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ORIGINAL ARTICLE

Vitamin B12 and folate during pregnancy and offspring motor, mental and social development at 2 years of age

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Insufficiency of vitamin B12 (B12) and folate during pregnancy can result in low concentrations in the fetus and have adverse effects on brain development. We investigated the relationship between maternal B12 and folate nutrition during pregnancy and offspring motor, mental and social development at two years of age (2 y). Mothers ($n = 123$) and their offspring (62 girls, 61 boys) from rural and middle-class urban communities in and around Pune city were followed through pregnancy up to 2 y. Maternal B12 and folate concentrations were measured at 28 and 34 weeks of gestation. At 2 y, the Developmental Assessment Scale for Indian Infants was used to determine motor and mental developmental quotients and the Vineland Social Maturity Scale for the social developmental quotient. Overall, 62% of the mothers had low B12 levels (<150 pmol/l) and one mother was folate deficient during pregnancy. Maternal B12 at 28 and 34 weeks of gestation was associated with offspring B12 at 2 y ($r = 0.29$, $r = 0.32$, $P < 0.001$), but folate was not associated with offspring folate. At 2 y, motor development was associated with maternal folate at 28 and 34 weeks of gestation. Mental and social development quotients were associated positively with head circumference and negatively with birth weight. In addition, pregnancy B12 and folate were positively associated with mental and social development quotients. Maternal B12 and folate during intrauterine life may favorably influence brain development and function. Pregnancy provides a window of opportunity to enhance fetal psychomotor (motor and mental) development.

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Key words: developmental quotients, folate, maternal vitamin B12, offspring

Introduction

The structure and function of the nervous system of the developing child may be altered permanently by suboptimal maternal nutrition during pregnancy and lactation.[1,2] Vitamin B12 (B12) and folate deficiencies can interfere in early brain development and function, often by restricting the myelination and synaptic connectivity that occur early in life.[3] Myelin is the supportive sheath that protects the nerve cells and facilitates transmission of neuronal signals. Synaptic connectivity depends on the quantities of neurotransmitters available and the density of their receptors. If this is altered, neuroanatomical, chemical or metabolic changes may be induced. Functional consequences of these alterations vary depending on the availability of B12 and folate at appropriate times during neurodevelopment.

There are not many reports of associations of maternal B12 and folate status during pregnancy with offspring psychomotor development. Case studies have shown delayed neurodevelopment in infants of vegan mothers and in mothers with pernicious anemia.[4] In the Mysore Parthenon study, higher maternal folate during pregnancy was associated with better cognitive function in the offspring in childhood.[5] Our earlier Pune Maternal Nutrition Study (PMNS) showed that maternal B12 concentrations during pregnancy were positively related to cognitive performance in the offspring at 9 years (9 y) of age.[6] A Mexican study[7] reported that maternal dietary B12 deficiency in the first trimester was associated with poor mental development of children in their first year of life. Folate deficiency was associated with poor cognition in children of mothers who had methyl tetra folate reductase-MTHFR677TT genotype. Studies in rodents have shown that maternal folate deficiency is associated with structural brain abnormalities[8] and poor postnatal learning ability in offspring.[9]

In India, where the majority of the population is vegetarian,[10] there is a high prevalence of B12 deficiency and folate status is adequate.[11] We now report the effect of B12 and folate nutrition during pregnancy on child B12 and folate status and motor, mental and social development at 2 y.

Design and methods

In this study, urban and rural women and their 2-year-old offspring were investigated. Details of the study design were published earlier.[12]

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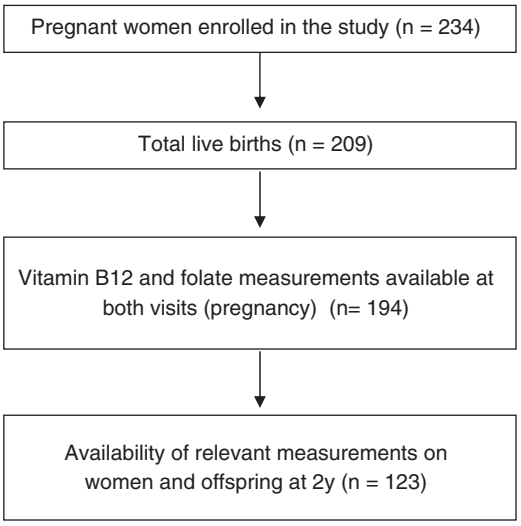

Fig. 1. Flow chart of study participants.

