Cobalamin supplementation improves motor development and regurgitations in infants: results from a randomized intervention study[1–4]

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ABSTRACT

Background: During infancy, minor developmental delays and gastrointestinal complaints are common, as is a biochemical profile indicative of impaired cobalamin status.

Objective: We investigated whether cobalamin supplementation can improve development or symptoms in infants with biochemical signs of impaired cobalamin function and developmental delay or feeding difficulties.

Design: Infants <8 mo of age ($n = 105$) who were referred for feeding difficulties, subtle neurologic symptoms, or delayed psychomotor development were assessed for cobalamin status [by the measurement of serum cobalamin, plasma total homocysteine (tHcy), and plasma methylmalonic acid (MMA)]. Infants with biochemical signs of impaired cobalamin function, defined as a plasma tHcy concentration ≥ 6.5 μmol/L ($n = 79$), were enrolled in a double-blind, randomized controlled trial to receive 400 μg hydroxycobalamin intramuscularly ($n = 42$) or a sham injection ($n = 37$). Motor function [Alberta Infants Motor Scale (AIMS)] and clinical symptoms (parental questionnaire) were recorded at entry and after 1 mo.

Results: During follow-up, cobalamin supplementation changed all markers of impaired cobalamin status (ie, plasma tHcy decreased by 54%, and MMA decreased by 84%), whereas no significant changes were seen in the placebo group ($P < 0.001$). The median (IQR) increase in the AIMS score was higher in the cobalamin group than in the placebo group [7.0 (5.0, 9.0) compared with 4.5 (3.3, 6.0); $P = 0.003$], and a higher proportion showed improvements in regurgitations (69% compared with 29%, respectively; $P = 0.003$).

Conclusions: In infants with biochemical signs of impaired cobalamin function, 1 intramuscular injection of cobalamin resulted in biochemical evidence of cobalamin repletion and improvement in motor function and regurgitations, which suggest that an adequate cobalamin status is important for a rapidly developing nervous system. This trial was registered at clinicaltrials.gov as NCT00710359 and NCT00710138. *Am J Clin Nutr* 2013;98:1233–40.

INTRODUCTION

An adequate cobalamin status is crucial for postnatal development of the central nervous system (1), as shown by the clinical picture presented in children with inborn errors of cobalamin metabolism and cobalamin deficiency (2–5). Although a rapid progress in motor development and improvement in clinical symptoms after cobalamin treatment have anecdotally been reported (5–7), cobalamin deficiency during infancy, even when optimally treated, may result in permanent developmental disabilities (8).

Common findings in severely cobalamin-deficient infants are feeding difficulties, developmental delay, and progressive neurologic symptoms (5, 9, 10). A clinical picture resembling these symptoms, albeit less severe, is frequently observed in young infants. Gastrointestinal complains are common but normally resolve without treatment during the first year of life (11). Because of large variations in normal psychomotor development and limitations of diagnostic tests used in this age group (12), subtle neurologic symptoms such as tremors or twitching and minor developmental delays may not be considered abnormal in young infants. Infants develop discontinuously, and there has been no clear agreement among parents and pediatricians concerning the degree of delay that deserves evaluation and intervention (13). As a consequence, cobalamin deficiency during infancy may be overlooked, or there may be a diagnostic delay (10).

A biochemical profile indicative of impaired cobalamin function that could be corrected by cobalamin supplementation has been observed in more than two-thirds of mainly breastfed Norwegian infants from 6 wk to 4 mo of age (14). Clinical and developmental consequences of these biochemical abnormalities have been debated (15).

We have conducted a randomized, double-blind, cobalamin-intervention study in infants referred for delayed psychomotor development or feeding difficulties. Total homocysteine (tHcy)[5] is considered the most sensitive metabolic marker of impaired cobalamin status in this age group (16, 17), and we selected infants with tHcy concentrations ≥ 6.5 μmol/L for intervention to assess

the effect of cobalamin supplementation on the subsequent development and symptoms.

