Vitamin B-12 Supplementation during Pregnancy and Early Lactation Increases Maternal, Breast Milk, and Infant Measures of Vitamin B-12 Status\textsuperscript{1,2}

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Abstract

Pregnant women in resource-poor areas are at risk of multiple micronutrient deficiencies, and indicators of low vitamin B-12 status have been associated with adverse pregnancy outcomes, including anemia, low birth weight, and intrauterine growth retardation. To evaluate whether daily oral vitamin B-12 supplementation during pregnancy increases maternal and infant measures of vitamin B-12 status, we performed a randomized, placebo-controlled clinical trial. Pregnant women <14 wk of gestation in Bangalore, India, were randomly assigned to receive daily oral supplementation with vitamin B-12 (50 \textmu g) or placebo through 6 wk postpartum. All women were administered iron and folic acid supplements throughout pregnancy. One hundred eighty-three women were randomly assigned to receive vitamin B-12 and 183 to receive placebo. Compared with placebo recipients, vitamin B-12-supplemented women had significantly higher plasma vitamin B-12 concentrations at both the second (median vitamin B-12 concentration: 216 vs. 111 pmol/L, \(P < 0.001\)) and third (median: 184 vs. 105 pmol/L, \(P < 0.001\)) trimesters. At 6 wk postpartum, median breast milk vitamin B-12 concentration was 136 pmol/L in vitamin B-12-supplemented women vs. 87 pmol/L in the placebo group (\(P < 0.0005\)). Among vitamin B-12–supplemented women, the incidence of delivering an infant with intrauterine growth retardation was 33 of 131 (25\%) vs. 43 of 125 (34\%) in those administered placebo (\(P = 0.11\)). In a subset of infants tested at 6 wk of age, median plasma vitamin B-12 concentration was 199 pmol/L in those born to supplemented women vs. 139 pmol/L in the placebo group (\(P = 0.01\)). Infant plasma methylmalonic acid and homocysteine concentrations were significantly lower in the vitamin B-12 group as well. Oral supplementation of urban Indian women with vitamin B-12 throughout pregnancy and early lactation significantly increases vitamin B-12 status of mothers and infants. It is important to determine whether there are correlations between these findings and neurologic and metabolic functions. This trial was registered at clinicaltrials.gov as NCT00641862. J. Nutr. 144: 758–764, 2014.

Introduction

Pregnant women in resource-poor areas are at risk of multiple micronutrient deficiencies, and diets that are low in animal products place women at increased risk of vitamin B-12 deficiency (1–6). Vitamin B-12 acts as a cofactor in the conversion of homocysteine to methionine, as well as the formation of succinyl-CoA from \(\text{t-methylmalonyl-CoA}\). Deficiency of vitamin B-12 causes macrocytic anemia, neurologic dysfunction, and biochemical abnormalities related to the accumulation of the precursor moieties (hyperhomocysteinemia and methylmalonic acidemia). In turn, hyperhomocysteinemia has been linked to a higher risk of multiple adverse outcomes of pregnancy, including delivering an infant small for gestational age or with intrauterine growth retardation (IUGR),\textsuperscript{12} low birth weight (LBW),...
pregnancy-induced hypertension, neural tube defects, and preterm delivery (7–9).

A number of surveys confirmed a high rate of LBW, IUGR, and other poor maternal and infant outcomes in India, with an incidence of small for gestational age of 44.5% in South Asia, accounting for >17 million deliveries (10). However, the relation between maternal vitamin B-12 status and birth outcomes has been the subject of limited studies. In pregnant women in Pune, India, total homocysteine (tHcy) concentrations in pregnancy were significantly and inversely associated with birth weight, adjusting for maternal height, gestational age, and infant sex (11).

