **Baseline Characteristics of the Participants**

	Control Group	Intervention Group	*P*
Infant data ($N = 107$)	$n = 53$	$n = 54$	
Male gender, *n* (%)	25 (47)	31 (57)	.29[a]
At birth			
Gestational age, mean ± SD, wk	39.8 ± 1.4	39.9 ± 1.2	.66[b]
Weight, mean ± SD, g	3543 ± 502	3577 ± 528	.73[b]
Length, mean ± SD, cm	50.5 ± 2.2	50.5 ± 1.9	.89[b]
Head circumference, mean ± SD, cm	35.0 ± 1.5	35.3 ± 1.4	.38[b]
At 6 wk			
Weight, mean ± SD, g	4921 ± 605	4924 ± 687	.99[b]
Length, mean ± SD, cm	55.9 ± 2.3	56.4 ± 2.4	.33[b]
Head circumference, mean ± SD, cm	38.6 ± 1.3	38.6 ± 1.1	.70[b]
Nutrition at 6 wk, *n* (%)			
Breastfed	38 (72)	48 (89)	.05[a]
Breastfed and formula-fed	13 (25)	4 (7)	
Formula-fed	2 (4)	2 (4)	
Additional solid food	0 (0)	0 (0)	
Maternal data at 6 wk ($N = 104$)	$n = 52$	$n = 52$	
Age, mean ± SD, y	31.4 ± 4.8	31.5 ± 4.9	.92[b]
BMI, mean ± SD	25.2 ± 3.9	24.6 ± 4.1	.71[b]
Para 0, *n* (%)	20 (39)	21 (40)	.75[a]
No. of children for para ≥1, mean ± SD	1.4 ± 0.6	1.5 ± 0.8	.54[b]
Daily vitamin supplementation, *n* (%)	18 (35)	17 (33)	.79[a]
Daily smoking, *n* (%)	4 (8)	5 (10)	.82[a]

[a] χ^2 test.
[b] Student's *t* test.

TABLE 2 **Characteristics of the Infants at 4 Months**

	Control Group ($n = 51$)	Intervention Group ($n = 52$)	*P*
Weight, mean ± SD, g	6862 ± 724	6736 ± 876	.43[a]
Length, mean ± SD, cm	64.0 ± 2.4	63.7 ± 2.5	.55[a]
Head circumference, mean ± SD, cm	42.0 ± 1.2	41.9 ± 1.2	.70[a]
Infant nutrition, *n* (%)			
Breastfed	38 (75)	43 (83)	.32[b]
Breastfed and formula-fed	7 (14)	7 (14)	
Formula-fed	6 (12)	2 (4)	
Additional solid food	8 (16)	6 (12)	.56[b]

[a] Student's *t* test.
[b] χ^2 test.

the outcome measure was the change in infant indices from 6 weeks to 4 months.

Two-sided *P* values of $<.05$ were considered statistically significant. SPSS 11 (SPSS, Chicago, IL) was used for all statistical analyses except for quantile regression analysis, for which R[37] was used.

RESULTS

Demographic Features and Nutrition

The total group included healthy term infants with birth weight (mean ± SD) of 3560 ± 513 g, birth length of 50.5 ± 2.0 cm, and head circumference at birth of 35.1 ± 1.5 cm. More infants were exclusively breastfed in the intervention group than in the control group ($P = .052$). Apart from this, there were no significant differences ($P = .29$-$.99$) between the infant groups with respect to baseline characteristics (Table 1).

At 6 weeks (Table 1), all infants were exclusively milk fed, 86 of 107 were exclusively breastfed, none received additional solid food, and daily multivitamin supplements (not including cobalamin) were given to only 1 infant. The 2 different kinds of milk formula used were both enriched with cobalamin, at 0.13 μg per 100 mL of prepared milk formula (Collet; Axellus, Norway) or 0.2 μg per 100 mL of prepared milk formula (Nan; Nestlé, Norway).

