Folic acid and vitamin B-12 supplementation and common infections in 6−30-mo-old children in India: a randomized placebo-controlled trial[1–3]

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ABSTRACT

Background: Young children in low- and middle-income countries frequently have inadequate vitamin B-12 (cobalamin) status. Poor folate status is also common and is associated with increased diarrheal and respiratory morbidity.

Objective: The objective was to measure the effect of folic acid and/or vitamin B-12 administration on the incidence of diarrhea and acute lower respiratory tract infections.

Design: One thousand North Indian children (6−30 mo of age) were enrolled in a randomized, double-blind, placebo-controlled trial to receive 2 times the Recommended Dietary Allowance of folic acid and/or vitamin B-12 or placebo daily for 6 mo. Children were individually randomly assigned in a 1:1:1:1 ratio in blocks of 16. Primary outcomes were the number of episodes of acute lower respiratory infections, diarrhea, and prolonged diarrhea.

Results: Folic acid and vitamin B-12 supplementation significantly improved vitamin B-12 and folate status, respectively. Neither folic acid nor vitamin B-12 administration reduced the incidence of diarrhea or lower respiratory infections. In comparison with placebo, children treated with folic acid alone or in combination with vitamin B-12 had a significantly higher risk of persistent diarrhea (OR: 2.1; 95% CI: 1.1, 3.8).

Conclusions: Folic acid or vitamin B-12 supplementation did not reduce the burden of common childhood infections. In view of the increased risk of diarrhea, the safety of folic acid supplements in young children should be further assessed. This trial was registered at www.clinicaltrials.gov as NCT00717730 and at www.ctri.nic.in as CTRI/2010/091/001090. *Am J Clin Nutr* 2013;98:731–7.

INTRODUCTION

Pneumonia and diarrhea are among the leading causes of morbidity and mortality in children younger than 5 y in low- and middle-income countries (1). Deficiencies of folate and vitamin B-12 are often part of general malnutrition and may, in part, be responsible for the excess morbidity and mortality seen in malnourished children (2). In a cohort study of nearly 2500 Indian children, we showed that those with poor folate status had a 77% higher incidence of persistent diarrhea and a 44% increased risk of pneumonia compared with those with normal folate status (3, 4).

The defense against infections relies on the ability of the immune cells to proliferate and differentiate and on the effective renewal of the epithelial linings. Folates and vitamin B-12 play a crucial role in DNA and protein synthesis, which suggest that processes in which cell proliferation is essential may be impaired by poor status. Macroscopic disruption of the epithelial linings occurs with antifolate treatment, and the immune system is affected by folate and vitamin B-12 deficiency (5–7). The phagocytic and bactericidal activity of polymorphonuclear leukocytes is poor in individuals with severe folate deficiency and improves with folate replenishment (8, 9). Furthermore, the thymus and cell-mediated immunity and the blastogenic response of T lymphocytes to several antigens is reduced in folate-deficient individuals (10). Thus, deficiencies of these nutrients may have negative consequences on the resistance against infections. Furthermore, in experimental animals, impaired intracellular folate activity due to antifolate treatment leads to gross impairment of the intestinal mucosa and to diarrhea (11).

Two small studies have examined folic acid in the treatment of diarrhea in young children; one found a reduction in diarrheal duration (12) and the other did not (13). Folic acid supplementation may lead to clinical improvement in adult tropical sprue (14); however the role of folates in the risk of infectious diseases is still unclear. We are not aware of any studies that have measured the efficacy of routine vitamin B-12 administration on reducing childhood infections.

Using a factorial design, we conducted a randomized placebo-controlled trial of folic acid and vitamin B-12 to assess whether daily administration to young children of either or both of these nutrients reduced the risk of diarrhea and respiratory infections.

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SUBJECT AND METHODS

Study population

The trial was conducted in the urban neighborhoods of Tigri, Dakshinpuri in New Delhi, India, comprising 300,000 inhabitants. Details of the study setting were described previously (15, 16). The study was approved by the ethic committees of the Society for Essential Health Action and Training, New Delhi, India; the Society for Applied Studies, New Delhi, India; the Christian Medical College, Vellore, India; and the Norwegian Regional Committee for Medical and Health Research Ethics.

