Cobalamin and folate status in infants and young children in a low-to-middle income community in India[1–3]

Sunita Taneja, Nita Bhandari, Tor A Strand, Halvor Sommerfelt, Helga Refsum, Per M Ueland, Jörn Schneede, Rajiv Bahl, and Maharaj Kishan Bhan

ABSTRACT

Background: Population-based data on the prevalence of cobalamin and folate deficiency in India are lacking.

Objective: The objective was to measure the prevalence of cobalamin and folate deficiency among children aged 6–30 mo residing in a low-to-middle income community in North India.

Design: Children aged 6–30 mo ($n = 2482$) were identified through a community survey in a low-to-middle socioeconomic area in New Delhi, India. Non-fasting venous blood samples were collected before enrollment in another trial.

Results: The median (interquartile range; IQR) cobalamin concentration in 6–11-mo-old children was substantially lower in breastfed (183; 120–263 pmol/L) than in nonbreastfed (334; 235–463 pmol/L) children. Cobalamin concentrations decreased progressively with increasing age in the nonbreastfed children. Median (IQR) plasma folate concentrations in the 6–11-mo-old group were higher in breastfed (20.3; 11.7–34.4 nmol/L) than in nonbreastfed (5.3; 3.4–7.7 nmol/L) children ($P < 0.001$). Folate concentrations decreased with increasing age in the breastfed children. In the nonbreastfed children, folate concentrations increased with increasing age. Low concentrations of plasma cobalamin (<150 pmol/L) were detected in 36% of breastfed and 9% of nonbreastfed children ($P < 0.001$). The proportions of children with plasma folate concentrations <5 nmol/L in these 2 subgroups were 6% and 33%, respectively ($P < 0.001$).

Conclusions: In north Indian preschool children, cobalamin and folate concentrations were commonly low and were associated with elevated total homocysteine and methylmalonic acid concentrations. Because low cobalamin and folate concentrations have functional consequences, population-based measures for improving cobalamin and folate concentrations need to be seriously considered. *Am J Clin Nutr* 2007;86:1302–9.

KEY WORDS Cobalamin, folate, homocysteine, methylmalonic acid, children, India

INTRODUCTION

Deficiency and inadequate dietary intake of several micronutrients, including iron, zinc, vitamin A, folate, and cobalamin, have been reported in India (1, 2). There is, however, a paucity of population-based data on the prevalence of cobalamin deficiency in children. This is surprising given the high likelihood of cobalamin deficiency occurring because of predominantly vegetarian diets and, hence, a low intake of dietary cobalamin throughout life (3). Most studies from India used serum or plasma measurements of the vitamins to assess cobalamin and folate status. These assays have limited sensitivity (4). Markers of cobalamin function, such as serum or plasma concentrations of total homocysteine (tHcy) or methylmalonic acid (MMA) may contribute to identifying mild-to-moderate cobalamin deficiency (5). Homocysteine remethylation to methionine requires 5-methyltetrahydrofolate as cosubstrate and cobalamin as a cofactor. Thus, deficiencies of folate or cobalamin will lead to elevated plasma tHcy concentrations. Cobalamin also functions as a cofactor in a second enzyme, methylmalonyl CoA mutase, which explains elevated concentrations of MMA in cobalamin deficiency. In subjects with normal renal function, elevation of both MMA and tHcy concentrations usually indicates a cobalamin deficiency, whereas normal concentrations of MMA and elevated tHcy concentrations in most cases indicate folate deficiency (5). Measurement of these indicators, therefore, provides a more comprehensive assessment of the folate and cobalamin status in a population. In the present study, we report biochemical evidence of cobalamin and folate deficiencies in 6–30-mo-old children residing in a low to midlevel socioeconomic urban community in Delhi.

[1] From the Department of Pediatrics, All India Institute of Medical Sciences, New Delhi, India (ST, NB, RB, and MKB); the Center for International Health, University of Bergen, Bergen, Norway (TAS and HS); the Institute of Basic Medical Sciences, Department of Nutrition, University of Oslo, Oslo, Norway (HR); the Oxford Centre for Gene Function, Department of Physiology, Anatomy & Genetics, Oxford University, Oxford, United Kingdom (HR); the Section for Pharmacology, Institute of Medicine, University of Bergen, Bergen, Norway (PMU); the Department of Clinical Chemistry, University of Umea, Umea, Sweden (JS); the Department of Biotechnology, New Delhi, India (MKB); and the Society for Applied Studies, New Delhi, India (ST and NB).

