Vitamin B12, Folate and Cognition in 6 to 9 year-olds: A Randomized Controlled Trial

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Data Sharing Statement: 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 upon 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. Sunita Taneja (sunita.taneja@sas.org.in)

Abbreviations: randomized controlled trials – RCT; total homocysteine – tHcy; Wechsler Intelligence Scale for Children 4th edition (India) - WISC-IV^{INDIA}; Crichton Vocabulary Scales – CVS

Table of Contents Summary

Although improving vitamin B12 and folate status in early childhood improves short-term neurodevelopment, we find no persistent impact on cognition in later childhood.

What`s known on This Subject

Vitamin B12 and folate are important for brain development and suboptimal status have been linked to poor neurodevelopment. The evidence from randomized trials points towards a positive effect of vitamin B12 supplementation in susceptible populations.

What This Study Adds

We find no persistent long-term impacts of vitamin B12 or folates in early childhood on cognitive outcomes in 6 to 9-year-old children. Vitamin B12 and folates are probably of limited public health relevance for cognitive functioning.

Contributors` Statement Page

Dr Kvestad and Prof Strand conceptualized and designed the study, carried out the initial analyses drafted the initial manuscript, and reviewed and revised the manuscript.

Prof Hysing conceptualized and designed the study, carried out the initial analyses and reviewed and revised the manuscript.

Dr Upadhyay carried out the initial analyses and reviewed and revised the manuscript.

Dr Bhandari and Dr Taneja conceptualized and designed the study, coordinated and supervised data collection, and critically reviewed the manuscript for important intellectual content.

All authors approved the final manuscript as submitted and agree to be accountable for all aspects of the work.

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Abstract

Background Vitamin B12 and folate are important for normal brain development.

Objective: To measure the effects of six months supplementation of vitamin B12 and/or folic acid in early childhood on cognition when the children are 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 randomized to receive placebo, 1.8 µg of vitamin B12, 150 mg of folic acid, or both daily for six months. After six years, we re-enrolled 791of these for cognitive assessments. We compared the scores of the main outcomes; the Wechsler Intelligence Scale for Children, 4th edition (WISC-IV ^{INDIA}), the Crichton Verbal Scale (CVS) 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 (tHcy) concentration in early childhood and the cognitive outcomes.

Results: There were no differences between the intervention groups and the placebo on any of the cognitive outcomes. Plasma cobalamin, folate and tHcy concentration in early childhood were associated with the cognitive outcomes at follow-up in the unadjusted models. These associations disappeared in models adjusting for relevant confounders.

Conclusion: Our findings, from both an observational and a randomized design suggest that vitamin B12 and folate in children 6 to 36 months have limited public health relevance for long-term cognition.

Key words: Vitamin B12: Folic acid: RCT: Long term effects: Cognition: Childhood: India

Introduction

Inadequate vitamin B12 status can impair important processes in the developing brain (1). Vitamin B12 and folate deficiency is widespread among children in South Asian, African and South American populations (2), and have been linked to neurodevelopment in observational studies, although results are inconclusive (1, 3-6). Two randomized controlled trials (RCT) in infants from clinical populations suggest a positive short-term effect of high dose injections of vitamin B12 on gross motor abilities (7, 8). One trial reports a positive effect of folic acid supplementation in combination with iron on gross motor function in early childhood (9). We have previously shown that young North Indian children who received vitamin B12 and folic acid supplements for six months had better scores on tests of gross motor abilities and problem-solving skills than children receiving placebo (10). This effect was most pronounced in stunted children, in those with elevated total homocysteine (tHcy), and who were less than 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 (11).

The original RCT in North Indian children described above was designed to measure the effect of six months vitamin B12 and/or folic acid supplementation on infections and growth (12, 13). Neurodevelopment was included as a secondary outcome (10). Approximately six 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 (14). The main aim of the current study is to examine the long-term effects of the six months 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 concentration) and later cognitive function.

Patients and 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 two recommended daily allowances (RDA) of vitamin B12 and/or folic acid supplementation on childhood infections and growth in New Delhi, India (12). 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 (10).