In a cohort of pregnant women in Bangalore, India, with a high incidence of vitamin B-12 deficiency, we reported previously that women in the lowest tertile for plasma vitamin B-12 concentration during all 3 trimesters of pregnancy had significantly higher risk of IUGR that persisted after adjusting for maternal age, weight, education, and parity (12). In addition, vitamin B-12 status in the mother was also related to neonatal vitamin B-12 status as measured by cord plasma vitamin B-12 concentrations; low neonatal vitamin B-12 concentrations were associated with LBW (13). To evaluate the effect of maternal supplementation of vitamin B-12 during pregnancy and lactation on maternal and infant biomarkers of vitamin B-12 status, we performed a randomized, blinded controlled clinical trial in urban pregnant south Indian women.

Materials and Methods

The study was a randomized, double-blind, placebo-controlled trial (registered at clinicaltrials.gov as NCT00641862). Recruitment and follow-up were conducted at Hosahalli Referral Hospital, a government maternity health care center predominantly catering to the needs of the women from the lower socioeconomic strata of urban Bangalore.

Pregnant women aged ≥18 y who presented for prenatal care before or at 14 wk of gestational age (as judged by the date of the last menstrual period) were eligible for inclusion. Excluded were those mothers who anticipated moving out of the area before study completion, who had twin or multiple pregnancies, who were treated for infertility, who tested positive for hepatitis B (hepatitis B surface antigen), HIV, or syphilis (Venereal Disease Research Laboratory test) infections, or who were taking daily vitamin supplements in addition to folic acid and iron. Women with a serious preexisting medical condition (defined as the need for chronic or daily medication use) were excluded, as were women with a history of previous caesarean section.

Sociodemographic information was obtained by interviews completed by trained research assistants. Gestational age (weeks) was calculated from the reported first day of the last menstrual period. Weights of all the mothers were recorded using a digital balance (Salter 9016; Tonbridge) to the nearest 100 g, and heights were measured using a stadiometer to the nearest 0.1 cm. BMI was calculated as weight in kilograms divided by the height in meters squared. LBW was defined as birth weight <2500 g, and IUGR was defined as birth weight less than the 10th percentile of norms for gestational age (14).

Treatment allocation. Women were randomly assigned to receive a daily oral dose of vitamin B-12 (50 μg) or a placebo identical in appearance (Cadila Pharmaceuticals) from enrollment through 6 wk postpartum. A randomization list from 1 to 370 was prepared by the study biostatistician using permuted blocks of variable size, with each number corresponding to a code denoting 1 of the 2 treatment groups. Onsite study pharmacists stored the coded randomization list in a locked file cabinet and concealed allocation by only displaying the woman’s identification number on the label of the bottle. Capsules of the regimen were distributed in bottles containing 40 each. The placebo and vitamin B-12 supplement were indistinguishable in terms of taste, smell, and appearance. Study physicians, research nurses, and participants were unaware of treatment groups.

 Provision of standard of care and follow-up. Each pregnant woman was followed up in the clinic once per month until week 32 of pregnancy, then once every 2 wk until week 36, and then once every week until delivery, as per the standard prenatal care visiting schedule in India. As part of standard medical care, all mothers were administered daily iron (60 mg) and folic acid (500 μg) supplementation, diagnosis and treatment for sexually transmitted infections, and prophylaxis. A clinical examination was performed that included vital signs and blood pressure, measurement of weight, height, triceps skin fold, and mid-upper arm circumference, obstetric examination (fetal movement, heart rate, presentation, lie, position), and reproductive and neurologic examinations.

Compliance with the daily regimen was measured by research nurses counting unused supplements. Compliance was then calculated by dividing the number of pills taken (as determined by pill counts at monthly follow-up visits) by the number of days on study (from random assignment to 6 wk postpartum). Mothers who were traveling out of Bangalore temporarily were provided with extra regimens to suffice until the next visit to the research clinic. Mothers were reimbursed for their travel expenses to the study clinic but received no other payment. Those who missed their monthly follow-up appointment were contacted by phone and/or visited at home and encouraged to return for their next visit.