At 4 months (Table 2), 72 of the 103 infants (39 from the intervention group and 33 from the control group) were exclusively breastfed. The remaining 31 infants (13 from the intervention group and 18 from the control group) received formula either additionally or exclusively and/or cereal. None of the cereals used was enriched with cobalamin. Only 1 infant (control group) received dinner, and daily multivitamin supplements were given to 2 infants (1 from each group); none of these supplements contained cobalamin.

Maternal age, BMI, parity, vitamin supplement use, and smoking habits at 6 weeks were not significantly different between the intervention group and the control group (Table 1). Two of the mothers were vegetarians, and the others claimed to have an omnivorous diet, although 9 mothers reported that they rarely or never ate meat ($n = 6$) or fish ($n = 3$).

Infant Blood Indices and Effects of Intervention

There were no gender differences in serum cobalamin or folate levels or the metabolic markers plasma tHcy, MMA, and cystathionine levels (all $P > 0.1$; data not shown), and the data for both genders were analyzed together. Before intervention, the concentrations of the B vitamins and metabolites were not significantly different between the intervention group and the control group (Table 3).

During the follow-up period (between 6 weeks and 4 months), median serum cobalamin levels increased markedly (from 172 to 421 pmol/L) in the infants given cobalamin and moderately (from 170 to 240 pmol/L) in the control infants. Serum folate levels increased in both groups during this period but less so in the intervention group (from 21.7 to 35.2 nmol/L), compared with the control group (from 23.7 to 47.9 nmol/L). At 4 months, the median serum folate level was significantly lower in the intervention group (Table 3 and Fig 1).

The most notable effects of cobalamin intervention were considerable reductions in plasma tHcy, MMA, and cystathionine levels during the follow-up period (Table 3 and Fig 1). In the intervention group, the median plasma tHcy level was reduced from 7.46 to 4.57 μmol/L (39%), plasma MMA level from 0.58 to 0.20 μmol/L (66%), and plasma cystathionine level from 0.46 to 0.16 μmol/L (65%). In the control group, plasma tHcy and MMA levels remained stable and the plasma cystathionine level decreased from 0.46 to 0.24 μmol/L (48%) (Table 3 and Fig 1).

We observed that, at 4 months, 35 of 51 control infants had plasma tHcy levels above the 97.5th percentile (6.50 μmol/L) of the tHcy levels in the supplement-

TABLE 3 **Vitamin and Metabolite Levels in Infants at 6 Weeks and 4 Months**

	Control Group	Intervention Group	*P*[a]
N			
6 wk	53	54	
4 mo	51	52	
Serum cobalamin level, median (interquartile range), pmol/L			
6 wk	170 (137–288)	172 (128–250)	.40
4 mo	240 (162–337)	421 (291–497)	<.001
P[b]	.002	<.001	
Serum folate level, median (interquartile range), nmol/L			
6 wk	23.7 (18.0–29.3)	21.7 (16.9–28.3)	.63
4 mo	47.9 (32.5–62.1)	35.2 (27.5–47.6)	.001
P[b]	<.001	<.001	
Plasma tHcy level, median (interquartile range), μmol/L			
6 wk	7.66 (6.10–9.11)	7.46 (6.20–9.31)	.78
4 mo	7.40 (6.02–8.70)	4.57 (4.02–5.05)	<.001
P[b]	.42	<.001	
Plasma MMA level, median (interquartile range), μmol/L			
6 wk	0.50 (0.27–2.44)	0.58 (0.28–0.97)	.29
4 mo	0.51 (0.23–1.55)	0.20 (0.15–0.43)	<.001
P[b]	.47	<.001	
Plasma cystathionine level, median (interquartile range), μmol/L			
6 wk	0.46 (0.36–0.54)	0.46 (0.33–0.59)	.70
4 mo	0.24 (0.21–0.32)	0.16 (0.13–0.20)	<.001
P[b]	<.001	<.001	

[a] Mann-Whitney *U* test.
[b] Wilcoxon signed-ranks test.

treated infants. Approximately the same proportion, 73 of 107 infants, had tHcy levels of >6.50 μmol/L at baseline. Therefore, more than two thirds of young infants had a metabolic profile consistent with impaired cobalamin status that could be corrected with cobalamin supplementation. No adverse effects of the cobalamin injections were reported.

