The Journal of Nutrition
Nutritional Epidemiology

Higher Maternal Plasma Folate but Not Vitamin B-12 Concentrations during Pregnancy Are Associated with Better Cognitive Function Scores in 9- to 10- Year-Old Children in South India[1–3]

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Abstract

Folate and vitamin B-12 are essential for normal brain development. Few studies have examined the relationship of maternal folate and vitamin B-12 status during pregnancy and offspring cognitive function. To test the hypothesis that lower maternal plasma folate and vitamin B-12 concentrations and higher plasma homocysteine concentrations during pregnancy are associated with poorer neurodevelopment, 536 children (aged 9–10 y) from the Mysore Parthenon birth cohort underwent cognitive function assessment during 2007–2008 using 3 core tests from the Kaufman Assessment Battery, and additional tests measuring learning, long-term storage/retrieval, attention and concentration, and visuo-spatial and verbal abilities. Maternal folate, vitamin B-12, and homocysteine concentrations were measured at 30 ± 2 wk gestation. During pregnancy, 4% of mothers had low folate concentrations (<7 nmol/L), 42.5% had low vitamin B-12 concentrations (<150 pmol/L), and 3% had hyperhomocysteinemia (>10 μmol/L). The children's cognitive test scores increased by 0.1–0.2 SD per SD increase across the entire range of maternal folate concentrations ($P < 0.001$ for all), with no apparent associations at the deficiency level. The associations with learning, long-term storage/retrieval, visuo-spatial ability, attention, and concentration were independent of the parents' education, socioeconomic status, religion, and the child's sex, age, current size, and folate and vitamin B-12 concentrations. There were no consistent associations of maternal vitamin B-12 and homocysteine concentrations with childhood cognitive performance. In this Indian population, higher maternal folate, but not vitamin B-12, concentrations during pregnancy predicted better childhood cognitive ability. It also suggests that, in terms of neurodevelopment, the concentration used to define folate deficiency may be set too low. J. Nutr. 140: 1014–1022, 2010.

Introduction

Folate and vitamin B-12 are important micronutrients, essential for neurodevelopment and function in the antenatal and early postnatal periods (1–3). The folate and vitamin B-12 supply to the growing fetus depends on maternal folate and vitamin B-12 status (4–6). Deficiencies of folate and vitamin B-12 are prevalent across the globe (7). A recent review reported that circulating levels of vitamin B-12 and total homocysteine (tHcy),[8] a functional measure of folate or vitamin B-12 status, drop during normal pregnancy (8). Folate deficiency has been reported in Indians (9–11). Vitamin B-12 deficiency is common in Indians, including pregnant women and children (9–13), and has been attributed mainly to a vegetarian diet, with or without malabsorption due to intestinal infections (14). Deficiency of these nutrients during pregnancy has been linked to fetal growth restriction and neural tube defects (8,15–18).

Literature reporting associations between maternal folate status during pregnancy and later cognitive ability in the offspring are few and are limited mainly to animal studies (19,20). Studies in rodents have shown associations of maternal folate deficiency with structural brain abnormalities (19) and poor postnatal learning ability in the offspring (20). In humans, maternal megaloblastic anemia has been associated with delayed or abnormal infant development (3) and poor mental development has been reported in children of genetically susceptible

[1] Supported by The Wellcome Trust grant 079877/Z/06/Z and by Medical Research Council grant G0400519 (ID no.71108).
[2] Author disclosures: S. R. Veena, G. V. Krishnaveni, K. Srinivasan, A. K. Wills, S. Muthayya, A. V. Kurpad, C. S. Yajnik, and C. H. D. Fall, no conflicts of interest.
[3] Supplemental Figure 1 and Supplemental Table 1 are available with the online posting of this paper at jn.nutrition.org.
[8] Abbreviations used: HMH, Holdsworth Memorial Hospital; SES, socioeconomic status; tHcy, total homocysteine; WISC-III, Wechsler Intelligence Scale for Children-III.
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Manuscript received October 27, 2009. Initial review completed November 24, 2009. Revision accepted February 20, 2010.
First published online March 24, 2010; doi:10.3945/jn.109.118075.

mothers with low dietary folate intakes (21). In relation to vitamin B-12 status during pregnancy, there are case reports of delayed neurodevelopment in infants born to mothers with pernicious anemia or among strict vegetarians (1,2,22,23). Two recent studies, from Mexico and India, have reported poor cognitive outcomes in children of vitamin B-12–deficient mothers (21,24).

There is recent interest in potential interactions between folate and vitamin B-12 status. Among elderly U.S. individuals, higher folate status in the presence of vitamin B-12 deficiency was associated with impaired cognitive function and anemia (25). A study in India reported that children of mothers with high folate and low vitamin B-12 status in pregnancy had increased insulin resistance (13). A recent trial in Nepal reported a reduced risk of metabolic syndrome in children of mothers supplemented during pregnancy with folic acid and vitamin A compared with children of control mothers who received vitamin A alone. This benefit was not apparent in children of mothers who received multiple micronutrients (which included folic acid and vitamin B-12), suggesting the possibility of nutrient interactions (26).

We examined associations between maternal plasma folate, vitamin B-12, and tHcy concentrations in pregnancy and cognitive performance in 9- to 10-y-old children from the Mysore Parthenon Study, India (27,28). Our main objective was to test the hypothesis that low maternal folate and/or vitamin B-12 concentrations are associated with lower offspring cognitive ability, independent of socioeconomic factors and the child's size and current vitamin B-12 and folate status. We also examined the associations of maternal plasma folate, vitamin B-12, and tHcy status across a whole range of concentrations with offspring cognitive ability.

Materials and Methods

Study population. The Mysore Parthenon study is a prospective birth cohort study initiated in 1997–1998, mainly to examine the incidence and determinants of gestational diabetes mellitus in India and its short- and long-term effects (27,28). Eight hundred and thirty women booking consecutively into the antenatal clinic at the Holdsworth Memorial Hospital (HMH), Mysore, India, and satisfying the eligibility criteria (no history of diabetes before pregnancy, planning to deliver at HMH, and having a singleton pregnancy of <32 wk gestation) participated in the study. Six hundred and seventy-four women delivered their babies at HMH (81% of the participants). Excluding 7 stillborn babies and 4 with major congenital anomalies, detailed newborn anthropometry was performed on 663 normal live-born babies according to a standard protocol, within 72 h of birth, as reported previously (27,28). Excluding 25 children who died and 8 with major medical problems, 630 healthy children were followed-up with repeat anthropometry annually until the age of 5 y and every 6 mo thereafter.

