Maternal intake of methyl-donor nutrients and child cognition at 3 years of age

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SUMMARY

Methyl-donor nutrients are substrates for methylation reactions involved in neurodevelopment processes. The role of maternal intake of these nutrients on cognitive performance of the offspring is poorly understood. We examined the associations of maternal intake of folate, vitamin B12, choline, betaine, and methionine during the first and second trimesters of pregnancy, with tests of cognitive performance in the offspring at 3 y of age using data from 1210 participants in Project Viva, a prospective pre-birth cohort study in Massachusetts. We assessed nutrient intake with the use of food frequency questionnaires. Children's cognition at age 3 y was evaluated with the Peabody Picture Vocabulary Test III (PPVT-III) and visual-motor skills with the Wide Range Assessment of Visual Motor Abilities (WRAVMA) test. In multivariable models adjusting for potential sociobehavioral and nutritional confounders, for each 600 μg/d increment in total folate intake during the first trimester, PPVT-III score at age 3 y was 1.6 points [95% CI: 0.1, 3.1; $P = 0.04$] higher. There was a weak inverse association between vitamin B12 intake during the second trimester and PPVT-III scores [−0.4 points per 2.6 μg/d; 95% CI: −0.8, −0.1; $P = 0.01$]. We did not find associations between choline, betaine, or methionine and cognitive outcomes at this age. Results of this study suggest that higher intake of folate in early pregnancy is associated with higher scores on the PPVT-III, a test of receptive language that predicts overall intelligence, at age 3 y.

Keywords

folate; vitamin B12; choline; methionine; pregnancy; cognition; children

INTRODUCTION

The development and normal functioning of the central nervous system depends on methylation reactions in several processes including myelination, production of monoamine neurotransmitters, dendritic arborization, and synaptic plasticity.[1, 2] These reactions involve the donation of methyl groups by S-adenosylmethionine (SAM) to various substrates. The production of SAM, which is generated from methionine, partly depends on the availability

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of methyl donor nutrients from the diet. These nutrients include folate and vitamin B12, which participate in the generation of methionine via the methionine-synthase pathway; and choline and betaine, which are substrates in the betaine:homocysteine methyltransferase pathway.

Epidemiological investigations suggest that inadequate intake of methyl donor nutrients may be associated with impaired neurocognitive performance in children.[3, 4] Longitudinal studies in adolescents have found inverse associations between vitamin B12 status and measures of neurocognitive performance,[5, 6] and vitamin supplements containing folate and vitamin B12 might have a beneficial effect on cognition among people with low intake of these nutrients.[7]

The role of methyl donor nutrients on neurocognitive function may be particularly relevant at early stages of development; yet relatively few studies have examined the associations between maternal status of these nutrients and cognitive performance in the offspring. Low dietary intake of folate in early pregnancy was related to behavioral problems among 4,214 infants in the Netherlands[8] and in 100 school-age children from the UK,[9] but possible effects on measures of cognition were not examined. In addition, studies from Spain[10] and Mexico[11] indicated that maternal folate status could be related to improved mental development of the infants. One potential limitation of these studies is lack of control for potential confounding by nutritional or other factors that are important for neurobehavioral development. In addition, while these studies focused on folate, the potential effects of other methyl donors such as maternal choline, betaine, and methionine intake on the offspring's cognitive performance have not been researched in humans.

We examined the associations of maternal dietary intake of methyl donor nutrients including folate, vitamin B12, choline, betaine, and methionine during the first and second trimesters of pregnancy with tests of cognitive performance in the offspring at age 3 y among participants of Project Viva, a prospective pre-birth cohort study.

