Cobalamin and folate status predicts mental development scores in North Indian children 12–18 mo of age[1–3]

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ABSTRACT

Background: Micronutrient deficiencies can affect cognitive function. Many young children in low- and middle-income countries have inadequate cobalamin (vitamin B-12) status.

Objective: The objective was to measure the association of plasma concentrations of folate, cobalamin, total homocysteine, and methylmalonic acid with cognitive performance at 2 occasions, 4 mo apart, in North Indian children aged 12–18 mo.

Design: Bayley Scales of Infant Development II were used to assess cognition. In multiple regression models adjusted for several potential confounders, we measured the association between biomarkers for folate and cobalamin status and psychomotor or mental development scores on the day of blood sampling and 4 mo thereafter.

Results: Each 2-fold increment in plasma cobalamin concentration was associated with a significant increment in the mental development index score of 1.3 (95% CI: 0.2, 2.4; $P = 0.021$). Furthermore, each 2-fold increment in homocysteine or methylmalonic acid concentration was associated with a decrement in mental development index score of 2.0 (95% CI: 0.5, 3.4; $P = 0.007$) or 1.1 (95% CI: 0.3, 1.8; $P = 0.004$) points, respectively. Plasma folate concentration was significantly and independently associated with mental development index scores only when children with poor cobalamin status were excluded, ie, in those who had cobalamin concentrations below the 25th percentile. None of these markers was associated with psychomotor scores in the multiple regression models.

Conclusions: Cobalamin and folate status showed a statistically significant association with cognitive performance. Given the high prevalence of deficiencies in these nutrients, folate and cobalamin supplementation trials are required to measure any beneficial effect on cognition. The study was registered at www.clinicaltrials.gov under the identifier number NCT00272116. *Am J Clin Nutr* 2013;97:310–7.

INTRODUCTION

Micronutrient deficiencies represent a major challenge to child health in many low- and middle-income countries and may be associated with suboptimal cognitive function (1). Elevated total homocysteine (tHcy)[4] in combination with poor folate or cobalamin (vitamin B-12) status has been associated with decreased cognitive performance, particularly among the elderly (2) but also in school-age children (3, 4). In infants of strict vegan mothers, growth and psychomotor development are delayed (5), possibly related to a high prevalence of low cobalamin status in these infants (3).

Elevated tHcy and poor cobalamin status are common in low- and middle-income countries because of the low dietary intake of animal products (6). Folates are provided through breast milk, fruit, green leafy vegetables, and legumes, and folate deficiency is probably somewhat less common (7). Cobalamin is required for the folate-dependent enzyme methionine synthase, which is necessary for the synthesis of methionine from homocysteine. Methionine in its activated form, *S*-adenosylmethionine, is the major methyl group donor used in human methylation reactions, including methylation of DNA and RNA. Deficiencies of cobalamin and folate, therefore, have similar consequences on cellular division and differentiation, and both result in elevated tHcy concentrations in blood (8).

We previously measured concentrations of cobalamin, folate, tHcy, and methylmalonic acid (MMA)—a marker of cobalamin status—in a cohort of Indian children aged 6–30 mo. In this slum area of New Delhi, where one-fifth of the children <5 y of age were wasted, we found that most children had low plasma concentrations of cobalamin (54% <221 pmol/L), which again explained their high plasma concentrations of tHcy and MMA

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[4] Abbreviations used: MDI, mental development index; MMA, methylmalonic acid; PDI, psychomotor development index; tHcy, total homocysteine.

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(9). We also found that some children had low plasma concentrations of folate (27% <7 nmol/L) and that having poor folate status was associated with an increased risk of infections over the next 4 mo (10). In those who were 12–18 mo of age at enrollment, we also measured cognitive performance on 2 occasions 4 mo apart. Here we present the associations between the above-mentioned markers of cobalamin and folate status on one hand and cognitive development scores on the other hand. We also measured the associations between these baseline markers and cognitive development scores 4 mo thereafter.