SUBJECTS AND METHODS

Patients

Between January 2008 and May 2010, recruitment was done 2 times/mo among referrals to the Pediatric Outpatient Clinic at Haukeland University Hospital, Bergen, Norway, for infants <8 mo of age with feeding difficulties, minor neurologic symptoms, or developmental delay. In 120 mothers who were contacted by phone, 105 mothers chose to participate, 13 mothers declined because their infants no longer had problems or symptoms, and 2 mothers declined because they wanted an ordinary pediatric consultation.

The infants underwent a pediatric examination, including a motor developmental assessment by using the Alberta Infants Motor Scale (AIMS) (18). Infants with signs of severe neurologic disease ($n = 3$) were excluded from additional participation.

Blood samples for the determination of cobalamin status [ie, serum cobalamin, plasma tHcy and plasma methylmalonic acid (MMA)] and serum folate were obtained from enrolled infants and mothers. Procedures for blood sampling and measurements of serum cobalamin and folate and plasma tHcy and MMA have been previously published (14).

In infants, cobalamin is the main determinant of plasma tHcy (16, 17). A plasma tHcy concentration of 6.5 μmol/L was chosen as a cutoff for the definition of impaired cobalamin function in infants. This cutoff represents the 97.5th percentile in 4-mo-old infants given a single intramuscular dose of 400 μg hydroxycobalamin at 6 wk (14) and is considered to be a vitamin-optimized value. Infants with a tHcy concentration >18 μmol/L were considered to have a more-severely impaired cobalamin function, and they were excluded from the study and received regular pediatric follow-up ($n = 2$). Infants with a tHcy concentration between 6.5 and 18.0 μmol/L ($n = 79$) were included in the intervention trial. The infants were invited back after 1 wk and were assigned by using block random assignment [envelopes with equal numbers ($n = 10$) of As (cobalamin) and Bs (placebo)] to receive either an intramuscular injection of 400 μg hydroxycobalamin (Vitamin B12 Depot; Nycomed Pharma) (cobalamin group: $n = 42$) or a sham injection (ie, the skin was punctured by a needle connected to a syringe) (placebo group: $n = 37$) (**Figure 1**). These procedures were performed by one physician (A-LB-M), and parents were blinded to whether their infants received cobalamin or not (both syringes were wrapped in aluminum foil to hide the content, and the parent was asked to turn her head away, to prevent the mother from observing whether the syringe was activated).

Infants with a tHcy concentration <6.5 μmol/L were considered to have adequate cobalamin status and were included as an additional comparison group (low-tHcy group: $n = 21$).

Clinical assessments

Assignments to placebo, treatment, and additional comparison groups were blinded to the pediatrician (IT), who performed all clinical and developmental assessments, and laboratory personnel. All infants were scheduled for follow-up 1 mo after the first examination. On entry and at follow-up, mothers completed
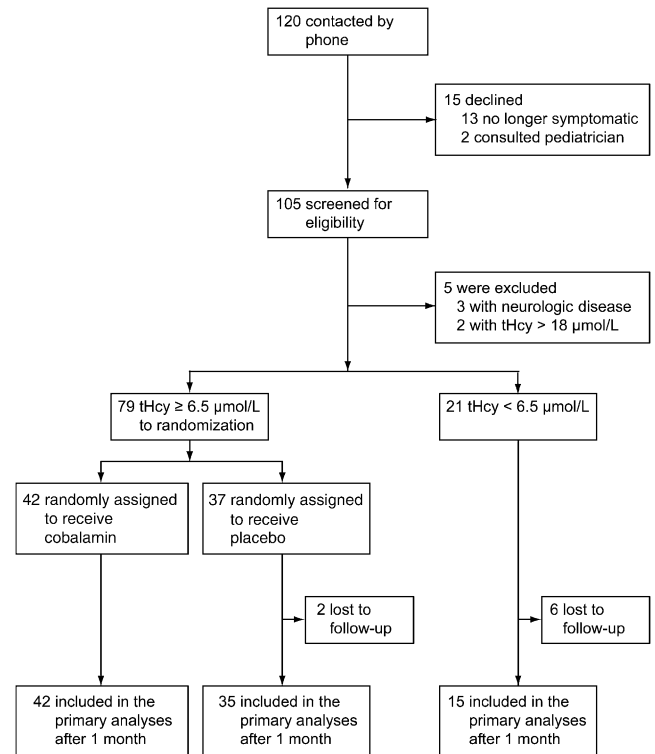
FIGURE 1. Flow of participants through the trial. A total of 105 infants were screened for eligibility, 5 infants were excluded (3 infants had neurologic disease, and 2 infants had tHcy concentrations >18 μmol/L). tHcy, total homocysteine.