Dietary data. A pretested interviewer-administered FFQ was used to assess habitual dietary intake for the 3 mo preceding the date of the participant’s enrollment in the study. Standard measures were placed before the respondent to quantify the portion size of each food item when administering the FFQ. The FFQ was adapted from 1 developed for an urban south Indian population (15) and has a food list of 127 items, derived from a food database developed from studies at St. John’s Medical College, Bangalore. Nutrient scores were computed by multiplying the relative frequency of consumption of each food item by nutrient content of the standard portion size. Nutrient information was obtained for 27 macronutrients and micronutrients. The total amount of food groups consumed (grams per day) was also calculated. Nutrient intakes were compared with published Indian recommendations (RDAs) (16).

Biochemical data. Approximately 10 mL of blood was drawn from participants by venipuncture and collected in both EDTA and plain vacutainers (BD Biosciences) that were kept on ice until separation in a refrigerated centrifuge, usually within 4 h. Hemoglobin and complete blood count were analyzed on whole-blood samples in an automated Coulter counter (ABX Pentra G+; Horiba Medicals). The plasma and RBCs were separated and stored at −80°C until analysis for vitamin B-12, tHcy, methylmalonic acid (MMA), and erythrocyte folate.

Plasma vitamin B-12 was measured by the electrochemiluminescence method (Elecsys 2010; Roche Diagnostics). A plasma concentration <150 pmol/L was considered deficient. Measurement of tHcy and MMA was performed by GC-MS (model 3800; Varian) (17). The intraday and interday assay CVs for vitamin B-12 were 0.54% and 2.44%, respectively. The interday assay CV for MMA and tHcy was 5.57% and 5.04%, respectively, whereas the intra- and interday assay CV was 6.92% and 5.60%, respectively. Erythrocyte folate was measured by a competitive immunoassay with direct chemiluminescence detection on an automated immunoanalyzer (ADVIA Centaur; Bayer Health Care Diagnostics) (18), with intra-assay and interassay variabilities of 1.9% and 5.2%, respectively. The folate concentration in the hemolysate was converted to values for whole blood by adjusting for the hematocrit.

For the 5,10-methylenetetrahydrofolate reductase (MTHFR) genotype analysis, DNA was extracted from theuffy coat by the phenolchloroform method, and the extracted DNA was purified by ethanol washing and quantified using a spectrophotometer (NanoDrop ND1000; Thermo Fisher Scientific). Primers for the MTHFR gene (rs1801133) were designed using Primer 3 plus software. The amplified products were sequenced using direct sequencing (Big Dye Terminator method; Applied Biosystems). The sequences were evaluated for variants with Finch
Breast milk was collected without restriction regarding time since last feed. After cleaning the nipple and before the mothers nursed the infants, 10 mL of breast milk was expressed manually from 1 breast into a prelabeled plastic opaque vial. The vials were inverted and shaken well by hand, and 2–3 mL was portioned into a screw-top polystyrene tube. Five such aliquots were made and stored at −80°C until they were shipped for analysis. Breast milk vitamin B-12 concentrations were determined by competitive protein binding immunoassay (Immulette 1000; Siemens) (19).

A single stool sample was collected at the baseline visit from the pregnant women and analyzed immediately for the presence of helminthic ova, cysts, and trophozoites by the wet mount method (20).

Data management and analysis. All study forms were checked for inconsistencies, and the data were double-entered into a structured query language database by 2 dedicated data entry operators. Discrepancies or mismatches in the data entry were corrected by the study coordinator. In nearly 10% of the participants, random data checks were conducted between firms that the 2 study groups were comparable on measures of age, socioeconomic characteristics, and nutritional status. Dietary intake was generally low, and intake of vitamin B-12 was lower than the RDA for Indian women in 44% of participants. Nearly one-third (30%) of women were anemic (hemoglobin < 11 g/dL) at baseline, 51% had low plasma concentrations of vitamin B-12 (<150 pmol/L), and 42% had impaired vitamin B-12 status (vitamin B-12 <150 pmol/L and MMA >0.26 μmol/L). Three-fourths of the women had elevated MMA, and one-fourth had elevated tHcy. *Giardia lamblia* was detected in 10 (3%) women.