Randomization and masking

Using a factorial design, we randomly assigned eligible children to 1 of 4 treatment groups: *1*) placebo (n = 249), *2*) 2 times the Recommended Dietary Allowance (RDA)[4] of folic acid (n = 249), *3*) 2 times the RDA of vitamin B-12 (n = 252), and *4*) a combination of both vitamin B-12 and folic acid (n = 250) in blocks of 16. A scientist at the University of Bergen, who was otherwise not involved in the study, provided the randomization scheme by using Stata Version 10 (StataCorp). The intervention was a lipid-based nutritional supplement prepared by NUTRISET Ltd and was provided in jars prelabeled with the subject identification number. The placebo and the vitamin supplements were identical in appearance and taste. The treatment allocation was masked to the study participants and the study team throughout the data collection period.

Procedures

Enrollment commenced in January 2010, and follow-up ended in September 2011. Children aged 6−30 mo of either sex were identified through a survey. Availability of informed consent and no plans to move away over the next 6 mo were considered for enrollment. We excluded children with severe systemic illness requiring hospitalization, those with severe acute malnutrition (weight-for-height z score less than −3), with severe anemia (hemoglobin <7 g/dL), and those who were using folic acid and/or vitamin B-12 supplements. Children with anemia or with acute infections that required medical treatment were enrolled after recovery if eligible.

Children were supplemented with 1 spoon (5 g) of the relevant supplement if they were 6−11 mo of age and with 2 spoons (10 g) if older than 12 mo. The supplement was administered daily by a fieldworker for 6 mo at home, except on Sundays and public holidays when the mother administered it herself. The supplement was packaged in 330-g jars; this supply was adequate for 1 mo for children aged >12 mo and for 2 mo for children aged 6−11 mo.

In addition to relevant vitamins, each 10-g of supplement (corresponding to the daily dose for children aged ≥12 mo) contained 54.1 kcal total energy, 0.7 g proteins, and 3.3 g fat. In addition, it also contained 150 μg folic acid for the groups that were randomly assigned to receive folic acid and contained 1.8 μg vitamin B-12 for the vitamin B-12 groups.

[4]Abbreviations used: ALRI, acute lower respiratory infection; RDA, Recommended Dietary Allowance; tHcy, total homocysteine.

A separate team of fieldworkers visited households twice weekly for 6 mo. At the visits, mothers were asked about diarrheal illness (number and consistency of stools), symptoms of respiratory illness (cough and fast or difficult breathing), and fever on any day since the last visit and whether treatment was sought for any illness. Respiratory rates were counted twice at each visit, temperature was measured, and the child was examined for signs of dehydration if diarrhea or vomiting was present. Children with cough and respiratory rates ≥35/min at age ≥12 mo and ≥45/min at <12 mo or lower chest indrawing were brought to the clinic for assessment by a study physician. We used a lower cutoff of 5 breaths/min below the WHO criteria for fast breathing to increase the sensitivity of detection of acute lower respiratory tract infections (ALRIs) and pneumonia. Children who visited the clinic spontaneously or sick children who were referred were treated according to WHO guidelines for Integrated Management of Childhood Illness (17). Anthropometric measures were assessed through weight and length measurements. Weight was measured to the nearest 50 g by using Digitron scales. Length was measured by using locally manufactured infantometers that read to the nearest 0.1 cm.

Sample size

Morbidity estimates were based on earlier studies carried out in the same population (15, 16). With an estimated rate of ALRIs in the community of 1.4 episodes per child-year and a 25% reduction, we needed a sample size of 420 per group (α error: 5%; power: 90%). Allowing for up to 15% attrition, we recruited 1000 children (500 per group) to compare the effect of folic acid or vitamin B-12 with placebo on ALRI incidence. The power to detect a difference in prolonged diarrhea (1.0 episodes per child-years in the placebo group) was lower (∼80% with the same assumptions). In these calculations, we assumed that there was no interaction between folic acid and vitamin B-12 administration.

Laboratory analysis

Blood samples were obtained at baseline from all children and at end of the 26-wk supplementation period in a subsample of 16 randomly selected blocks (256 children); 3 mL blood was collected into an evacuated tube containing EDTA (Becton Dickinson). The plasma was centrifuged at ∼450 × g at room temperature for 10 min, separated, and transferred into storage vials and stored at −20°C until analyzed. Total homocysteine (tHcy) was analyzed by using commercial kits from Abbott Laboratories (18). Plasma concentrations of vitamin B-12 and folate were measured by using microbiological assays with a chloramphenicol-resistant strain of *Lactobacillus casei* and a colistin sulfate–resistant strain of *Lactobacillus leichmannii*, respectively (19).

Definitions

Diarrhea was defined as the passage of ≥3 loose or watery stools in a 24-h period. Two different episodes of diarrhea were separated by ≥3 d (72 h) without diarrhea. Prolonged diarrhea and persistent diarrhea were defined as episodes that lasted for ≥7 or ≥14 d, respectively.