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.ctri.nic.in as CTRI/2016/11/007494 in November 2016, and received approval from the ethics committee of Society for Applied Studies (India) and from Norwegian Regional Committee for Medical and Health Research Ethics (REK VEST) 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 randomized in a 1:1:1:1 ratio in blocks of 16 to receive placebo, vitamin B12, folic acid or vitamin B12 and folic acid for six months (12). A scientist not otherwise involved in the study provided the randomization scheme using Stata Version 10 (Stata corporation, College Station, TX). The intervention was a lipid-based

nutritional supplement prepared by NUTRISET, Ltd (Malaunay France) provided in jars that were pre-labelled with the subject identification number. The four 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 one spoon (5 g) if they were 6 to 11 months, and two spoons (10 g) if they were 12 months and above. Each 10 g of supplement (dose for children aged ≥ 12) contained 54.1 kcal total energy, 0.7 g proteins and 3.3 g fat. For the groups that were assigned to receive B-vitamins, the supplement also contained 1.8 μg vitamin B12 or 150 mg folic acid, or both constituting 2 RDAs (15). 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 two psychologists of whom one performed the assessments and the other observed and scored. From these double scorings, we attained a kappa coefficient of above 96% indicating excellent inter-rater agreement.

Wechsler Intelligence Scale for Children 4th edition (WISC-IV^{INDIA}) is an assessment tool of intellectual ability in children validated for the Indian population with Indian norms (16). We conducted seven subtests that summed up to three index scores; the Perceptual Reasoning, Processing Speed and Working Memory (table 1). We did not conduct tests included in the Verbal Comprehension index since these tests require English language skills (16).

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 score (18) (table 1).

NEPSY-II is a neuropsychological test battery for children aged 3 to 16 years with American norms (19). We administered seven 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/height at enrolment. Weight was measured to the nearest 50 g using Digitron scales. Length/height was measured 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, if 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 based on 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 five wealth quintiles; poorest, very poor, poor, less poor and least poor (21).

Laboratory analyses

Three mL blood was obtained from all children at enrolment and collected into evacuated tubes containing EDTA (BD, Franklin Lakes, NJ, USA). Immediately following blood sampling, plasma was separated by centrifugation at room temperature (450 x g x 10 min),

transferred into storage vials and stored at -20 0C until analysis. Plasma tHcy was analysed using commercial kits (Abbott Park, IL, USA) (22). Plasma concentrations of vitamin B12 and folate were determined by microbiological assays using a chloramphenicol-resistant strain of *Lactobacillus casei* and colistin sulfate-resistant strain of *Lactobacillus leichmannii*, respectively (23).

Statistical considerations

Infant's baseline height-for-age (HAZ), weight-for-height (WHZ) and weight-for-age z-scores (HAZ, WHZ and WAZ) were calculated based on WHO growth standards (24). Scores on the cognitive tests were calculated based on available norms (table 1). We calculated a combined WISC-IV^{INDIA} and CVS z-score based on converted z-scores on each subtest, and a combined NEPSY-II z-score based on converted z-scores in seven subtests (IN-Naming vs. Inhibition Contrast Scaled Score, Design Fluency Total Scaled Score, Word Generation-Semantic vs. Initial Letter Contrast Scaled Score, Visuo-motor 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 [standard deviation (SD)] scores for the cognitive tests in the intervention groups and compared the intervention groups with placebo in linear regression models, where the intervention groups were included as dummy variables and compared against the placebo. We also compared the combined WISC-IV^{INDIA} and CVS and the combined NEPSY-II scores of the intervention groups with placebo in predefined subgroups based on the following baseline characteristics: age <19 months (less than 24 months when receiving the supplementation), stunting (< -2 z scores height/length-for-age) and high plasma tHcy (>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 years, 6-12 years and > 12 years), which school the child attends (private, governmental or none), number of children in the family (1-10) and parents` assistance with homework (yes/no).