METHODS

Study population

We conducted analyses among participants of Project Viva, an observational longitudinal study designed to examine pregnancy and child health outcomes in relation to multiple prenatal exposures. Detailed descriptions of the cohort have been published before.[12] In brief, we invited pregnant women who were receiving care at eight offices from a large group practice in eastern Massachusetts (Harvard Vanguard Medical Associates) to enroll at their first prenatal visit. Exclusion criteria were multiple pregnancy, inability to answer questions in English, planning to move out of the study area before delivery, and gestational age >22 completed weeks. At the time of recruitment, we collected data on sociodemographic and anthropometric characteristics and health status through a brief interview and a take-home self-administered questionnaire. This information included maternal race/ethnicity, age, smoking during pregnancy, pre-pregnancy weight, height, and education, and parity, father's education, child's sex and primary language, and household income. In addition, the interview included detailed questions regarding intake of nutritional supplements (frequency, timing, brand/type, and dosage) from 3 months before the participant learned she was pregnant until the interview. The self-administered questionnaire included a 166-item semi-quantitative food-frequency questionnaire (FFQ) slightly modified for use in pregnancy from a commonly used adult FFQ,[13] to assess the woman's diet since her last menstrual period (first trimester FFQ). During a second study visit at 26–28 weeks' gestation, participants completed another FFQ to assess dietary intake during the previous three months (second trimester FFQ). The second trimester FFQ was the same as the first

trimester except for a briefer assessment of nutritional supplements, which was included in the FFQ. Further details of the FFQ have been published.[14]

We obtained information on neurocognitive outcomes of the children at a follow-up visit that was scheduled at 3 y postpartum. Among 2128 women with live births, 1579 were eligible for the 3-y follow-up by virtue of having completed at least one of the FFQs during pregnancy, and having consented for the children's follow-up. At age 3 y, 1292 children (82%) underwent in-person examinations. During that visit, research assistants administered the Peabody Picture Vocabulary Test III (PPVT-III) and the Wide Range Assessment of Visual Motor Abilities (WRAVMA) following standardized protocols. They also administered the adult version of PPVT-III to the mothers. The PPVT-III is a test of receptive language that strongly correlates with tests of intelligence.[15] The WRAVMA[16] assesses visual-motor, visual-spatial, and fine motor abilities through line drawing, picture-matching, and pegboard subtests. These tests were completed by 1210 children.

Of the 1210 children with neurocognitive assessments at age 3 y and data on maternal diet, 1148 had first trimester and 1083 had second trimester maternal intake data. Compared to the 369 children who were eligible but did not complete the cognitive evaluations, the 1210 participants in this analysis were more likely to have mothers of white race (74% v. 53%), or who had college or graduate education (72% v. 54%).

Data analysis

Definition of exposures—Primary exposures of interest were average daily intake of folate, vitamin B12, betaine, choline, and methionine during the first and second trimesters of pregnancy, as estimated from the FFQs. We estimated total intake of these nutrients (from foods and supplements) by multiplying the frequency of intake of standard portion sizes of each food item or supplements times its nutrient content, according to the Harvard nutrient composition database used for several large cohort studies.[17, 18] Next, we adjusted intake of each nutrient by total energy intake, using the nutrient residuals method.[19] We separately considered intake of each nutrient during the first and second trimesters. In addition, we considered peri-conceptional intake of folate and vitamin B12 from supplements only, by using the information collected in the detailed baseline interview that inquired about supplement use between the last menstrual period date and 4 wk of gestation.

Definition of outcomes—Primary outcomes were PPVT-III and total WRAVMA scores at age 3 y. Secondary endpoints were scores for the line drawing, picture-matching, and pegboard subtests of WRAVMA. We treated all neurocognitive outcomes as continuous variables. Each has a standardized population mean of 100 and SD of 15.

Analyses—We estimated unadjusted mean changes in cognitive scores per unit of daily intake of each nutrient with the use of linear regression models. For folate, vitamin B12, and choline, we used the recommended dietary allowances (RDA) during pregnancy[20] as the daily intake unit to estimate the corresponding change in the outcomes; whereas for betaine and methionine, we used the value of one standard deviation of the distribution in the study sample because RDA have not been set. Next, we estimated adjusted mean changes and 95% confidence intervals (CI) with the use of multivariable linear regression models in which each score was the outcome and predictors included all five methyl-donor nutrients of interest in the model at the same time plus potential confounders including maternal race/ ethnicity, age, parity, smoking during pregnancy, prepregnancy body mass index, PPVT-III score, education, total energy, fish, and iron intake; paternal education; household income; and child's sex, and primary language. Models were run separately for each trimester of pregnancy. In addition, we ran models for peri-conceptional intake of folate and vitamin

B12 from supplements. We also explored interactions between early folate and vitamin B12 intake on the cognitive endpoints by including into the models a cross-product term between the nutrients. Because the use of nutrient intake values as continuous predictors assumes that the associations with the outcomes follow a linear shape, we conducted supplemental analyses in which nutrients were divided into quartiles of the population distribution. Since the results were consistent with a linear relation, we present results only with continuous measures of nutrient exposures. All analyses were carried out with the use of Statistical Analysis System software (version 9.2; SAS Institute, Inc).