a questionnaire concerning nutrition, growth, and symptoms of their infants, and the pediatrician performed an AIMS test and a standard pediatric examination. If the infant still had symptoms of concern after 1 mo, he or she was referred to an ordinary pediatric follow-up.

Questionnaire on infant symptoms

Mothers completed a questionnaire that addressed specific neurologic symptoms, such as twitching or tremors (19), and gastrointestinal symptoms [ie, the occurrence of regurgitations (defined as ≥2 instances/d; 20)] and number of regurgitations and food refusal), which are symptoms commonly reported in infants with severe cobalamin deficiency (8, 21, 22). The severity of symptoms was described as no, minor, and major problems. On follow-up, changes in symptoms were reported as no improvement or an improvement.

AIMS

Gross motor development was assessed by using the AIMS. This scale is a norm-referenced observational tool designed for the evaluation of gross motor development in infants from birth to 18 mo of age (18). Assessment was based on the free observation of the child in different positions according to the age of the child (prone, supine, sitting, and standing). The obtained score (0–60 points) was converted to a normative age-dependent percentile rank (5th to 90th percentiles). A score less than the 10th percentile was classified as possibly delayed motor development (18).

Ethics

Written informed consent was obtained from the mothers before enrollment, and ethical approval of the protocol was granted by the Regional Committee on Medical Research Ethics. A sham injection in the placebo group was advised by the ethical committee. All infants got sugar water for pain relief before and during injection and blood sampling (23).

Statistics

The calculation of the sample size was based on data from our previous study on cobalamin status in infants (14), with the assumption that cobalamin supplementation would result in tHcy concentrations <6.5 μmol/L for infants at 4 mo of age. A calculated sample size of 65 infants (ie, 33 infants in each group) would have given the study a statistical power >90% to detect a 25% relative reduction in tHcy concentrations at a 5% significance level. However, on the basis of our experience in earlier studies, a dropout of ~40–50% was expected, and a total of ~100 infants should have been included.

Analyses were based on the intention-to-treat principle. All analyses were conducted according to the treatment assignment, and all available data were incorporated. We telephoned and sent a letter with a new appointment ≤1–3 d to mothers who did not come back for follow-up. No assumptions were made for missing biochemical data because these were continuous. We investigated the potential effect of missing clinical data by assuming an outcome.

Results are presented as medians and IQRs or means ± SDs. Medians were compared by using Wilcoxon's signed-rank test and the Mann-Whitney U test, and means were compared by using Student's t test. Differences in categorical variables were tested by using the chi-square test. Predictors of infant cobalamin status were assessed by using multiple-linear regression models including maternal vitamin status, infant nutrition and sex, and the intervention as independent variables.

Two-sided P values <0.05 were considered statistically significant. The SPSS statistical package (version 18; SPSS Inc) was used for all statistical analyses.

RESULTS

Infant characteristics

Of 79 infants included in intervention trial and 21 infants included in the low-tHcy comparison group (total $n = 100$), 92 infants came back for follow-up assessment after 1 mo (Figure 1). Of the 8 infants lost to follow-up, 2 infants were from the placebo group, and 6 infants were from the low-tHcy comparison group, and they showed no significant differences in baseline characteristics compared with those of the 92 infants who came back (all $P > 0.25$).