We also examined the associations between markers for B-vitamin status; log2 transformed plasma cobalamin, folate and tHcy concentration at enrolment in the original trial and the z-scores of the combined WISC-IV^{INDIA} 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 (supplementary table 1). We then included each variable one-by-one 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 more than 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 as 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). Baseline WAZ was not included due to such collinearity. In addition to the crude model (model 1), we present two adjusted models; one model without growth variables (model 2) and one with HAZ and WHZ (model 3). The statistical analyses were performed in Stata, version 15 (Stata Corporation, College Station, TX).

Results

Figure 1 shows the flow of the participants through the study. 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 four intervention groups are similar (table 2). 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 one subscale of the NEPSY-II in the vitamin B12 group, there were no differences in means of the intervention groups compared to placebo. In the subgroup analyses, there were no significant differences in any of the subgroups between the intervention groups compared to placebo, with one exception. Children without 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, -0.08), p=0.013 compared to placebo.

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^{INDIA} and CVS z-scores [0.10 (95% CI 0.01, 0.18), p= 0.021] and the NEPSY-II z-scores [0.12 (95% CI 0.03, 020), p=0.007)] in crude models, but not in the adjusted models. Folate concentration was associated with the WISC-IV^{INDIA} and CVS z-score [0.08 (95% CI 0.02, 0.14), p=0.014)] 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 was associated with the combined WISC-IV^{INDIA} and CVS z-scores and the combined NEPSY-II z-scores in the crude models [-0.31 (95% CI 1.42, 0.21) and -0.33 (95% CI 0.44, 0.23) ($p<0.001$ 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 concentration was associated with a decrease of 0.11 (95% CI 0.01, 0.21), p=0.028 WISC-IV^{INDIA} and CVS z-score and a decrease of 0.12 (95% CI 0.01, 0.22), p=0.030 NEPSY-II z-score. Adjusting for growth resulted in a 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-score and not the WHZ-score. The R^{2}`s in model 3 was 0.36 for the combined WISC-IV^{INDIA} and CVS z-scores and 0.25 for the combined NEPSY-II z-scores for all markers.

Discussion

We examined the effects of six months supplementation of vitamin B12 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 while early plasma cobalamin, folate and tHcy concentration were associated with later cognitive functioning in crude models, these associations disappeared in models adjusting for relevant confounders such as socioeconomic factors, stimulation and learning opportunities and early childhood growth.

This is the first follow-up study to measure the long-term effects of vitamin B12 and/or folic acid supplementation in early childhood on later cognitive function. In spite of previous findings of a beneficial short-term effect of the B-vitamin supplementation on early child development (10), 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 following supplementation resulted in an expected metabolic response (12), and improved growth (13) and neurodevelopment (10) immediately following the 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 concentration in early pregnancy to offspring neurodevelopment (4, 26-30). We cannot rule out this effect based on 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 the vitamin B supplementation on later cognition (31).

In the subgroup analyses, we found that children with normal tHcy concentration and who received vitamin B12 and folic acid supplementation, had lower NEPSY-II z-scores than children who received placebo. This is in contrast to the subgroup analyses from the original study (10), and as one out of many subgroup comparisons quite 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 following adjustments for attained growth, socioeconomic status, and factors related to stimulation and learning opportunities for the child. There are observational studies that have demonstrated a link between early vitamin B12 status and cognition in later childhood such as in Dutch adolescents (6) and Nepalese 5-year olds (11). 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 five years later (11). THcy concentration is often considered a marker for both vitamin B12 and folate status (32). The biomarker is unspecific, however, and could also be a marker for other factors reflecting poor health and illness important for brain development (32, 33). In the current study, the R^2s 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 (34).

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 reflected in an expected response in plasma cobalamin, folate and tHcy from the supplementation (12). Furthermore, we were able to include 80% of the children from the original cohort after more than 5 years, with no differences between the children who were included in the follow-up and not. Limitation includes 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 inter-rater agreement between the examiners, variability in the testing may occur within the administrators due to subjectivity in the administration i.e. depending on the child being assessed. The fact that several of the participants were not deficient, reduces the 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 since neurodevelopment is measured on a continuous scale the sample size needed to detect differences in cognition is less than for infections.

