Vitamin B<sub>12</sub>, Folate, and Cognition in 6- to 9-Year-Olds: A Randomized Controlled Trial

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**Abstract**

**Background and Objectives:** Vitamin B<sub>12</sub> and folate are important for normal brain development. Our objective for this study was to measure the effects of 6-month supplementation of vitamin B<sub>12</sub> and/or folic acid in early childhood on cognition when the children were 6 to 9 years old.

**Methods:** The study is a follow-up of a factorial randomized, double-blind, placebo-controlled trial in 1000 North Indian children. Children 6 to 30 months of age were randomly assigned to receive a placebo or 1.8 µg of vitamin B<sub>12</sub>, 150 mg of folic acid, or both daily for 6 months. After 6 years, we re-enrolled 791 of these children for cognitive assessments. We compared the scores of the main outcomes (the Wechsler Intelligence Scale for Children, Fourth Edition [India], the Crichton Verbal Scale, and subtests of the NEPSY-II) between the study groups. We also measured the associations between markers of the B vitamins (plasma cobalamin, folate, and total homocysteine concentrations) in early childhood and the cognitive outcomes.

**Results:** There were no differences between the intervention groups and the placebo group on the cognitive outcomes. Plasma cobalamin, folate, and total homocysteine concentrations in early childhood were associated with the cognitive outcomes at follow-up in the unadjusted models. These associations disappeared in models adjusted for relevant confounders.

**Conclusions:** Our findings, from both an observational and a randomized design suggest that vitamin B<sub>12</sub> and folate in children 6 to 36 months have limited public health relevance for long-term cognition.

**What’s Known on this Subject:** Vitamin B<sub>12</sub> and folate are important for brain development, and suboptimal status has been linked to poor neurodevelopment. The evidence from randomized trials suggests a positive effect of vitamin B<sub>12</sub> supplementation in susceptible populations.

**What This Study Adds:** We find no persistent long-term impacts of vitamin B<sub>12</sub> or folate in early childhood on cognitive outcomes in 6- to 9-year-old children. Vitamin B<sub>12</sub> and folate are probably of limited public health relevance for cognitive functioning.


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Drs Kvestad and Strand conceptualized and designed the study, conducted the initial analyses, drafted the initial manuscript, and reviewed and revised the manuscript; Ms Hysing conceptualized and designed the study, conducted the initial analyses, and reviewed and revised the manuscript; Dr Upadhyay conducted the initial analyses and reviewed and revised the manuscript; Drs Bhandari and Taneja conceptualized and designed the study, coordinated and supervised data collection, and critically reviewed the manuscript for important intellectual content; and all authors approved the final manuscript as submitted and agree to be accountable for all aspects of the work.

Deidentified individual participant data (including data dictionaries) will be made available in addition to study protocols, the statistical analysis plan, and the informed consent form. The data will be made available after publication to researchers who provide a methodologically sound proposal for use in achieving the goals of the approved proposal. Proposals should be submitted to Dr Taneja (sunita.taneja@sas.org.in).
Inadequate vitamin B₁₂ status can impair important processes in the developing brain. Vitamin B₁₂ and folate deficiency is widespread among children in South Asian, African, and South American populations and has been linked to neurodevelopment in observational studies, although results are inconclusive. Two randomized controlled trials in infants from clinical populations suggest a positive short-term effect of high-dose injections of vitamin B₁₂ on gross motor abilities. There are reports of a positive effect of folic acid supplementation in combination with iron on gross motor function in early childhood. We have previously shown that young North Indian children who received vitamin B₁₂ and folic acid supplements for 6 months had better scores on tests of gross motor abilities and problem-solving skills than children receiving a placebo. This effect was most pronounced in children who were stunted, in those with elevated total homocysteine (tHcy) concentrations, and in those who were <24 months of age when receiving the supplements. Studies on the long-term impact of B-vitamin deficiency or repletion on cognition are scarce. In a cohort of Nepalese children, we found that vitamin B₁₂ status in infancy predicted cognitive function when the children were 5 years old.