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[3] Reprints not available. Address correspondence to MK Bhan, Department of Pediatrics, All India Institute of Medical Sciences, Ansari Nagar, New Delhi 110029, India. E-mail: community.research@cih.uib.no.

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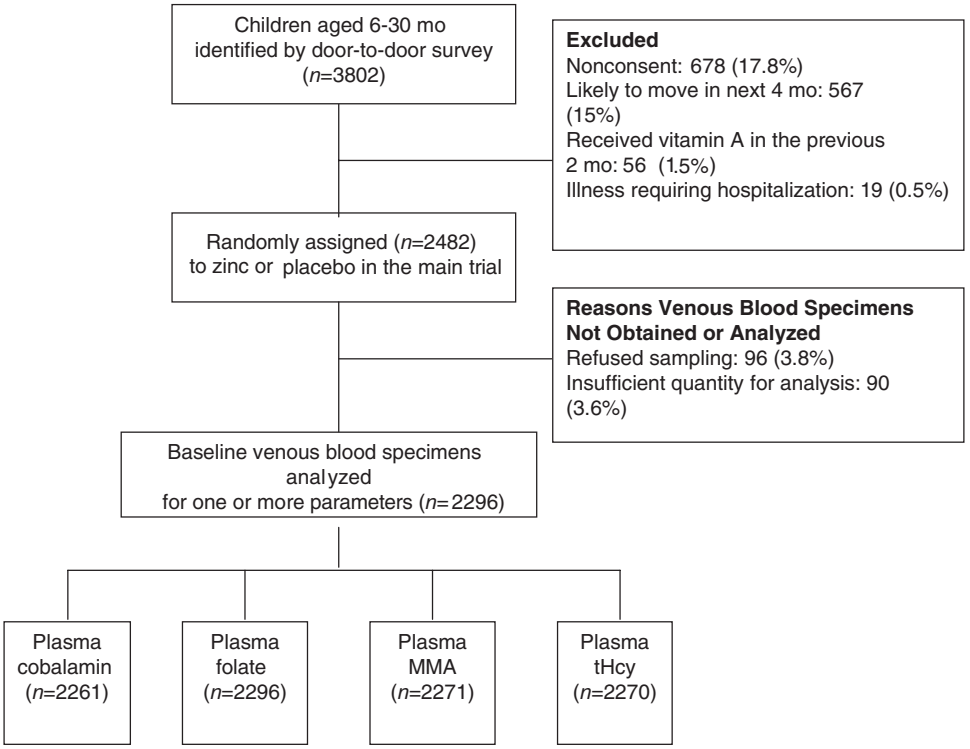

FIGURE 1. Trial profile.

SUBJECTS AND METHODS

Study population

Children were identified through a survey for a double-blind, randomized, placebo-controlled trial aimed to assess the effect of daily zinc supplementation for 4 mo on diarrhea and pneumonia (6, 7). Children included in the trial were aged 6–30 mo and were residents of the urban community Dakshinpuri, which comprised a population of ≈75 000 individuals residing in 15 000 households. More details of the study setting, design, and recruitment procedures were described previously (6, 7).

Briefly, a total of 3802 children aged 6–30 mo were identified through a door-to-door survey. Exclusion criteria followed the protocol of the trial, and the reasons for exclusion are shown in **Figure 1**. Of the 2482 children randomly assigned into the main trial, 96 (3.8%) of the parents refused sampling, and blood was insufficient for analysis in another 90 (3.6%) children. The current analysis is accordingly based on 2296 children (Figure 1) whose blood specimens were collected before initiation of zinc supplementation or any other intervention. The procedures were in accordance with the ethical standards of the All India Institute of Medical Sciences.