Determinants of Infant B Vitamin Status Before and After Intervention

In the multivariate linear regression analyses (Table 4), maternal cobalamin level was a strong predictor of infant serum cobalamin and plasma tHcy levels before intervention (6 weeks). At 4 months, cobalamin intervention was by far the strongest predictor of infant cobalamin status, as determined by cobalamin, tHcy, and MMA levels. Intervention also predicted serum folate and plasma cystathionine concentrations (Table 4).

In the control group at 4 months, maternal cobalamin level and infant nutrition were strong predictors of infant serum cobalamin ($P < .001$ and $P < .001$, respectively) and plasma tHcy ($P < .001$ and $P = .008$, respectively) levels. Maternal cobalamin level was also a predictor of cystathionine levels ($P = .001$) (data not shown).

Determinants of Infant Metabolic Responses

Changes in infant cobalamin, folate, tHcy, and MMA levels from 6 weeks to 4 months according to intervention, concentrations of these indices at 6 weeks, infant nutrition at 4 months, and maternal cobalamin and folate levels at 4 months were determined through quantile regression (Fig 2). Separate analyses were conducted for each infant blood index.

Intervention was the strongest predictor of changes for all blood indices. The largest increase in cobalamin levels and the largest decreases in tHcy and MMA levels were observed in the upper quantiles of these variables, whereas the folate responses (reduction) were similar throughout the folate level distribution. Cobalamin, folate, tHcy, and MMA levels at 6 weeks showed a weak positive relationship with changes in these indices from 6 weeks to 4 months; the strongest associations were observed at the highest quantiles of folate, tHcy, and MMA levels. Infant nutrition had a moderate positive association with changes in cobalamin levels and a minor effect on the other indices. Maternal cobalamin and folate levels showed no or only weak associations with changes in infant indices (Fig 2).

DISCUSSION

Overall Findings

Apart from case reports on cobalamin-deficient infants,[38–40] no cobalamin intervention study with infants or younger children has been published previously. In the present study of predominantly breastfed, healthy, term infants, an intramuscular injection of 400 μg of cobalamin at 6 weeks of age resulted in higher serum cobalamin levels and markedly lower plasma tHcy and MMA concentrations at 4 months of age, compared with control values. Folate and cystathionine levels were lower at 4 months among those who received cobalamin. Our results show that cobalamin supplementation can normalize a metabolic profile consistent with impaired cobalamin status in young infants.

Study Design and Limitations

It is a formal weakness of the study design that the control group did not receive placebo medication and the investigators and the mothers were not blinded to the cobalamin injection. These decisions were attributable to ethical constraints.

From a theoretical perspective, it would have been desirable to study whether cobalamin supplementation had an effect on psychomotor development. Neurodevelopmental assessment was not attempted, however, because even detailed examinations are not sufficiently accurate to detect mild or moderate developmental delays in young infants.[41]