There were no significant differences in clinical characteristics between cobalamin and placebo groups (**Table 1**). The mean (±SD) age of the 79 infants in the intervention trial was 4.0 ± 2.0 mo (total range: 3 wk to 8 mo); 15 infants were <2 mo of age. Infant nutrition at each visit was categorized as exclusive breast-milk or mixed feeding, which included breastfeeding combined with formula, exclusive formula feeding, or either of these combined with solid foods. Three different kinds of milk formula were used, all of which were enriched with cobalamin (0.13–0.20 μg cobalamin/100 mL prepared milk). At inclusion and follow-up, only 2 infants received multivitamin supplements, none of which contained cobalamin. Between entry and follow-up, there were no significant differences in the mean increase in weight, length,

TABLE 1
Demographic data at inclusion

| | Trial groups (6.5–18.0 μmol tHcy/L)[1] | | | | |
	Cobalamin group ($n = 42$)	Placebo group ($n = 37$)	P[2]	Low-tHcy comparison group (<6.5 μmol tHcy/L) ($n = 21$)	P[3]
Infant data					
Sex (M) [n (%)]	18 (43)	22 (59)	0.14	14 (67)	0.19
Gestational age (wk)	39.0 ± 1.9[4]	39.6 ± 1.4	0.14	39.2 ± 2.6	0.90
Birth weight (g)	3347 ± 637	3572 ± 550	0.10	3527 ± 758	0.63
Age at inclusion (mo)	4.1 ± 2.0	4.0 ± 2.1	0.76	4.8 ± 1.7	0.14
Weight (g)	6480 ± 1839	6423 ± 1661	0.89	6869 ± 1105	0.30
Length (cm)	62.7 ± 5.3	63.0 ± 4.8	0.84	65.8 ± 4.6	0.02
Head circumference (cm)	41.1 ± 2.5	41.1 ± 2.3	0.97	42.3 ± 2.0	0.04
Exclusively breastfed [n (%)]	20 (48)	20 (54)	0.57	2 (10)	0.001
Maternal data					
Age (y)	31.3 ± 5.2	30.6 ± 5.0	0.58	30.1 ± 4.6	0.49
BMI (kg/m^2)	24.6 ± 4.6	25.5 ± 4.8	0.37	24.4 ± 5.0	0.59
Para 0 [n (%)]	20 (48)	13 (35)	0.26	7 (33)	0.48
Daily smoking [n (%)]	3 (7)	6 (16)	0.22	2 (10)	0.79
Daily multivitamin use [n (%)]	19 (45)	12 (32)	0.21	8 (38)	0.89

[1] Infants with plasma tHcy concentrations from 6.5 to 18.0 μmol/L were assigned by block random assignment to receive either an intramuscular injection of 400 μg hydroxycobalamin (cobalamin group) or a sham injection (placebo group). tHcy, total homocysteine.

[2] Cobalamin group compared with placebo group. The chi-square test was used for data given as numbers (percentages), and Student's t test was used for data given as means ± SDs.

[3] Cobalamin group compared with low-tHcy comparison group and placebo group compared with low-tHcy comparison group. The chi-square test was used for data given as numbers (percentages), and Student's t test was used for data given as means ± SDs.

[4] Mean ± SD (all such values).

or head circumference between cobalamin or placebo groups ($P > 0.45$).

At inclusion, the 21 infants in the low-tHcy comparison group tended to be slightly older and less often exclusively breastfed than were trial infants (Table 1).

Infant cobalamin and folate status and effect of cobalamin intervention

At inclusion, there were no significant differences in concentrations of metabolites, cobalamin, or folate between cobalamin and placebo groups (**Table 2**). In the cobalamin group, tHcy decreased by 54%, MMA decreased by 84%, and cobalamin increased 3-fold, whereas in the placebo group, no significant changes were observed for tHcy and MMA, and cobalamin increased by merely 20% (Table 2). Vitamins and metabolites remained stable during follow-up in the low-tHcy comparison group (Table 2). No adverse effects from the cobalamin injections were reported.

Determinants of infant cobalamin status

Of 79 infants in the intervention trial, the maternal cobalamin concentration, which was given as quartiles, was the strongest predictor (assessed by using multiple linear regression including infant sex and infant nutrition) of infant cobalamin status at inclusion (cobalamin: $B = 46$, $P = 0.002$; tHcy: $B = -0.36$, $P = 0.07$; MMA: -0.27, $P = 0.16$), whereas the cobalamin intervention was the main predictor after 1 mo (cobalamin: $B = 612$, $P < 0.001$; tHcy: $B = -3.90$, $P < 0.001$; MMA: B $= -0.77$, $P = 0.001$).