Maternal folate and vitamin B-12 concentrations. Maternal micronutrient (folate, vitamin B-12, and tHcy) concentrations were measured using stored plasma samples. Maternal blood samples collected at 30 ± 2 wk of gestation were immediately centrifuged after venipuncture and frozen at –80°C within 1 h in lightproof boxes and remained at or below that temperature throughout their time in storage (8 y). Children's blood samples collected at 9.5 y and stored in a similar way were analyzed within 1 y of collection to measure folate and vitamin B-12 concentrations. Laboratory analyses were performed using microbiological assays (Wallac Victor 1420, PerkinElmer Life Sciences) at the Diabetes Research Centre, KEM Hospital, Pune, India. Plasma vitamin B-12 was measured using a colistin sulfate-resistant strain of *L. leichmanii* (29), plasma folate was measured using a chloramphenicol-resistant strain of *L. casei* (30), and plasma tHcy was measured by fluorescence polarization immunoassay (Abbott) (31). Intra- and inter-assay CV were <8% for the folate and vitamin B-12 assays and for the tHcy assay, between-day CV were <3%. Low folate and vitamin B-12 status were defined as concentrations <7 nmol/L (32) and <150 pmol/L (33), respectively, and hyperhomocysteinemia as a concentration >10 μmol/L (34).

Folic acid and vitamin B-12 supplementation. General practitioners and obstetricians routinely prescribe folic acid and/or multivitamin supplements to pregnant women. We collected data on the intake of multivitamin supplements at the time of recruitment, but not subsequently, and therefore no information is available on their use when blood samples were collected or at term. Approximately 70% of women were recruited at <24 wk gestation and 30% were recruited between 24 and 32 wk.

Study sample for cognitive function assessment. At 9–10 y of age (September 2007 to May 2008), children were invited for assessment of their cognitive function. Of the 630 children, 61 were unwilling, 17 had moved away from Mysore, and 10 were untraceable. The remaining 542 (86%) underwent cognitive testing. Six children were excluded because maternal folate and vitamin B-12 concentrations were not available. The current analysis is restricted to 536 children (boys, n = 259, girls, n = 277) (**Supplemental Fig. 1**).

Tests of cognitive function. The cognitive measures consisted of a series of neuropsychological tests applicable for use in school-aged children and related to specific cognitive domains (memory, attention, fluid reasoning) consistent with the Carroll model (35). They included 3 core tests from the Kaufman Assessment Battery for children (36) and additional tests (37–40) that underwent extensive adaptation to the local cultural context and the adapted version was validated (41,42). These cognitive tests covered the domains of learning, long-term memory and retrieval ability (Atlantis), short-term memory (word order), reasoning ability (pattern reasoning), language production (verbal fluency), visuo-spatial ability (Kohs' block design), and visuo-motor processing speed and coordination, attention, and concentration [coding-Wechsler Intelligence Scale for Children-III (WISC-III)]. The descriptions of these cognitive tests are summarized in **Supplemental Table 1.** All the tests were administered to each child in a single session of 60–90 min at the Epidemiology Research Unit, HMH, in separate rooms, free from distraction, by 1 of 2 trained masters' level child psychologists (unaware of the maternal folate and vitamin B-12 status of study children) in the local Kannada language.

Covariates and confounders. We considered the following as important covariates and potential confounding variables: parental factors included maternal age, parity, BMI, and height in pregnancy, maternal and paternal educational attainment (years completed), rural/urban residence, and current socioeconomic status (SES), assessed using the Standard of Living Index (43). None of the mothers had ever smoked or consumed alcohol. Infant factors included the child's sex, gestational age at birth, newborn weight, and head circumference. Child factors included current age, BMI, head circumference, educational level, and folate and vitamin B-12 concentrations at the time of cognitive testing.

The research ethics committee of the HMH approved the study and informed verbal consent was obtained from parents and children.

Statistical methods. Variables with skewed distributions were either log-transformed (maternal BMI, vitamin B-12, tHcy, and Kohs block design score) or square root transformed (pattern reasoning score). Scores of cognitive tests and maternal folate, vitamin B-12, and tHcy concentrations were Z-standardized to facilitate interpretation of regression models. To describe the current BMI and height of the children with reference to international standards, we derived their Z-score using the WHO growth reference (44). Comparisons of means and percentages between groups were made using t tests and chi-square tests, where appropriate. Correlations between maternal folate, vitamin B-12, and tHcy and the children's current folate and vitamin B-12 were examined using Pearson correlation coefficients. Associations of covariates and confounders with maternal folate and vitamin B-12

concentrations (exposure) and cognitive scores (outcomes) were initially examined using multiple linear regression adjusting for sex and current age. For categorical covariates/confounders (religion), the largest category was used as the reference and tests of general association (Wald) were performed. Associations of maternal folate and vitamin B-12 concentrations [as binary variables (low compared with normal concentrations) and as continuous variables] with cognitive scores were then analyzed using multiple linear regression adjusting for covariates/confounders that were significantly associated with either maternal folate and vitamin B-12 concentrations or cognitive outcomes. A series of models was considered to examine whether any associations found were acting through socioeconomic factors, fetal growth, and/or the child's current size and vitamin B-12 and folate status. Interaction terms were used to test for differences in associations between boys and girls and to examine interactions between maternal folate and vitamin B-12 concentrations, in relation to cognitive scores. Data presented in the text include percentages, mean ± SD, and median (interquartile range) values, correlation coefficients (r), and regression coefficients (β) (95% CI). For all tests, $P < 0.05$ was considered significant. Stata (version 10.0, Stata Corporation) was used for all analyses.