SUBJECTS AND METHODS

Study population

This study was nested within a zinc supplementation trial in which we measured the effect of daily zinc administration on the incidence of illness in children and on development scores in young children (11). Briefly, we identified children through a door-to-door survey and randomly assigned them to receive oral zinc or placebo daily for 4 mo. Data collection took place from February 1998 to September 2000. The trial site was the urban community of Dakshinpuri in New Delhi, which had a population of ~75,000 residing in ~15,000 dwellings. Children were identified through a survey and were included if their caregivers gave consent and were excluded if they were likely to move out of the study area within the next 4 mo or if they were sick. All children received a massive dose of vitamin A (200,000 IU). Assessment of the association between markers of cobalamin and folate status and cognitive development were predefined secondary objectives of this project. The inclusion and exclusion criteria, the effect of zinc administration, and baseline concentrations of plasma folate, cobalamin, tHcy, and MMA are described in detail elsewhere (9, 12, 13). There was no measurable effect of zinc administration on cognitive development. Among 2482 children included in the zinc trial, 650 children were in the age range 12–18 mo and were recruited into the cognitive substudy. Details of the study were given in writing and also read to the parents in the presence of a witness. Signatures or thumb impressions were obtained on a consent form. The ethics committee of the All India Institute of Medical Sciences in New Delhi approved the study.

Blood collection and biochemical analyses

We collected nonfasting venous blood samples (~5 mL) in heparinized polypropylene tubes (Sarstedt) between 0900 and 1200. The samples were centrifuged and the plasma divided and stored into polypropylene vials (Eppendorf) at −20°C until analyzed. All samples were analyzed at the University of Bergen, Norway (Section for Pharmacology, Institute of Medicine). Plasma cobalamin and plasma folate concentrations were measured with microbiological assays by using a chloramphenicol-resistant strain of *Lactobacillus casei* and colistin sulfate–resistant strain of *Lactobacillus leichmannii*, respectively (14, 15). Both assays were adapted to a microtiter plate format and carried out by a robotic workstation (16). Plasma MMA and tHcy were analyzed by using a modified gas chromatography–mass spectrometry method based on ethylchloroformate derivatization (17). Ferritin was analyzed by a turbidimetric immunoassay and C-reactive protein by immunoassay in a Roche/Hitachi Modular Analyzer.

Development assessment

The infants' development was assessed on the day of blood sampling and after 4 mo by using the mental development index (MDI) and the psychomotor development index (PDI) subscales of the Bayley Scales of Infant Development (version II) according to the instruction manual (18). Some of the test items had to be modified to be used in an Indian setting. This cultural adaptation was done with help from child psychologists.

Children were tested in the presence of their mothers at the clinic. If the child was uncooperative, too restless, crying, or sleepy during the assessment, the child was reassessed the next day. Three attempts on different days were made before the child was labeled as uncooperative. Children who were sick at the time of assessment were tested after recovery. Assessments were done in a well-lit, ventilated room, free of distractions, and the assessment was initiated only when the child was comfortable. The age was estimated by subtracting the date of birth from the test date. For premature births, the months and days by which the child was premature were subtracted from the chronologic age to compute a corrected age. Children were given credits for administered tasks only when they were able to complete them.

The Bayley Scales II were administered by the second author (ST) and a clinical psychologist. After several practice sessions, we conducted standardization exercises in 100 children in which we compared the scores obtained by the 2 assessors. Interobserver agreement during the study was ascertained by testing 10% of the baseline and end-of-study assessments in duplicate and thereby comparing the MDI and PDI scores obtained by the first and the second assessors. Intrarater agreement was high, with κ coefficients of 0.88 and 0.86 for the MDI and PDI scores, respectively.

Data management and statistical analyses

The data entry forms were designed with FoxPro for Windows (Microsoft Corporation), with range and consistency checks incorporated. Double data entry by 2 data encoders followed by validation was completed within 48 h after the forms were completed in the field, which enabled quick correction of any erroneous entries. Anthropometric measures were made by calculating height-for-age, weight-for-age, and weight-for-height z scores based on WHO Child Growth Standards (19). We used a chi-square test, t test, or Mann-Whitney U test to compare groups (**Table 1**).

We identified predictors for MDI and PDI scores using a stepwise modeling approach; all variables listed in the baseline table (Table 1) were included in this process. The variables were coded as in this table. These predictors were included in multiple regression models that measured the associations between the markers of folate and cobalamin status at enrollment and cognitive development. In these models, log (base 2)-transformed plasma folate, cobalamin, tHcy, and MMA concentrations were used. We used interaction terms in the linear regression models to assess whether or not sex, breastfeeding, or zinc supplementation status modified the associations between the biomarkers and cognitive scores.