**TABLE 1** Baseline characteristics of pregnant Indian women enrolled in the vitamin B-12 supplementation trial

<table>
<thead>
<tr>
<th>Measure</th>
<th>Vitamin B-12 group</th>
<th>Placebo group</th>
</tr>
</thead>
<tbody>
<tr>
<td>Age, y</td>
<td>22 (20, 24)</td>
<td>22 (20, 24)</td>
</tr>
<tr>
<td>Gestational age, wk</td>
<td>11.0 (8.2, 13.0)</td>
<td>11.3 (9.1, 13.3)</td>
</tr>
<tr>
<td>Monthly household income, INR</td>
<td>6000 (4500, 9600)</td>
<td>6000 (4000, 9000)</td>
</tr>
<tr>
<td>Anthropometry</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Weight, kg</td>
<td>47.6 (42.8, 54.9)</td>
<td>46.3 (40.5, 52.4)</td>
</tr>
<tr>
<td>Height, cm</td>
<td>153 (149, 157)</td>
<td>153 (150, 157)</td>
</tr>
<tr>
<td>Dietary intake</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Energy, kcal/d</td>
<td>1700 (1510, 1960)</td>
<td>1880 (1410, 2030)</td>
</tr>
<tr>
<td>Protein, g/d</td>
<td>51.4 (43.6, 57.1)</td>
<td>50.3 (41.1, 61.2)</td>
</tr>
<tr>
<td>Fat, g/d</td>
<td>46.8 (39.0, 54.2)</td>
<td>45.5 (36.8, 56.8)</td>
</tr>
<tr>
<td>Vitamin B-6, μg/d</td>
<td>1.66 (1.37, 1.96)</td>
<td>1.62 (1.29, 2.00)</td>
</tr>
<tr>
<td>Folate, mg/d</td>
<td>273 (230, 312)</td>
<td>273 (219, 319)</td>
</tr>
<tr>
<td>Vitamin B-12, μg/d</td>
<td>1.22 (0.90, 1.85)</td>
<td>1.40 (0.84, 2.11)</td>
</tr>
<tr>
<td>Iron, mg/d</td>
<td>14.5 (12.3, 17.2)</td>
<td>14.3 (12.1, 17.4)</td>
</tr>
<tr>
<td>Biochemical data</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Hemoglobin, g/dl</td>
<td>11.6 (10.6, 12.3)</td>
<td>11.7 (10.8, 12.6)</td>
</tr>
<tr>
<td>Hematocrit, %</td>
<td>34.9 (32.0, 38.9)</td>
<td>35.4 (32.5, 37.6)</td>
</tr>
<tr>
<td>Mean corpuscular volume, fl</td>
<td>83 (75, 87)</td>
<td>84 (78, 87)</td>
</tr>
<tr>
<td>Plasma vitamin B-12, pmol/L</td>
<td>160 (110, 226)</td>
<td>141 (109, 190)</td>
</tr>
<tr>
<td>&lt;150 pmol/L, n (%)</td>
<td>80 (46)</td>
<td>100 (56)</td>
</tr>
<tr>
<td>Plasma MMA, μmol/L</td>
<td>0.44 (0.28, 0.65)</td>
<td>0.49 (0.28, 0.70)</td>
</tr>
<tr>
<td>&gt;0.26 μmol/L, n (%)</td>
<td>135 (76)</td>
<td>138 (76)</td>
</tr>
<tr>
<td>Plasma tHcy, μmol/L</td>
<td>8.89 (5.24, 14.43)</td>
<td>9.41 (6.05, 15.76)</td>
</tr>
<tr>
<td>&gt;15.0 μmol/L, n (%)</td>
<td>43 (24)</td>
<td>48 (28)</td>
</tr>
</tbody>
</table>