Results

Characteristics of the study cohort are summarized in **Table 1**. During pregnancy, 22 (4%) women had low folate concentrations, 228 (42.5%) had low vitamin B-12 concentrations, and 18 (3.4%) had hyperhomocysteinemia. At 9.5 y, <1% of the children had low folate and 2.6% had low vitamin B-12 concentrations. Folate and vitamin B-12 concentrations and the prevalence of low concentrations were not significantly different between mothers whose children were tested for cognitive function and mothers whose children did not take part (data not shown).

Girls scored better than boys in tests of word order (short-term memory; $P = 0.01$), pattern reasoning (planning and fluid reasoning, $P = 0.004$), verbal-fluency-names (broad retrieval ability and speed and flexibility of verbal thought process; $P < 0.0001$), and coding-WISC-III (attention and concentration; $P < 0.0001$) (Table 1). Approximately 5% of the children were in the third y at school, 38% in the fourth y, 50% in the fifth y, and 5% in the sixth y (this variation in a sample of children of the same age arises because of different admission guidelines in different schools and was not generally due to children being held back for poor academic performance). One percent of mothers were illiterate, ~35% had received only primary school education, 50% had completed secondary school education, and 14% were graduates or postgraduates and/or professionals. Corresponding figures for fathers were 3, 35, 39, and 23%, respectively. Approximately 73% of the families were from urban areas and 27% from rural areas.

Associations of maternal plasma folate and vitamin B-12 concentrations with covariates and confounders. Higher maternal folate concentration was associated with lower parity, larger child size at birth and 9.5 y, and higher SES and parental education (**Table 2**). The prevalence of low plasma folate concentrations was higher among Muslim women (6.0%) with a concentration (mean ± SD) of 28.5 ± 17.9 nmol/L than in Hindu (3.2%, 37.9 ± 19.1 nmol/L) and Christian (2.2%, 38.4 ± 19.9 nmol/L) women. The mother's folate concentration was positively correlated with her vitamin B-12 concentrations ($r = 0.1$; $P = 0.015$) and with the child's folate concentration at 9.5 y ($r = 0.2$; $P < 0.0001$).

As already reported (45), there was an inverse association between maternal vitamin B-12 concentration and maternal BMI in pregnancy (Table 2). Hindu women had a higher prevalence of low vitamin B-12 concentrations [50.5%, median (interquartile range) 149 (117, 209) pmol/L] than Muslims [30.2%, 180 (135, 224)] and Christians [37.8%, 186 (134, 229)]. Maternal vitamin B-12 correlated with the child's vitamin B-12 concentration at 9.5 y ($r = 0.2$; $P < 0.0001$).

The maternal tHcy concentration was inversely correlated with maternal folate ($r = -0.3$; $P < 0.0001$) and vitamin B-12 ($r = -0.2$; $P < 0.0001$). Offspring birth weight decreased with increasing maternal tHcy concentrations ($P = 0.003$) (Table 2). Compared with Hindu women, Muslim women had higher tHcy concentrations.

At the time of recruitment, 156 (29%) women reported taking multivitamin supplements containing both folic acid and vitamin B-12. Of these, 89 (57%) were recruited at <24 wk gestation and 67 (43%) between 24 and 32 wk gestation. Forty-eight women (9%) were taking folic acid alone, of whom 38 (79%) were recruited before 24 wk gestation and 10 (21%) between 24 and 32 wk gestation. There was no significant association of supplement status at recruitment either before 24 wk or between 24 and 32 wk with folate and vitamin B-12 concentrations at 30 ± 2 wk of gestation.

Associations of cognitive outcomes with covariates and confounders. Cognitive scores tended to be lower in the children of mothers of higher parity and to increase with increasing maternal age and children's birth size (**Table 3**). The children's current BMI and head circumference and parental educational level and SES were strongly and positively related to all the cognitive outcomes. The cognitive ability of urban children was significantly better than rural children. Muslim children performed less well compared with other children in tests of pattern reasoning, verbal fluency, Kohs block design (visuo-spatial ability), and coding. The children's current folate, but not vitamin B-12, concentration was positively associated with all the cognitive outcomes except word order and coding. Coding test scores were positively related to the children's current educational level (Table 3).

Associations of maternal plasma folate and vitamin B-12 concentrations with cognitive outcome. Low maternal folate status was not associated with any of the cognitive outcomes, although cognitive scores were lower in the small group of children whose mothers had low folate status compared with children of women with normal status (**Table 4**). However, there were positive associations, across the whole range of maternal folate concentrations, with scores for all the cognitive outcomes [adjusted for sex and current age (model 1)] (Table 4). All these associations, except for word order and pattern reasoning, were reduced but remained significant after further adjustment for SES, parent's education, rural/urban residence, religion, parity, maternal pregnancy age and BMI, gestation, and child's birth size (model 2). Further adjusting for the children's current size, educational level, and folate and vitamin B-12 concentrations at 9.5 y (model 3) showed independent associations of maternal folate with the cognitive measures of learning ability and long-term storage and retrieval, visuo-spatial ability, and attention and concentration.

Lower maternal vitamin B-12 status was associated with higher verbal-fluency scores (Table 4). There were no associations between maternal vitamin B-12 concentrations across the whole range and the cognitive test scores (Table 4). Maternal tHcy concentrations and most of the cognitive test scores were not associated (data not shown). Higher maternal tHcy concen-

TABLE 1 General characteristics of the study cohort[1]