ALRI was defined as cough or difficult breathing with an elevated respiratory rate above the age-specific cutoff values (≥50 breaths/min in infants and ≥40 breaths/min in older children) according to WHO criteria (19) or cough or difficult breathing and lower chest indrawing.

Clinical pneumonia was defined either by a combination of cough with crepitations or bronchial breathing by auscultations or as an episode of ALRI associated with at least one of the following features: lower chest indrawing, convulsions, inability to drink or feed, extreme lethargy, restlessness or irritability, nasal flaring, or abnormal sleeping and difficulty in waking.

Statistical analysis

The forms for the study were designed in Visual Basic.net with range and consistency checks incorporated. Double data entry by 2 data clerks followed by validation was completed within 72 h. In the table of baseline data, continuous variables were reported as means or medians as appropriate and categorical variables as proportions. We log transformed the plasma folate, vitamin B-12, and tHcy concentrations. The mean differences and the corresponding 95% CIs were compared by using *t* tests, and differences between the groups are expressed as geometric mean ratios. The 6-mo intervention was divided into 26 periods of 7 d for each child. For a period to be included in the analyses, we required information on ≥4 d of the given 7-d period. To account for the interdependence of multiple observation periods in the same child, regression models were fitted with generalized estimating equations by using an exchangeable covariance-variance matrix. In the generalized estimating equations model, occurrence of a new episode of diarrhea, ALRI, or pneumonia in a child period was modeled as a binomial dependent variable and group allocation as the independent variable. The model used a logit link, binomial variance, and exchangeable correlation to yield ORs. In the main analyses we compared each of the intervention groups (folic acid, vitamin B-12, and folic acid + vitamin B-12) with the group that received only placebo. In this analysis, each treatment arm contained ∼250 children. We also explored the effects of the interventions in various predefined subgroups. In these subgroup analyses, we compared those who received folic acid (with or without vitamin B-12; n = 499) with those who received placebo without folic acid (with or without vitamin B-12; n = 501) and those who received vitamin B-12 (n = 502) with those who received placebo without vitamin B-12 (n = 498). The dichotomous variables that indicated whether vitamin B-12 or folic acid was given were included in the multiple models simultaneously. In these subgroup analyses, we also included age, breastfeeding status, and sex in the models. We also included interaction terms in the models to measure whether the effects between the subgroups were significantly different. In these models we also measured the interaction between folic acid and vitamin B-12 supplementation. Statistical analyses were performed with Stata, version 12 (StataCorp). All analyses were done by intention-to-treat.

RESULTS

Of 1377 eligible children aged 6−30 mo, we randomly assigned 1000 children into the study. The flow of the participants throughout the study is shown in **Figure 1**. Baseline characteristics of the enrolled children are shown in **Table 1**. The baseline features were similar in the 4 groups. Approximately 72% were still breastfeeding, 36.5% of the children were stunted, 34% were underweight, and 12% were wasted. Approximately 70% of the children were anemic (Table 1). One-third of the children had a vitamin B-12 concentration ≤200 pmol/L and almost one-third had a baseline folate concentration <7.5 nmol/L. Elevated tHcy was seen in >50% of the children (**Table 2**).

Of the randomly assigned children, 99% were available for the last scheduled visit, ie, only 7 children withdrew. Data on morbidity were available from 25,495 of 26,219 (97%) weekly follow-up periods. Compliance was good, and 96% of the scheduled doses were reportedly ingested. Compliance was reflected in the change in plasma concentrations of folate, vitamin B-12, and tHcy from baseline to the end of the study (Table 2). At the end of the study, the children who received vitamin B-12 supplements (±folic acid) had a geometric mean vitamin B-12 concentration that was 1.28 times (95% CI: 1.14, 1.44) that of children in the placebo±folic acid groups. Similarly, the geometric mean folate concentration was 3.14 (95% CI: 2.56, 3.86) higher among the folic acid recipients. Either vitamin B-12 or folic acid supplementation alone resulted in a decreased tHcy concentration, with geometric mean ratios of 0.78 (95% CI: 0.72, 0.83) and 0.83 (95% CI: 0.77, 0.89), respectively.

The effects of folic acid or vitamin B-12 on diarrhea and ALRIs are shown in **Tables 3** and **4**. Neither folic acid nor vitamin B-12 administration reduced the incidence of diarrhea or ALRI. Children in the 2 folic acid groups had more episodes of diarrhea lasting >3 d and of persistent diarrhea than did the placebo group. The same negative effect of folic acid supplementation was seen when all 499 children who were given folic acid were compared with all those who were not given folic acid (data not shown).