We also (a priori) categorized the children into having low vitamin concentrations (<25th percentile) or high metabolite concentrations (>75th percentile) and used these categories in the multiple regression analyses, including the same exposure variables as mentioned above. In children with cobalamin deficiency,

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TABLE 1
Baseline characteristics of children 12–18 mo of age in a study on cognition and folate and cobalamin status

	Cobalamin <150 pmol/L		Cobalamin ≥150 pmol/L	
Baseline characteristics	Total *n*	Value	Total *n*	Value
Age (mo)	145	14.8 ± 2.0[1]	393	15.0 ± 2.0
Breastfed [*n* (%)]	145	138 (95.2)	393	259 (65.9)
Mean weight (kg)[2]	145	8.1 ± 1.0	393	8.3 ± 1.1
Mean length (cm)[2]	145	72.9 ± 3.6	393	73.9 ± 3.5
Weight-for-length *z* score	145	−1.2 ± 0.9	393	−1.1 ± 1.0
<−2 [*n* (%)]	145	23 (15.9)	393	69 (17.6)
Length-for-age *z* score[2]	145	−2.0 ± 1.1	393	−1.7 ± 1.1
<−2 [*n* (%)][2]	145	72 (49.7)	393	134 (34.1)
Weight-for-age *z* score[2]	145	−1.9 ± 1.0	393	−1.6 ± 1.0
<−2 [*n* (%)]	145	62 (42.8)	393	136 (34.6)
Boys [*n* (%)]	145	79 (54.5)	393	214 (54.5)
Annual income (in 1000 Indian rupees)	145	46.4 ± 40.1	393	48.6 ± 39.0
Living in joint family [*n* (%)]	145	80 (55.2)	393	183 (46.6)
Family size (*n*)	145	5.8 ± 2.2	393	5.9 ± 2.5
Age of mother (y)	145	24.9 ± 4.2	392	24.7 ± 4.0
Age of father (y)	145	29.2 ± 5.3	390	28.8 ± 5.0
Duration of mother's schooling (y)	145	4.6 ± 4.5	392	6.1 ± 4.5
Duration of father's schooling (y)	145	7.9 ± 4.3	389	8.9 ± 4.0
Mothers who work [*n* (%)]	145	9 (6.2)	393	33 (8.4)
Has television at home [*n* (%)][2]	145	123 (84.8)	393	361 (91.9)
Born in hospital [*n* (%)][2]	145	59 (40.7)	393	239 (60.8)
Attending an Anganwadi center [*n* (%)]	145	6 (4.1)	393	12 (3.1)
Father consumes alcohol [*n* (%)]	144	76 (52.8)	391	193 (49.4)
Packed cell volume, hematocrit (%)	145	32.8 ± 2.9	392	32.5 ± 3.4
Biochemical markers				
Cobalamin concentration (pmol/L)	145	107.8 (85.5–129.3)[3]	393	259.2 (197.0–341.0)
<150 [*n* (%)]	145	145 (100.0)	393	0 (0.0)
Folate concentration (nmol/L)[2]	145	16.2 (10.7–26.7)	389	9 (5.7–15.5)
<5 [*n* (%)][2]	145	5 (3.4)	389	78 (20.1)
Homocysteine concentration (μmol/L)[2]	144	14.7 (11.2–20.8)	390	9.5 (7.7–11.9)
>10 [*n* (%)][2]	144	119 (82.6)	390	166 (42.6)
Methylmalonic acid concentration (μmol/L)[2]	144	1.2 (0.7–2.4)	390	0.5 (0.3–0.8)
>0.4 [*n* (%)][2]	144	131 (91.0)	390	239 (61.3)
Ferritin concentration (μg/L)	106	7 (5.0–9.0)	254	6 (5.0–10.0)
<12 [*n* (%)]	106	87 (82.1)	254	205 (80.7)
CRP[4] concentration (mg/L)	109	0 (0.0–1.0)	254	0 (0.0–2.0)
>10 [*n* (%)]	106	6 (5.5)	254	11 (4.3)

[1] Mean ± SD (all such values).
[2] Significant difference between the groups, $P < 0.05$.
[3] Median; IQR in parentheses (all such values).
[4] CRP, C-reactive protein.