No significant predictors for cobalamin status were observed in the low-tHcy comparison group, either at inclusion or after 1 mo (all $P > 0.3$).

At inclusion, there were no significant differences in median (IQR) concentrations of cobalamin [442 pmol/L (379, 563 pmol/L)], folate [5.4 nmol/L (3.9, 11.9 nmol/L)], tHcy [8.44 μmol/L (7.05, 10.42 μmol/L)], and MMA [0.16 μmol/L (0.13, 0.19 μmol/L)] between mothers in the cobalamin, placebo, and low-tHcy groups [$P > 0.11$ (Kruskal-Wallis test)].

Clinical symptoms and cobalamin intervention

At inclusion, there were no significant differences in reported symptoms between cobalamin and placebo groups or between the trial group and low-tHcy comparison group (**Table 3**). At follow-up, mothers of all groups generally reported improvements in infant symptoms, in particular of twitching and tremors (Table 3).

A larger proportion of infants in the cobalamin group (69%) than placebo group (29%) ($P = 0.003$) showed an improvement in regurgitations. No significant difference between groups was observed for food refusal (Table 3). Two infants in the placebo group had had regurgitations problems at inclusion and were lost to follow-up. There was still a significantly better outcome in cobalamin group than placebo group, even with the assumption that these infants had an improvement in regurgitations ($P = 0.01$). In the low-tHcy comparison group, 45% of infant had an improvement in regurgitations (Table 3).

TABLE 2
Infant vitamin and metabolite concentrations at inclusion and follow-up[1]

| | Trial groups (6.5–18.0 μmol tHcy/L)[2] | | | | |
	Cobalamin group (n = 42/42)	Placebo group (n = 37/35)[6]	P[3]	Low-tHcy comparison group (<6.5 μmol tHcy/L) (n = 21/15)[4]	P[5]
Plasma total homocysteine (μmol/L)					
At inclusion	8.74 (7.18, 10.42)	7.88 (7.32, 9.52)	0.48	5.43 (4.87, 6.01)	<0.001
At follow-up	4.04 (3.47, 4.74)	8.14 (7.43, 9.13)	<0.001	5.33 (4.82, 5.90)	<0.001/<0.001
P[7]	<0.001	0.37	—	0.87	—
Plasma methylmalonic acid (μmol/L)					
At inclusion	0.85 (0.26, 2.15)	0.43 (0.27, 1.11)	0.20	0.21 (0.19, 0.38)	0.002
At follow-up	0.14 (0.13, 0.20)	0.46 (0.24, 1.39)	<0.001	0.21 (0.16, 0.46)	0.005/0.05
P[7]	<0.001	0.75	—	0.53	—
Serum cobalamin (pmol/L)					
At inclusion	317 (204, 390)	271 (206, 347)	0.58	423 (351, 665)	<0.001
At follow-up	836 (663, 1141)	326 (216, 493)	<0.001	425 (363, 540)	<0.001/0.01
P[7]	<0.001	<0.001	—	0.98	—
Serum folate (nmol/L)					
At inclusion	28.7 (16.1, 52.7)	20.0 (12.9, 34.0)	0.06	17.5 (14.1, 26.1)	0.15
At follow-up	27.8 (18.9, 38.6)	22.7 (15.5, 35.7)	0.17	17.3 (12.8, 27.6)	0.06/0.42
P[7]	0.16	0.28	—	0.98	—

[1] All values are medians; IQRs in parentheses. tHcy, total homocysteine.

[2] Infants with plasma tHcy concentrations from 6.5 to 18.0 μmol/L were assigned by block random assignment to receive either an intramuscular injection of 400 μg hydroxycobalamin (cobalamin group) or a sham injection (placebo group).

[3] Cobalamin group compared with placebo group (Mann-Whitney U test).

[4] Six infants missing at follow-up.

[5] Total trial group compared with low-tHcy comparison group at inclusion (Mann-Whitney U test); cobalamin group compared with low-tHcy comparison group at follow-up/placebo group compared with low-tHcy comparison group at follow-up (Mann-Whitney U test).