No substantial or significant differences in incidence of ALRI or clinical pneumonia were found between the intervention groups. The effects of folic acid and vitamin B-12 supplementation on diarrhea and on ALRI in various subgroups are shown in **Figure 2** and in **Figure 3**, respectively. We were not able to identify any subgroups that benefitted significantly from folic acid or vitamin B-12 administration.

DISCUSSION

We here report the effect of folic acid and vitamin B-12 supplementation on the risk of common infections in young children. In this population with poor folate and vitamin B12 status, we have previously found an association between poor plasma folate status and risk of ALRI and persistent diarrhea (3, 4). However, in this study, treatment with 2 times the RDA of folic acid or vitamin B-12, alone or in combination, had no measurable beneficial effect on respiratory or diarrheal morbidity. In contrast, we observed a modest but significant increase in risk of diarrhea in those receiving folic acid with or without vitamin B-12. We found no interaction between administration of folic acid and vitamin B-12 on any of our outcomes.

The apparent lack of beneficial effects on the incidence of infections can have several, not mutually exclusive explanations. For example, the doses of vitamin B-12 and folic acid may not have been sufficiently high. However, both vitamin B-12 and folic acid administration resulted in a substantial reduction in plasma tHcy concentration—a marker of folate or vitamin B-12

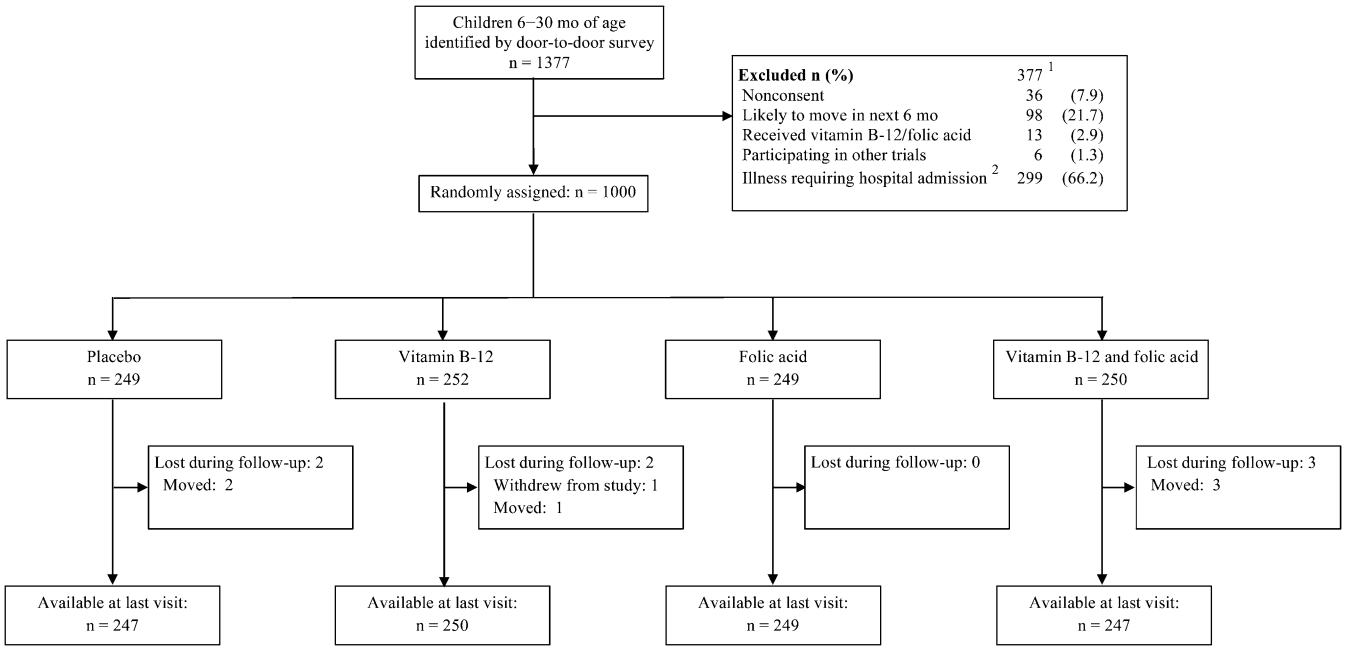

FIGURE 1. Trial profile of a randomized, placebo-controlled trial on the effect of folic acid and/or vitamin B-12 administration in 6–30-mo-old North Indian children. [1]For 60 children, there were multiple reasons for exclusion. Fever (n = 70), ALRI (n = 92), hemoglobin (n = 73), diarrhea (12), vomiting (6), lethargy (1), dysentery (3), dehydration (3), hepatitis (1), extensive skin infection (n = 1), hydrocephalus (n = 2), hepatosplenomegaly (n = 1), coronary heart disease (n = 1), nephrotic syndrome (n = 1), wasting (n = 7), mastoid (n = 1), length z score less than −3 SD (n = 22), and cause unknown (n = 2).