The original randomized controlled trial in North Indian children described above was designed to measure the effect of 6-month vitamin B₁₂ and/or folic acid supplementation on infections and growth. Neurodevelopment was included as a secondary outcome. Approximately 6 years after the study was completed, we contacted the children when they were in early school age and conducted a comprehensive assessment of cognitive function. Our main aim for the current study is to examine the long-term effects of the 6-month supplementation of vitamin B₁₂ and/or folic acid in early childhood on cognition at age 6 to 9 years. Secondarily, we will examine the associations between early markers of the B vitamins (plasma cobalamin, folate, and tHcy concentrations) and later cognitive function.

METHODS

Study Design and Participants

The children in the follow-up study previously participated in a factorial randomized, double-blind, placebo-controlled trial (N = 1000) on the effect of 2 recommended daily allowances of vitamin B₁₂ and/or folic acid supplementation on childhood infections and growth in New Delhi, India. The study enrolled children from January 2010 to September 2011. Neurodevelopment was added during the first phase as a secondary outcome. We were only able to include the last 422 children for these assessments.

In September 2016, we attempted to approach all the children in the original trial. Families were initially contacted by phone and invited to participate in the study. If no contact could be made, a physical visit was made to the family’s address. Families that had moved were requested to come to the study clinic for a day. On the day of assessment, consent was taken from the children’s caregiver and demographic information was ascertained through a questionnaire.

The follow-up was registered at www.ctrini.c.in (CTRI/2016/11/007494) in November 2016 and received approval from the Ethics Committee of the Society for Applied Studies (India) and from the Norwegian Regional Committee for Medical and Health Research Ethics in 2016.

Randomization, Blinding, and Intervention

In the original trial, children were recruited at age 6 to 30 months from low- to middle-socioeconomic class families living in New Delhi and randomly assigned in a 1:1:1:1 ratio in blocks of 16 to receive placebo, vitamin B₁₂, folic acid, or vitamin B₁₂ and folic acid supplements for 6 months. A scientist not otherwise involved in the study provided the randomization scheme using Stata version 10 (Stata Corp, College Station, TX). The intervention was a lipid-based nutritional supplement prepared by Nutriset, Ltd (Malaunay, France) that was provided in jars prelabeled with the subject identification number. The 4 different interventions were identical both in appearance and taste and were offered daily to the children by field workers according to the serial numbers provided by the producer. Children were supplemented with 1 spoon (5 g) if they were 6 to 11 months, and 2 spoons (10 g) if they were ≥12 months. Each 10 g of the supplement (dose for children aged ≥12) contained 54.1 kcal total energy, 0.7 g of protein and 3.3 g of fat. For the groups that were assigned to receive B vitamins, the supplement also contained 1.8 µg of vitamin B₁₂ or 150 mg of folic acid or both, constituting 2 recommended daily allowances.

Study participants and personnel were blinded to the intervention group throughout the period of data collection in the original trial.

Outcomes

The cognitive assessments for the follow-up study were conducted at the field clinic in well-lit rooms with minimal distractions. Five psychologists blinded to the intervention groups undertook all assessments after training and standardization in 20 children per tester. Ten percent of all assessments were scored by 2 psychologists, of
whom 1 performed the assessments and the other observed and scored. From these double scorings, we attained a κ coefficient of >96%, indicating excellent interrater agreement.

The Wechsler Intelligence Scale for Children, Fourth Edition (India) (WISC-IVINDIA) is an assessment tool of intellectual ability in children validated for the Indian population with Indian norms.16 We conducted 7 subtests that summed up to 3 index scores: Perceptual Reasoning, Processing Speed, and Working Memory (Table 1). We did not conduct tests included in the Verbal Comprehension Index because these tests require English-language skills.16

The Crichton Vocabulary Scales (CVS) covers verbal skills in children 4 to 11 years.17 The CVS is translated to Hindi, with Indian norms providing a standardized total score18 (Table 1).