Blood sampling procedures

Between 0900 and 1200, nonfasting venous blood specimens were collected by physicians into heparinized polypropylene tubes (Sarstedt, Numbrecht, Germany). The heparinized blood was centrifuged at 447 × *g* for 10 min at room temperature within 10 min of collection with portable centrifuges placed at the site of collection, and plasma was transferred into polypropylene vials (Eppendorf, Hinz, Germany) and stored at −20 °C until analyzed.

Plasma concentrations of cobalamin ($n = 2261$) and folate ($n = 2296$) were determined by microbiological assays using a chloramphenicol-resistant strain of *Lactobacillus casei* and colistin sulfate–resistant strain of *Lactobacillus leichmannii*, respectively (8, 9). Both assays were adapted to a microtiter plate format and carried out by a robotic workstation (10). Plasma concentrations of MMA ($n = 2271$) and tHcy ($n = 2270$) were analyzed by a modified gas chromatography–mass spectrometry (GC-MS) method based on ethylchloroformate derivatization (11).

Data entry and statistical analysis

Data were double entered independently by 2 data clerks into databases (FoxPro; Microsoft Corporation, Redmond, WA) with range, consistency, and logic checks. The 2 data sets were merged after validation, and a backup was kept offsite.

Statistical analysis was performed with STATA (version 8; Statacorp, College Station, TX). All observations were displayed in scatter plots to identify any outliers. Means, medians, SDs, and interquartile ranges (IQRs) were estimated. For comparison of vitamin concentrations in breastfed and nonbreastfed children, nonparametric tests were used. Median concentrations of vitamins and metabolites across age categories in breastfed and nonbreastfed children were compared by using a Kruskal-Wallis test. The interactions between various categories of cobalamin and folate as dependent variables and other subgroups as explanatory variables were examined by multivariate logistic regression. In addition to all the explanatory variables as the main effects, all interaction terms up to the second order were included in the multivariate logistic regression model. The effect of interaction term was assessed by the level of significance of each interaction term.

TABLE 1
Baseline characteristics of the study children aged 6–30 mo, who resided in a low-to-middle income community of Delhi, India

Characteristics	Total group (n = 2296)
Age (mo)	15.3 ± 7.5[1]
Male [n (%)]	1205 (52.5)
Breastfed [n (%)]	1585 (69.0)
Weight (kg)	8.1 ± 1.6
Length (cm)	72.7 ± 7.2
Mother literate [n (%)]	1473 (64.4)
Annual family income (rupees)[2]	36 000 (24 000, 54 000)[3]

[1] $\bar{x}$ ± SD (all such values).
[2] One US dollar = 44 rupees.
[3] Median; interquartile range in parenthesis.

We used generalized additive model (GAM) plots in the package "mgcr" in the statistical software R (12, 13) to depict the dose-response relation between age, breastfeeding status, and vitamin or metabolite concentrations. The significance level was set at 0.05.

RESULTS

The demographic characteristics of the included children are shown in **Table 1**. The mean age at enrollment was 15.3 mo; ≈50% of the children were boys and 69% were breastfed. There was a significant interaction of age and breastfeeding with cobalamin, folate, tHcy, and MMA ($P < 0.001$ in each instance). The median and interquartile ranges of cobalamin, folate, tHcy, and MMA values are given in **Table 2** by breastfeeding status and age.

Overall in the breastfed children, median cobalamin concentrations were lower (178.3 compared with 277), whereas folate (14.3 compared with 6.3), tHcy (12 compared with 9.2), and MMA (0.9 compared with 0.4) concentrations were higher than in nonbreastfed children ($P < 0.001$ for all comparisons).

Across the age categories 6–11, 12–23, and 24–30 mo, median plasma folate, tHcy, and MMA were significantly different ($P < 0.001$ for each) in breastfed children. In the nonbreastfed children, all of these variables and cobalamin concentrations were different across the same age subgroups ($P < 0.001$ for each).