[6] Two infants missing at follow-up.

[7] Wilcoxon's signed-rank test.

Motor development and cobalamin intervention

AIMS data from both the inclusion and follow-up were available for 89 of 92 infants; the 3 infants with missing AIMS data were from the placebo group. Scores at inclusion were highly correlated to age in both trial groups and the low-tHcy comparison group ($B >$ 0.9, $P = 0.001$). At inclusion, 19% of infants (17 of 89) scored below the 10th percentile, and 71% of infants (63 of 89) scored below the 50th percentile. There was no significant difference in score distributions between cobalamin and placebo groups.

The AIMS score increased in all groups from inclusion to follow-up; however, the median (IQR) score increase was significantly higher in the cobalamin group [7.0 (5.0, 9.0)] than placebo group [4.5 (3.3, 6.0)] ($P = 0.003$) (**Figure 2**), and more infants in the cobalamin group than placebo group attained higher AIMS percentiles (Table 3). The median percentile in the cobalamin group increased from the 25th to 50th percentile, whereas it remained stable at the 25th to 50th percentile in the placebo group ($P <$ 0.001). There was still a significantly better outcome in the cobalamin group than placebo group, even with the assumption that the 3 infants with missing AIMS data and the 2 infants lost to follow-up (all 5 infants were from the placebo group) showed an increment in the AIMS percentile ($P = 0.01$). The lowest median (IQR) score increment was seen in the low-tHcy comparison group [4.0 (4.0, 7.0)], and their median percentile actually declined from the 25th to 50th percentile to the 25th percentile (Figure 2, Table 3).

DISCUSSION

In this study, $\sim 80\%$ of young infants referred for pediatric assessment because of feeding difficulties or developmental delay had a biochemical profile indicative of mildly impaired cobalamin function. Within this group, cobalamin treatment resulted in a normal cobalamin status and significant improvement in regurgitations and motor function, whereas no significant improvements in cobalamin markers or clinical symptoms were observed in infants randomly assigned to receive the placebo. These results indicated that the commonly occurring abnormal profile of cobalamin biomarkers in infants (14) reflects an impaired vitamin function to the extent that it may adversely affect infant development. Infants with similar symptoms but

TABLE 3
Clinical symptoms and motor development at inclusion and follow-up[1]

	Trial groups (6.5–18.0 μmol tHcy/L)[2]				
	Cobalamin group (n = 42/42)	Placebo group (n = 37/35)[6]	P[3]	Low-tHcy comparison group (<6.5 μmol tHcy/L) (n = 21/15)[4]	P[5]
Regurgitation [n (%)]					
At inclusion: infants with an event/total number of infants	32/42 (76)	26/37 (70)	0.55	15/21 (71)	0.68/0.93
At follow-up: infants with improvement/infants with an event	22/32 (69)	7/24 (29)[7]	0.003	5/11 (45)[8]	0.17/0.35
Food refusal [n (%)]					
At inclusion: infants with an event/total number of infants	17/42 (40)	17/37 (49)	0.62	11/21 (52)	0.37/0.64
At follow-up: infants with improvement/infants with an event	12/17 (71)	11/17 (65)	0.71	2/7 (29)[9]	0.06/0.11
Twitching [n (%)]					
At inclusion: infants with an event/total number of infants	8/42 (19)	9/37 (24)	0.57	2/21 (10)	0.33/0.17
At follow-up: infants with improvement/infants with an event	6/8 (75)	8/9 (89)	0.45	2/2 (100)	0.43/0.62
Change in AIMS percentile from inclusion to follow-up [n (%)]					
Infants with an increment/infants with an AIMS evaluation	34/42 (81)	13/32 (41)[10]	0.002	4/15 (27)[5]	<0.001/0.04
Infants with no change/infants with an AIMS evaluation	6/42 (14)	15/32 (47)[10]		4/15 (27)[5]	
Infants with a decline/infants with an AIMS evaluation	2/42 (5)	4/32 (13)[10]		7/15 (47)[5]	