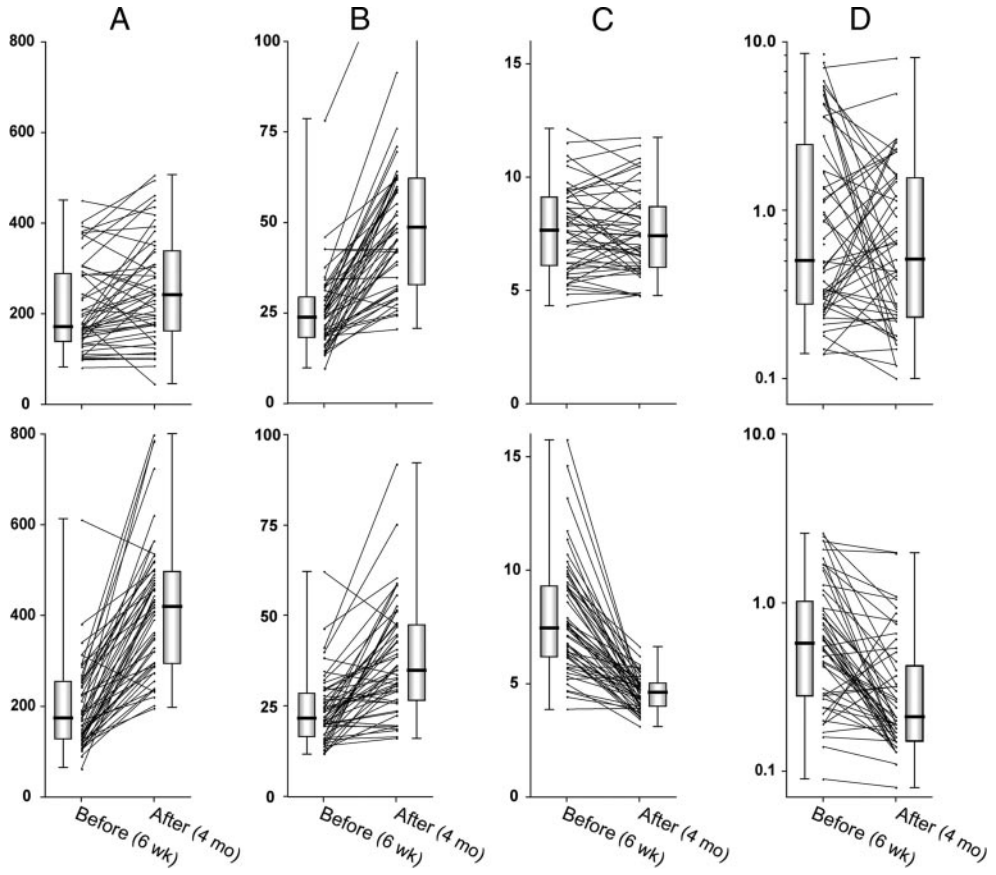

FIGURE 1

Effects of cobalamin supplementation on infant blood indices. The graph shows changes in cobalamin (A), folate (B), tHcy (C), and MMA (D) levels from 6 weeks to 4 months in 51 infants who received no cobalamin (control group) (upper row) and 52 infants who were given injections of 400 μg of hydroxycobalamin at 6 weeks (treatment group) (lower row). The dots connected by thin lines show the longitudinal individual changes, whereas the overall changes are summarized as boxplots. The horizontal lines across the boxes represent the medians, and the upper and lower hinges the 75th and 25th percentiles, respectively. The vertical lines cover the ranges.

Vitamin and Metabolite Concentrations at Baseline

The metabolic profile at 6 weeks of age (ie, low cobalamin and high folate, tHcy, and MMA levels) (Table 3) was consistent with previous reports for infants.[23–25] During the first months of life of term infants, serum cobalamin levels decrease,[23,42,43] whereas serum folate levels tend to increase.[26,44,45] Compared with data for children 1 to 10 years of age,[26] the median serum level of cobalamin at 6 weeks was low (170 vs 551 pmol/L), whereas the median folate level was high (22.2 vs 14.9 nmol/L). The plasma concentrations of tHcy at 6 weeks were in the range of 6 to 9 μmol/L, as observed previously for young infants,[23,25,26,46,47] and were much higher than the concentrations of 3 to 8 μmol/L found for 1- to 10-year-old children.[26,48–51] In the present study and in previous studies,[23,26] the median plasma level of MMA in infants was 2 times higher than the upper reference limit of 0.28 μmol/L established for adults[52] and was considerably higher than MMA levels reported for 1- to 10-year-old children (median: 0.13 μmol/L; range: 0.11–0.17 μmol/L).[26] High urinary MMA excretion and plasma MMA levels have been reported consistently for breastfed infants up to the age of 6 to 12 months[23,26,53] and infants of mothers on a vegetarian diet.[54] Data on plasma cystathionine levels in infants are scarce, but the observed median plasma cystathionine levels at 6 weeks (0.46 μmol/L) were comparable to reported concentrations in cord serum at birth.[55]