In simple correlation analysis, the cobalamin concentration was inversely associated with tHcy in breastfed ($r = -0.53$, $P < 0.001$) and nonbreastfed ($r = -0.24$, $P < 0.001$) children. The cobalamin concentration was also inversely associated with MMA concentrations in breastfed ($r = -0.44$, $P < 0.001$) and nonbreastfed ($r = -0.39$, $P < 0.001$) children. Folate concentrations showed an inverse correlation with tHcy ($r = -0.35$, $P < 0.001$) and MMA ($r = -0.07$, $P = 0.061$) in the nonbreastfed children. However, in breastfed children there was a significant positive association of folate with tHcy ($r = 0.28$, $P < 0.001$) and MMA ($r = 0.34$, $P < 0.001$). The correlation between folate and MMA and between folate and tHcy in nonbreastfed children was significantly different ($P < 0.001$) from the respective correlation in breastfed children (14).

Plasma cobalamin and folate

Various reference limits for cobalamin and folate were used previously, but none are available for tHcy and MMA in children. Many studies have used either 150 or 200 pmol/L as cutoffs for defining cobalamin deficiency and 5 or 7.5 nmol/L for defining folate deficiency (15–17). Twenty-eight percent (breastfed: 36%; nonbreastfed: 9%) of the children had plasma cobalamin concentrations <150 pmol/L, 48% (breastfed: 58%; nonbreastfed: 24%) had concentrations <200 pmol/L, and 64% (breastfed: 73%; nonbreastfed: 43%) had concentrations <250 pmol/L.

TABLE 2
Plasma cobalamin, folate, methylmalonic acid (MMA), and total homocysteine (tHcy) concentrations in the study children at different ages according to breastfeeding status[1]

	Breastfed children			Nonbreastfed children		
	6–11 mo	12–23 mo	24–30 mo	6–11 mo	12–23 mo	24–30 mo
Cobalamin (pmol/L)						
n	776	609	178	144	310	244
Median	183.8	171.6	180.5	334.0	284.5	230.9
IQR	120–263	120–250	132–264	234–463	212–381	178–321
Folate (nmol/L)						
n	791	614	180	143	316	252
Median	20.2	11.9	9.3	5.3	5.8	7.4
IQR	11.7–34.4	7.9–18.9	6.6–13.6	3.4–7.7	4.3–8.2	5.3–10.1
tHcy (μmol/L)						
n	780	609	179	142	313	247
Median	12.6	11.5	10.3	10.7	9.1	9.1
IQR	9.2–18.1	8.9–16.1	8.5–13.5	8.2–13.9	7.5–11.2	7.2–11.1
MMA (μmol/L)						
n	770	609	178	142	314	247
Median	1.03	0.79	0.60	0.44	0.37	0.41
IQR	0.53–2.08	0.45–1.48	0.36–1.02	0.31–0.71	0.25–0.53	0.26–0.64

[1] IQR, interquartile range. For cobalamin, folate, tHcy, and MMA, the interaction between breastfeeding and age was significant ($P < 0.001$ for each); test of interaction. Median values across age categories are significantly different for folate, tHcy, and MMA in the breastfed children ($P < 0.001$ for each) and for all 4 metabolites in the nonbreastfed children ($P < 0.001$ for each); Kruskal-Wallis test. Values were significantly different between breastfed and nonbreastfed children, $P < 0.001$ (k sample equality median test).

TABLE 3
Differences in baseline variables by plasma cobalamin concentration in the study children[1]

Characteristics	Categories of plasma cobalamin (pmol/L) <150 (n = 639)	≥150 to 200 (n = 445)	>200 (n = 1177)	P[2]
Age (mo)	14.6 ± 7.2[3]	15.4 ± 7.6	15.6 ± 7.6[4]	0.018
Age 6–11 mo [n (%)]	284 (44.4)	181 (40.7)	455 (38.7)	0.057
Age 12–30 mo [n (%)]	355 (55.5)	264 (59.3)	722 (61.3)	
Sex [n (%)]				
Male	308 (48.2)	223 (50.1)	651 (55.3)[4]	0.009
Female	331 (51.8)	222 (49.9)	526 (44.7)	
Breastfeeding status [n (%)]				
Breastfed	572 (89.5)	341 (76.6)[4]	650 (55.2)[4,5]	<0.001
Nonbreastfed	67 (10.5)	104 (23.3)	527 (44.8)	
Plasma folate (nmol/L)	23.0 ± 16.9 [635]	16.7 ± 14.3[4] [44]	11.5 ± 10.1[4,5] [1172]	<0.001
<5 nmol/L [n (%)]	31 (4.9)	52 (11.8)	250 (21.3)[4,5]	<0.001
≥5 nmol/L [n (%)]	604 (95.1)	390 (88.2)	922 (78.7)	
Plasma tHcy (μmol/L)	18.0 ± 9.1 [632]	12.0 ± 4.8[4] [442]	10.1 ± 3.9[4,5] [1165]	<0.001
Plasma MMA (μmol/L)	2.33 ± 3.35	1.02 ± 1.21[4]	0.77 ± 1.99[4]	<0.001