NEPSY-II is a neuropsychological test battery for children aged 3 to 16 years with American norms.19 We administered 7 age-appropriate subscales (Table 1). These were piloted in terms of suitability; no modifications and adjustments were made for the study.

### Covariates

In the original trial, trained field supervisors measured weight and length or height at enrollment. Weight was measured to the nearest 50 g by using Digitron scales. Length or height was measured by using locally manufactured infantometers, reading to the nearest 0.1 cm.

At the follow-up, caregivers reported on their socioeconomic status, such as parental years of schooling and assets owned by the household, and on the home environment of the child, such as number of children in the home, whether parents read books for the child and assist with homework, and which school the child attends.20 The wealth of the family was determined by a wealth index created through a principal component analysis on the basis of assets, such as televisions and bicycles; materials used for housing construction; and types of water and sanitation facilities. The wealth index places the individual household on a continuous scale of relative wealth, and the participants are divided further into 5 wealth quintiles: poorest, very poor, poor, less poor, and least poor.21

### Laboratory Analyses

Three milliliters of blood was obtained from all children at enrollment and collected into evacuated tubes containing EDTA (BD, Franklin Lakes, NJ). Immediately after blood sampling, plasma was separated by centrifugation at room temperature (450 × g × 10 minutes), transferred into storage vials and stored at −20°C until analysis. Plasma tHcy was analyzed by using commercial kits (Abbott Laboratories, Abbott Park, IL).22 Plasma concentrations of cobalamin and folate were determined by microbiologic assays by using a chloramphenicol-resistant strain of Lactobacillus casei and a colistin sulfate–resistant strain of Lactobacillus leichmannii, respectively.23

### Statistical Considerations

The infant’s baseline height-for-age z score (HAZ), weight-for-height z score (WHZ), and weight-for-age z score

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—, not applicable.
WAZ were calculated on the basis of the World Health Organization growth standards.24 Scores on the cognitive tests were calculated on the basis of available norms (Table 1). We calculated a combined WISC-IVINDIA and CVS z score on the basis of converted z scores on each subtest and a combined NEPSY-II z score on the basis of converted z scores in 7 subtests (Inhibition-Naming versus Inhibition Contrast Scaled Score, Design Fluency Total Scaled Score, Word Generation-Semantic versus Initial Letter Contrast Scaled Score, Visuomotor Precision Combined Scaled Score, Manual Motor Sequences Total Score [raw score], Affect Recognition Total Scaled Score, and Geometric Puzzles Total Scaled Score).

We present mean (SD) scores for the cognitive tests in the intervention groups. We compared the intervention groups with the placebo group in predefined subgroups on the basis of the following baseline characteristics: age <19 months (<24 months when receiving the supplementation), stunting (<−2 height or length-for-age z scores), and high plasma tHcy concentration (>10 μmol/L). Subgroups were determined by the same criteria as used in the original study.10 In these regression models, we adjusted for the wealth quintile (poorest, very poor, poor, less poor, and least poor), maternal years of schooling (no schooling, 1–5, 6–12, and >12 years), which school the child attended (private, governmental, or none), the number of children in the family (1–10), and parents’ assistance with homework (yes or no).

We also examined the associations between markers for B-vitamin status: log2-transformed plasma cobalamin, folate, and thcy concentrations at enrollment in the original trial and the z scores of the combined WISC-IVINDIA and CVS and the combined NEPSY-II in multiple linear regression models. We present both crude and adjusted models. For the adjusted models, we first selected the variables that could be related both to the B-vitamin markers and the cognitive outcomes (Supplemental Table 5). We then included each variable 1 by 1 in the crude models with the B-vitamin markers as the exposure and the cognitive z scores as the outcome. We kept the variables that changed the regression coefficients by >15% in the multiple linear regression models.25 We repeated this process for each of the markers and outcomes. Sex and age at baseline were included in all models independent of this process. We did not include growth measured after enrollment because these measures could be in the causal pathway between the exposures and the cognitive outcomes. In the models, we carefully considered the collinearity of the included variables through the variance inflation factor (vif command in Stata). The baseline WAZ was not

FIGURE 1
Trial profile of 1000 North Indian young children. a Two were not enrolled because of long-term illness.
included because of such collinearity. In addition to the crude model (model 1), we present 2 adjusted models: 1 model without growth variables (model 2) and 1 with the HAZ and WHZ (model 3). The statistical analyses were performed in Stata version 15 (Stata Corp).