Effects of Intervention on B Vitamin Status

Whereas the metabolic markers of cobalamin status were unchanged in the control subjects between 6 weeks and 4 months, the reductions in tHcy (39%) and MMA (66%) concentrations in the cobalamin supplement-treated infants were remarkable (Table 3 and Fig 1). These findings are in accordance with reports of lower tHcy and MMA levels in infants given cobalamin supplements or formula, which usually contains higher cobalamin concentrations than human milk.[24,25,53,56]

The infants given cobalamin had lower folate levels, compared with the control subjects, at 4 months (Table 3). High serum folate levels have been observed in early cobalamin deficiency in adults[57] and have been attributed to so-called methylfolate trapping. This is explained by increased 5-methyltetrahydrofolate levels in serum/plasma because of inhibition of the cobalamin-dependent enzyme methionine synthase.[58]

TABLE 4 **Nutritional and Maternal Factors as Determinants of Vitamin and Metabolite Levels in Infants in Multivariate Linear Regression Analyses**

Independent Variables	6 wk ($N = 107$)		4 mo ($N = 103$)	
	B	*P*	*B*	*P*
Serum cobalamin level (pmol/L)				
Vitamin B_{12} intervention[a]			183	<.001
Infant nutrition[b]	54	.002	75	.001
Maternal serum cobalamin level[c]	32	<.001	43	<.001
Serum folate level (nmol/L)				
Vitamin B_{12} intervention[a]			−10.4	.003
Infant nutrition[b]	−0.7	.74	−1.3	.66
Maternal serum cobalamin level[c]	0.3	.76	−3.7	.02
Maternal serum folate level[c]	1.5	.10	2.3	.13
Plasma tHcy level (μmol/L)				
Vitamin B_{12} intervention[a]			−3.03	<.001
Infant nutrition[b]	−0.13	.76	−0.54	.02
Maternal serum cobalamin level[c]	−0.46	.02	−0.46	<.001
Maternal serum folate level[c]	−0.33	.09	−0.19	.10
Plasma MMA level (μmol/L)				
Vitamin B_{12} intervention[a]			−0.70	.001
Infant nutrition[b]	0.10	.77	−0.20	.27
Maternal serum cobalamin level[c]	−0.02	.91	−0.09	.34
Plasma cystathionine level (μmol/L)				
Vitamin B_{12} intervention[a]			−0.09	<.001
Infant nutrition[b]	0.001	.99	−0.002	.85
Maternal serum cobalamin level[c]	−0.01	.60	−0.02	.006
Maternal serum folate level[c]	−0.02	.33	−0.003	.67

The regression model contained gender as an independent variable, in addition to the parameters listed in the table. *B* is a regression coefficient.

[a] Vitamin B_{12} intervention indicates no additional vitamins and 400 μg of hydroxycobalamin administered intramuscularly.

[b] Infant nutrition was categorized as breastfed, combined breastfed and formula-fed, or formula-fed.

[c] Quartiles of maternal cobalamin and folate levels at 6 weeks and 4 months, respectively.

We observed a larger reduction in cystathionine levels in the cobalamin supplement-treated infants (65%), compared with the control subjects (48%) (Table 3). Cystathionine levels were reported to be elevated in 87% of adults with cobalamin deficiency and 95% of adults with folate deficiency and were found to decrease in response to cobalamin or folate treatment in some[22] but not all[59] studies. Increased urinary cystathionine levels were reported for an infant with dietary cobalamin deficiency[60] and a child with a cobalamin absorption defect.[61]

Possible Mechanisms

The metabolic profile commonly observed in infants has been attributed to nutritional, developmental, and physiologic factors. The higher MMA levels in breastfed infants in particular may be related to enhanced production of MMA or its precursors by intestinal microorganisms[62,63] and formation of MMA from odd-chain fatty acids,[64] which are known to be abundant in human milk.[65] In addition, low clearance of MMA attributable to immaturity of enzyme or organ systems may predispose infants to high MMA levels.

However, the observation that the period with elevated MMA levels coincides with low serum cobalamin levels, high tHcy levels, and high folate levels, with the latter suggesting a folate-trapping mechanism, supports the possibility of impaired cobalamin status.[2] This explanation agrees with our previous observation that markers of cobalamin status in infants are related to infant nutrition and maternal cobalamin status,[23] as confirmed for the control group in the present study.

