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Maternal plasma vitamin B_{12} concentrations during pregnancy and infant cognitive outcomes at 2 years of age

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Abstract

Evidence on long-term influences of maternal vitamin B_{12} deficiency or concentrations on infant cognition is limited. We examined associations between maternal plasma vitamin B_{12} and cognitive development in 24-month-old infants. Maternal plasma vitamin B_{12} concentrations were measured at 26–28 weeks' gestation; infant cognitive development was assessed with the Bayley Scales of Infant and Toddler Development-III at 24 months, for 443 mother–infant pairs from the Growing Up in Singapore Towards Healthy Outcomes cohort. Linear regressions adjusted for key confounders examined associations of maternal vitamin B_{12} with cognitive, receptive and expressive language, fine and gross motor subscales. Co-occurrence of maternal vitamin B_{12} with folate or vitamin B_6 insufficiencies on child's cognition was explored. Average maternal plasma vitamin B_{12} concentrations was 220·5 ± 80·5 pmol/l; 15 % and 41 % of mothers were vitamin B_{12} deficient (<148 pmol/l) and insufficient (148–220·9 pmol/l), respectively. Infants of mothers with vitamin B_{12} deficiency had 0·42 (95 % CI −0·70, −0·14) SD lower cognitive scores, compared with infants of mothers with sufficient vitamin B_{12}. Co-occurrence of maternal vitamins B_{12} and B_6 insufficiencies was associated with 0·37 (95 % CI −0·69, −0·06) SD lower cognitive scores in infants compared with infants of mothers sufficient in both vitamins. No significant associations were observed with other subscales. Study findings suggest the possible need to ensure adequate vitamin B_{12} during pregnancy. The impact of co-occurrence of maternal B-vitamins insufficiencies on early cognitive development warrants further investigation.

Key words: **Vitamin B_{12}: Pregnancy: Cognition: Infants: Asian populations**

Adequate maternal nutrition is important for normal fetal growth and development, as the mother's nutrient stores are the only source of nutrition for the growing fetus[(1)]. There is an increasing interest in recent years to examine influences of maternal nutrition on cognitive development in infants, due to the growing body of literature showing a connection

Abbreviations: BSID, Bayley Scales of Infant and Toddler Development; GUSTO, Growing Up in Singapore Towards healthy Outcomes.

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between improved maternal nutrition and structural changes and maturation of the infant brain[2], which may subsequently affect early childhood cognitive function due to the strong link between anatomical changes of the brain and cognitive development[3]. However, there is still a limited understanding on the specific nutrients important for neurodevelopment *in utero*, and their subsequent longer-term effects on children's cognitive function.

Vitamin B_{12} plays an important role in neural myelination, synaptogenesis and neurotransmitter synthesis[4]. Myelination and synaptogenesis begin *in utero* and continue to influence neuronal development in offspring during the first few years of life[5]; thus maternal vitamin B_{12} has the potential to affect cognitive development and function in early childhood. For example, maternal vitamin B_{12} deficiency may result in a disruption in myelination and synaptic connectivity in the fetal brain[4]. If the development of the hippocampus, the auditory and visual cortices is impacted, memory, language and visual processing in children will consequently be affected[6].

The evidence supporting the role of maternal vitamin B_{12} in cognitive development in children is growing. Observational studies in 1–2-year-old infants found maternal vitamin B_{12} deficiency to be associated with poorer mental development measured with the Bayley Scale of Infant and Toddler Development (BSID)[7,8], but one other observational study and a randomised controlled trial found no significant association of maternal vitamin B_{12} status[9] or effect of maternal vitamin B_{12} supplementation[10], with/on infants' cognition measured with BSID. Two studies in older children of 7–8 years of age found no significant associations between maternal vitamin B_{12} and child's intelligence quotient[11,12]. In stark contrast, two studies reported higher maternal vitamin B_{12} concentrations or intakes to be associated with lower receptive vocabulary[13] or verbal ability[14] in 3- and 10-year-old children, respectively, while another study had conflicting findings, reporting children of mothers in the lowest decile of vitamin B_{12} concentrations to perform poorer in a working memory task but performed better in a sustained-attention task at 9 years of age, compared with children of mothers in the highest decile of concentrations[15]. Taken together, the evidence on maternal vitamin B_{12} and child's cognitive outcomes is inconclusive. It was also noted that majority of studies were conducted in Western settings[7,9,11,12,13] or from a developing Asian country – India[8,14,15]. No studies have been conducted in a multi-ethnic (Chinese, Malay and Indian) Asian population of a developed nation which differ in socio-demographic structure, cultural environment and dietary practices.