the plasma folate concentration may be high because of the folate trap phenomenon (20, 21). Because of this, the interpretation of plasma folate can be obscured when cobalamin is low. In this population we found that the folate concentration started to rise when the cobalamin concentration was below the 25th percentile (9). We therefore assessed whether the association between folate and cognitive scores was different in those with low (<25 percentile) and adequate (≥25 percentile) cobalamin status by including an interaction term in the multiple linear regression models. We used STATA version 12 (Stata Corporation) for most statistical analyses. We also used generalized additive models in the statistical software R version 2.0. (The R Foundation for Statistical Computing) to explore nonlinear associations between the exposure variables and the outcome variables after adjustment for potential confounders (22). We considered an association to be statistically significant when $P < 0.05$.

RESULTS

Only 8 children were uncooperative for assessment, and we were unable to measure one of the biomarkers of folate and cobalamin status in 32 children. The flow of children into the study is depicted in **Figure 1**. The baseline features of the participants are described in Table 1. Mean age was 14.9 mo, 74% were still breastfed, 45.2% were stunted, and 17.0% were wasted. The mean cobalamin concentration was 212 pmol/L, and 27% had a concentration <150 pmol/L. The mean folate concentration was 11 nmol/L, and 15.5% had a concentration <5 nmol/L. Plasma tHcy and MMA were generally high (53.5% had tHcy values >10 μmol/L and 70% had MMA values >0.4 μmol/L), which was mainly explained by their poor cobalamin status (9, 12, 13). The plasma concentrations of these markers in relation to age and breastfeeding status were published elsewhere (9, 12, 13). In this sample, the median plasma concentration of folate was 13 (IQR:

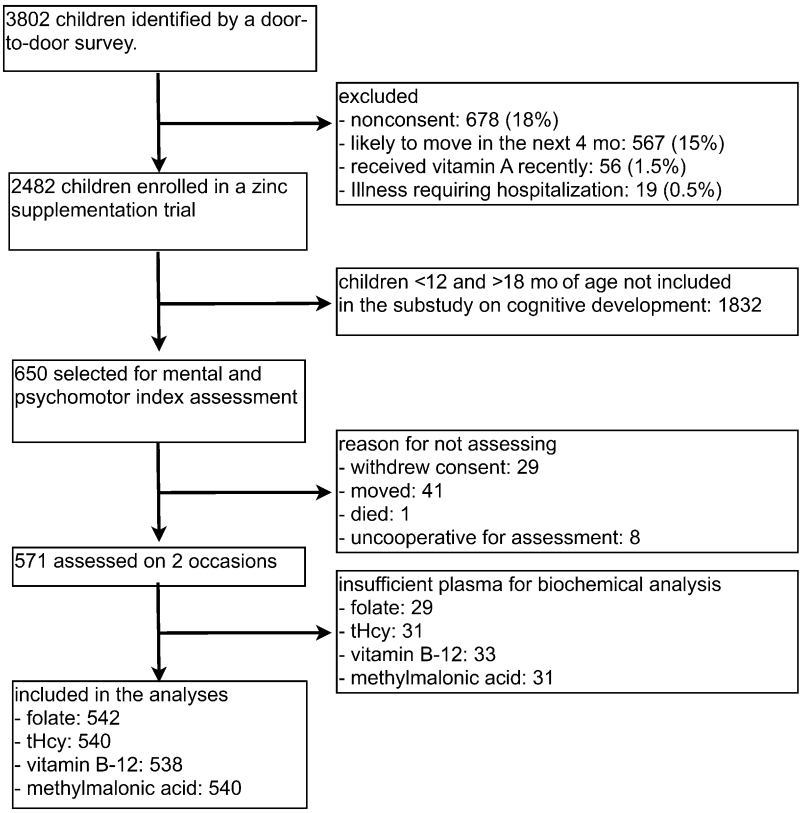

FIGURE 1. Flowchart for a study on the association between folate and cobalamin status in young North Indian children. tHcy, total homocysteine.

9–22) in those who were breastfed and 6 (IQR: 4–8) in those who were not breastfed. For cobalamin, the median concentration was higher in those who were not breastfed (310; IQR: 239–415) than in those who were breastfed (182; IQR: 127–263). Mean PDI and MDI scores are shown in **Table 2**. Predictors for PDI and MDI scores are shown in **Table 3**.