[1] AIMS, Alberta Infant Motor Scale; tHcy, total homocysteine.
[2] Infants with plasma tHcy concentrations from 6.5 to 18.0 μmol/L were assigned by block random assignment to receive either an intramuscular injection of 400 μg hydroxycobalamin (cobalamin group) or a sham injection (placebo group).
[3] Cobalamin group compared with placebo group (chi-square test).
[4] Six infants missing at follow-up.
[5] Cobalamin group compared with low-tHcy comparison group/placebo group compared with low-tHcy comparison group (chi-square test).
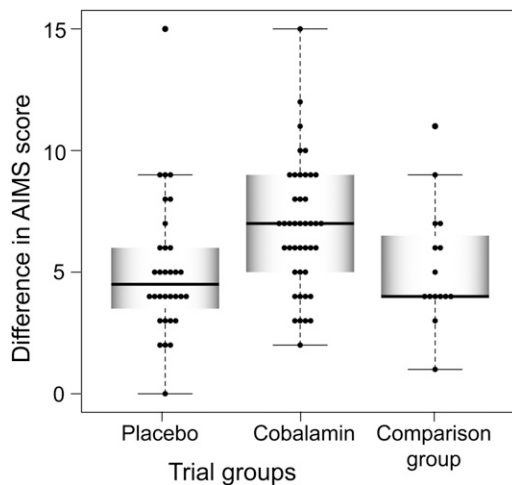[6] Two infants missing at follow-up.
[7] Two infants with an event missing at follow-up.
[8] Three infants with an event missing at follow-up.
[9] Four infants with event missing at follow-up.
[10] Two infants missing at follow-up and an additional 3 infants with a missing AIMS evaluation.

FIGURE 2. Differences between AIMS scores at inclusion and follow-up for trial groups [placebo group ($n = 35$): 4.5 (IQR: 3.3, 6.0); cobalamin group ($n = 42$): 7.0 (IQR: 5.0, 9.0)] ($P = 0.003$; Mann-Whitney-U test) and the low–total homocysteine comparison group ($n = 15$) [4.0 (IQR: 4.0, 7.0)] ($P = 0.05$ for the low–total homocysteine comparison group compared with the cobalamin group; $P = 0.70$ for the low–total homocysteine comparison group compared with the placebo group; Mann-Whitney-U test). Results are presented as box plots. Horizontal lines across boxes represent the medians, and upper and lower hinges represent 75th and 25th percentiles, respectively. Vertical lines cover ranges. AIMS, Alberta Infants Motor Scale.

with normal cobalamin status did not show any improvements during the same observation period of 1 mo, which suggested that their symptoms were not related to cobalamin status.

Study design and limitations

The given dose of 400 μg hydroxycobalamin represented approximately twice the total amount of cobalamin considered necessary for the first year of life on the basis of an adequate intake for cobalamin of 0.4 μg/d for the first 6 mo of life and 0.5 μg/d for the next 6 mo of life (24). The consecutive recruitment of patients referred for pediatric assessment from primary care was a strength to the study, and the similar clinical characteristics of the cobalamin and placebo groups suggested that the random assignment was appropriate.

A follow-up period of 1 mo may be considered too short for the evaluation of potential effects of cobalamin supplementation on development and nonspecific symptoms. However, the short period was chosen for 2 reasons; first, the infants were admitted for pediatric assessment and treatment, and therefore, a short observational period was necessary for clinical reasons, and second, a short observation period was desirable to minimize confounding (eg, from changes in feeding practices, which may have affected cobalamin status).

A psychomotor assessment of young infants is challenging (25) but necessary for the evaluation of the intervention effect from a nutrient known to interfere with neurodevelopment (26). The AIMS test is considered to be among the most reliable tests for the assessment of gross motor function, which is a major developmental function in early infancy (12, 18, 27) and known to be related to micronutrient status (28, 29). However, it might be difficult for practitioners to apply AIMS endpoints to know if an improvement has occurred. Each infant was evaluated according to the deviation from its original percentile trajectory, which was an approach conditioned on age. The maternal assessment of infant

symptoms was, to a large extent, subjective. To minimize the bias related to maternal assessment, we used a simple questionnaire that addressed specific gastrointestinal and neurologic symptoms and a simple description of the change at follow-up (ie, improvement or no improvement).