In short, women attending the King Edward Memorial (KEM) Hospital, Pune and the Vadu Rural Health Centre antenatal clinics were enrolled between May 2004 and February 2006 in the study. We screened records of over 995 pregnant women who attended the antenatal clinic before 28 weeks of gestation. Women with a gestation period of longer than 28 weeks, a multiple or an abnormal pregnancy, adverse obstetric history (previous cesarean section, intrauterine growth retardation, fetal death, neonatal death, preeclampsia, twin pregnancy) or a chronic medical condition (diabetes, hypertension, chronic infectious illness) were excluded, and of those remaining ($n = 234$) agreed to participate. Two hundred and nine of these women delivered at the study centers. Of these, 123 singleton pregnancies with serial measurements at 28 (range 24–30) and 34 (32–36) weeks of gestation, at birth and 2 y follow-up were analyzed (Fig. 1). In addition to demographic and clinical details, blood was sampled at 28 and 34 weeks of gestation for plasma B12 and folate measurements. Socioeconomic status (SES) was measured using the Indian standard of living index (SLI) scale.[13] Nutritional assessment was done by a trained nutritionist. Maternal diet during pregnancy (at 28 and 34 weeks of gestation) and the child's diet at 2 y were assessed using a semi-quantitative food frequency questionnaire based on local practices (investigated through focus group discussion) to obtain the frequency of consumption of foods that contain B12 and folate.

At delivery, the child's weight (to the nearest 0.01 kg) and head circumference (to the nearest 0.1 cm; Pedobaby, ETS JMB, Brussels, Belgium) were measured. At 2 y, offspring were followed up for measurements of hemoglobin, B12 and folate, and motor, mental and social development tests were conducted.

The study was approved by the KEM Hospital Research Centre's ethics committee and informed consent was signed by all participants.

Biochemical measurements

Hemoglobin was measured on a Beckman Coulter Analyzer (A$^{C\cdot}$T diff ^{TM}Analyzer, Miami, Florida, USA). The remaining blood was centrifuged (4°C, 2500 ***g*** × 15 min) within an hour of collection, and plasma was stored at −70°C until further analysis. Plasma B12 was measured by a microbiological assay using a colistin sulfate-resistant strain of *Lactobacillus Leichmanii*.[14,15] Plasma folate was measured by a microbiological assay using a chloramphenicol-resistant strain of *Lactobacillus Casei*.[16,17]

Psychomotor development

For psychomotor assessment, the developmental assessment scale for Indian infants (DASII) was used. DASII[18] is an Indian adaptation of Bayley's scale[19] for infants and was administered by a trained psychologist. The motor scale includes 67 items and the mental scale 163 items, which progressively test developmental milestones from birth to 36 months of age. The motor development assessment includes the child's movement from supine to erect posture and locomotion and basic locomotive skills, such as climbing, jumping and skipping. It also includes the record of manipulatory behavior such as reaching, handling and manipulating objects. Mental development items record the child's cognizance of objects in the surrounding environment, perceptual pursuit and exploration to meaningful manipulation. It also covers communication, language comprehension, spatial skills, manual dexterity and social interaction. The number of items achieved is matched to the median motor or mental age for this level for a reference child.[18] Motor and mental age are converted into motor and mental developmental quotients by the formula: (motor or mental age/chronological age) × 100.

Social development

The revised Vineland Social Maturity Scale[20] was used to assess social development. Social maturity is an age-dependent measure of the ability to communicate and interact, and milestones include day-to-day activities such as dressing, drinking, eating with hands and talking in short sentences. The Vineland Social Maturity Scale questionnaire was completed by the researcher during a face-to-face interview of the mother with clarification and further explanation of the questions and answers as necessary. Social age and quotient are derived from the raw scores as for the motor and mental scores.