Variable	Boys	Girls	All
n	259	277	536
Maternal characteristics in pregnancy			
Age, *y*	24.0 ± 4.2	23.8 ± 4.2	23.9 ± 4.2
Parity, *n (%)*			
0	125 (48.3)	147 (53.1)	272 (50.8)
1	88 (34.0)	88 (31.8)	176 (32.8)
≥2	46 (17.8)	42 (15.2)	88 (16.4)
Height, *cm*	154.1 ± 5.5	154.5 ± 5.3	154.3 ± 5.4
BMI, *kg/m²*	22.9 (21.0, 25.6)	23.5 (21.0, 26.5)	23.2 (21.0, 26.1)
Plasma folate, *nmol/L*	34.1 ± 19.2	35.4 ± 19.3	34.7 ± 19.2
Low folate, *n (%)*	11 (4.3)	11 (4.0)	22 (4.1)
Plasma vitamin B-12, *pmol/L*	183.0 (126.0, 226.0)	153.0 (123.0, 207.0)	162.5 (124.0, 220.0)
Low vitamin B-12, *n (%)*	98 (37.8)	130 (46.9)	228 (42.5)
Plasma homocysteine, *μmol/L*	6.0 (5.0, 6.9)	6.0 (5.1, 7.1)	6.0 (5.1, 7.0)
Hyperhomocysteinemia, *n (%)*	8 (3.1)	10 (3.7)	18 (3.4)
Children's characteristics			
Tests of cognitive function (score)			
Atlantis	67.5 ± 17.8	67.6 ± 16.7	67.6 ± 17.2
Word order	16.1 ± 2.5	16.7 ± 2.6	16.4 ± 2.5
Pattern reasoning	9.0 (4.0, 13.0)	11.0 (6.0, 14.0)	10.0 (5.0, 14.0)
Verbal fluency, animals	11.9 ± 3.2	12.2 ± 3.4	12.0 ± 3.3
First names	14.7 ± 4.1	17.4 ± 5.3	16.1 ± 4.9
Kohs block design	76.9 (63.7, 88.0)	76.2 (63.1, 88.4)	76.6 (63.3, 88.4)
Coding-WISC-III	30.1 ± 7.5	35.0 ± 8.0	32.7 ± 8.1
At birth			
Gestational age, *wk*	39.1 ± 1.7	39.5 ± 1.5	39.3 ± 1.6
Birth weight, *kg*	2.908 ± 0.471	2.840 ± 0.437	2.873 ± 0.454
Head circumference, *cm*	34.1 ± 1.4	33.5 ± 1.3	33.8 ± 1.4
At the time of testing			
Age, *y*	9.7 ± 0.3	9.7 ± 0.3	9.7 ± 0.3
BMI, *kg/m²*	14.5 ± 1.7	14.6 ± 2.0	14.6 ± 1.8
BMI, *WHO Z-score*	−1.3 ± 1.2	−1.2 ± 1.2	
Height, *cm*	131.1 ± 5.5	130.3 ± 6.0	130.7 ± 5.8
Height, *WHO Z-score*	−0.6 ± 0.8	−0.8 ± 0.9	
Head circumference, *cm*	50.7 ± 1.4	50.5 ± 1.5	50.6 ± 1.4
Plasma vitamin B-12, *pmol/L*	338.9 ± 160.1	351.3 ± 169.5	345.4 ± 165.0
Low vitamin B-12, *n (%)*	5 (2.1)	8 (3.1)	13 (2.6)
Plasma folate, *nmol/L*	28.6 ± 15.3	29.1 ± 14.8	28.8 ± 15.0
Low folate, *n (%)*	0 (0.0)	1 (0.4)	1 (0.2)
Parents' status			
Standard of living index, score	36.1 ± 7.9	36.3 ± 8.6	36.2 ± 8.3
Maternal education, *n (%)*			
<10 y completed	106 (41.1)	88 (31.8)	194 (36.3)
−10 y completed	74 (28.7)	91 (32.9)	165 (30.8)
>10 y completed	78 (30.2)	98 (35.4)	176 (32.9)
Paternal education, *n (%)*			
<10 y completed	105 (40.7)	100 (36.1)	205 (38.3)
−10 y completed	92 (35.7)	116 (41.9)	20 (38.9)
>10 y completed	61 (23.6)	61 (22.0)	122 (22.8)
Residence, *n (%)*			
Rural	77 (29.7)	70 (25.3)	147 (27.4)
Urban	182 (70.3)	207 (74.7)	389 (72.6)
Religion, *n (%)*			
Hindu[2]	145 (56.0)	164 (59.2)	309 (57.7)
Muslim[3]	90 (34.8)	92 (33.2)	182 (34.0)
Christian[4]	24 (9.2)	21 (7.6)	45 (8.4)

[1] Values are mean ± SD or median (interquartile range) or *n* (%).
[2] Maternal folate and Vitamin B-12 deficiency among Hindus (3.2 and 50.5%, respectively).
[3] Maternal folate and Vitamin B-12 deficiency among Muslims (6.0 and 30.2%, respectively).
[4] Maternal folate and Vitamin B-12 deficiency among Christians (2.2 and 37.8%, respectively).

TABLE 2 Associations of covariates or confounders with maternal plasma folate and vitamin B-12 concentrations

Covariates/confounders	β[1] (95% CI)[2,3]		
	Maternal folate	Maternal vitamin B-12	Maternal homocysteine
	nmol/L	*pmol/L*	*μmol/L*
Maternal age, *y*	0.18 (−0.20, 0.57)	−0.004 (−0.01, 0.004)	0.004 (−0.002, 0.01)
Maternal parity, 0, 1, and ≥2	−3.8 (−5.7, −1.87)***	0.003 (−0.04, 0.04)	0.01(−0.02, 0.03)
Maternal BMI in pregnancy, *kg/m²*	2.75 (−8.26, 13.76)	−0.56 (−0.79, −0.33)***	0.05 (−0.10, 0.21)
Birth weight, *kg*	3.89 (0.28, 7.50)*	−0.01(−0.09, 0.06)	−0.08 (−0.13, −0.03)**
Head circumference at birth, *cm*	1.29 (0.10, 2.49)*	−0.02 (−0.04, 0.01)	−0.01 (−0.03, 0.002)
Child's BMI at 9.5 y, *kg/m²*	1.14 (0.25, 2.03)*	−0.01(−0.03, 0.01)	−0.0004 (−0.01, 0.01)
Child's head circumference at 9.5 y, *cm*	2.11 (0.97, 3.26)***	−0.01 (−0.04, 0.01)	−0.002 (−0.02, 0.01)
Standard of living index, *score*	0.54 (0.35, 0.73)***	0.0004 (−.004, 0.005)	−0.002 (−0.004, 0.001)
Maternal education, *y completed*	1.05 (0.58, 1.51)***	−0.007 (−0.02, 0.003)	0.001 (−0.006, 0.008)
Paternal education, *y completed*	0.96 (0.60, 1.32)***	−0.007 (−0.01, 0.001)	0.001 (−0.004, 0.006)
Residence: urban 0, rural 1	−1.79 (−5.5, 1.88)	−0.05 (−0.13, 0.03)	−0.01 (−0.06, 0.04)
Religion: Hindu vs. Muslim	−9.3 (−12.8, −5.9)***,†	0.12 (0.05, 0.20)**,†	−0.11 (−0.16, −0.06)***,†
Hindu vs. Christian	0.62 (−5.26, 6.50)	0.13 (0.004, 0.26)*,†	−0.06 (−0.14, 0.03)