**RESULTS**

The flow of the participants through the study is shown in Fig 1. Of the 1000 children in the main study, we established contact with 798 children, of whom 791 children consented to participate. Demographic characteristics in the full baseline sample and the follow-up sample and between the 4 intervention groups are similar (Table 2). The mean (SD) age at follow-up was 7.4 (0.7) years, ranging from 6 to 9 years.

Means (SD) of the cognitive outcomes by intervention groups are shown in Table 3. Except for 1 subscale of the NEPSY-II in the vitamin B12 group, there were no differences in means of the intervention groups compared with the placebo group. In the subgroup analyses, there were no significant differences in any of the subgroups between the intervention groups and the placebo group, with 1 exception. Children without an elevated baseline tHcy concentration who received vitamin B12 and folic acid (n = 266) had a significant decrease in the combined NEPSY-II z score of −0.38 (−0.68 to −0.08;
The associations between the vitamin B markers at baseline and the cognitive z scores at follow-up are shown in Table 4. Baseline plasma cobalamin concentration was associated with the WISC-IV INGINDIA and CVS z scores (0.10 [95% confidence interval (CI) 0.01 to 0.18]; \( P = .021 \)) and the NEPSY-II z scores (0.12 [95% CI 0.03 to 0.20]; \( P = .007 \)) in crude models but not in the adjusted models. Folate concentration was associated with the WISC-IV INGINDIA and CVS z scores (0.08 [95% CI 0.02 to 0.14]; \( P = .014 \)) but not with the combined NEPSY-II z scores in the crude models and not with the cognitive outcomes in the adjusted models. Baseline tHcy concentration was associated with the combined WISC-IV INGINDIA and CVS z scores and the combined NEPSY-II z scores in the crude models (−0.31 [95% CI 1.42 to 0.21] and −0.33 [95% CI 0.44 to 0.23]; \( P < .001 \) for both). Adjusting for confounders (model 2) resulted in more than a halving of these estimates and increasing \( P \) values. Still, a twofold increase of tHcy concentrations was associated with a decrease of 0.11 (95% CI 0.01 to 0.21; \( P = .028 \)) in the WISC-IV INGINDIA and CVS z scores and a decrease of 0.12 (95% CI 0.01 to 0.22; \( P = .030 \)) in the NEPSY-II z scores. Adjusting for growth resulted in further decrease of the coefficients, and the associations were no longer significant (Table 4). The attenuation of the coefficients was mainly caused by the HAZ and not the WHZ. The \( R^2 \) in model 3 was 0.36 for the combined WISC-IV INGINDIA and CVS z scores and 0.25 for the combined NEPSY-II z scores for all markers.

**DISCUSSION**

We examined the effects of 6-month supplementation of vitamin \( B_{12} \) and/or folic acid in early childhood on cognitive outcomes when the children had reached school age. There were no differences in the cognitive outcomes between the intervention groups overall or in the predefined subgroups. In an observational design, we found that although early plasma cobalamin, folate, and tHcy concentrations were associated with later cognitive functioning in crude models, these associations disappeared in models adjusted for later cognitive functioning in crude models, these associations disappeared in models adjusted for relevant confounders such as socioeconomic factors, stimulation and learning opportunities, and early childhood growth.