RESULTS

Characteristics of women with data on dietary intake during the first or second trimester are presented in Table 1. Correlations between intake of the methyl donor nutrients during the first and second trimesters were 0.32, 0.19, 0.55, 0.51, 0.53 for folate, vitamin B12, choline, betaine, and methionine, respectively. Only folate intake increased substantially from first to second trimesters. Correlations between the methyl donor nutrients were relatively low, except for that of choline and methionine. During the first trimester, the largest Pearson correlation coefficients were as follows: choline and methionine = 0.75; folate and betaine = 0.27; folate and vitamin B12 = 0.14. All others were <0.10. During the second trimester, Pearson correlations were: choline and methionine = 0.70; choline and vitamin B12 = 0.26; folate and betaine = 0.21; folate and vitamin B12 = 0.18. Others were <0.17. Mean (SD) PPVT-III and total standardized WRAVMA scores at age 3 y were 103.9 (14.3) and 102.3 (11.2), and mean scores for the WRAVMA drawing, matching, and pegboard subtests were 99.4 (11.2), 108.0 (13.6), and 98.4 (10.7), respectively.

In unadjusted analyses, intake of folate and betaine during the first or second trimesters was directly related to PPVT-III scores (Table 2). After adjustment for potential socioeconomic, educational, and dietary confounders, however, the associations with both nutrients were attenuated and only the relation between folate intake during the first trimester and PPVT-III scores remained statistically significant (Table 2). Every increment of 600 μg/d of folate intake from foods and supplements during the first trimester was associated with a 1.6 point [95% CI: 0.1, 3.1; $P = 0.04$] higher PPVT-III score at age 3 y. The estimate for 600 μg/d folate from peri-conceptional supplements only was 1.2 points [95% CI: −0.1, 2.4; $P = 0.07$]; from supplements only during the first trimester, 1.7 points [95% CI: 0.2, 3.3; $P = 0.03$]; and from foods only during the first trimester, −0.2 points [95% CI: −4.1, 3.7; $P = 0.92$]. There was a weak, inverse association between vitamin B12 intake during the second trimester and PPVT-III score; every 2.6 μg/d increment was related to a 0.4 points [95% CI: −0.8, −0.1; $P = 0.01$] lower PPVT-III. There were no interactions between intakes of folate and vitamin B12. Intake of methyl donor nutrients during pregnancy was not associated with total WRAVMA standard scores (Table 2), or with the drawing, matching, or pegboard subtests (data not shown). Additional adjustment for maternal alcohol intake at each of the exposure periods considered yielded essentially identical results.

DISCUSSION

In this longitudinal study, maternal folate intake during early pregnancy was positively related to PPVT-III scores at age 3 y, independent of nutritional and sociobehavioral potential confounders. On the other hand, there was a small, inverse association between intake of vitamin B12 during the second trimester and the child's performance on the PPVT-III. We did not find associations between maternal intake of choline, methionine, or betaine and tests of cognitive performance in the offspring.