1 Data are medians (quartile 1, quartile 3) or n (%); n = 183 in both the vitamin B-12 and placebo groups, except for biochemical data (n = 174–183). INR, Indian rupees (100 Indian rupees = −$2 at the time of the study); MMA, methylmalonic acid; tHcy, total homocysteine.
The effect of vitamin B-12 supplementation on maternal plasma concentrations during pregnancy is shown in Figure 2. Compared with the women who were administered placebo, vitamin B-12–supplemented women had significantly higher plasma vitamin B-12 concentrations in both the second [median vitamin B-12 concentration: 216 pmol/L (n = 119) vs. 112 pmol/L (n = 119), P < 0.001] and third [median: 184 pmol/L (n = 102) vs. 105 pmol/L (n = 102), P < 0.001] trimesters. The median change in maternal vitamin B-12 status from the first to third trimester in the supplemented arm was 3.0 (−54.8, 83.5) pmol/L, and this was significantly different (P < 0.001) from the decline in the placebo arm, −37.6 (−66.5, −10.9) pmol/L. The median change in maternal vitamin B-12 status from the first to second trimester in the supplemented arm was 36.5 (22.6, 50.2) pmol/L, and this was significantly different (P < 0.001) from the decline in the placebo arm, −46.3 (−66.7, −25.9) pmol/L. No significant group differences in maternal MMA, tHcy, or prevalence of anemia (hemoglobin < 11 g/dL) were noted at the second or third trimester time points. Among the women who were administered vitamin B-12, the mean ± SD compliance rate was 69 ± 17%, and among those who were administered placebo, it was 70 ± 13% (P = 0.74).

Vitamin B-12 concentrations in breast milk among 68 vitamin B-12–supplemented mothers was significantly higher than that of 73 women who were administered placebo when supplementation ended at 6 wk postpartum (Table 2). However, breast milk vitamin B-12 concentrations at 3 and 6 mo postpartum were not significantly different between the treatment groups.

Figure 3 shows plasma vitamin B-12, MMA, and tHcy concentrations obtained from a subset of infants at 6 wk of age. Compared with infants whose mothers were administered placebo (n = 34), infants of the vitamin B-12–supplemented women (n = 43) had significantly higher plasma vitamin B-12 concentrations (median vitamin B-12 concentration: 199 vs. 139 pmol/L, P < 0.001), as well as lower MMA (0.09 vs. 0.16 μmol/L, P = 0.022) and tHcy (10.9 vs. 21.0 μmol/L, P < 0.001) concentrations.

Clinical outcomes of the pregnancies were recorded, although the trial was not powered to detect differences in these. In the vitamin B-12 arm, 24 of 131 (18%) mothers underwent elective caesarean section vs. 13 of 125 (10%) in the placebo group (P = 0.06). The frequency of LBW was not statistically significantly different between the 2 arms (12% in the vitamin B-12 group vs. 16% in the placebo group, P = 0.38), nor was mean birth weight (2.85 ± 0.46 kg in the vitamin B-12 group vs. 2.83 ± 0.45 kg in the placebo group). Among the vitamin B-12–supplemented women, the incidence of delivering an infant with IUGR was 33 of 131 (25%) vs. 43 of 125 (34%) in placebo recipients (P = 0.11).

Stratified analyses were performed to assess whether vitamin B-12 supplementation was more effective in women with baseline vitamin B-12 deficiency, baseline body weight < 50 kg, or low dietary intake of vitamin B-12. There was no evidence of a differential effect of the intervention by baseline vitamin B-12 concentration or body weight on the second and third trimester plasma vitamin B-12 concentration, maternal weight gain, or birth weight (P > 0.30 for tests of interaction). Similarly, there was no evidence for differential effects of the intervention on the outcomes of preterm birth, LBW, or IUGR. Among women whose dietary intake of vitamin B-12 was less than the Indian RDA during pregnancy (1.2 μg/d) in the first trimester, the frequency of IUGR was 17 of 61 (28%) in the vitamin B-12 group and 17 of 50 (34%) in the placebo group (P = 0.14 by χ² test).