Vitamin B_{12} is interconnected with folate and vitamin B_6 in the one-carbon metabolism[16]. As such, synthesis and metabolism of vitamin B_{12} may be influenced by the availability of these other B vitamins. There is evidence to suggest that vitamin B_{12} deficiency co-occurs with other B-vitamin deficiencies[17], while several other studies reported high maternal folate coupled with low vitamin B_{12} to be associated with a number of infant health outcomes[18]. Few studies to date have accounted for the influence of other B vitamins when examining maternal vitamin B_{12} and offspring cognition. Those that do would adjust for maternal folate in statistical model or examine interactions between maternal folate and vitamin B_{12}[13,14], but have found to not change the associations. Interestingly, the effects of co-occurrence of maternal B-vitamin deficiencies on cognitive function in children have not been well elucidated.

In view of the aforementioned reasons, we aim to: (1) associate maternal vitamin B_{12} concentrations with offspring cognitive, language and motor outcomes at 24 months of age in a developed country of multi-ethnic Asians – Singapore; and (2) explore the effects of combinations of maternal vitamin B_{12} and folate or vitamin B_6 status on child's cognitive development.

Methods

Subjects

We used data from the GUSTO (Growing Up in Singapore Towards healthy Outcomes) study, a mother–offspring cohort study which has collected lifestyle and health information from pregnant women and their offsprings from birth onward. The GUSTO methodology has been published in detail elsewhere[19]. In summary, pregnant women aged 18–50 years (n 1247) were recruited in their first trimester from the KK Women's and Children's Hospital and National University Hospital in Singapore from June 2009 to September 2010. Inclusion criteria included the following: intention to live in Singapore for the following 5 years and to deliver in one of the two study maternity units; willingness to donate birth tissues; and homogeneous ethnicity of the participants' and spouse's parents. The major exclusion criterion was having a pre-pregnancy health condition such as type 1 diabetes, undergoing chemotherapy, or receiving psychotropic drugs. The GUSTO cohort study was conducted according to the guidelines laid down in the Declaration of Helsinki, and all procedures were approved by the National Healthcare Group Domain Specific Review Board (reference D/09/021) and the SingHealth Centralised Institutional Review Board (reference 2009/280/D). Written informed consent was obtained from all participants before being enrolled into the study.

Participants for the current study were limited to the subset of mother–offspring pairs in which the mothers had plasma B-vitamin concentrations measured at 26–28 weeks' gestation, and their offspring completed the cognitive test at 24 months of age. Due to limited manpower and available test slots, priority in scheduling to complete the cognitive test was given to infants who had participated in neurodevelopmental assessments prior to 24 months, or infants whose parents expressed interest to participate[20]. Those who did not participate were generally due to busy schedules, lack of interest, inability to contact the participants or participant dropping out from the GUSTO study. Further detail on the sample selection has been previously described[20].

Maternal plasma B vitamins

Pregnant women underwent a venipuncture in a fasting state during the 26–28 weeks' gestation clinic visit. The blood samples were processed within 4 hours and stored at −80°C before analysis. Plasma vitamin B_{12} and folate were assessed by competitive electrochemiluminescence immunoassay (ADVIA Centaur

Immunoassay System; Siemens) at the NUH Referral laboratory. Between-assay CV for plasma vitamin B_{12} and folate were 4–9 % and 6–11 % respectively. Plasma vitamin B_6 was analysed by using the reverse-phase HPLC method with post–column derivatisation and fluorimetric detection (MRC Human Nutrition Research, Elsie Widdowson Laboratory). Between-assay CVs was <5 %.