Cobalamin status was associated with cognitive performance (**Table 4**). In the multiple regression analysis (adjusted for several confounders), each 2-fold increment in the plasma cobalamin concentration was associated with an increment in the MDI score of 1.3 (95% CI: 0.2, 2.4; $P = 0.021$). Furthermore, each 2-fold increase in tHcy or MMA concentration was associated with a decrease in MDI score of −2.0 (95% CI: −3.4, −0.5; $P = 0.007$) and −1.1 (95% CI: −1.8, −0.3; $P = 0.004$) points, respectively. For MMA and cobalamin, these significant associations were only seen 4 mo after the time of blood sampling. For plasma tHcy, however, the adjusted association with the MDI score was significant at baseline and at the end of the study. The plasma folate concentration was associated with MDI scores in the crude analyses but not in the adjusted analyses, when all observations were included. Cobalamin status, however, modified the association between folate and MDI scores (**Table 5**). In the subgroup consisting of those with a cobalamin concentration above the 25th percentile, the folate concentration was positively and significantly associated with the MDI scores at both time points, ie, at baseline ($P = 0.020$) and 4 mo thereafter ($P = 0.003$). Sex, breastfeeding status, or zinc supplementation status did not modify these associations.

In the multiple regression models, children who were breastfed had significantly higher PDI scores than did those who were not breastfed, both at baseline and 4 mo later (Table 3). The effect of breastfeeding on the PDI scores was substantially attenuated when plasma folate concentration was included in the model (data not shown). None of the measured markers of folate and cobalamin status were associated with PDI scores in the adjusted statistical models.

The results from the generalized additive models showed no nonlinear associations between the markers of cobalamin status and cognitive scores. For folate, the graph indicates that the folate concentration and MDI scores are positively associated when the folate concentration is below the median (11 nmol/L). For MDI scores, we depicted these associations in **Figure 2**.

TABLE 2
Bayley II psychomotor and mental index scores in 542 children 12–18 mo of age in a study on cognition and folate and cobalamin status[1]

	Value
Psychomotor index score, baseline	98.9 ± 14.4 (49–138)
Psychomotor index score, end of study	93.4 ± 11.2 (50–125)
Mental index score, baseline	99.7 ± 11.5 (57–141)
Mental index score, end of study	92.1 ± 11.0 (54–122)

[1] All values are means ± SDs; ranges in parentheses.

DISCUSSION

In this study, all 3 markers of cobalamin status—serum cobalamin, tHcy, and MMA—were independently associated with a change in MDI but not in PDI scores. However, although statistically significant, the observed changes in cognitive scores per doubling of the biomarker concentrations were small and, for cobalamin and MMA, were only significant when concentrations

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TABLE 3
Predictors of Bayley developmental index scores in children 12–18 mo of age in a study on cognition and folate and cobalamin status[1]

	Psychomotor development index						Mental development index					
	Baseline			End of study			Baseline			End of study		
	Coefficient	95% CI	*P*	Coefficient	95% CI	*P*	Coefficient	95% CI	*P*	Coefficient	95% CI	*P*
Age of child (mo)	−1.1	(−1.6, −0.5)	0.0002	−0.9	(−1.4, −0.5)	<0.001	−2.2	(−2.6, −1.8)	<0.001	−1.3	(−1.7, −0.9)	<0.001
Sex (0 = male, 1 = female)	5.1	(2.7, 7.4)	<0.0001	3.5	(1.6, 5.3)	<0.001	3.2	(1.5, 4.9)	<0.001	3.7	(2.0, 5.4)	<0.001
Breastfed (0 = no, 1 = yes)	3.7	(1.2, 6.2)	0.0041	2	(0.0, 4.0)	0.048						
Mother's age (y)				0.2	(0.0, 0.4)	0.043				0.3	(0.1, 0.5)	<0.001
Any school, mother (no = 0, yes = 1)				1.9	(−0.1, 3.9)	0.057	3.7	(1.9, 5.5)	<0.001	3.5	(1.7, 5.3)	<0.001
Packed cell volume unit	0.5	(0.2, 0.9)	0.0016	0.3	(0.1, 0.6)	0.019	0.4	(0.1, 0.6)	0.002	0.5	(0.3, 0.8)	<0.001
Father drinks alcohol (no = 0, yes = 1)				−1.6	(−3.3, 0.1)	0.064				−1.9	(−3.5, −0.3)	0.0215
Mother works (no = 0, yes = 1)							2.8	(−0.1, 5.8)	0.06			
Length-for-age *z* score unit	3.6	(2.6, 4.6)	<0.0001	2.5	(1.8, 3.3)	<0.001	1.9	(1.2, 2.6)	<0.001	1.9	(1.2, 2.6)	<0.001
Weight-for-length *z* score unit	1.6	(0.4, 2.7)	0.0063	1.3	(0.4, 2.1)	0.006	0.8	(0.0, 1.7)	0.046			
Born in a hospital (no = 0, yes = 1)				1.5	(−0.2, 3.3)	0.088	2.6	(0.9, 4.3)	0.003	2.7	(1.1, 4.3)	0.001
Income in rupees log− (base 2)				0.8	(−0.1, 1.8)	0.097				1.1	(0.2, 1.9)	0.021
Attended by Anganwadi worker (no = 0, yes = 1)							3.9	(−0.5, 8.3)	0.083			
Constant	97	(82.6, 111.5)		78.8	(59.5, 98.0)		115.8	(104.9, 126.8)		67.3	(49.3, 85.2)	
R^2		0.19			0.21			0.29			0.25	