The MTHFR C677T single nucleotide polymorphism was screened in a total of 359 mothers. The frequencies of MTHFR 677C and 677T alleles were found to be 93% and 7%, respectively. The frequency of MTHFR homozygous genotype 677CC was 89% in the vitamin B-12 group and 85% in the placebo group.

**TABLE 2** Concentrations of vitamin B-12 in breast milk among women randomly assigned to be administered either vitamin B-12 or placebo during pregnancy

<table>
<thead>
<tr>
<th>Participants</th>
<th>Value</th>
<th>Participants</th>
<th>Value</th>
<th>P</th>
</tr>
</thead>
<tbody>
<tr>
<td>Vitamin B-12 group</td>
<td>Value</td>
<td>Placebo group</td>
<td>Value</td>
<td></td>
</tr>
<tr>
<td>n pmol/L</td>
<td></td>
<td>n pmol/L</td>
<td></td>
<td></td>
</tr>
<tr>
<td>6 wk postpartum</td>
<td>68 136 (93, 203)</td>
<td>73 87 (44, 127)</td>
<td>&lt;0.001</td>
<td></td>
</tr>
<tr>
<td>3 mo postpartum</td>
<td>47 97 (63, 146)</td>
<td>57 68 (37, 102)</td>
<td>0.65</td>
<td></td>
</tr>
<tr>
<td>6 mo postpartum</td>
<td>37 106 (65, 160)</td>
<td>44 80 (61, 113)</td>
<td>0.77</td>
<td></td>
</tr>
</tbody>
</table>

1 Data are medians (quartile 1, quartile 3) compared using Mann-Whitney U test.
and low breast milk vitamin B-12 correlated significantly with infant urinary MMA (21). A limited number of studies addressed vitamin B-12 status among Indian women and their infants. In 1 early study in Mumbai, India, mean serum vitamin B-12 concentration in lacto-vegetarian women was lower than that of nonvegetarian women (71.8 vs. 134.0 pmol/L, respectively, \( P < 0.01 \)) (22). In another study from Mumbai, mean plasma vitamin B-12 in pregnant nonanemic women was 50.2 vs. 131.3 pmol/L in nonpregnant women (\( P < 0.001 \)) (23). Of note, vitamin B-12 concentrations in all of the groups reported in these studies were generally low.

Although to our knowledge this is the first trial of supplemental vitamin B-12 among pregnant Indian women, 2 previous randomized trials of vitamin B-12 supplementation in India are relevant to our findings. In Pune, nonpregnant vegetarian women responded to 500 \( \mu \)g of vitamin B-12, with a mean increase in serum vitamin B-12 over 2 wk of 125–215 pmol/L (\( P < 0.05 \)) and reduced tHcy concentrations (18.0–13.0 \( \mu \)mol/L, \( P < 0.05 \)) (24). In a longer trial of lower doses of vitamin B-12 (0, 2, and 10 \( \mu \)g) with or without 200 \( \mu \)g of folic acid among families in Pune, 12 mo of 10 \( \mu \)g of vitamin B-12 increased mean serum vitamin B-12 concentrations from 159 to 307 pmol/L (\( P < 0.0001 \)) and reduced tHcy concentrations from 18.5 to 11.6 \( \mu \)mol/L (\( P < 0.0001 \)) (25). However, both of these trials excluded pregnant women, in whom progressive declines in vitamin B-12 plasma concentrations are known to occur, and neither reported clinical outcomes. In conjunction with an observational study among pregnant rural and urban Indian women (26) wherein a cumulative dose of vitamin B-12 >1000 \( \mu \)g during pregnancy was associated with lower tHcy concentrations at 34 wk, it seems likely that vitamin B-12 supplementation in pregnant Indian women is an effective way to improve vitamin B-12 status.