We also measured plasma homocysteine, a functional marker of vitamin B_{12} status, as it has been identified to be a more sensitive indicator of vitamin B_{12} deficiency. Plasma homocysteine was determined using HPLC (1100 series, Agilent Technologies) and mass-spectrometry (API 3000, AB Sciex) as described by Midttun *et al.*[21] at the Bevital AS laboratory. The between-assay CV was <2 %.

Maternal dietary intake

Maternal diet during pregnancy (at 26–28 weeks' gestation) was assessed using a 24-hour recall by trained clinical staff to obtain intakes of foods high in vitamin B_{12} (animal-based protein foods, e.g. poultry, meat, eggs, fish and seafood; dairy products, e.g. milk, yoghurt and cheese), and to assess overall diet quality with the Healthy Eating Index for pregnant women in Singapore (HEI-SGP)[22].

Cognitive outcomes in infants

The Bayley Scales of Infant and Toddler Development, Third Edition (BSID-III)[23], was administered to infants at 24 (± 1) months. It is a standardised test that assesses development of children 1–42 months of age in the following domains: cognitive, receptive and expressive language, and fine and gross motor[23]. The test was performed in homes when infants were likely to be alert. Distractions were kept to a minimum (e.g. television off, a quiet space), and there were at least one parent or guardian present.

The BSID-III was administered in English, Chinese, Malay or Tamil languages depending on the child's dominant language. As per common practice by Singapore's clinical psychologists, the BSID-III was informally adapted into Chinese, Malay and Tamil equivalents, and scored as follows: a correct score is given for responses in a dominant language, a mix of dominant or non-dominant languages, or entirely in a non-dominant language[24]. Previous study has shown minimal influence of cultural or language bias on test performance[24]. Administration and scoring was performed by research coordinators of the same ethnicity to the child, and they were trained by the head psychologist from KKH in accordance to the manual. Training details have been described elsewhere[24]. Raw test scores were used as age-specific norms were not available for our population.

Covariates

Covariates were selected based on previous literature[11,12,13,14]. Information on maternal age and self-reported ethnicity and highest education attained was collected during recruitment visit (<14 weeks' gestation). At the 26–28 weeks' gestation clinic visit, information on antenatal mental well-being assessed with the Edinburgh Postnatal Depression Scale[25] (Cronbach's alpha for internal reliability = 0·82) and the State-Trait Anxiety Inventory [26] (Cronbach's alpha for internal reliability = 0·91); an oral-glucose-tolerance test was also administered, and the diagnosis of gestational diabetes mellitus was based on the 1999 WHO criteria[27]. Maternal pre-pregnancy BMI was based on self-reported pre-pregnancy weight and height measured with a stadiometer (SECA model 213) at the 26–28 weeks' gestation clinic visit, calculated as weight divided by height squared (kg/m^2). Maternal parity and infant sex were retrieved from hospital delivery records.

Statistical analysis

The BSID-III raw scores were converted to standard deviation scores to facilitate comparison across the domain subscales. Maternal vitamin B_{12} statuses during pregnancy were categorised as follows: deficient (<148 pmol/l), insufficient (148 to <221 pmol/l) and sufficient (≥221 pmol/l), based on commonly used cut-offs in literature[17,28]. For maternal homocysteine, the top 75th percentile of the study sample was used to define high concentrations of homocysteine (≥5·5 μmol/l) as none of the mothers had plasma homocysteine concentrations above the cut-off for elevated homocysteine (>10 μmol/l)[14,15,29].