[1] The predictors were identified in a stepwise process in multiple linear regression models.

TABLE 4
Associations between the $\log_2$ transformed concentrations of markers of folate and cobalamin status and psychomotor and mental development index scores in children 12–18 mo of age[1]

	Psychomotor development score						Mental development score					
	Baseline[2]			End of study[3]			Baseline[2]			End of study[3]		
	Coefficient	95% CI	*P*	Coefficient	95% CI	*P*	Coefficient	95% CI	*P*	Coefficient	95% CI	*P*
Folate												
Crude	1.8	(0.8, 2.9)	0.001	1.3	(0.5, 2.2)	0.002	1.5	(0.6, 2.4)	0.001	1.4	(0.5, 2.2)	0.001
Adjusted	0.3	(−0.9, 1.6)	0.586	0.4	(−0.5, 1.4)	0.3581	0.6	(−0.2, 1.4)	0.1306	0.7	(−0.2, 1.6)	0.128
tHcy												
Crude	−1.2	(−3.2, 0.8)	0.231	−1.5	(−3.0, 0.1)	0.058	−2.6	(−4.2, −1.0)	0.002	−3.1	(−4.6, −1.6)	<0.001
Adjusted	−1.2	(−3.1, 0.7)	0.216	−0.6	(−2.1, 0.9)	0.46	−1.7	(−3.1, −0.2)	0.0227	−2.0	(−3.4, −0.5)	0.007
Cobalamin												
Crude	−0.1	(−1.6, 1.4)	0.890	0.2	(−0.9, 1.4)	0.712	0.7	(−0.5, 1.9)	0.253	1.6	(0.4, 2.7)	0.006
Adjusted	0.1	(−1.4, 1.6)	0.933	−0.4	(−1.5, 0.9)	0.5949	0.0	(−1.0, 1.1)	0.9722	1.3	(0.2, 2.4)	0.021
MMA												
Crude	0.1	(−0.9, 1.0)	0.963	−0.5	(−1.2, 0.3)	0.222	−0.8	(−1.6, −0.1)	0.037	−1.2	(−1.9, −0.5)	0.001
Adjusted	0.0	(−1.0, 1.0)	0.665	−0.2	(−0.9, 0.6)	0.6776	−0.4	(−1.1, 0.3)	0.2278	−1.1	(−1.8, −0.3)	0.004

[1] Adjusted for the predictors listed in Table 3; all adjusted models also included age, breastfeeding status, height-for-age *z* scores, and weight-for-length *z* scores. MMA, methylmalonic acid; tHcy, total homocysteine.
[2] Development scores were measured on the same day as the collection of blood specimens for vitamin marker measurements.
[3] Development scores were measured 4 mo after the collection of blood specimens for vitamin marker measurements.

were compared with MDI scores 4 mo after blood sampling. Plasma tHcy was also associated with MDI scores when it was measured on the day of blood sampling.

Several studies have shown that poor cobalamin status is associated with cognitive deficits in the elderly, even at concentrations above established cutoffs for deficiency (2), and a low concentration of folate is associated with cognitive decline and dementia (23). Studies in children are limited, particularly in early childhood.