Statistical methods

Data are presented as arithmetic mean (S.D.) for normally distributed variables, as median (25th, 75th centiles) for skewed variables and as number and percentage (%) for categorical variables. Skewed variables, maternal B12 at 34 weeks, child's B12 and folate at 2 y, were normalized using natural logarithmic transformations. Gender differences were analyzed using Student's unpaired *t*-test. Correlations between maternal B12, folate and offspring psychomotor and social development are reported as

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Pearson's or Spearman's correlation coefficients. The prediction of the child's mental and social developmental quotient was by backward regression using the independent variables: birth weight and neonatal head circumference, child's current B12 and folate, separately, maternal B12 and folate status during pregnancy (at 28 and 34 weeks of gestation) adjusted for gender. Maternal age, education, SLI and parity did not have any association with outcomes, and hence were not included in the models reported. This approach was not possible for the motor developmental quotient because of the skewed distribution. A binary group variable for the motor developmental quotient was defined as <115 and $\geqslant 115$ was created. Differences between groups were analyzed by Student's unpaired *t*-test. Statistical analysis was carried out using SPSS 16 (SPSS Inc., Chicago, IL, USA).

Results

Maternal characteristics

Briefly, for the purpose of this report, 123 pregnancies were included in the analysis (70 rural, 53 urban) who had all measurements available at 28 (range 24–30) and 34 (32–36) weeks of pregnancy and for the child at 2 y follow-up. Women included in the analysis ($n = 123$) were not different ($P > 0.05$) in weight, height, hemoglobin at 34 weeks of gestation and SES compared with those not included ($n = 86$; data not shown).

As a group, the mothers were young, short and light (Table 1). At 28 weeks of gestation, 78% women had low hemoglobin (<110 g/l), 62% had low B12 concentrations (<150 pmol/l) and only one woman had a low folate concentration (Table 1).

Offspring characteristics

There were 61 boys and 62 girls: 78 were first born (63%), 7 (6%) were delivered preterm and 18 (15%) by the cesarean section. Twenty five percent (31) had low birth weight (<2.5 kg).

New born were small and light (Table 2). At 2 y (2.00, 2.04), the children were 1.2 standard deviation scores (SDS) lighter and 0.8 SDS shorter compared with the World Health Organization reference child.[21] Seventy eight (63.4%) children had low hemoglobin (<110 g/l).

Maternal B12 at 28 and 34 weeks of gestation was associated with offspring B12 at 2 y ($r = 0.29$; $r = 0.32$; $P < 0.001$), but maternal folate was not associated with offspring folate. At 2 y, median motor and mental development quotients were 115.4 (95% CI 92.4, 115.4) and 100.0 (95% CI 81.3, 117.9), respectively, and social quotient of the offspring was 94.5 (95% CI 77.1, 100.0). Compared with Indian norms,[18] the average motor development quotient was on the 84th percentile and mental development quotient on the 50th percentile. Social quotient reference values are not available; a value of more than 100 would be considered 'advanced'. There was no gender difference for any of the developmental quotients. The only effect of maternal age, education or SES on developmental quotients was that children with a lower SES had a lower motor quotient than children with a higher SES (114.1 *v.* 115.4, $P = 0.006$, Mann–Whitney *U*-test). The urban children performed better than the rural children on motor development quotient (113.7 *v.* 110.8, $P < 0.01$, Mann–Whitney *U*-test).

Table 1. *Maternal characteristics at 28 and 34 weeks and 2 y after delivery (n = 123)*

	28 weeks gestation	34 weeks gestation	2 y after delivery
Age (y)	22.9 ± 3.7		
Education (y)	11 (9, 12)		
SES[a]	37.3 ± 8.4		
Height (cm)	154.7 ± 5.5		
Weight (kg)	52.6 ± 7.5	54.6 ± 7.8	50.1 ± 9.2
Diet [*n* (%)][b]			
Nonvegetarian food	43 (35)	46 (37)	
Milk	70 (60)	71 (58)	
GLV	109 (89)	87 (71)	
Milk products	80 (65)	79 (64)	
Biochemistry			
Hb (g/l)	98 ± 13	104 ± 14	117 ± 15
<110 g/l (%)	96 (78)	77 (62.6)	29 (23.6)
Vitamin B12 (pmol/l)	141.1 ± 52.8	120.0 (95.0, 153.0)	171.0 (129.0, 241.0)
<150 pmol/l	76 (61.8)	90 (73.2)	
Folate (nmol/l)	29.9 ± 18.7	33.8 ± 22.0	18.8 (14.4, 25.7)
<7 nmol/l	1 (0.8)	1 (0.8)	

SES, socioeconomic status; GLV, green leafy vegetables; Hb, hemoglobin.
[a] Measured as the Indian standard of living index,[13] values are median (25th–75th centiles), mean ± S.D., *n* (%).
[b] More than twice a week.