[1] β is the effect size on exposure (maternal plasma folate, vitamin B-12, and homocysteine concentrations) per unit change in covariates/confounders derived using multiple linear regression adjusted for the child's sex and current age and using all variables as continuous.
[2] *P* values (*$P < 0.05$; ** $P < 0.01$; $P < 0.001$***) derived using multiple linear regression adjusted for the child's sex and current age.
[3] *P*-value (†$P < 0.01$) derived using a test of general association (Wald) among categories of religion, adjusted for the child's sex and current age.

trations were associated with higher scores for verbal fluency-first names (β = 0.10; 95% CI: 0.02, 0.19; P = 0.02 in the fully adjusted model).

There were no significant interactions between maternal folate and vitamin B-12 groups in relation to cognitive scores. All the cognitive test scores tended to increase with increasing thirds of folate concentrations in children of mothers with low as well as normal vitamin B-12 status (data not shown).

The effects of maternal folate and vitamin B-12 concentrations on cognitive scores were similar in boys and girls except for the association of maternal folate with verbal fluency-first names, which was stronger among girls than boys (P for interaction = 0.003).

Discussion

To our knowledge, no previous study has examined associations between maternal plasma folate, vitamin B-12, and tHcy concentrations during pregnancy and cognitive performance in children in a large unselected sample of healthy mothers and children. We found that in our sample, there was a high prevalence of low vitamin B-12 concentrations (43%), whereas few women had low folate concentrations (4%). Higher maternal folate concentration was associated with better cognitive performance in the children. This effect occurred across the whole range of maternal folate concentrations, with no apparent threshold at the level used to define deficiency. The associations with learning ability and long-term storage and retrieval, visuo-spatial ability, and attention and concentration were independent of all the confounding factors measured and of the children's current folate and vitamin B-12 status. There were no consistent associations between maternal vitamin B-12 or tHcy concentrations and cognitive ability. There were no interactions between maternal folate and vitamin B-12 groups in relation to cognitive scores.

Strengths of this study were that in a large sample of children, we measured a battery of cognitive function tests specifically adapted for, and validated in, a South Indian population and also collected data on a range of important confounding factors. A limitation was that maternal micronutrient assays were performed using plasma samples stored for 8 y. However, vitamin B-12 and folate have been shown to be stable following long-term storage at lower temperatures (46). The interpretation of the concentrations of these vitamins in pregnancy is complicated because of hemodilution, raised glomerular filtration rate, and complex physiological storage mechanisms for both vitamins (especially vitamin B-12) resulting in lower concentrations of these vitamins during pregnancy (8,47). There is no universally agreed-to cut-off value to define deficiency in pregnancy; however, our definition has been used in earlier studies (48). Other limitations were a lack of data on maternal diet, the use of folate and vitamin B-12 supplements at the time of sample collection, and a lack of information on parental IQ and the home environment.

A high prevalence of low vitamin B-12 concentrations has been observed in earlier studies in India (9–13). Consistent with these, 43% of the women in our study had low vitamin B-12 status. Animal products are the main dietary source of vitamin B-12 and the lowest concentrations were found among Hindus, who are mainly lacto-vegetarians (with milk and milk products being the main nonvegetarian food source). Despite being nonvegetarians, 30% Muslims and 37% Christians also had low vitamin B-12 concentrations, probably due to poor economic conditions. In comparison to rural women in Pune, India, fewer Mysore women had low vitamin B-12 concentrations (Mysore, 43% vs. Pune, 71%) (13). This is probably due to a higher proportion of Muslim women, higher consumption of nonvegetarian food, and possibly higher intakes of fermented foods like idli, dosa, and yogurt, which are commonly eaten in southern India and which favor bacterial vitamin B-12 production. Few (4%) women had low folate concentrations; the lowest concentrations were found among Muslims, who are mainly nonvegetarians with low vegetable intakes. Apart from dietary factors, adiposity (higher BMI) has been shown to be associated with lower micronutrient concentrations, possibly due to disruption in absorption, higher excretion, fat sequestra-

TABLE 3 Association of covariates or confounders with children's cognitive function score