The magnitudes of the observed reductions in tHcy, MMA, and cystathionine levels in response to 1 dose of cobalamin exceed the effects observed in most published intervention studies in adults.[66] A meta-analysis of 12 randomized trials of vitamin supplementation to decrease homocysteine levels in adults showed that folic acid reduced homocysteine levels by 25% and cobalamin by an additional 7%.[66]

Because the enzyme methylmalonyl-CoA mutase is not fully saturated with its cofactor adenosylcobalamin under physiologic conditions, it should be possible to decrease MMA formation through cobalamin supplementation even in the absence of cobalamin deficiency.[67] Therefore, a reduction in plasma MMA levels with cobalamin supplementation does not necessarily reflect restoration of cobalamin status. In contrast, methionine synthase, which catalyzes the conversion of homocysteine to methionine, is fully saturated with its cobalamin cofactor under normal conditions and is not stimulated by exogenous cobalamin.[68,69] Consequently, a concurrent reduction in tHcy levels with cobalamin supplementation, as seen for the supplement-treated infants, strongly suggests the presence of impaired cobalamin status. This interpretation is supported by the significantly lower serum folate levels in the intervention group, which indicate reversal of the methylfolate trap.

Implications

Our results demonstrate that two thirds of young infants have biochemical evidence of impaired cobalamin status, which responds to cobalamin supplementation. This observation supports the idea that cobalamin deficiency may be common among apparently healthy infants in developed countries[2,23] and may not be confined to sporadic cases related to exclusive breastfeeding combined with poor maternal cobalamin status[13,15] or to breastfed infants in developing countries.[1,4] In our study population, exclusive breastfeeding was common (80% at 6 weeks and 70% at 4 months), which may predispose infants to a negative cobalamin balance. The cobalamin content of milk is reflected by the maternal cobalamin concentration in blood[27] and, without exception, reported cases of symptomatic cobalamin deficiency have involved exclusively breastfed infants.[12,13,15,70,71]

No detailed assessment of developmental status was conducted in the present study, and we observed no relationships between gross growth parameters and infant cobalamin indices. However, the reports on delayed