function (21). One can only speculate whether increasing the dose of either of these vitamins would lead to a further decrease in the tHcy concentration and other potential beneficial outcomes. The mean plasma concentrations of vitamin B-12 or folate were significantly higher in those who received either of these nutrients than in those who did not. However, the mean

TABLE 1
Baseline characteristics of 1000 children 6–30 mo of age[1]

Characteristics	Placebo (n = 249)	Vitamin B-12 (n = 252)	Folate (n = 249)	Vitamin B-12 + folate (n = 250)
Age at enrollment (mo)	16.4 ± 7.1[2]	15.9 ± 6.9	16.2 ± 7.3	15.9 ± 7.0
Proportion of children [n (%)]				
<12 mo	80 (32.1)	80 (31.8)	81 (32.5)	80 (32.0)
12–23 mo	118 (47.4)	128 (50.8)	115 (46.2)	123 (49.2)
24–30 mo	51 (20.5)	44 (17.5)	53 (21.3)	47 (18.8)
Boys [n (%)]	135 (54.2)	115 (45.6)	125 (50.2)	132 (52.8)
Ever breastfed [n (%)]	248 (99.6)	248 (98.4)	243 (97.6)	247 (98.8)
Currently breastfed [n (%)][3]	181 (72.7)	188 (74.6)	172 (69.1)	182 (72.8)
Prevalence in previous 24 h [n (%)]				
Diarrhea	13 (5.2)	12 (4.8)	14 (5.6)	13 (5.2)
Cough or difficult breathing or fast breathing	76 (30.5)	77 (30.6)	84 (33.7)	83 (33.2)
Literate mother [n (%)]	185 (74.3)	183 (72.6)	196 (78.7)	192 (76.8)
Annual family income (INR)	84,000 (53,000–144,000)[4]	72,000 (48,000–120,000)	72,000 (60,000–156,000)	76,000 (60,000–140,000)
Weight (kg)	8.6 ± 1.5	8.4 ± 1.5	8.7 ± 1.6	8.5 ± 1.6
Length (cm)	74.4 ± 7.1	73.8 ± 6.8	74.4 ± 7.2	74.3 ± 7.1
WHZ	−0.90 ± 0.89	−0.93 ± 0.94	−0.73 ± 1.0	−0.90 ± 0.89
HAZ	−1.64 ± 1.14	−1.68 ± 1.25	−1.55 ± 1.25	−1.54 ± 1.17
WAZ	−1.53 ± 0.98	−1.58 ± 1.09	−1.36 ± 1.13	−1.48 ± 1.04
Wasted, < −2 WHZ [n (%)]	28 (11.2)	32 (12.7)	23 (9.2)	22 (8.8)
Stunted, < −2 HAZ [n (%)]	97 (39.0)	93 (36.9)	91 (36.6)	84 (33.6)
Underweight, < −2 WAZ [n (%)]	80 (32.1)	81 (32.1)	75 (30.1)	73 (29.2)
Hemoglobin (mL)	10.0 ± 1.4	10.1 ± 1.5	10.2 ± 1.5	10.1 ± 1.4
<11 g/dL [n (%)]	181 (72.7)	170 (67.5)	161 (64.7)	184 (73.6)

[1] HAZ, height-for-age z score; INR; Indian rupee; WAZ, weight-for-age z score; WHZ, weight-for-height z score.
[2] Mean ± SD (all such values).
[3] Missing information for 5 children.
[4] Median; IQR in parentheses (all such values).