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Table 2. *Characteristics of children at birth and 2 y (n = 123)*

	At birth	At 2 y	Boys at 2 y (61)	Girls at 2 y (62)
Gestation (weeks)	39.2 ± 1.3			
Age (y)		2.0 ± 0.1		
Birth weight (kg)	2.710 ± 0.403			
Weight (kg)		10.3 ± 1.2	10.6 ± 1.5	10.0 ± 1.2b
Length (cm)	48.2 ± 1.9	84.6 ± 3.5	85.5 ± 3.4	83.7 ± 3.5b
Head circumference (cm)	33.2 ± 1.2	46.3 ± 1.4	46.7 ± 1.3	45.9 ± 1.3a
Developmental quotients				
Motor quotient		115.4 (110.2, 115.4)	115.4 (108.7, 115.4)	115.4 (110.8, 115.4)
Mental quotient		100.0 (95.8, 107.5)	98.9 (94.8, 107.5)	100.4 (96.3, 106.6)
Social quotient	–	94.5 (88.5, 97.0)	93.0 (85.5, 97.0)	94.5 (88.5, 97.0)
Biochemistry	–			
Hb (g/l)		103 ± 15	103 ± 14	102 ± 16
B12 (pmol/l)		330.0 (257.0, 440.0)	316.5 (255.2, 405.5)	336.0 (256.5, 487.5)
<150 pmol/l		4 (3.3)		
Folate (nmol/l)		23.7 (16.5, 32.6)	24.2 (18.3, 32.7)	22.7 (16.0, 33.5)
<7 nmol/l		2 (1.6)		

Hb, hemoglobin.
Values are median (25th, 75th centiles) or mean ± S.D.
[a] Different by gender, unpaired *t*-test ($P < 0.05$).
[b] Different by gender, unpaired *t*-test ($P < 0.01$).

Table 3. *Child motor development quotient group (low and high) at 2 y and differences in maternal vitamin B12 and folate concentrations at 28 and 34 weeks of pregnancy*

Motor development quotient	Low <115 $n = 50$	High ⩾115 $n = 73$	P[a]
Maternal B12 at 28 weeks (pmol/l)	137.9 ± 45.6	143.3 ± 57.4	0.58
Maternal B12 at 34 weeks (pmol/l)	120.5 (97.5, 147.5)	120.5 (90.5, 156.5)	0.77
Maternal folate at 28 weeks (nmol/l)	25.1 ± 16.9	33.2 ± 19.2	0.018
Maternal folate at 34 weeks (nmol/l)	29.1 ± 19.7	37.1 ± 23.0	0.05

[a] Unpaired Student's *t*-test, mean ± S.D. and median (95% confidence interval).

Motor development associations

We found that at 2 y, motor development quotient was not associated with any of the child's current or birth measurements. It was associated with maternal folate at 28 and 34 weeks of gestation (Spearman's $r = 0.29$; $P = 0.001$ and $r = 0.22$; $P = 0.016$) but not with B12. Motor development quotient was reclassified into a binary variable (motor development quotient <115 and ⩾115). The only difference found between these groups was that pregnancy folate at 28 and 34 weeks was significantly high in the higher scoring children (Table 3).

Mental development associations

Two-year mental development quotient was not associated with any of the measurements at 2 y or at birth, but was positively associated with maternal B12 at 28 weeks ($r = 0.19$, $P = 0.041$). Mental development quotient in children of B12 sufficient mothers (>150 pmol) was higher than that in the children of B12 deficient mothers (101 *v.* 98, $P = 0.035$). The child's mental development quotient was also associated positively with head circumference at birth but negatively with birth weight (Table 4). In backward regression models, the mental development quotient was positively and separately associated with maternal B12 at 28 weeks of gestation and folate at 28 and 34 weeks of gestation. A maximum of 12% of the variation was explained by B12 at 28 weeks of gestation (Table 4, model 1).