Conclusion

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 no longer significant after adjusting for relevant confounder. 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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Table 1. Overview of measures in the follow up study of 791 North Indian children 6 to 9 years

Assessment tool	Domain	Subscales	Subtests	Scores	Mean	Range	Norms
WISC IV INDIA	*Perceptual reasoning*	Perceptual Reasoning Index	Block design, Picture concept and Matrix reasoning	composite score	100		Indian
	Processing speed	Processing Speed Index	Symbol search and Letter-number sequences	composite score	100		
	Working memory	Working Memory Index	Digit span and Coding	composite score	100		
Crichton Verbal Scale	*Verbal*			standard score	50		Indian
NEPSY-II	*Executive function*	Inhibition	IN-Naming vs. Inhibition Contrast Scaled Score	scaled score	10	1-19	American
			IN-inhibition vs. Switching Contrast Scaled Score	scaled score	10	1-19	
			Inhibition Total Errors Scaled Scores	scaled score	10	1-19	
		Design Fluency	Design Fluency Total Scaled Score	scaled score	10	1-19	
	Language	Word Generation	Word Generation-Semantic vs. Initial Letter Contrast Scaled Score	scaled score	10	1-19	
	Sensorimotor	Visuomotor Precision	Visuo-motor Precision Combined Scaled Score	scaled score	10	1-19	
		Manual Motor Sequences	Manual Motor Sequences Total Score - raw score	raw score			
	Social perception	Affect Recognition	Affect Recognition Total Scaled Score	scaled score	10	1-19	
	Visuospatial	Geometric Puzzles	Geometric Puzzles Total Scaled Score	scaled score	10	1-19	

Table 2. Demographic information of the children in the original study (N=1000) when they were 6 to 30 months and in the follow up study (N=791) when they were 6 to 9 years old.

	Original	Follow-up				
	Total sample	**Total sample**	**Placebo**	**B12**	**Folic Acid**	**B12 & Folic acid**
	N=1000	N=791	n=202	n=201	n=204	n=184
Child characteristics at baseline (6 to 30 months)						
Age baseline mean (SD)	16.1 (7.1)	16.2 (7.0)	16.3 (7.0)	15.9 (6.9)	16.4 (7.2)	16 (7.0)
Boys, n (%)	507 (50.7%)	399 (50.4%)	108 (53.5%)	88 (43.8%)	101 (49.5%)	102 (55.4%)
Still breastfed, n (%)	798 (80.2%)	632 (80.1%)	159 (78.7%)	164 (82%)	160 (78.8%)	149 (81%)
Growth Z scores						
Weight-for-height (WHZ)	-0.9 (0.9)	-0.9 (0.9)	-0.9 (0.9)	-0.9 (1.0)	-0.8 (1.0)	-0.9 (0.9)
Height-for-age (HAZ)	-1.8 (1.2)	-1.8 (1.7)	-1.8 (1.8)	-1.8 (1.7)	-1.8 (1.2)	-1.7 (1.1)
Weight-for-age (WAZ)	-1.6 (1.1)	-1.6 (1.1)	-1.7 (1.0)	-1.6 (1.1)	-1.5 (1.1)	-1.6 (1.0)
Biomarkers[a]						
Cobalamin < 200 pmol/L, n (%) [b]	328 (32.8%)	256 (32.4%)	64 (31.7%)	69 (34.3%)	74 (36.5%)	49 (26.6%)
Folate < 7.5 nmol/L, n (%) [c]	303 (30.3%)	256 (32.4%)	57 (28.2%)	64 (31.8%)	62 (29.9%)	73 (39.6%)
tHcy > 10 µmol/L, n (%) [d]	628 (62.8%)	498 (63.4%)	133 (66.2%)	120 (59.7%)	129 (64.2%)	116 (63.7%)
Child characteristics at follow up (6 to 9 years)						
Age at follow up (yrs) mean (SD)	na	7.4 (0.7)	7.3 (0.7)	7.4 (0.7)	7.4 (0.7)	7.4 (0.7)
Schooling						
Governmental, n (%)	na	475 (60%)	107 (53%)	116 (57.7%)	137 (67.2%)	115 (62.5%)
Private, n (%)	na	302 (38.2%)	91 (45%)	81 (40.3%)	65 (31.9%)	65 (35.3%)
No school, n (%)	na	14 (1.8%)	4 (2%)	4 (2%)	2 (0.9%)	4 (2.2%)
Family characteristics at follow up						
Wealth quintile						
Poorest, n (%)	na	159 (20.2%)	42 (20.8%)	42 (20.9%)	39 (19.1%)	36 (19.6%)