A study in the Netherlands showed that infants of macrobiotic mothers had delayed motor and language development compared with infants of omnivores (3). At age 12 y, the children still had higher plasma MMA concentrations and scored lower than the omnivores on cognitive tests (24). Cognitive performance was also measured in Guatemalan school-age children with and without cobalamin deficiency. Cobalamin deficiency was associated with poor cognitive and neuromotor performance along with lower academic performance, lower teacher ratings, and more attentional problems (25). Furthermore, in a cohort of young Nepalese children, intake of meat and other animal products, which are good sources of cobalamin, was positively associated with walking acquisition (26). In a study in Kenyan schoolchildren, cobalamin was the only detectable nutrient that responded to meat or milk supplementation (27).

Several studies, particularly in developed countries, have shown that the duration of breastfeeding is positively associated with cognitive test results (28, 29). This was also confirmed in a recent cluster-randomized trial in Belarus, where children whose mothers were randomly assigned to exclusive breastfeeding promotion scored higher in cognitive tests than did those in the control group (30). In our study, the folate concentration was positively and significantly associated with the MDI scores when children with poor cobalamin status were excluded. Breast milk is an important source of folate, and breastfeeding was the most important predictor of plasma folate concentration in this population (9). Thus, the positive effect of breastfeeding shown in several observational studies, and in the aforementioned study from Belarus, may have been due to the folate content of breast milk. Indeed, we found that children who were breastfed had higher PDI scores than did those who were not, and this association was attenuated when the plasma folate concentration was included in the statistical model, which indicated that folate could mediate the effect of breastfeeding on cognition in our population. Cobalamin status was substantially poorer in those who were breastfed than in those who were not breastfed, which is consistent with results from other populations (31). It is also possible that breastfeeding was associated with socioeconomic status and education. However, the described associations were not substantially altered when we adjusted for breastfeeding status or years of schooling of mothers or fathers.

Because of the folate trap phenomenon, plasma folate becomes artificially high among those who are cobalamin deficient and

TABLE 5
Associations between the $\log_2$ transformed concentrations of folate on mental development scores according to cobalamin status in children 12–18 mo of age[1]

	Mental development score					
	Baseline			End of study		
	Coefficient	95% CI	P^2	Coefficient	95% CI	P^2
Folate, adjusted						
Cobalamin <25%	−1.5	(−2.9, 0.6)	0.2	−0.5	(−2.2, 1.1)	0.052
Cobalamin >25%	1.3	(0.2, 2.4)	0.02	1.6	(0.6, 2.7)	0.003

[1] Adjusted for age, sex, breastfeeding status, height-for-age *z* score, weight-for-length *z* score, weight-for-height *z* score, income (log), nuclear or joint family, number of family members, years of schooling of parents, age of parents, attended by Anganwadi worker, born in hospital, and packed cell volume.
[2] *P*-interactions between low cobalamin status at baseline and the end of study were 0.017 and 0.03, respectively.