The finding that maternal supplementation during pregnancy and lactation significantly increased vitamin B-12 concentrations in breast milk is novel and potentially important. Early studies of well-nourished women did not confirm that vitamin B-12 supplementation increased breast milk concentrations of this nutrient (27). However, among U.S. women of low socioeconomic status, 8 \( \mu \)g of vitamin B-12 provided during lactation increased vitamin B-12 milk concentration to 0.79 \( \mu \)g/L at 6 wk postpartum compared with 0.55 \( \mu \)g/L in unsupplemented women (\( P < 0.05 \)) (28). These studies used an older radioisotope dilution method for measuring milk vitamin B-12 concentrations, the validity of which has been questioned (29), as opposed to our chemiluminescence method, so direct comparisons are difficult to make. Nonetheless, it does seem that maternal supplementation of vitamin B-12 among women of marginal vitamin B-12 status is an effective method to increase vitamin B-12 breast milk concentration. Of note, the effect of supplementation may not persist after the period of supplementation; breast milk vitamin B-12 concentrations in our study were significantly higher in the vitamin B-12 group compared with the placebo group only at 6 wk postpartum, the time when supplementation ended. At subsequent time points in the postpartum period, values of breast milk vitamin B-12 concentration were higher in the vitamin B-12 group, but this difference did not reach statistical significance.

In our trial, vitamin B-12–supplemented mothers had higher blood vitamin B-12 concentrations during pregnancy but not significantly lower MMA or tHcy blood concentrations. In contrast, all 3 measures of vitamin B-12 nutritional status were improved in infants. These findings are difficult to interpret clearly. One possibility is that supplementary vitamin B-12 was preferentially shunted to fetal tissues and therefore did not result in improved maternal vitamin B-12 status. Possibly supportive

**FIGURE 3** Effect of dietary supplementation with vitamin B-12 in pregnant Indian women compared with placebo on infant plasma concentrations of vitamin B-12 (A), MMA (B), and Hcy (C) at 6 wk after birth. Values are medians and IQRs, \( n = 43 \) in the vitamin B-12 group and \( n = 34 \) in the placebo group. *B-12 different from \( P, P < 0.001 \) by Mann-Whitney \( U \) test. B12, Vitamin B-12 group; Hcy, homocysteine; MMA, methylmalonic acid; P, placebo group.

placebo group (\( P = 0.18 \)), and the frequency of homozygous 677TT was 0.6% in both groups (\( P = 1.00 \)).

**Discussion**

In this randomized, blinded clinical trial of vitamin B-12 supplementation during pregnancy and early lactation, we found that urban south Indian women responded with higher plasma and breast milk concentrations of vitamin B-12. In addition, infants born to vitamin B-12–supplemented mothers had higher plasma vitamin B-12 concentrations and lower tHcy and MMA concentrations, indicative of improved vitamin B-12 status.

Several studies suggested that vitamin B-12 status in women in resource-limited countries is poor. Among 113 Guatemalan women, plasma vitamin B-12 was deficient or low in nearly 50%, breast milk vitamin B-12 concentration was low in 31%, and low breast milk vitamin B-12 correlated significantly with vitamin B-12 breast milk concentration. Of note, vitamin B-12 concentrations in all of the groups reported in these studies were generally low.