TABLE 2
Plasma vitamin B-12, folate, tHcy, and hemoglobin concentrations in the study population[1]

	Placebo (n = 249)	Vitamin B-12 (n = 252)	Folate (n = 249)	Vitamin B-12 + folate (n = 250)
Baseline				
Plasma vitamin B-12 (pmol/L)	266 (165−381)	253 (172−404)	265 (176−425)	277 (189−416)
<150 [n (%)]	48 (19.3)	45 (17.9)	38 (15.3)	37 (14.9)
≥150 to ≤200 [n (%)]	34 (13.7)	41 (16.3)	51 (20.5)	33 (13.2)
>200 [n (%)]	167 (67.1)	166 (65.9)	159 (64.1)	179 (71.9)
Plasma folate (nmol/L)	11.4 (6.8−19.5)	10.6 (6.6−20.8)	11.5 (6.7−20.8)	11.1 (6.3−20.5)
<5 [n (%)]	28 (11.2)	26 (10.3)	30 (12.2)	38 (15.3)
≥5 to ≤7.5 [n (%)]	43 (17.3)	49 (19.4)	45 (18.1)	51 (20.5)
>7.5 [n (%)]	178 (71.5)	177 (70.2)	172 (69.6)	160 (64.3)
Plasma tHcy (μmol/L)	11.9 (9.1−16.9)	11.2 (8.9−16.1)	11.5 (8.7−15.3)	11.6 (8.9−15.4)
End of study[2]				
Plasma vitamin B-12 (pmol/L)	318 (191−404)	381 (282−567)	317 (248−492)	455 (307−605)
<150 [n (%)]	7 (10.9)	3 (4.6)	2 (3.0)	1 (1.5)
≥150 to ≤200 [n (%)]	10 (15.6)	3 (4.6)	8 (11.9)	2 (3.0)
>200 [n (%)]	47 (73.4)	59 (90.8)	57 (85.1)	63 (95.5)
Plasma folate (nmol/L)	15.3 (9.8−21.5)	13.3 (8.5−18)	46.5 (27.9−64.3)	47.7 (30.7−67)
<5 [n (%)]	1 (1.6)	4 (6.2)	1 (1.5)	—
≥5 to ≤7.5 [n (%)]	8 (12.5)	10 (15.4)	1 (1.5)	1 (1.5)
>7.5 [n (%)]	55 (85.9)	51 (78.5)	65 (97.0)	65 (98.5)
Plasma tHcy (μmol/L)	10.7 (8.5−13.9)	8.5 (6.8−10.5)	7.3 (6.1−9.6)	6.8 (5.7−9.4)
Change from baseline to end of study				
Vitamin B-12 (pmol/L)	45.5 (1.5−90)	180 (43−252)	79 (12−209)	177 (78−205)
Folate (nmol/L)	2.9 (−2.1 to 5.9)	1.7 (−3.0 to 7.2)	35 (9.9−49.4)	31.7 (15.6−58.9)
tHcy (μmol/L)	−1.3 3 (−4.5 to 0.24)	−3.1 (−5.3 to −0.5)	−3.5 (−5.4 to −2.0)	−5.3 (−8.3 to −3.0)

[1] Values are medians (IQRs) unless otherwise specified. tHcy, total homocysteine.
[2] n = 64, 65, 67, and 66 for the placebo, vitamin B-12, folate, and vitamin B-12 + folate groups, respectively.

values were still relatively low. This indicates that the doses, particularly of vitamin B-12, could have been increased beyond 2 times the RDA. Note, however, that the values of these nutrients during early childhood vary substantially by age and breastfeeding status. Interpretation of the concentrations, changes in concentrations, and proportions with low concentrations should be done with caution.

Another possible reason for the lack of effect on infections is that the children were not sufficiently deficient to benefit from supplementation. It is possible that children have to have an even lower status of these vitamins in order for the supplements to work. If so, we would expect to find a beneficial effect in the subgroups of children with poor folate or vitamin B-12 status. However, when examined, poor status did not predict a beneficial effect on infections. Note, however, that this study was not powered to measure subgroup specific effects.

There may also have been deficiencies of other limiting nutrients. Zinc deficiency increases the risk of diarrhea and pneumonia. We previously showed that deficiency is common in this population and that zinc administration reduces the burden of

TABLE 3
Effect of daily administration of folic acid and/or vitamin B-12 for 6 mo on incident diarrhea episodes in children 6–36 mo of age[1]