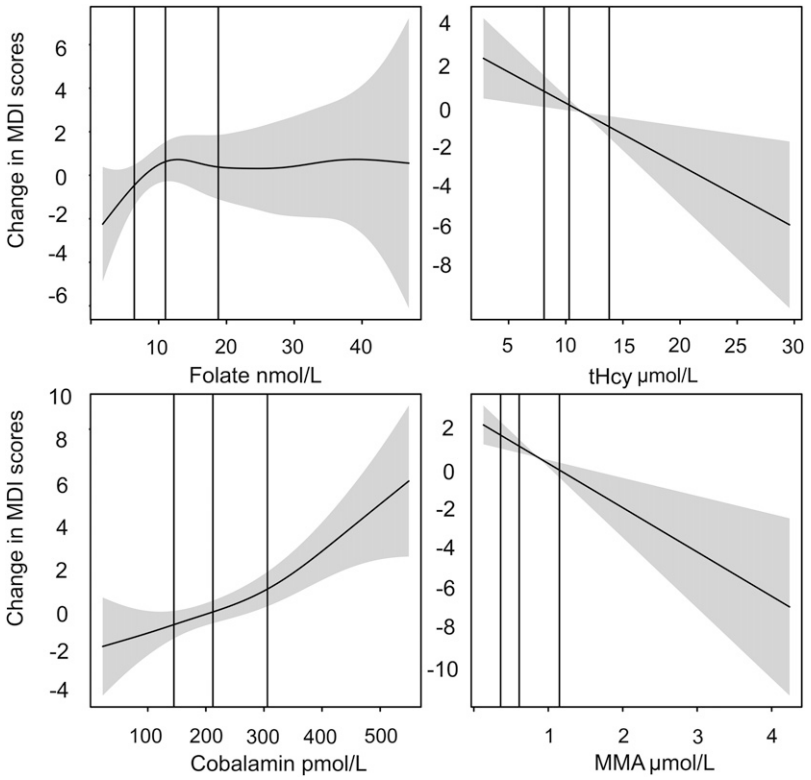

FIGURE 2. Associations between markers of cobalamin and folate status and relative changes in MDI scores (Bayley II) in North Indian children. The vertical lines depict the 25th, 50th, and 75th percentiles of the plasma concentrations of the vitamins or metabolites. The concentration-developmental score responses were obtained from generalized additive models in R (r-project.org). In these models we adjusted for age, breastfeeding status, growth, and other predictors for developmental scores. The shaded area represents the 95% CI of the regression line/curve. The *y* axis is centered around the mean; thus, each plot represents how MDI scores changed relative to its mean with changes in the log-transformed concentrations of folate, cobalamin, tHcy, and MMA. MDI, mental development index; MMA, methylmalonic acid; tHcy, total homocysteine.

may obscure the assessment of folate status in these subjects. This can explain why we only found an association between folate and MDI scores in those with a cobalamin concentration above the 25th percentile (>145 pmol/L).

It is an inherent weakness of cohort studies that they identify associations rather than causality and that the observed associations can be due to confounding. Several demographic, clinical, and socioeconomic variables were collected at baseline, and we assessed whether these variables confounded the associations of interest. The final models included socioeconomic status, maternal and paternal age and education, nutritional status of the child, breastfeeding status, whether or not the children attended an Anganwadi center (a daycare center for young children), and income. The associations between markers of cobalamin status and MDI scores were maintained after adjustment for these factors. However, residual confounding or confounding by variables that we did not measure cannot be ruled out. One such source of confounding could be related to the quality of mother and child interaction in early childhood, including breastfeeding patterns, which we did not properly assess. Note that such confounding also could underestimate the association between cognition and vitamin status. It is also possible that poor cobalamin and folate status are markers of overall malnutrition or impaired status of other nutrients, which again could explain our findings. Randomized, placebo-controlled trials are needed to establish causality between folate or cobalamin status and cognitive functioning.

We believe that the Bayley Scales of Infant Development is the most appropriate available tool to measure the relation between cognitive functioning and nutritional status in young children. It is one of the most used and best-validated tests for research in children <3 y of age (18). Furthermore, in the current study, the theoretically expected variables predicted the MDI and PDI scores (Table 3), which also lend credibility to the usefulness of this method in this population. The fact that all 3 markers of cobalamin status were independently associated with MDI scores also strengthens our interpretation of the findings.

The association between cognition and the concentrations of cobalamin or folate has several, not mutually exclusive, explanations. Folate and cobalamin are necessary cofactors in the synthesis of RNA and DNA (32), and both are necessary for maintaining the nervous system (2). These micronutrients are accordingly essential to the rapid growth and development during the early years of life. Poor cobalamin status leads to a deficiency of *S*-adenosylmethionine, which impairs methylation reactions, including the methylation of myelin basic protein in the central and peripheral nervous system (2). The production of myelin is a key component of brain development from early childhood to well into middle age and is highly related to cognitive function. Severe functional folate deficiency of the central nervous system also impairs myelin metabolism, which can be reversed by folic acid administration (33, 34).

We showed that poor cobalamin status is common in these Indian children. Reduced cognitive potential that may be associated

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with poor folate or cobalamin status may result in constrained learning ability and impaired school achievements later in life (35). It is probably the most marginalized children who face the highest risk of malnutrition. Their parents may not have resources to provide an optimal developmental environment, and they may have a higher burden of infections. All these factors acting in early childhood may result in poorer chances to later success in school and work (36). Our findings should motivate the undertaking of clinical trials on the efficacy of folate and cobalamin supplementation on cognitive development in populations in which deficiencies of these vitamins are prevalent.

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