Maternal and infant characteristics according to maternal vitamin B_{12} status were compared using the χ^2 test for categorical variables, and using one-way ANOVA or Kruskal–Wallis tests for continuous variables with normal or skewed distribution, respectively. Associations between maternal vitamin B_{12} and homocysteine status and scores of each BSID-III subscale in the infants were examined using linear regressions. Several statistical models were employed: Model 1 – basic model with adjustment for infant's exact age at cognitive testing; Model 2 – additional adjustment for maternal age, ethnicity, education, pre-pregnancy BMI, parity, gestational diabetes mellitus status and antenatal depression and anxiety levels; and Model 3 – further adjustment for maternal plasma folate and vitamin B_6 concentrations, and additionally for maternal plasma vitamin B_{12} concentrations for homocysteine analysis. Potential effect modifications by infant sex on the associations between maternal vitamin B_{12} and infant's BSID-III outcomes were also explored.

As vitamin B_{12} deficiency tends to co-occur with folate and vitamin B_6 deficiencies, we further explored combinations of maternal vitamin B_{12} and vitamin B_6 or folate status in relation to infant BSID-III subscale scores. For a simpler analysis, we re-classify mother–infant pairs into two groups of maternal vitamin B_{12} status: insufficient B_{12} (<221 pmol/l) and sufficient B_{12} (≥221 pmol/l). Mother–infant pairs were also grouped according to maternal vitamin B_6 and folate status: insufficient B_6 (<20 nmol/l) and sufficient B_6 (>20 nmol/l)[30]; insufficient folate (<13·6 nmol/l) and sufficient folate (≥13·6 nmol/l)[31]. For combinations of maternal vitamin B_{12} and B_6 statuses, the reference group is mothers who were sufficient in both B_{12} and B_6, while the comparison groups are mothers who were (i) insufficient in both B_{12} and B_6 and (ii) sufficient in B_6 but insufficient in B_{12}. For combinations of maternal vitamin B_{12} and folate statuses, the reference group is mothers who were sufficient in both vitamin B_{12} and folate, while the comparison groups are mothers who were (i) insufficient in both B_{12} and

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folate and (ii) sufficient in folate but insufficient in B_{12}. Groups with very small sample sizes, which we hypothesised to be the following: (i) sufficient in vitamin B_{12} but insufficient in folate and (ii) sufficient in vitamin B_{12} but insufficient in vitamin B_6, will be excluded from the analysis of B_{12}–B_6 and B_{12}–folate combinations. The statistical models for this analysis were adjusted for covariates as per Model 3 discussed earlier.

Missing data for covariates were imputed using multiple imputation techniques with chained equations (twenty times). All analyses were performed using Stata version 14 (StataCorp LP). The significance level was set at $P<0{\cdot}05$.

Results

Of the 1247 pregnant women initially recruited, 70 dropped out during pregnancy due to personal reasons or family disapproval, or loss to follow-up; eighty-five conceived through *in-vitro* fertilisation or gave birth to twins and were excluded. A total of 1092 of remaining women conceived naturally with singleton foetuses, and 998 provided sufficient blood for assays of plasma vitamin B_{12}, folate and vitamin B_6 concentrations. A subset of their offspring (*n* 443) completed the BSID-III at 24 months of age (Fig. 1). This subset of mother–offspring pairs was included in the maternal vitamin B_{12} and offspring neurocognitive outcomes. The 555 mother–offspring pairs who did not participate in the BSID-III were comparable in characteristics to those who participated (online Supplementary Table S1). The analysis for maternal homocysteine was performed in 436 mother–offspring pairs as seven mothers had no measurement for homocysteine. The analysis examining combinations of maternal vitamin B_{12} and folate or vitamin B_6 status was performed in 436 and 426 mother–offspring pairs respectively; seven mothers who were vitamin B_{12} sufficient but folate insufficient and seventeen mothers who were vitamin B_{12} sufficient but vitamin B_6 insufficient were excluded.