	Placebo	Vitamin B-12	Folic acid	Vitamin B-12 + folic acid	Placebo vs vitamin B-12 OR (95% CI)	P	Placebo vs folic acid OR (95% CI)	P	Placebo vs vitamin B-12 + folic acid OR (95% CI)	P
No. of children	249	252	249	250						
Total child-years of follow-up (y)	122.1	123.6	122.1	122.6						
7-d child periods (n)	6355	6446	6354	6340						
Episodes of diarrhea (n)	540	569	595	567	1.0 (0.9, 1.2)	0.67	1.1 (1.0, 1.2)	0.14	1.1 (0.9, 1.3)	0.41
Episodes of diarrhea lasting (n)										
≥3 d	214	221	261	251	1.1 (0.8, 1.3)	0.70	1.3 (1.0, 1.6)	0.04	1.24 (1.0, 1.6)	0.07
≥5 d	121	120	158	149	0.9 (0.7, 1.3)	0.67	1.3 (1.0, 1.7)	0.07	1.28 (1.0, 1.7)	0.09
≥7 d	82	75	101	86	0.9 (0.6, 1.3)	0.45	1.2 (0.9, 1.7)	0.29	1.13 (0.8, 1.6)	0.52
≥14 d	17	16	38	27	0.7 (0.3, 1.7)	0.49	2.0 (1.1, 3.8)	0.03	1.61 (0.8, 3.1)	0.16
Episodes of diarrhea with ≥6 stools/d (n)	162	150	160	159	0.8 (0.6, 1.1)	0.23	1.0 (0.7, 1.3)	0.87	1.0 (0.8, 1.4)	0.85

[1] ORs were calculated by using generalized estimating equations with a logit link function.

TABLE 4
Effect of daily administration of folic acid and/or vitamin B-12 for 6 mo on ALRI in children 6−30 mo of age[1]

	Placebo	Vitamin B-12	Folic acid	Vitamin B-12 + folic acid	Placebo vs vitamin B-12		Placebo vs folic acid		Placebo vs vitamin B-12 + folic acid	
					OR (95% CI)	*P*	OR (95% CI)	*P*	OR (95% CI)	*P*
Child-based analysis										
No. of children	249	252	249	250						
≥1 episode of ALRI [*n* (%)]	96 (38.6)	101 (40.1)	111 (44.6)	103 (41.2)	1.0 (0.8, 1.3)	0.76	1.2 (0.9, 1.4)	0.18	1.1 (0.9, 1.3)	0.50
≥1 episode of clinical pneumonia [*n* (%)]	74 (29.7)	89 (35.3)	76 (30.5)	84 (33.6)	1.2 (0.9, 1.5)	0.19	1.0 (0.8, 1.3)	0.84	1.14 (0.9, 1.5)	0.32
Admitted to hospital for any cause [*n* (%)]	1	1	1	1						
Person time-based analysis (*n*)										
7-d child periods	6355	6446	6354	6340						
Episodes of ALRI	171	174	193	170	1.0 (0.8, 1.3)	0.97	1.1 (0.9, 1.5)	0.35	1.0 (0.8, 1.3)	0.97
Episodes of clinical pneumonia	107	130	113	115	1.2 (0.9, 1.6)	0.24	1.1 (0.8, 1.4)	0.74	1.1 (0.8, 1.5)	0.63

[1] ORs were calculated by using generalized estimating equations with a logit link function. ALRI, acute lower respiratory infection.

diarrhea and lower respiratory infections (15, 16). We have also shown that there is an interaction between zinc and vitamin B-12 status, ie, poor vitamin B-12 status predicts the efficacy of zinc (4). Deficiencies of other limiting nutrients, such as zinc, could explain the lack of effect of these nutrients.

It is also possible that folate and vitamin B-12 deficiencies are not important risk factors for these illnesses and that the previously described associations between folate status and infections (3, 4) are a result of confounding. Folates and vitamin B-12 are essential for nucleotide synthesis, cell growth, and thereby for the normal function of the immune system (21). Poor folate and vitamin B-12 status therefore increases the risk of many infections; however, we do not know at what level of deficiency or for how long an individual needs to be deficient before it becomes clinically relevant. Most of the infections that we detected were mild, and the risk of these infections may be independent of immune status. Furthermore, folic acid and vitamin B-12 administration may be beneficial to some and harmful to others, which will shift the overall effect estimates toward the null.

We observed a modest but significant increased risk of diarrhea lasting ≥3 d, including persistent diarrhea, in children who received folic acid compared with those who did not. This finding contrasts with our previous observational study (4), in which children with poor folate status had the highest risk of persistent diarrhea. If this is correct, we can only speculate about potential mechanisms. For instance, it has been suggested that high concentrations of circulating folates and unmetabolized folic acid can impair natural killer cell function and cell-mediated toxicity (22), which in turn may increase the risk of persistent diarrhea (23). Another possibility is that the administration of folic acid impairs the absorption of other nutrients such as zinc