[1] n values in brackets. tHcy, total homocysteine; MMA, methylmalonic acid.
[2] Chi-square test used for proportions and ANOVA used for means.
[3] $\bar{x}$ ± SD (all such values).
[4] Significantly different from subjects with a cobalamin concentration of <150 pmol/L, $P < 0.015$ (Bonferroni correction).
[5] Significantly different from subjects with a cobalamin concentration of 150–200 pmol/L, $P < 0.015$ (Bonferroni correction).

With regard to folate, 15% (breastfed: 6%; nonbreastfed: 33%) had concentrations <5 nmol/L, 32% (breastfed: 18%; nonbreastfed: 63%) had concentrations <7.5 nmol/L, and 46% (breastfed: 30%; nonbreastfed: 82%) had concentrations <10 nmol/L.

The distribution of plasma cobalamin concentrations in relation to several baseline characteristics of the children is shown in **Table 3**. Of the subgroup with plasma cobalamin concentrations <150 pmol/L, only 31 (4.9%) had folate concentrations <5 nmol/L, and 83 (13.1%) had concentrations <7.5 nmol/L (data not shown).

The distribution of plasma folate concentrations by age, breastfeeding status, and metabolite concentrations is shown in **Table 4**. Overall, 31 of the 2296 study children had both plasma cobalamin concentrations <150 pmol/L and folate concentrations <5 nmol/L. In a multivariate logistic model, the interaction among all explanatory variables shown in Table 4 was determined, and there was a significant interaction only of breastfeeding with age and with tHcy across various categories of plasma folate ($P < 0.001$; data not shown).

We used GAM to describe the associations between vitamin concentrations and the metabolites adjusted for age in the breastfed and nonbreastfed children (**Figure 2**). In relation to folate, the GAM curves showed a marked difference between breastfed and nonbreastfed children. In breastfed children, folate was positively associated with tHcy, with no apparent threshold effects. In nonbreastfed children, the expected inverse association between folate and tHcy was apparent (Figure 2). For cobalamin, the GAM curves showed that in breastfed children the metabolite concentrations start to decline at lower cobalamin concentrations and more sharply than in nonbreastfed children. The thresholds were less distinct for the nonbreastfed children.

DISCUSSION

Assuming that our cutoff values for defining deficiency are appropriate, the current study highlights the very common occurrence of cobalamin and folate deficiency in young Indian children. Cobalamin deficiency affected nearly 1 of every 3 children, and some of the others might well have milder degrees of deficiency. Several studies in India have reported cobalamin and folate deficiency, hyperhomocysteinemia, and elevated plasma MMA concentrations in 50% to 66% of adults in different parts of India (16, 18, 19). However, few population-based estimates of cobalamin and folate deficiency among preschool children are available. The available studies in children who were either attending school or visiting hospitals (20–24) have shown that ≈33% have low plasma cobalamin and 5–20% have low plasma folate (23, 24) concentrations. A poor cobalamin status of mothers may be an important factor in the high prevalence of cobalamin deficiency observed in the present study. This may be caused by limited cobalamin transfer across the placenta leading to low cobalamin stores in newborns and by a low content in breast milk (25, 26).

Dietary habits were not documented in the current study. According to previous studies conducted in this population, most families are vegetarian (27). Milk cobalamin concentrations are lower in women consuming a strict vegetarian diet than in those consuming an omnivorous diet (28). In this setting, breastfeeding during the first year is generally the rule, the introduction of complementary foods is delayed, and the intake of such foods is low (29, 30). Foods of animal origin are very uncommonly offered to young children. The consumption of fortified cereals and dairy products is low in both mothers and children (2). Gastrointestinal infections, intestinal bacterial overgrowth, and giardiasis are common (31–33).