Infants develop discontinuously, and it might be difficult to select those infants who need additional evaluation and intervention. Regurgitations are common in young infants and are usually a self-limiting problem that resolves by age 12–18 mo (30). However, in some infants, regurgitations may cause reduced weight gain, respiratory symptoms, irritability, and crying, and regurgitations are one of the most common causes for physician consultation (30). The study population included infants admitted to a pediatric outpatient clinic because of feeding difficulties or delayed neurodevelopment, and one may assume that these infants were more than usually troubled. The evaluation of both inclusion criteria and confirmation of the findings should be done in a larger study that also includes infants from primary care.

Markers of cobalamin status in infants

There is no clear agreement of how to define moderate cobalamin deficiency in adults (31–33), and the establishment of adequate cutoffs for various cobalamin biomarkers is even more challenging in infants because this period is characterized by substantial changes in markers of cobalamin status (34). Associations of cobalamin and folate with the metabolic markers tHcy and MMA differ between infants and older children. In the first year of life, folate concentrations are high, and plasma tHcy is strongly correlated to serum cobalamin, whereas the relation to serum folate is weak or absent (16, 17, 35, 36). In infants, MMA is inversely related to cobalamin, but concentrations are higher through the range of cobalamin concentrations than in older children (34). The chosen cutoff for defining cobalamin deficiency in infants of a plasma tHcy concentration of 6.5 μmol/L represented the 97.5 percentile in 4-mo-old infants given a single intramuscular dose of 400 μg hydroxycobalamin at 6 wk, which rendered them cobalamin optimized (14).

Effect of cobalamin intervention

The observed metabolic effects in the intervention group essentially confirmed results of published studies (14) and supported the notion that cobalamin supplementation normalizes the cobalamin biomarkers in these young infants.

Several case reports have described gastrointestinal and neurologic symptoms and delayed psychomotor development in severely cobalamin-deficient children (6, 7, 9, 37–42). Symptoms and signs observed in the infants with a biochemical profile indicative of a moderately impaired cobalamin function in the current study were similar, albeit weaker, than those reported for severely cobalamin-deficient infants.

Gastrointestinal and minor neurologic complaints are common in young infants and are often a self-limiting problem (11, 20), as illustrated by the considerable reduction over time in twitching in the trial group and the low-tHcy comparison group. However, cobalamin supplementation during a short follow-up period significantly improved the distinct gastrointestinal problem of regurgitations and enhanced motor development. Our results were in accordance with published case studies that showed improvements of hypotonia, increased physical activity, and the ability to roll over

within the first week of cobalamin treatment in severely deficient infants (5–7).

Possible pathophysiologic mechanisms

The pathophysiologic mechanisms of cobalamin dysfunction or deficiency in the central nervous system are not fully understood (10) but involve a reduced supply of methyl groups as a result of the inadequate remethylation of homocysteine to methionine and inappropriate conversion of methylmalonyl CoA to succinyl CoA that results in excess propionyl CoA and an altered and deranged myelin structure (43, 44). Myelination is rendered an indicator of functional brain maturation and is correlated to psychomotor development (45, 46). Delayed myelination has been documented in infants with cobalamin deficiency in magnetic resonance studies (47, 48). However, improved myelination cannot be the only neurologic effect from cobalamin supplementation because significant improvements have been reported within days in severely deficient infants (5, 49).

In conclusion, during infancy, a biochemical profile indicative of a moderately impaired cobalamin function is common (14) as are gastrointestinal complaints and minor delays in psychomotor development (11, 12). However, this randomized, double-blind, intervention study shows that cobalamin supplementation improved biochemical status, gastrointestinal symptoms, and motor development in moderately cobalamin-deficient infants, which were results that may imply that such moderate symptoms and delays are not always innocuous. Cobalamin is important for a rapidly developing nervous system (26). Long-term effects of a temporary moderate deficiency are unknown, but cobalamin deficiency should be considered in young, mainly breastfed infants with feeding difficulties, subtle neurologic symptoms, and delayed motor development.

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