Social development

Two-year social development quotient was not associated with the child's current or birth measurements, but it was positively associated with maternal B12 at 28 weeks of gestation, folate at

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Table 4. *Determinants of child's mental development quotient at 2 y (n = 123)*

	Model 1	Model 2	Model 3	Model 4
	Week 28 B12	Week 34 B12	Week 28 folate	Week 34 folate
Independent variable	Stdβ (*P*-value)	Stdβ (*P*-value)	Stdβ (*P*-value)	Stdβ (*P*-value)
Gender				
Child B12 at 2 y				
Child folate at 2 y				
Birth weight (kg)	−0.353 (0.002)	−0.304 (0.008)	−0.323 (0.004)	−0.349 (0.002)
Birth head circumference (cm)	0.333 (0.004)	0.247 (0.030)	0.265 (0.019)	0.257 (0.022)
Maternal B12 at 28 weeks (pmol/l)	0.253 (0.005)			
Maternal B12 at 34 weeks (pmol/l)				
Maternal folate at 28 weeks (nmol/l)			0.178 (0.045)	
Maternal folate at 34 weeks (nmol/l)				0.199 (0.027)
R^2 total (%)	12.2	8.1	9.5	10.0

stdβ, standardized beta.

Models determined by backward regression. The effect of adding maternal vitamin B12 and folate at 28 and 34 weeks pregnancy is separately explored in models 1–4.

Model 1: gender, child B12 at 2 y, birth weight, neonatal head circumference, maternal B12 at 28 weeks.

Model 2: gender, child B12 at 2 y, birth weight, neonatal head circumference, maternal B12 at 34 weeks.

Model 3: gender, child folate at 2 y, birth weight, neonatal head circumference, maternal folate at 28 weeks.

Model 4: gender, child folate at 2 y, birth weight, neonatal head circumference, maternal folate at 34 weeks.

both 28 ($r = 0.18$, $P = 0.05$) and 34 weeks of gestation ($r = 0.21$, $P = 0.02$) and the child's hemoglobin at 2 y ($r = 0.34$; $P < 0.001$). Social development quotient in children of B12 sufficient mothers (>150 pmol/l) was higher than that in the children of B12 deficient mothers (93 *v.* 91, $P = 0.029$). Girls performed better than boys on social development. In backward regression (Table 5), social development was predicted positively by hemoglobin and negatively by child B12 at 2 y. Social development was also associated with birth measurements. Positive association was found with head circumference and negative association with birth weight. On maternal parameters, there was a positive association with maternal B12 at 28 (Table 5, model 1) and 34 (Table 5, model 2) weeks of gestation. Girls performed better than boys in folate models as well, and social developmental quotient was associated negatively with birth weight, positively with head circumference at birth and maternal folate at 28 (Table 5, model 3) and 34 weeks of gestation (Table 5, model 4). If child hemoglobin at 2 y was entered into the folate models, folate was no longer a predictor.

Discussion

Our study demonstrates, in women with low B12 status and adequate folate, a positive association between maternal B12 and folate status during pregnancy with offspring motor, mental and social development at 2 years of age. Our previous study (PMNS) found that lower maternal B12 during pregnancy was associated with lower scores for sustained attention and short-term memory in offspring at 9 years of age.[6] The present study adds to the evidence that maternal B12 insufficiency is associated with lower scores in mental development on the DASII scale. In addition, it demonstrates that motor development at 2 y was predicted by maternal folate during pregnancy. These findings are independent of child's B12 and folate status at 2 y.

Social development quotient was positively associated with hemoglobin at 2 y and maternal B12 at 34 weeks, but negatively with child B12 at 2 y. This is in agreement with a recent report[22] of cognitive measures in 6- to 10-year-old children, where a positive association with hemoglobin and a negative association with current B12 was shown. We extend this finding to demonstrate the favorable influence of pregnancy B12 on child development measured at 2 y. We also showed that at 2 y head circumference at birth was positively associated with mental and social development, which may reflect the effect of 'head-sparing' in these relatively small offspring. In other words, as the degree of growth retardation increases, head circumference proportionally increases and this increase in size may be associated with higher levels of mental and social development.[23] Our finding that maternal B12 status predicts offspring B12 status at 2 y is confirmed by other studies of B12 deficiency in infants.[24,25] Maternal B12 concentrations in our study were comparable to those in other studies in India,[26] although lower than those in other populations that are predominantly nonvegetarian.[27] Low maternal B12 levels can result in low levels in infants[26] with resultant poor brain development[28] and cognitive impairment. Case studies have shown that maternal B12 deficiency

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Table 5. *Determinants of child's social quotient at 2 y (n = 123)*