In our trial, vitamin B-12–supplemented mothers had higher blood vitamin B-12 concentrations during pregnancy but not significantly lower MMA or tHcy blood concentrations. In contrast, all 3 measures of vitamin B-12 nutritional status were improved in infants. These findings are difficult to interpret clearly. One possibility is that supplementary vitamin B-12 was preferentially shunted to fetal tissues and therefore did not result in improved maternal vitamin B-12 status. Possibly supportive...
of this hypothesis is the finding that infants whose mothers were administered placebo had generally higher tHcy concentrations (median: 21.0 μmol/L) than reported as normal amounts in healthy infants (30) (6–9 μmol/L at ages 6 wk to 6 mo). Among rural Indian newborns with high rates of LBW, median (IQR) concentrations of tHcy have been reported as 11.7 (8.3–14.7) μmol/L (31). Previous data demonstrating unbilical cord vitamin B and related metabolite amounts 2- to 6-fold higher than maternal serum concentrations are also suggestive of a high fetal demand for these nutrients (32). Another possibility is that declines in tHcy and MMA observed during pregnancy are phenomena not clearly related to deficiency status and that vitamin B-12 supplementation increased maternal blood vitamin B-12 concentration, as well as infant markers of vitamin B-12 status. Better biochemical assays (e.g., holo-transcobalamin II) and functional outcome measures in mothers and children would help address this possibility.

The relation between maternal and infant vitamin B-12 status has been the subject of limited studies (33). Although fetal and maternal plasma concentrations of vitamin B-12 are reported to correlate strongly (34), some suggest that maternal dietary intake during pregnancy is a stronger determinant of infant vitamin B-12 status than are maternal vitamin B-12 stores (35). Among breast-fed Norwegian infants administered i.m. vitamin B-12 injections at 6 wk of age, higher vitamin B-12 and lower tHcy and MMA concentrations were noted up to age 4 mo, suggesting that marginal vitamin B-12 status may be observed in breast-fed infants whose mothers are presumably eating a Western diet (36). Our data suggest that oral maternal vitamin B-12 supplementation is effective at improving infant vitamin B-12 status, as well (37). In addition to its effect on child vitamin B-12 status, vitamin B-12 status in pregnancy may also have an effect on long-term child health. In the Pune cohort, infants of mothers who were in the lowest decile of vitamin B-12 concentrations at 28 wk of gestation performed less well on tests of attention and memory compared with infants born to mothers of the highest decile (38). This group also reported that low maternal vitamin B-12 plasma concentrations in pregnancy were correlated with insulin resistance, as measured by the HOMA-IR in children aged 6 y (39). This observed relation between low vitamin B-12 concentrations in pregnancy and child insulin resistance was also supported by data from Nepal, but supplementation of pregnant women with vitamin B-12–containing micronutrients was not found to affect child HOMA-IR (40). Recent data linked higher child vitamin B-12 concentrations, as well as lower tHcy and MMA concentrations, with improved mental development scores at 12–18 mo (41). A recent study also showed that infant vitamin B-12 supplementation was associated with improved gross motor function and less frequent gastrointestinal regurgitation compared with placebo (42). Our findings of higher maternal vitamin B-12 concentration after supplementation and the finding of increased breast milk and infant plasma vitamin B-12 concentrations suggest that supplementation with vitamin B-12 during pregnancy can have an effect on infant vitamin B-12 status and health outcomes. Longer follow-up of our cohort for cardio-metabolic and neurodevelopmental status will allow us to determine whether these other important child outcomes are affected by maternal supplementation during pregnancy and early lactation.

Our study has several limitations. Our moderate sample size may have limited our ability to detect clinical differences in the study arms, and we were not powered to detect differences in birth outcomes, including the frequency of LBW and IUGR. In addition, some suggested that serum holo-transcobalamin may be a more valid marker of vitamin B-12 status during pregnancy than the markers we used (43). Finally, not all infants underwent blood drawing to assess vitamin B-12 status.

In summary, maternal supplementation during pregnancy and early lactation with 50 μg of daily oral vitamin B-12 significantly improved maternal plasma and breast milk measures of vitamin B-12 status, as well as multiple measures of infant vitamin B-12 status. Determining the clinical and long-term effects of these biochemical findings will be of great interest.

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