Some previous studies have suggested that higher intake of folate during early pregnancy could be associated with improved cognitive or behavioral performance in the offspring. For example, among Spanish women, intake of folic acid from supplements during the first trimester of pregnancy was associated with higher verbal and motor performance scores and lower risk of inattention symptoms in the offspring at 4 y of age, independent of sociodemographic characteristics and iron supplementation.[10] In the "Generation R" study of Rotterdam mothers and their children, intake of folate from supplements during the first 10 weeks of pregnancy was related to decreased risk of behavioral problem syndromes (emotionally reactive, anxious/depressed, somatic complaints, withdrawn, attention problems, and aggressive behavior) in the children at age 18 mo, after adjusting for maternal age, country of origin, education, and psychopathology.[8] Total dietary folate intake and erythrocyte folate concentrations during the first trimester of pregnancy were inversely related to scores of hyperactivity and peer problems in the offspring at 8 y of age in a cohort of Southampton, UK,[9] independent of maternal smoking and alcohol or iron intake. Among Mexican mothers with a polymorphism of the methylenetetrahydrofolate reductase enzyme, low total dietary intake of folate during the first trimester of pregnancy was related to lower mental development scores during the babies' first year of age.[11] Despite differences in the domains and test batteries of cognitive and behavioral function that have been examined across studies, our finding of a positive association between folate intake from foods and supplements during the first trimester and PPVT-III scores at 3 y is consistent with those reported from other settings. Because our analyses were adjusted for many important confounders, especially the mother's own PPVT-III score and sociodemographic characteristics including parity, the estimate of association between folate intake and PPVT-III is likely to be less biased than those reported before.

The association of folate intake and PPVT-III was observed for total folate intake and for folic acid from supplements but not for folate from foods alone. Mean folate intake from foods during the first trimester, 367 μg/d, was lower than the recommended 600 μg/d. When folic acid from supplements was considered, total folate intake increased to more than twice the mean value from foods alone. This suggests that a potential effect of folate on cognitive function may be observed only at high intake. This high level of intake might only be achievable through folic acid supplementation. Not only was intake from foods lower than that from supplements in this population, but the bioavailability of folate from foods is only about half that of the folic acid present in supplements [21,22]. Folic acid bioavailability from supplements may have been underestimated in comparison to that of folate from foods in this study, since the calculation of folate intake from supplements was not adjusted for differences in bioavailability between food and supplement sources.

We did not find associations of folate intake during the second trimester of pregnancy with cognitive outcomes. Previous observational studies of cognition in relation to maternal folate status in the second trimester reported protective effects;[23, 24] nevertheless, folate status indicators used in these studies could be surrogates of folate at earlier stages. Folate status after the first trimester was not associated with neurocognitive development measurements in a study of low income children from the US[25] and current evidence from intervention trials does not support an effect of folate alone administered after the first trimester of pregnancy on neurocognitive function.[26, 27] A potential effect of folate on cognition could be specific to early pregnancy. The biological mechanisms underlying this effect are speculatory; they could be related to folate-mediated alterations in DNA methylation that may result in differential expression of proteins related to production of neurotransmitters, synaptic formation, or myelination in the central nervous system.

We found a small, albeit statistically significant, inverse relation between vitamin B12 intake during the second trimester and PPVT-III scores at age 3 y. In a previous in

India, 9-y old children whose mothers had high vitamin B12 serostatus at gestation week 28 scored 6% lower in the Raven's Colored Progressive Matrices test, a proxy for intelligence, than did children born to mothers with low vitamin B12.[28] That difference, however, was not statistically significant; on the other hand, there were direct associations between maternal vitamin B12 and other cognitive outcomes including sustained attention (13% difference) and short-term memory (12% difference). In a cross-sectional study of Indian school-age children, serum levels of vitamin B12 were inversely related to short-term memory and a mental processing index [29]. The nature of the inverse relation between vitamin B12 status and child cognition reported in some studies is unclear at this point and warrants further examination in additional studies.

In our study, maternal intake of other nutrients involved in methyl donation pathways, including choline, betaine, and methionine, was not related to cognitive outcomes of the offspring. In a different cohort, maternal serum choline concentrations during the second or third trimesters were also unrelated to intelligence in the children at 5 years of age.[30] Results from these studies are in contrast with evidence from animal research, which consistently indicates that choline is an essential nutrient for adequate neurodevelopment.[31] Women in our study were not receiving supplements of these nutrients; their mean intake of choline in first (332 mg/d) and second (325 mg/d) trimesters was below the 450 mg/d currently recommended during pregnancy and variability seemed relatively low. Whether doses above usual dietary intakes could have an impact on neurocognitive outcomes deserves additional investigation. It is also possible that the cognitive effects of these nutrients become evident at later ages.