Characteristics of mother–offspring pairs

Maternal and infant characteristics according to maternal vitamin B_{12} status are presented in Table 1. A total of 15·6 % of mothers were vitamin B_{12} deficient and 41·8 % of mothers were vitamin B_{12} insufficient. Mothers who were vitamin B_{12} deficient were more likely to belong to the Indian ethnic group, tended to have higher concentrations of homocysteine and more likely to have lower concentrations of vitamin B_6 and folate as well as a greater proportion of them having insufficient vitamin B_6 and folate. These mothers were also observed to have higher pre-pregnancy BMI and tended to be primi- or multi-parous. In addition, mothers with vitamin B_{12} deficiency or insufficiency tended to have lower intakes of meat, eggs or animal-based products and dairy products, although the groups of mothers did not differ in their overall diet quality.

There were missing observations for the following variables: *n* 2 maternal education, *n* 8 antenatal depression, *n* 7 antenatal anxiety, *n* 36 maternal pre-pregnancy BMI, *n* 14 maternal gestational diabetes mellitus, *n* 3 animal-based protein foods and *n* 3 dairy products.

Maternal vitamin B_{12}, homocysteine and cognitive outcomes in infants

Compared with infants of mothers with sufficient vitamin B_{12}, infants of mothers with vitamin B_{12} deficiency had 0·42 (95 % CI −0·70, −0·14) SD lower cognitive scores, upon adjusting for

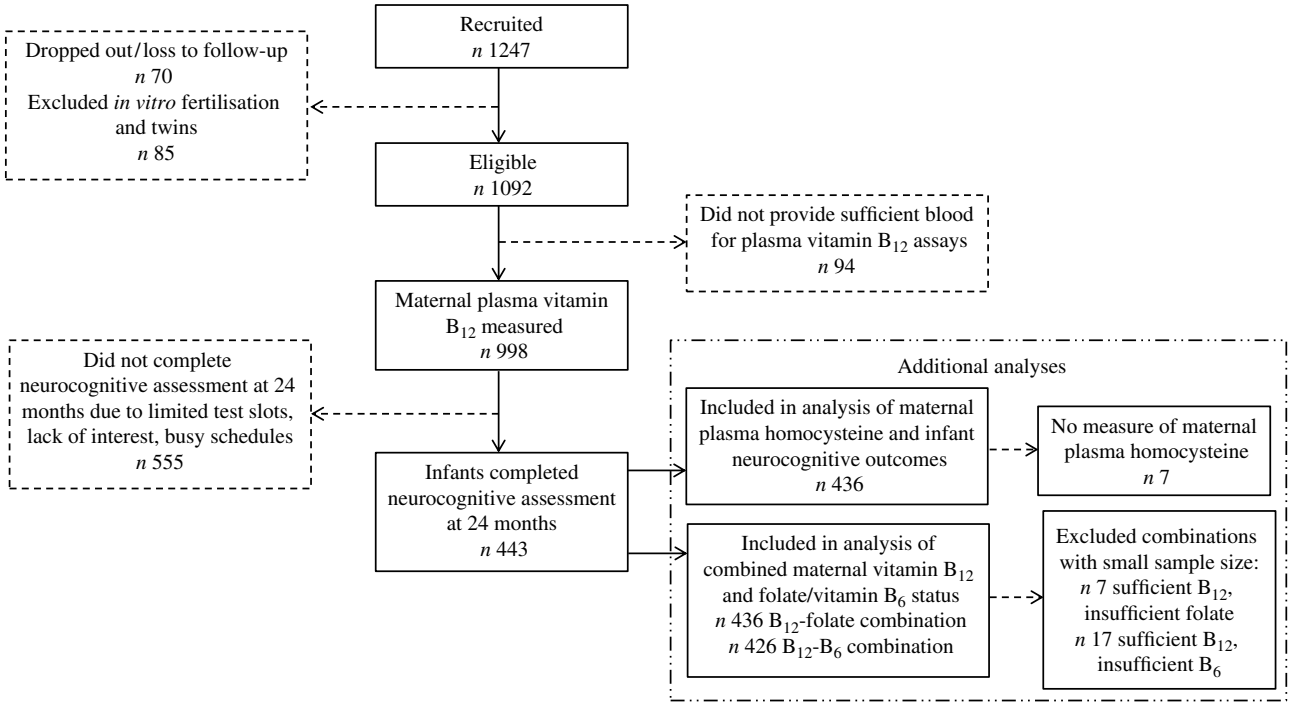

Fig. 1. Participant flow diagram for analysis of associations between maternal plasma vitamin B_{12} concentrations and infant cognitive development in the Growing Up in Singapore Towards healthy Outcomes study.