Covariates/confounders	Atlantis	Word order	Pattern reasoning	Verbal fluency, animals	Verbal fluency, first names	Kohs block design	Coding-WISC-III
				β[1] (95% CI)[2,3]			
Maternal age, *y*	0.35 (0.01, 0.70)*	0.05 (−0.003, 0.10)	0.03 (0.01, 0.06)**	0.04 (−0.03, 0.11)	0.03 (−0.07, 0.12)	0.08 (0.003, 0.01)**	0.16 (0.01, 0.31)*
Maternal parity, 0, 1 and 2	−2.2 (−3.9, −0.50)*	−0.35 (−0.60, −0.10)**	−0.16 (−0.27, −0.05)**	−0.40 (−0.73, −0.07)*	−0.36 (−0.83, 0.11)	−0.01 (−0.04, 0.01)	0.006 (−0.75, 0.76)
Maternal BMI in pregnancy, *kg/m²*	4.35 (−5.48, 14.20)	0.05 (−1.40, 1.50)	0.28 (−0.33, 0.89)	0.61 (−1.29, 2.50)	−0.51 (−3.21, 2.18)	0.09 (−0.03, 0.22)	3.67 (−0.65, 7.99)
Birth weight, *kg*	3.66 (0.43, 6.88)*	0.46 (−0.02, 0.93)	0.24 (0.04, 0.44)*	0.52 (−0.11, 1.14)	0.10 (−0.078, 0.99)	0.07 (0.03, 0.12)***	1.34 (−0.08, 2.76)
Head circumference at birth, *cm*	1.65 (0.59, 2.72)**	0.22 (0.06, 0.37)**	0.08 (0.01, 0.14)*	0.14 −0.07, 0.34)	0.14 (−0.16, 0.43)	0.03 (0.01, 0.04)***	0.35 (−0.12, 0.83)
Child's BMI at 9.5 y, *kg/m²*	1.77 (0.99, 2.55)***	0.19 (0.08, 0.31)**	0.11 (0.06, 0.16)***	0.32 (0.17, 0.47)***	0.32 (0.10, 0.54)**	0.01 (0.001, 0.02)*	0.69 (0.34, 1.04)***
Child's head circumference at 9.5 y, *cm*	2.49 (1.48, 3.50)***	0.39 (0.24, 0.54)***	0.15 (0.09, 0.21)***	0.37 (0.17, 0.57)***	0.39 (0.11, 0.67)**	0.03 (0.01, 0.04)***	0.96 (0.51, 1.41)***
Child's current educational level, *y*	0.57 (−1.8, 3.03)	0.32 (−0.04, 0.68)	0.14 (−0.008, 0.30)	0.10 (0.37, 0.58)	0.47 (−0.21, 1.14)	0.02 (−0.01, 0.05)	1.21 (0.14, 2.29)*
Child's folate at 9.5 y, *nmol/L*	0.11 (0.01, 0.21)*	0.01 (−0.002, 0.03)	0.01 (0.003, 0.02)**	0.02 (0.01, 0.04)*	0.06 (0.03, 0.08)***	0.002 (0.0003, 0.003)*	0.03 (−0.02, 0.07)
Child's vitamin B-12 at 9.5 y, *pmol/L*	0.001 (−0.08, 0.01)	0.0003 (−0.001, 0.002)	−0.0002 (−0.001, 0.0003)	−0.001 (−0.002, 0.001)	−0.001 (−0.003, 0.002)	0.00001 (−0.0001, 0.0001)	−0.004 (−0.01, 0.0003)
Standard of living index, *score*	0.46 (0.29, 0.64)***	0.07 (0.04, 0.09)***	0.03 (0.02, 0.05)***	0.08 (0.05, 0.12)***	0.11 (0.06, 0.15)***	0.01 (0.004, 0.008)***	0.19 (0.11, 0.26)***
Maternal education, *y completed*	1.02 (0.61, 1.44)***	0.20 (0.14, 0.26)***	0.08 (0.06, 0.11)***	0.19 (0.11, 0.27)***	0.24 (0.13, 0.36)***	0.02 (0.01, 0.02)***	0.43 (0.25, 0.62)***
Paternal education, *y completed*	0.89 (0.57, 1.21)***	0.12 (0.08, 0.17)***	0.07 (0.05, 0.09)***	0.14 (0.08, 0.21)***	0.18 (0.09, 0.27)**	0.01 (0.008, 0.02)***	0.35 (0.21, 0.50)***
Residence: urban 0, rural 1	6.8 (3.62, 10.07)***	0.70 (0.22, 1.18)**	0.34 (0.14, 0.54)**	0.08 (−0.56, 0.71)	0.03 (−0.87, 0.93)	0.07 (0.03, 0.11)**	0.67 (−0.77, 2.11)
Religion: Hindu vs. Muslim	−1.86 (−4.99, 1.26)	−0.23 (−0.70, 0.23)	−0.40 (−0.60, −0.21)***,†	−1.51 (−2.10, −0.92)***,†	−2.99 (−3.82, −2.17)***,†	−0.05 (−0.09, 0.01)**,†	−2.46 (−3.82, −1.10)***,†
Hindu vs. Christian	8.03 (2.69,13.40)**,†	0.61 (-0.19, 1.40)	0.40 (0.08, 0.73)*,†	1.16 (0.15, 2.16)*,†	0.22 (−1.19, 1.63)	0.11 (0.05, 0.18)**,†	2.74 (0.41, 5.07)*,†

[1] β is the effect size on cognitive scores per unit change in covariates/confounders, derived using multiple linear regression adjusted for the child's sex and current age, and using all variables as continuous.
[2] *P* values (* $P < 0.05$; ** $P < 0.01$; *** $P < 0.001$) derived by multiple linear regression adjusted for the child's sex and current age.
[3] *P* value († $P < 0.01$ derived using a test of general association (Wald) among categories of religion, adjusted for the child's current age and sex.

tion, increased catabolism, and lower dietary intakes (49,50). Our finding of no significant associations between intake of vitamin supplements and vitamin concentrations is possibly due to a lack of complete information on supplement intake, because our study was not originally designed to examine maternal folate and vitamin B-12 status and we recorded intake of vitamin supplements only at the time of recruitment. Among women recruited between 24 and 32 wk gestation, very few were taking supplements. Women who were taking supplements in early pregnancy might have stopped taking them by 30 wk and women who were not taking supplements at recruitment may have been prescribed them later in pregnancy.

We found that the children's cognitive performance increased with increasing maternal folate concentrations across the whole range. However, there were no associations at the level of folate deficiency, although cognitive scores were lower in children of mothers with low compared with normal folate concentrations, probably because of a lack of statistical power, as the low-folate status group was small. A recent study from Birmingham, Alabama, reported no association between maternal folate status at 37 wk gestation and mental or psychomotor development of the children at 5 y (51). In this study, 14.0% of mothers had poor folate status (plasma folate concentrations $\leq$ 11.0 nmol/L). It was carried out among socially disadvantaged families and the author concluded that the severity of socioeconomic factors overwhelmed any effect of maternal folate status in the first 5 y of life. Animal studies examining maternal folate status during pregnancy and neurobehavioral development in the offspring have reported a positive relationship (19,20). Rats born to dams fed on a diet low in folate during pregnancy and reared on the same diet postnatally demonstrated poor maze-learning ability (20) and adverse effects on the developing brain were found among rats born to folate-deficient dams (19). Data from human studies are limited. A case study reported abnormal or delayed development in infants born to mothers with severe folate deficiency (megaloblastic anemia) during pregnancy (3). Another recent study, based on maternal first trimester dietary data, found that a low dietary intake of folate (<400 μg/d) was associated with a lower mental development index only among children of genetically susceptible mothers [carriers of the TT genotype (MTHFR*677C > T*)] (21). Thus, most of the published literature on this topic has examined the effects of maternal deficiency. If the associations in our study are causal, our data suggests that, in terms of cognitive development, the concentration used to define folate deficiency is set too low.