Very poor, n (%)	na	157 (19.8%)	43 (21.3%)	50 (24.9%)	34 (16.7%)	30 (16.3%)
Poor, n (%)	na	158 (20%)	37 (18.3%)	42 (20.8%)	35 (17.2%)	44 (23.9%)
Less poor, n (%)	na	158 (20%)	44 (21.8%)	30 (14.9%)	50 (24.5%)	34 (18.5%)
Least poor, n (%)	na	158 (20%)	36 (17.8%)	36 (17.9%)	46 (22.5%)	40 (21.7%)
Parents regularly assist and follow up with the child`s studies	na	680 (87.1%)	175 (88.4%)	162 (81.8%)	179 (88.6%)	164 (89.6%)
Mothers years of schooling						
No schooling, n (%)	na	214 (28.3%)	59 (30.7%)	59 (31.4%)	52 (26.7%)	44 (24.3%)
Primary (1- 5 years), n (%)	na	101 (13.3%)	24 (12.5%)	24 (12.8%)	27 (13.8%)	26 (14.4%)
Middle (6-12 years), n (%)	na	346 (45.8%)	90 (46.9%)	83 (44.1%)	85 (43.6%)	88 (48.6%)
Higher (> 12 years), n (%)	na	95 (12.6%)	19 (9.9%)	22 (11.7%)	31 (15.9%)	23 (12.7%)
Fathers occupation						
Government job or private services, n (%)	na	429 (55.1%)	110 (55.0%)	105 (54.1%)	113 (56.2%)	101 (54.9%)
Self-employed, n (%)	na	204 (26.2%)	46 (23.0%)	54 (27.8%)	52 (25.9%)	52 (28.3%)
Daily wager/farming, n (%)	na	120 (15.4%)	39 (19.5%)	25 (12.9%)	28 (13.9%)	28 (15.2%)
No job/other, n (%)	na	26 (3.3%)	5 (0.5%)	10 (5.2%)	8 (4%)	3 (1.6%)

[a] plasma, non-fasting

[b] data available in 999 children at baseline, and in 790 children at follow-up

[c] data available in 999 children at baseline and in 791 at follow up

[d] data available in 994 children at baseline and in 785 children at follow up

Table 3. Mean (SD) cognitive scores by study group at follow up in 791 North Indian children 6 to 9 years [a]