This is the first follow-up study in which the long-term effects of vitamin \( B_{12} \) and/or folic acid supplementation in early childhood on later cognitive...
function are measured. Despite previous findings of a beneficial short-term effect of B-vitamin supplementation on early child development, we did not find long-term effects on the cognitive outcomes in the full sample or in the predefined subgroups when the children were 6 to 9 years old. The change in infant biomarker status after supplementation resulted in an expected metabolic response and improved growth immediately after supplementation. The present results suggest, however, that the improved status in early childhood did not lead to a change in cognition in early school age, when cognitive measures are considered more stable than in early childhood. The public health relevance of vitamin B12 and folic acid administration in early childhood to improve long-term cognitive function is accordingly questionable. It should be noted that there are studies linking maternal cobalamin, folate, and tHcy concentrations in early pregnancy to offspring neurodevelopment. We cannot rule out this effect on the basis of our findings. A different timing of the intervention, for instance, at the time of neurogenesis early in pregnancy, could have yielded a beneficial effect of vitamin B supplementation on later cognition.

In the subgroup analyses, we found that children with normal tHcy concentrations who received vitamin B12 and folic acid supplementation had lower NEPSY-II z scores than children who received the placebo. This is in contrast to the subgroup analyses from the original study and, as one of many subgroup comparisons, is likely a chance finding.

In an observational design, we find that plasma cobalamin, folate, and tHcy concentrations in early childhood are associated with the cognitive outcomes in crude models but not in models adjusted for confounders. For tHcy, the associations disappeared after adjustments for attained growth, socioeconomic status, and factors related to stimulation and learning opportunities for the child. There are observational studies that have revealed a link between early vitamin B12 status and cognition in later childhood, such as in Dutch adolescents and Nepalese 5-year-old children. Differences in study design, in age at exposure and outcome measurements, in limiting nutrients, and in sociodemographic factors could explain the contrasting results. In the Nepalese study, infant tHcy concentration was associated with cognitive functioning 5 years later. tHcy concentration is often considered a marker for both vitamin B12 and folate status. The biomarker is unspecific, however, and could also be a marker for other factors reflecting poor health and illness important for brain development. In the current study, the R² values of 25% and 36% in the multiple regression models suggest that factors such as socioeconomic status, stimulation and learning opportunities, and early growth are important determinants of cognition when the children are 6 to 9 years old, which is in accordance with findings from when the children were in early childhood.

The strengths of the study include the high quality and comprehensive assessment of cognitive function with...
validated tests with Indian norms in a large sample of children in early school age when cognitive outcomes are considered to be more stable and with greater predictive value than in early childhood. Compliance to the supplementation was excellent and was reflected in an expected response in plasma cobalamin, folate, and tHcy concentrations from the supplementation. Furthermore, we were able to include 80% of the children from the original cohort after 5 years, with no differences between the children who were included in the follow-up and who were not. One limitation is that the timing and length of the supplementation may not have been ideal to detect long-term differences in cognition. Although we have measures of excellent interrater agreement between the examiners, variability in the testing may occur within the administrators because of subjectivity in the administration (ie, depending on the child being assessed). The fact that several of the participants were not deficient reduces the expected effect size and statistical power. Finally, this is a secondary outcome of a study intended to measure the effect of infections in early childhood. We believe, however, that because neurodevelopment is measured on a continuous scale, the sample size needed to detect differences in cognition is less than that needed for infections.

CONCLUSIONS
We find no persistent long-term effects of early vitamin B12 and/or folic acid supplementation on cognition. Associations between vitamin B12 and folate status in early childhood and cognition in school age are not significant after adjusting for relevant confounders. In view of our findings, vitamin B12 and folate are probably of limited public health relevance for the developing brain and long-term cognitive functioning.

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ABBREVIATIONS
- CI: confidence interval
- CVS: Crichton Vocabulary Scales
- HAZ: height-for-age z score
- tHcy: total homocysteine
- WAZ: weight-for-age z score
- WHZ: weight-for-height z score
- WISC-IVINDIA: Wechsler Intelligence Scale for Children, Fourth Edition (India)

This trial has been registered at www.clinicaltrials.gov (identifier NCT00717730) and www.ctri.nic.in (identifiers CTRI/2010/091/001090 and CTRI/2016/11/007494).

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