TABLE 4 Associations between maternal plasma folate and vitamin B-12 concentrations during pregnancy and children's cognitive performance[1]

	n	Atlantis	Word order	Pattern reasoning	Verbal fluency, animals	Verbal fluency, first names	Koh's block design	Coding-WISC-III
Maternal folate status					*Score*			
Normal (>7 nmol/L)	514	67.9 ± 17.0	16.4 ± 2.5	10.0 (5.0, 14.0)	12.1 ± 3.3	16.1 ± 4.9	76.9 (63.7, 88.3)	32.7 ± 8.1
Low (<7 nmol/L)	22	60.7 ± 20.4	16.4 ± 3.0	5.0 (3.0, 13.0)	11.2 ± 4.0	17.5 ± 5.5	71.0 (57.2, 90.3)	31.3 ± 7.8
					β[2] *(95% CI)*			
Model 1[3]		−0.40 (−0.83, 0.03)	0.01 (−0.41, 0.44)	−0.37 (−0.80, 0.05)	−0.25 (−0.68, 0.18)	0.32 (−0.10, 0.73)	−0.22 (−0.65, 0.21)	−0.10 (−0.50, 0.30)
Model 2[4]		−0.33 (−0.74, 0.08)	0.04 (−0.37, 0.45)	−0.32 (−0.72, 0.08)	−0.21 (−0.63, 0.22)	0.33 (−0.07, 0.74)	−0.20 (−0.61, 0.24)	−0.13 (−0.53, 0.26)
Model 3[5]		−0.32 (−0.73, 0.09)	0.12 (−0.31, 0.54)	−0.23 (−0.64, 0.19)	−0.13 (−0.56, 0.31)	0.22 (−0.19, 0.64)	−0.14 (−0.57, 0.28)	−0.02 (−0.38, 0.42)
Maternal folate in quartiles, *nmol/L*					*Score*			
≤17.4	134	63.5 ± 17.3	16.1 ± 2.3	8.0 (4.0, 13.0)	11.1 ± 3.3	14.9 ± 4.7	74.0 (62.0, 85.2)	30.4 ± 7.6
17.5–33.9	134	67.3 ± 17.1	16.2 ± 2.4	9.0 (4.0, 14.0)	12.1 ± 3.2	15.9 ± 4.4	73.1 (57.7, 83.4)	32.6 ± 8.3
34–50.6	134	66.9 ± 16.8	16.7 ± 2.5	10.0 (6.0, 14.0)	12.3 ± 3.1	16.6 ± 4.9	77.2 (64.8, 88.7)	33.4 ± 8.1
≥50.7	134	72.7 ± 16.4	16.8 ± 2.9	11.0 (7.0, 15.0)	12.6 ± 3.5	16.9 ± 5.4	81.9 (71.0, 92.6)	34.3 ± 8.1
					β[6] *(95% CI)*[7]			
Model 1[3]		0.17 (0.08, 0.25)***	0.10 (0.02, 0.18)*	0.16 (0.07, 0.24)***	0.17 (0.09, 0.26)***	0.15 (0.07, 0.24)***	0.17 (0.08, 0.25)***	0.17 (0.09, 0.25)***
Model 2[4]		0.10 (0.01, 0.18)*	0.01 (−0.07, 0.10)	0.06 (−0.03, 0.14)	0.10 (0.01, 0.19)*	0.09 (0.003, 0.17)*	0.09 (0.005, 0.18)*	0.12 (0.04, 0.20)**
Model 3[5]		0.10 (0.01, 0.19)*	−0.001 (−0.09, 0.09)	0.05 (−0.03, 0.14)	0.06 (−0.03, 0.15)	0.08 (−0.003, 0.17)	0.10 (0.01, 0.19)*	0.10 (0.02, 0.18)*
Maternal vitamin B-12 status					*Score*			
Normal (>150 pmol/L)	308	66.3 ± 17.5	16.3 ± 2.5	9.0 (4.0, 13.0)	11.8 ± 3.4	15.7 ± 4.5	76.8 (63.6, 87.7)	32.3 ± 8.0
Low (<150 pmol/L)	228	69.3 ± 16.7	16.6 ± 2.6	10.5 (6.0, 14.0)	12.4 ± 3.2	16.7 ± 5.4	76.5 (63.1, 89.3)	33.2 ± 8.2
					β[2] *(95% CI)*[7]			
Model 1[3]		0.17 (−0.001, −0.34)	0.12 (−0.05, 0.29)	0.14 (−0.03, 0.31)	0.20 (0.02, 0.37)*	0.16 (−0.004, 0.33)	0.01 (−0.16, 0.19)	0.06 (−0.10, 0.22)
Model 2[4]		0.15 (−0.02, 0.32)	0.09 (−0.09, 0.26)	0.06 (−0.11, 0.23)	0.15 (−0.02, 0.33)	0.09 (− 0.08, 0.26)	−0.06 (−0.23, 0.12)	0.01 (−0.16, 0.17)
Model 3[5]		0.12 (−0.05, 0.30)	0.03 (−0.15, 0.22)	0.03 (−0.14, 0.21)	0.19 (0.01, 0.37)*	0.09 (−0.09, 0.27)	−0.06 (−0.24, 0.12)	−0.007 (−0.18, 0.16)
Maternal vitamin B-12 in quartiles, *pmol/L*					*Score*			
≤124	138	69.6 ± 16.9	16.5 ± 2.6	10.5 (6.0, 15.0)	12.4 ± 3.3	16.6 ± 4.0	76.6 (63.0, 88.3)	33.2 ± 7.8
125–162	130	69.0 ± 17.1	16.5 ± 2.5	10.0 (7.0, 13.0)	12.2 ± 3.4	16.3 ± 5.5	73.4 (62.5, 87.8)	33.3 ± 8.8
163–220	134	64.6 ± 16.8	16.3 ± 2.4	9.0 (5.0, 13.0)	11.5 ± 2.8	15.4 ± 4.3	77.2 (66.3, 88.9)	31.8 ± 8.3
>220	134	67.2 ± 17.7	16.3 ± 2.6	10.0 (4.0, 14.0)	12.2 ± 3.7	16.1 ± 4.7	77.2 (62.5, 88.4)	32.4 ± 7.6
					β[6] *(95% CI)*			
Model 1[3]		−0.08 (−0.17, 0.004)	−0.03 (−0.11, 0.06)	−0.07 (−0.16, 0.01)	−0.04 (−0.13, 0.04)	−0.04 (−0.12, 0.05)	−0.01 (−0.10, 0.07)	−0.04 (−0.12, 0.04)
Model 2[4]		−0.08 (−0.17, 0.0004)	−0.02 (−0.10, 0.07)	−0.05 (−0.13, 0.03)	−0.03 (−0.12, 0.06)	−0.003 (−0.09, 0.08)	0.01 (−0.08, 0.09)	−0.03 (−0.11, 0.06)
Model 3[5]		−0.08 (−0.17, 0.009)	0.007 (−0.08, 0.10)	−0.03 (−0.12, 0.06)	−0.05 (−0.14, 0.04)	−0.006 (−0.09, 0.08)	0.02 (−0.07, 0.11)	−0.02 (−0.11, 0.06)