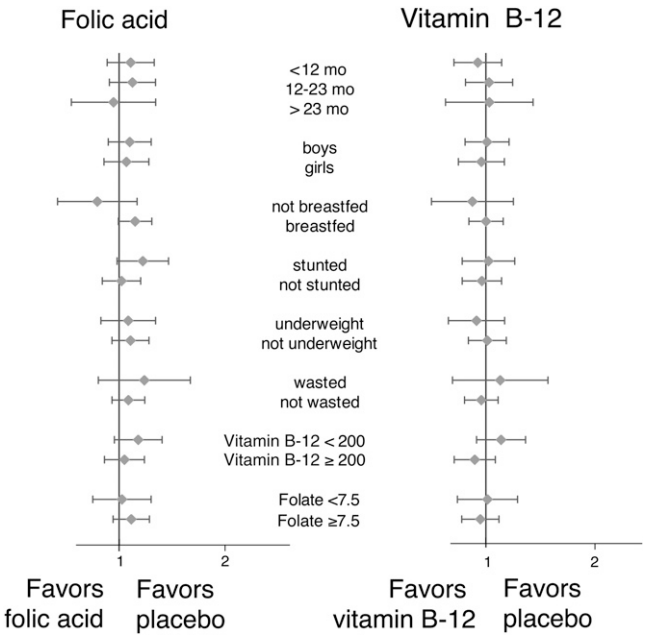

FIGURE 2. The effects (ORs) of folic acid or vitamin B-12 supplementation on episodes of diarrhea in North Indian children 6–30 mo of age in various subgroups based on baseline characteristics.

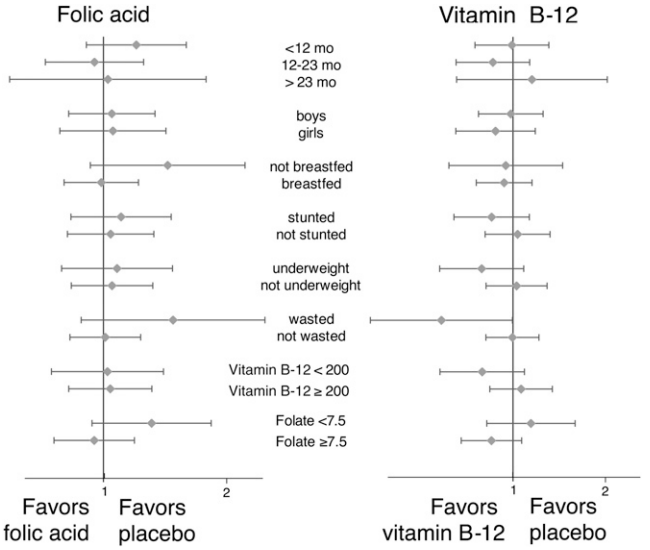

FIGURE 3. The effects (ORs) of folic acid or vitamin B-12 supplementation on episodes of acute lower respiratory infection in North Indian children 6–30 mo of age in various subgroups based on baseline characteristics.

(24), although clinical trial data are missing. Finally, the observation of an increased risk of diarrhea in the folic acid intervention groups may very well be a chance finding, but could also add support to the hypothesis that folic acid combinations in young children may increase the risk of infection (25).

The study was not powered to measure subgroup effects, and we did not observe significant results in the investigated subgroups. However, such analyses suggest that, if anything, folic acid intervention increases the risk of ALRI in children who are not breastfeeding or have low plasma folate, which is contrary to our original hypothesis based on our previous cohort study (3). In relation to the effect of the vitamin B-12 intervention on common infections, the observed effects were in the right direction in some subgroups, but were not significant.

The strength of our study was that it was a well-conducted randomized, placebo-controlled trial with very few losses to follow-up. Furthermore, compliance was excellent, as reflected by expected changes in plasma concentrations of vitamin B-12, folate, and tHcy at the end of the study. The disease burden was in line with the sample size calculations. However, somewhat fewer children had vitamin B-12 deficiency compared with our data from 1998 to 2000 (20). If we had targeted only those with poor vitamin status, our results may have been different.

In summary, we showed in a population of North Indian preschoolers that administration of 2 times the RDA of folic acid or vitamin B-12 did not significantly reduce the risk of respiratory or diarrheal illnesses. Vitamin B-12 intervention may be more effective against common childhood infections in certain subgroups, such as malnourished children with low vitamin B-12 status; however, this theory needs to be tested in large-scale studies or in more targeted interventions in populations of interest. The use of folic acid should be further assessed in light of the potential increased risk of diarrheal illnesses. However, we cannot rule out that poor folate and vitamin B-12 status have consequences on other outcomes such as cognitive development, growth, or metabolic disorders later in life.

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