TABLE 4
Differences in baseline variables by plasma folate concentration in the study population[1]

Characteristics	Categories of plasma folate (nmol/L)			
	<5 (n = 335)	≥5 to ≤7.5 (n = 409)	>7.5 (n = 1552)	P^2
Age (mo)	16.5 ± 6.9[3]	18.9 ± 7.3[4]	14.1 ± 7.4[4,5]	<0.001
Age 6–11 mo [n (%)]	100 (29.9)	92 (22.5)	742 (47.8)	<0.001
Breastfed	7.9 ± 1.8 [32]	8.1 ± 1.7 [54]	7.5 ± 1.7 [705]	0.595
Nonbreastfed	8.1 ± 1.8 [68]	8.1 ± 1.7 [38]	8.2 ± 1.9 [37]	0.908
Age 12–30 mo [n (%)]	235 (70.2)	317 (77.5)	810 (52.5)	<0.001
Breastfed	19.5 ± 4.8 [69]	21.1 ± 4.6 [136]	18.8 ± 4.9[5] [589]	0.671
Nonbreastfed	20.3 ± 4.8 [166]	22.7 ± 5.0[4] [181]	23.4 ± 4.6[4] [221]	0.449
Sex [n (%)]				
Male	183 (54.6)	217 (53.1)	805 (51.9)	0.636
Female	152 (45.4)	192 (46.9)	747 (48.1)	
Breastfeeding status [n (%)]				
Breastfed	101 (30.1)	190 (46.4)	1294 (83.4)	<0.001
Nonbreastfed	234 (69.8)	219 (53.5)	258 (16.6)	
Plasma cobalamin (pmol/L)	307.7 ± 149.9 [333]	281.3 ± 132.7 [400]	209.7 ± 128.9[4,5] [1016]	<0.001
<150 pmol/L [n (%)]	31 (9.3)	52 (13.0)	552 (36.4)	
≥150 to ≤200 pmol/L [n (%)]	52 (15.6)	71 (17.8)	319 (21.0)	
>200 pmol/L [n (%)]	250 (75.1)	277 (69.3)	646 (42.6)	
Plasma tHcy (μmol/L)	11.7 ± 4.7 [332]	10.6 ± 4.0 [404]	13.5 ± 7.7[4,5] [1524]	<0.001
Breastfed	11.7 ± 3.8 [100]	11.4 ± 4.6 [189]	14.5 ± 8.0[4,5] [1270]	<0.001
Nonbreastfed	11.8 ± 5.1 [232]	9.9 ± 3.3[4] [215]	8.8 ± 3.1[4] [254]	
Plasma MMA (μmol/L)	0.65 ± 0.96	0.68 ± 0.78	1.58 ± 2.91[4,5]	<0.001

[1] n values in brackets. tHcy, total homocysteine; MMA, methylmalonic acid. Comparison of all 3 folate and cobalamin categories: $P < 0.001$ (overall chi-square test for proportions). Interaction of breastfeeding with age and tHcy across plasma folate categories: $P < 0.001$ (multivariate logistic model).
[2] Chi-square test used for proportions and ANOVA used for means.
[3] $\bar{x}$ ± SD (all such values).
[4] Significantly different from subjects with a folate concentration of <5 nmol/L, $P < 0.015$ (Bonferroni correction).
[5] Significantly different from subjects with a folate concentration of 5–7.5 nmol/L, $P < 0.015$ (Bonferroni correction).

These factors have been reported to be contributory factors to cobalamin deficiency.

The present study showed the importance of breastfeeding in protecting against folate deficiency. Folate concentrations in breast milk are usually quite high (34), and folate is the only vitamin of the B group whose concentration in breast milk is independent of maternal intake and status (35). Note, however, that this is generally true in folate-replete populations, and it is not clear whether it would hold true for a folate-deplete population.