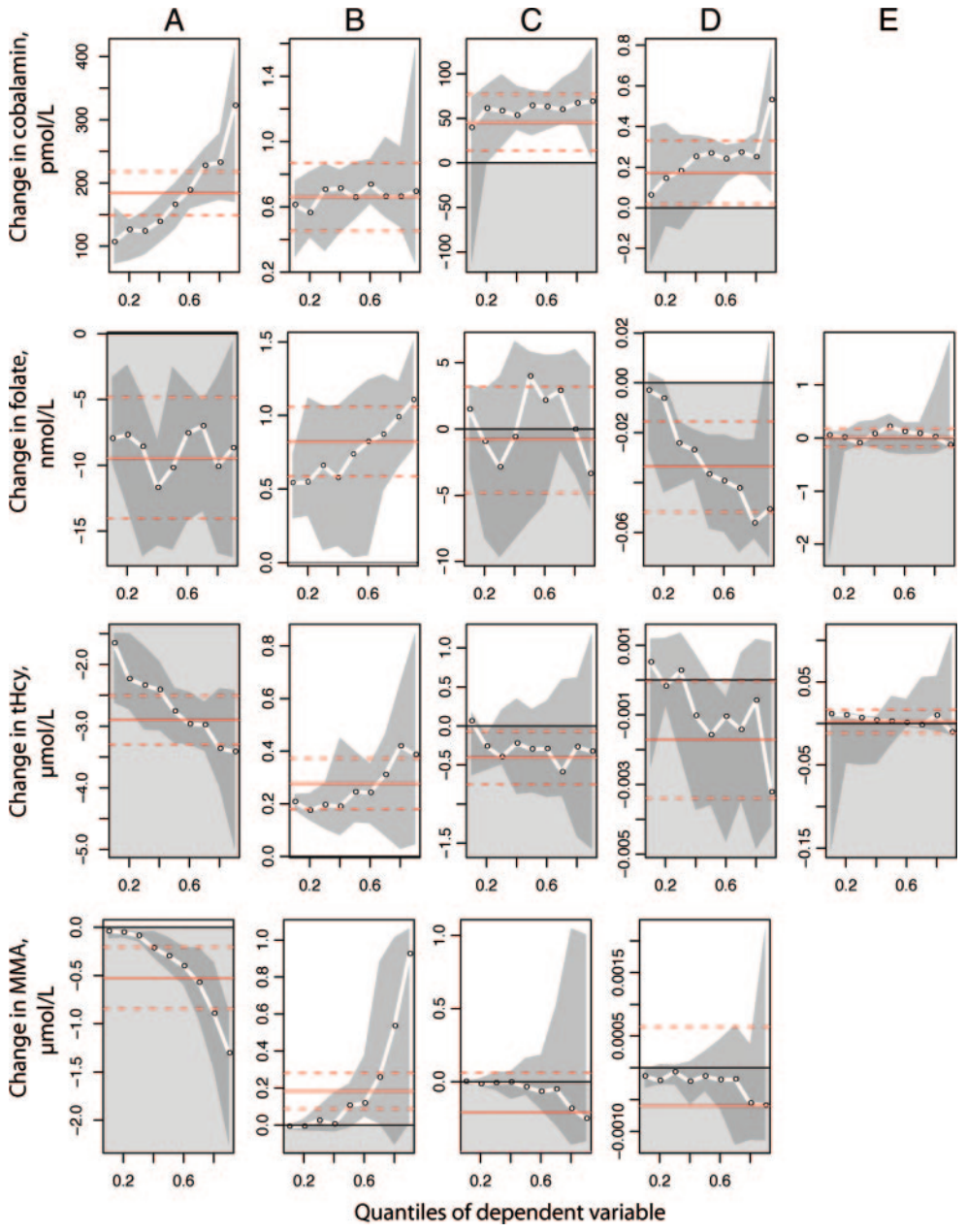

FIGURE 2

Changes in infant blood indices from 6 weeks to 4 months through quantile regression. A, Intervention; B, infant variable at 6 weeks; C, infant diet at 4 months; D, maternal cobalamin at 4 months; E, maternal folate at 4 months. The points represent quantile regression fits, dark shaded gray zones represent the 90% pointwise confidence intervals for the estimates, the dark horizontal lines no (zero) changes in biomarkers, and the light shading negative (inverse) changes. An upward or downward slope indicates the highest or lowest response at the upper or lower tail, respectively, of the distribution of the dependent variable, whereas a horizontal graph below or above 0 indicates similar effects through the whole distribution. The horizontal red solid lines represent the ordinary least-squares estimates of the conditional mean effects, and the red dotted lines represent the conventional 90% confidence intervals for the least-squares estimates. Infant nutrition at 4 months was categorized as breastfed, breastfed plus formula-fed, or formula-fed.

neurodevelopment and long-term neurologic effects related to cobalamin deficiency in young infants demonstrate the importance of adequate cobalamin status during the first months of life.[12–19]

To optimize compliance, cobalamin was given as an intramuscular injection. One would expect the biochemical response to oral supplements to be equal to the response to parenteral cobalamin administration in this age group, because better cobalamin status was reported for infants fed formula, which provides a greater oral cobalamin supply than breast milk.[24,25,53] Our data should strongly encourage advice on cobalamin intake for mothers in the preconceptional, prenatal, and postpartum periods.[30,31,71]

CONCLUSIONS

Cobalamin supplementation for infants changed all markers of impaired cobalamin status (low cobalamin levels, high tHcy levels, and high MMA levels) toward a

profile observed in cobalamin-replete older children and adults. Therefore, high tHcy and MMA levels reported for a large proportion of infants do not reflect organ immaturity but indicate insufficient cobalamin to fully sustain cobalamin-dependent reactions. Clinicians and researchers should address the possible developmental and clinical consequences of this prevalent metabolic evidence of cobalamin deficiency in infants.

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