[1] Values are mean ± SD or median (interquartile range) unless otherwise stated.
[2] β is the effect size (SD) on outcome (cognitive scores) per unit change in maternal folate or vitamin B-12 status derived by multiple linear regression.
[3] Model 1 adjusted for child's sex and age at the time of study.
[4] Model 2 model 1 parameter + gestational age, SES, parent's education, religion, rural/urban residence, parity, maternal pregnancy age, and BMI, child's newborn weight and head circumference.
[5] Model 3 adjusted for model 2 parameters + children's current head circumference, BMI, education, and vitamin B-12 and folate concentrations at 9.5 y.
[6] β is the effect size (SD) on outcome (cognitive scores) per SD increase in maternal plasma folate and vitamin B-12 concentration derived by multiple linear regression.
[7] *P*-values (*$P < 0.05$; **$P < 0.01$; ***$P < 0.001$) derived by multiple linear regression.

In our study, neither maternal vitamin B-12 status (low vs. normal) nor the range of vitamin B-12 concentrations was associated with cognitive performance in the children, except that verbal ability scores were higher in children of mothers with low vitamin B-12 compared with children of mothers with normal concentrations. Data on maternal vitamin B-12 deficiency and childhood cognitive development come mainly from case reports of maternal deficiency and small observational studies. Infants of mothers with untreated anemia and a strict vegetarian lifestyle were irritable, anorexic, and failed to thrive (1,2,22,23). In The Netherlands, infants of macrobiotic mothers had delayed motor and language development compared with infants of omnivores (23) and scored lower at age 12 y, even though their current diet contained the recommended daily intake of vitamin B-12 (52). A recent study in Mexico reported that deficient maternal dietary intakes of vitamin B-12 in the first trimester were associated with impaired mental development in early childhood (21). Comparing our study with these studies is difficult due to differing age groups of children and different measures of nutritional status. In contrast to our findings, a recent study in Pune, India reported that children of mothers with very low plasma vitamin B-12 concentrations (<77 pmol/L) during pregnancy performed poorly in tests of sustained attention and short-term memory compared with children of mothers with high (>224 pmol/L) vitamin B-12 concentrations (24). None of our mothers had such extremely low vitamin B-12 concentrations, which may explain the difference in findings.

In our study, cognitive scores were positively related to maternal folate concentrations even in children of mothers with low vitamin B-12 concentrations. A study in an elderly population in the US (25) reported that normal/high folate status in individuals with vitamin B-12 deficiency was associated with

anemia and cognitive impairment. It was suggested that folate may have damaging effects on cognitive function in individuals with low vitamin B-12 status. We did not find evidence for such an effect in the children in our study.

There are few data on maternal homocysteine and offspring cognitive function. Consistent with our findings of a positive association between maternal homocysteine and the children's verbal fluency, a recent study in the US reported that, at age 5 y, children of mothers with high plasma tHcy (>7.0 μmol/L; 8.4%) at 26 wk of gestation had better manual dexterity than children of mothers with normal homocysteine (51). In contrast, an animal study in Turkey demonstrated poor memory and maze-learning ability in adult offspring born to rats with methionine-induced hyperhomocysteinemia during pregnancy (53).

Mechanisms linking folate and vitamin B-12 status with neurocognitive development and function are not well understood. Shared metabolism between folate and vitamin B-12 means that deficiency of one vitamin may alter the metabolism of the other. Possible mechanisms by which deficiencies could interfere with brain development include delayed myelination or demyelination of nerves, altering methionine synthesis from homocysteine, imbalance in tissue levels of neurotransmitters (neurotrophic and neurotoxic cytokines), and/or accumulation of lactate in brain cells (1,2,22). The functional consequences could vary depending on the specific nutrient deficiency and its timing relative to the processes of development, which starts in early gestation and continues throughout childhood.

In conclusion, in this Indian population, we found that maternal plasma folate concentrations were mainly in the normal range, but they strongly predicted cognitive function in the children independently of a range of potential confounding factors. The data suggest that folate may be required not only for the prevention of neural tube defects but also for optimal early brain growth and cognitive development. It also suggests that, in terms of neurodevelopment, current definitions of folate deficiency may be set too low. In contrast, although many mothers had low vitamin B-12 concentrations, maternal vitamin B-12 and homocysteine concentrations were largely unrelated to the children's cognitive function. It is possible that only extremely low maternal vitamin B-12 status may result in cognitive impairment and/or that this population has adapted over centuries to low intakes of vitamin B-12. Our findings add to the few reports on this topic and more studies from different populations are required.

Acknowledgments

S.R.V. designed and conducted research, data analysis, and interpretation of the data and drafted the manuscript; G.V.K. recruited the mothers, carried out maternal and newborn measurements, and assisted in the manuscript preparation; A.K.W. assisted in statistical analysis, data interpretation, and drafting of the manuscript; A.V.K., K.S., and S.M. helped in designing cognitive function assessment and also in manuscript preparation; C.S.Y. helped in interpretation of the data and drafting of the manuscript; C.H.D.F. designed the baseline and follow-up study, contributed to the data interpretation and drafting of the manuscript and had primary responsibility for final content. All authors read and approved the final manuscript.

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