The public health significance of cobalamin deficiency is not well recognized despite its high prevalence. Studies in India and Nepal show that this may be because severe cobalamin deficiency can occur without the classic signs of anemia or macrocytosis (16, 36–39). The possibility that adequate folate intake protects against anemia in some subjects with cobalamin deficiency and, therefore, masks its other biological effects has been proposed (40). Leukocytosis and thrombocytopenia have been frequently reported in children and in adults with low cobalamin concentrations (24, 36). Interestingly, in a rotavirus vaccine trial at the same site where we undertook the current study, leukopenia was reported to be unexpectedly common among otherwise healthy infants aged 6–8 wk (41). Symptoms attributed to cobalamin deficiency include failure to thrive, movement disorders, psychomotor developmental delay and regression, and megaloblastosis, but neurologic symptoms can develop even without hematologic abnormalities (26). In apparently healthy children from macrobiotic families, who adhere to a strict vegetarian diet, metabolic signs of persistent cobalamin deficiency and impaired cognitive performance may prevail during adolescence and even after initiating consumption of animal products (42, 43).

A recent report on the incidence of neural tube defects in village clusters in one of the most underdeveloped areas of India showed an incidence of 6.7 to 8.2 per thousand live births (44). This risk is one of the highest in the world. Potential etiologic factors are deficiency of folate and possibly of cobalamin or vitamin B-6 (45). Furthermore, hyperhomocysteinemia has been reported in nearly 75% of Indian adults, and vegetarianism and several poverty-related factors may be important in its etiology (16, 46).

Several potential limitations of this study are noteworthy. The estimate of deficiency across studies may vary because of factors other than actual differences. These factors include the nature of the assays used, the fasting or nonfasting status at the time of specimen collection, and incipient dehydration as a result of mild diarrheal illnesses that are common in developing countries. Considerable variation is reported for folate estimates by various assays and across laboratories.

In general, microbiological assays have tended to have larger CVs than radioimmunoassays. This variability must invariably influence the comparison of folate deficiency estimates across studies (47). In the current study, blood was obtained in a nonfasting state because of the young age of the children, which