Our study has several strengths. The prospective nature of the design precludes reverse causation. While misclassification of nutrient intakes is possible, random error in the assessment of the exposure would likely lead to an attenuation of the underlying effect. We were able to control for major potential confounders in the analysis, including measures of maternal intelligence, socioeconomic status, and dietary intake of other nutrients that are relevant for neurodevelopment. The study also has some limitations. The reference amount of folate intake we used to estimate the effect on cognition, 600 μg/d, is relatively high and likely achievable only through supplement use. The effect associated with this amount might be considered modest, about 1.5 points in the PPVT-III score scale; nevertheless, the underlying effect could be higher in the absence of measurement error. Iron intake is a potential confounder of the association between folate and cognition because it may be found in the same multimicronutrient supplements as folate and has well-known effects on cognitive development. Although we adjusted for iron intake, some residual confounding by iron cannot be completely ruled out. Generalizability of results may be limited since cohort participants in whom neurodevelopmental testing was performed differed from those who were excluded with regard to race and education level. Finally, because several comparisons were made, chance cannot be fully discarded as a potential explanation of the findings.

In conclusion, folate intake during early pregnancy, particularly from supplements, among participants of Project Viva was associated with increased PPVT-III in their children at age 3 y. Intake of other methyl donor nutrients at levels provided by usual diet were not related to neurocognitive performance, except for a small inverse relationship of vitamin B12 intake during the second trimester with PPVT-III. Because folate is a substrate for methylation reactions, future studies should examine whether DNA methylation, a mechanism of epigenetic regulation, is associated with neurocognitive development indicators in the offspring. The potential effects of supplemental doses of other methyl donor nutrients deserve future investigation. Whether increasing periconceptional folate intake over current recommendations improves neurocognitive performance of the offspring is an open research question that requires testing in intervention studies.

Acknowledgments

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Table 1

Characteristics of participants with either first or second trimester dietary intake assessments (n = 1210)

Characteristics	N	% or mean ± SD
Mother and Family		
Maternal age at enrollment, y	1210	32.5 ± 5.0
Race/Ethnicity		
White	892	73.8%
Black or African American	139	11.5%
Hispanic or Latina	67	5.5%
Other	111	9.2%
Parity		
Nulliparous	577	47.7%
1	434	35.9%
2	153	12.6%
3	46	3.8%
Pre-pregnancy body mass index (kg/m^2)	1206	24.6 ± 5.1
Smoking during index pregnancy		
No	1062	89.7%
Yes	122	10.3%
Mother's PPVT-III score[a]	1181	106.1 ± 14.5
Highest grade level completed by mother		
Some college or less	340	28.1%
College graduate or more	869	71.9%
Highest grade level completed by father		
Some college or less	345	30.7%
College graduate or more	778	69.3%
Maternal dietary intake[b]		
Folate, periconceptional supplements[c] (μg/d)	1148	405 ± 405
Vitamin B12, periconceptional supplements (μg/d)	1148	4.5 ± 14.0
First Trimester		
Total energy intake (kcal/d)	1148	2090 ± 655
Folate, total (μg/d)	1148	949 ± 390
Folate, food sources (μg/d)	1148	367 ± 126
Vitamin B12, total (μg/d)	1148	10.8 ± 15.7
Vitamin B12, food sources (μg/d)	1148	6.3 ± 4.0
Choline (mg/d)	1148	332 ± 63
Betaine (mg/d)	1148	243 ± 108
Methionine (mg/d)	1148	2053 ± 408
Iron (mg/d)	1148	34.2 ± 17.6
Fish (servings/wk)	1148	1.7 ± 1.4
Second Trimester		
Total energy intake (kcal/d)	1083	2139 ± 607