	Placebo N=203		B12 N=200		Folic acid N=204		B12&Folic acid N=184	
	Mean	SD	Mean	SD	Mean	SD	Mean	SD
Combined WISC-IV INDIA and CVS z-score	-0.02	1.01	-0.12	1.07	0.08	0.98	0.06	0.93
WISC-IV INDIA subscales								
Perceptual Reasoning Index [b]	95.83	16.22	94.77	17.03	97.60	16.51	97.18	16.26
Processing Speed Index	98.10	15.18	97.12	15.94	98.29	14.36	98.28	15.06
Working Memory Index	93.02	19.29	92.48	21.24	95.78	18.93	95.4	19.1
Crichton Verbal Scale [c]	100.20	13.76	98.26	12.78	100.84	12.49	100.13	11.56
Combined NEPSY-II z-score	-0.03	1.00	-0.05	1.03	0.05	1.01	0.04	0.96
NEPSY-II subtests [d]								
Executive function								
IN-Naming vs. Inhibition Contrast Scaled Score	8.11	3.32	8.29	3.28	8.29	3.31	8.49	3.20
IN-inhibition vs. Switching Contrast Scaled Score	8.73	2.47	9.31	2.86	9.06	2.86	8.72	2.87
Inhibition Total Errors Scaled Scores	8.53	3.21	9.40 [e]	3.71	8.97	3.47	8.84	3.81
Design Fluency Total Scaled Score	7.97	2.76	7.84	2.93	8.33	2.68	8.33	2.57
Language								
Word Generation-Semantic vs. Initial Letter Contrast Scaled Score	7.62	2.62	7.54	2.62	7.60	2.59	7.97	3.07
Sensorimotor								
Visuo-motor Precision Combined Scaled Score	9.30	2.80	9.11	2.71	9.44	2.97	9.24	2.73
Manual Motor Sequences Total Score - raw score	38.54	10.22	38.93	10.06	39.04	10.32	39.01	9.14
Social perception								
Affect Recognition Total Scaled Score	9.98	2.38	10.01	2.44	10.18	2.21	10.38	2.25
Visuospatial								
Geometric Puzzles Total Scaled Score	10.35	3.40	9.99	3.33	10.25	3.55	10.14	2.89

[a] intervention groups are compared with placebo in linear regression models, coding the intervention groups with dummy variables

[b] WISC-IV index scores have a mean of 100, data is available in 774 children

[c] CVS total standard score has a mean of 50, data is available in 781 children
[d] NEPSY-II scaled scores have a mean of 10, range from 1-19, data is available in 782 children
[e] p=0.038, for all other comparisons p>0.01

Table 4. Associations between plasma cobalamin, folate and total homocysteine (tHcy) concentration in early childhood and cognitive scores in North Indian children 6 to 9 years.

		Combined WISC-IV [INDIA] and CVS z-score					Combined NEPSY-II z-score				
		N	Coef.[e]	95% CI		P	N	Coef.	95% CI		P
Vitamin B12[a]	Model 1[b]	769	0.10	0.01	0.18	0.021	768	0.12	0.03	0.20	0.007
	Model 2[c]	738	0.00	-0.07	0.07	0.994	732	0.01	-0.06	0.09	0.727
	Model 3[d]	738	-0.02	-0.10	0.05	0.521	732	0.01	-0.09	0.07	0.874
Folate[a]	Model 1	768	0.08	0.02	0.14	0.014	767	-0.01	-0.07	0.06	0.878
	Model 2	737	0.03	-0.03	0.09	0.314	731	0.02	-0.04	0.08	0.555
	Model 3	737	0.02	-0.04	0.08	0.443	731	0.01	-0.05	0.08	0.700
Total Homocysteine[a]	Model 1	765	-0.31	-0.42	-0.20	<0.001	764	-0.33	-0.44	-0.23	<0.001
	Model 2	734	-0.11	-0.21	-0.01	0.028	728	-0.12	-0.22	-0.01	0.030
	Model 3[f]	734	-0.07	-0.17	0.03	0.146	728	-0.08	-0.19	0.02	0.126

[a]log2 transformed.
[b] Model 1; unadjusted
[c] Model 2; Combined WISC-IV [INDIA] and CVS z-score: adjusted for sex, baseline age, maternal education at follow up, wealth quintile at follow up, family regularly buy a newspaper, number of children in the home, child reads storybook and which school (private, governmental or none). Combined NEPSY-II z-score: adjusted for sex, baseline age, maternal education at follow up, wealth quintile at follow up, family regularly buy a newspaper, number of children in the home, which school (private, governmental or none) and parents assist with homework
[d] Model 3; the variables in model 2 and baseline HAZ and WHZ for both outcomes
[e] Unstandardized regression coefficients, multiple linear regression model
[f] R^2 for model 3: Combined WISC-IV [INDIA] and CVS z-score: 0.36 for all markers, and Combined NEPSY-II z-score: 0.25 for all markers

Figure 1. Trial profile of 1000 North Indian young children