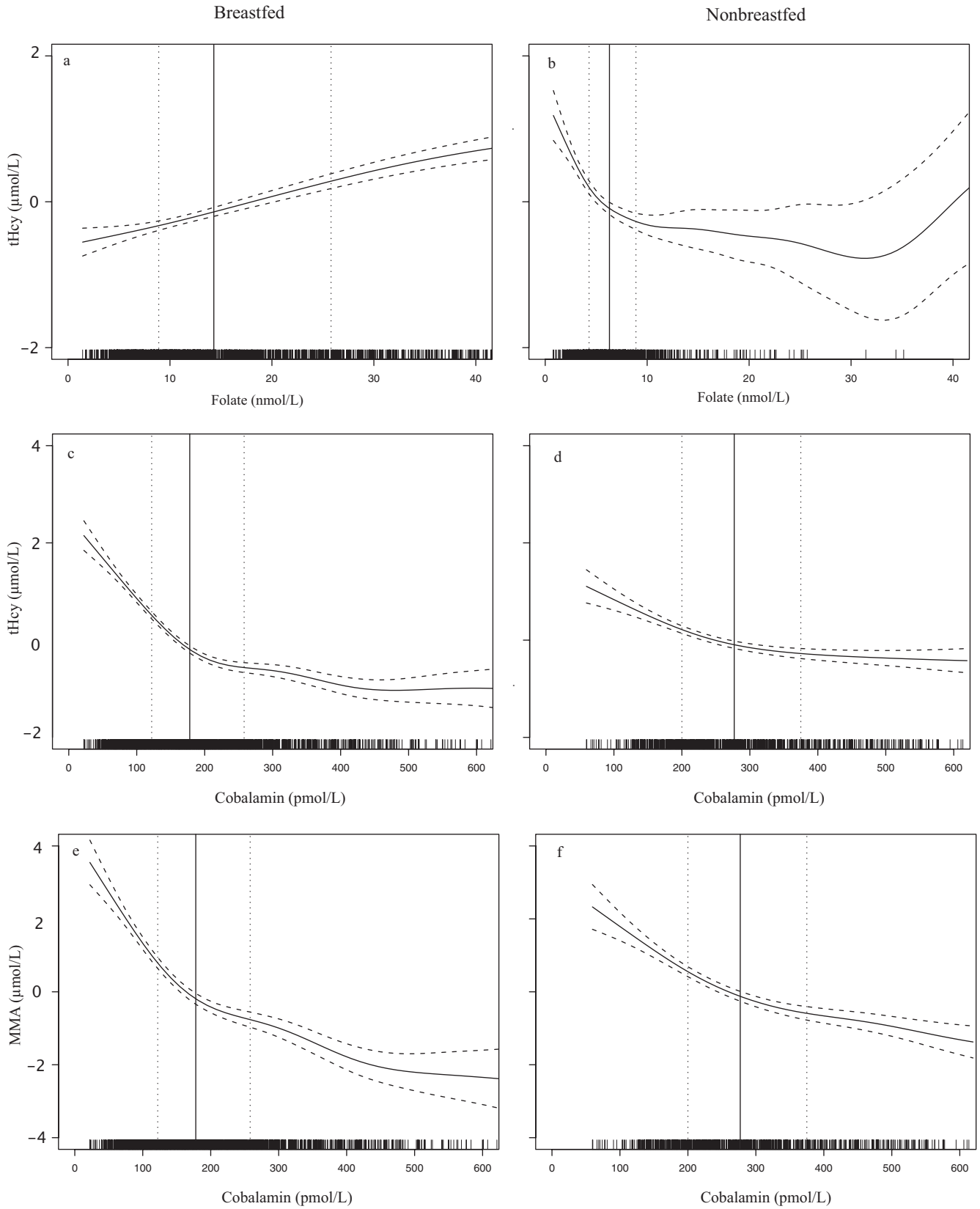

FIGURE 2. Relation between total homocysteine (tHcy) and methylmalonic acid (MMA) concentrations with cobalamin and folate concentrations in breastfed and nonbreastfed children. The graphs were made by using generalized additive models in R (13). The results are adjusted for age. The solid lines represent the estimated dose-response curves; the dashed lines represent the 95% CIs. The horizontal lines depict suggested thresholds. For the relations between cobalamin and MMA and tHcy, the thresholds are not clearly distinct; therefore, the estimates of proportion with low cobalamin concentrations are more uncertain. In the regression models that were the basis for these dose-response graphs, the *P* value for the interaction of breastfeeding and cobalamin with MMA and tHcy and the *P* value for the interaction of breastfeeding and folate with tHcy were both <0.001.

could have falsely elevated plasma folate concentrations and led to an underestimation of the prevalence of folate deficiency. Although mild diarrheal illness in a proportion of children at the time of blood collection cannot be totally ruled out, it is unlikely to be a major factor because children were considered for enrollment only after recovery from any significant illness. We did not specifically monitor the use of iron or folic acid supplements by children. However, previous program assessments have shown that compliance with iron–folic acid programs is very low for young children. Nevertheless, these factors could potentially lead to an underestimate of folate deficiency. The availability of detailed dietary intakes could have facilitated better interpretation of the study findings, but such data are regrettably not available. The availability of blood counts would have helped to provide clinical validity to the vitamin-deficiency data. Finally, we did not obtain vitamin B-6 data and, therefore, cannot comment on any possible effect of its deficiency on the observed homocysteine values.

On the other hand, despite these potential limitations, this study has yielded one of the very few population-based estimates of cobalamin and folate deficiency levels in Indian children. Plasma concentrations of folate and tHcy are usually inversely correlated (48). Thus, a surprising finding in our population was the positive association between folate and tHcy in the breastfed children. One possible explanation could be that the breastfed children had a cobalamin deficiency. Elevated folate concentrations in cobalamin-deficient subjects has been reported earlier (49) and has been explained by the so called "folate trap" mechanism (50). Furthermore, this phenomenon may obscure the assessment of folate status in cobalamin-deficient subjects (51).

The findings of this study, when viewed together with those of other studies in Indian adults and children, suggest that cobalamin and folate deficiencies are important public health problems that merit priority attention and effective control. Possible remedial measures include dietary modification, supplementation, and fortification of specific foods, mainly those that are commercially processed.

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