Characteristics	N	% or mean ± SD
Folate, total (μg/d)	1083	1272 ± 381
Folate, food sources (μg/d)	1083	367 ± 128
Vitamin B12, total (μg/d)	1083	10.6 ± 6.4
Vitamin B12, food sources (μg/d)	1083	6.3 ± 3.6
Choline (mg/d)	1083	325 ± 64
Betaine (mg/d)	1083	234 ± 103
Methionine (mg/d)	1083	2065 ± 375
Iron (mg/d)	1083	49.4 ± 24.2
Fish (servings/wk)	1083	1.6 ± 1.4
Child		
Sex, % female (n)	608	50.3%
Age at 3 y testing (mo)	1210	39.5 ± 4.1
Speaks English as a second language, % (n)	41	3.4%
Cognitive tests scores at age 3 y		
PPVT-III	1186	103.9 ± 14.3
WRAVMA		
Total standard	1147	102.3 ± 11.2
Drawing	1195	99.4 ± 11.2
Matching	1167	108.0 ± 13.6
Pegboard	1192	98.4 ± 10.7

[a]PPVT, Peabody Picture Vocabulary Test

[b]Nutrient values are adjusted for total-energy intake (residuals method)

[c]Intake from supplements at 0–4 wk of gestation

Table 2

Differences in cognitive test scores at age 3 according to maternal intake of methyl donor nutrients during pregnancy[a]

	PPVT-III		Total WRAVMA	
	Unadjusted difference [95% CI]	Adjusted difference[b] [95% CI]	Unadjusted difference [95% CI]	Adjusted difference[b] [95% CI]
Folate, per 600 μg/d				
Early pregnancy supplements	4.4 [3.2, 5.6]	1.2 [−0.1, 2.4]	1.3 [0.4, 2.3]	0.1 [−1.0, 1.1]
1st trimester	5.3 [4.0, 6.5]	1.6 [0.1, 3.1]	1.9 [0.9, 2.9]	0.3 [−1.0, 1.6]
2nd trimester	3.0 [1.6, 4.4]	0.9 [−0.5, 2.2]	0.4 [−0.6, 1.5]	−0.7 [−1.9, 0.6]
Vitamin B12, per 2.6 μg/d				
Early pregnancy supplements	0.2 [0.1, 0.4]	0.1 [−0.1, 0.2]	0.1 [−0.1, 0.2]	0.0 [−0.1, 0.2]
1st trimester	0.1 [−0.1, 0.2]	0.0 [−0.1, 0.1]	0.1 [−0.1, 0.2]	0.0 [−0.1, 0.2]
2nd trimester	−0.4 [−0.8, −0.1]	−0.4 [−0.8, −0.1]	−0.1 [−0.3, 0.2]	0.0 [−0.3, 0.2]
Choline, per 450 mg/d				
1st trimester	−1.7 [−7.6, 4.2]	−3.1 [−11.0, 4.8]	0.1 [−4.6, 4.9]	−2.8 [−9.9, 4.3]
2nd trimester	−0.4 [−6.5, 5.7]	0.8 [−7.4, 9.0]	0.3 [−4.6, 5.2]	−0.4 [−7.6, 6.8]
Betaine, per 100 mg/d				
1st trimester	2.2 [1.4, 2.9]	0.4 [−0.4, 1.1]	0.6 [0.0, 1.2]	0.0 [−0.6, 0.7]
2nd trimester	2.2 [1.4, 3.0]	0.4 [−0.3, 1.2]	0.9 [0.2, 1.5]	0.1 [−0.6, 0.8]
Methionine, per 400 mg/d				
1st trimester	0.2 [−0.6, 1.0]	0.4 [−0.7, 1.5]	0.4 [−0.2, 1.0]	0.5 [−0.5, 1.5]
2nd trimester	0.2 [−0.7, 1.1]	−0.3 [−1.5, 0.9]	0.4 [−0.4, 1.1]	0.0 [−1.1, 1.1]

[a] Intake includes both food and supplement sources. Intake of each nutrient during 1st or 2nd trimester was adjusted for total energy using the method of the residuals. Sample sizes for the 1st and 2nd trimester are 1148 and 1083, respectively.

[b] From trimester-specific linear regression models with cognitive test scores as the outcome and covariates that included all five nutrients in the table at the same time plus maternal race/ethnicity, age, parity, smoking during pregnancy, prepregnancy BMI, PPVT, education, total energy, fish, and iron intake; paternal education; household income; and child's sex, and English as primary language. Models for the periconceptional period did not include choline, betaine, or methionine.