	Model 1	Model 2	Model 3	Model 4
	Week 28 B12	Week 34 B12	Week 28 folate	Week 34 folate
Independent variable	Stdβ (*P*-value)	Stdβ (*P*-value)	Stdβ (*P*-value)	Stdβ (*P*-value)
Gender	0.200 (0.016)	0.207 (0.015)	0.183 (0.039)	
Child Hb at 2 y (g/l)	0.244 (0.005)	0.293 (0.001)		
Child B12 at 2 y (pmol/l)	−0.182 (0.039)	−0.158 (0.082)		
Child folate at 2 y (pmol/l)				
Birth weight (kg)	−0.240 (0.025)	−0.210 (0.052)	−0.227 (0.044)	−0.255 (0.024)
Birth head circumference (cm)	0.341 (0.002)	0.309 (0.006)	0.288 (0.011)	0.278 (0.014)
Maternal B12 at 28 weeks (pmol/l)	0.273 (0.004)			
Maternal B12 at 34 weeks (pmol/l)		0.179 (0.058)		
Maternal folate at 28 weeks (nmol/l)			0.194 (0.028)	
Maternal folate at 34 weeks (nmol/l)				0.223 (0.013)
R^2 total (%)	24.8	21.6	11.6	12.5

Hb, hemoglobin; stdβ, standardized beta.

Backward regression, stdβ. The effect of adding maternal vitamin B12 and folate at 28 and 34 weeks pregnancy is separately explored in models 1–4.

Model 1: gender, child B12 at 2 y, birth weight, neonatal head circumference, maternal B12 at 28 weeks.

Model 2: gender, child B12 at 2 y, birth weight, neonatal head circumference, maternal B12 at 34 weeks.

Model 3: gender, child folate at 2 y, birth weight, neonatal head circumference, maternal folate at 28 weeks.

Model 4: gender, child folate at 2 y, birth weight, neonatal head circumference, maternal folate at 34 weeks.

is associated with offspring neurological damage.[29,30] A recent study found that lower folate status in early pregnancy might impair fetal brain development and can be associated with hyperactivity/inattention and peer problems in childhood.[31]

To our knowledge, ours is one of very few studies that have examined both maternal B12 and folate status and their association with early development of the offspring. Adolescents from macrobiotic families in the Netherlands were reported to have impaired cognitive performance.[32] In these children, B12 deficiency was associated with a suboptimal performance on intelligence tests. B12 deficient schoolchildren in Guatemala had slower reaction times on psychological tests, lower academic performance, attention problems and delinquent behavior.[33] The Mysore Parthenon study found higher maternal folate and not B12 during pregnancy to be associated with better cognitive function in 536 offspring at 10 years of age,[5] whereas our study finds an association with maternal B12 as well as folate. Direct comparison of our study with other studies is difficult due to different age groups, sample size and measures used.

The possible mechanism linking B12 and folate with psychomotor development is the critical role that both B12 and folate have in one carbon metabolism: methylation, nucleotide synthesis and cell division and differentiation.[34] The shared metabolism means that deficiencies in one vitamin will alter the metabolism of the other.[35] In nervous tissue, under conditions of low B12 status, this results in the accumulation of methylmalonyl CoA and the formation and incorporation of nonphysiologic fatty acids into neuronal lipids including those in myelin sheaths.[4,35] Neurotransmission may also be affected because methylcobalamin is the obligate carrier of methyl groups for the synthesis of choline, the precursor of the neurotransmitter acetylcholine.[36]

The strength of our study is its prospective design and long-term follow-up. Other factors known to affect infant motor and mental development are maternal education, SES, length of gestation, birth weight and head circumference, and these were included in the multivariate analysis. The limitations of our study include lack of data on maternal cognitive measures and availability of the relevant data only on a part of the population.

Early development of competent motor skills, perception and cognition are vital for the future success of the child. Our study provides evidence that maternal B12 and folate concentrations influence offspring brain development and function in addition to increased risk for cardiovascular disease and type 2 diabetes that has been previously reported by our group.[37] Ideally, women should conceive with adequate B12 and folate status and this should be maintained throughout pregnancy and breast feeding. In India, B12 deficiency is common, and given its association with neural tube defects[38] several experts call for B12 fortification of the food supply. However, the interdependence of folate and B12 status on the methylation of DNA with potential lifelong epigenetic programming effects means that an intervention study needs to be designed and carefully evaluated before national fortification can be recommended. Before the introduction of food fortification of B12,

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advice to women of child-bearing age should be to increase their intake of B12 foods such as animal origin foods, including milk, and consider consumption of supplements particularly before and during pregnancy and lactation.

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