Table 1. Maternal and infant characteristics according to maternal vitamin B_{12} status in 443 mother–offspring pairs of the Growing Up in Singapore towards healthy Outcomes (GUSTO) cohort
(Numbers of participants and percentages; mean values and standard deviations; medians and interquartile ranges (IQR))

	Total		B_{12}-deficient (<148 pmol/l)		B_{12}-insufficient (148 to <221 pmol/l)		B_{12}-sufficient (≥221 pmol/l)		
	n 443*		*n* 69		*n* 185		*n* 189		
	n, mean, median	%, SD, IQR	*n*, mean, median	%, SD, IQR	*n*, mean, median	%, SD, IQR	*n*, mean, median	%, SD, IQR	*P*†
Maternal characteristics									
Age (years)	30·9	5·1	31·3	5·0	30·6	4·9	31·0	5·3	0·57
Ethnicity (*n*)									0·002
Chinese	246	55·5	35	50·7	98	53·0	113	59·8	
Malay	120	27·1	12	17·4	53	28·6	55	29·1	
Indian	77	17·4	22	31·9	34	18·4	21	11·1	
Education (*n*)									0·86
Secondary or lower	120	27·2	19	27·5	48	26·1	53	28·2	
Post-secondary	162	36·7	23	33·3	73	39·7	66	35·1	
University or higher	159	36·1	27	39·1	63	34·2	69	36·7	
Recruitment sites (*n*)									
KK Hospital	347	78·3	48	69·6	146	78·9	153	80·9	0·140
National University Hospital	96	21·7	21	30·4	39	21·1	36	19·1	
Plasma homocysteine concentrations (μmol/l)	5·0	1·1	5·5	1·4	4·9[a]	1·0	4·9[a]	1·0	<0·001
Plasma vitamin B_6 concentrations (nmol/l)	59·9	24·8, 108·3	36·4[a]	20·5, 94·8	53·5[a]	23·4, 104·2	78·8	33·6, 113·0	0·001
Vitamin B_6-insufficient (*n*)	66	14·9	17	24·6	32	17·3	17	9·0	0·004
Plasma folate concentrations (nmol/l)	34·0	24·5, 46·0	30·4	18·8, 39·2	35·6[a]	21·1, 47·8	34·0[a]	26·7, 45·6	0·030
Folate-insufficient (*n*)	46	10·4	11	15·9	28	15·1	7	3·7	<0·001
Pre-pregnancy BMI (kg/m^2)	21·9	19·7, 25·4	23·4[a]	20·5, 26·7	22·1[a]	19·9, 25·9	20·8	19·2, 23·9	<0·001
EPDS score	7·7	4·4	7·2	4·5	7·7	4·3	7·9	4·5	0·58
STAI-state score	33·8	10·1	33·5	10·4	33·5	10·2	34·1	9·9	0·80
Gestational diabetes (*n*)									0·51
Yes	79	18·4	15	22·7	34	18·9	30	16·4	
No	350	81·6	51	77·3	146	81·1	153	83·6	
Parity (*n*)									0·018
Nulliparous	192	43·3	21	30·4	77	41·6	94	49·7	
Primi/multiparous	251	56·7	48	69·6	108	58·4	95	50·3	
Maternal diet									
Diet quality (HEI-SGP)	52·4	13·5	52·5	12·0	52·3	14·0	52·3	13·7	0·99
Animal-based protein foods (g)	158	86, 236	155[a]	77, 217	142[a]	65, 233	170	102, 249	0·039
Dairy products (g)	250	0, 323	150[a]	0, 250	213[a]	0, 300	250	6·1, 400	0·009
Infant characteristics									
Age at cognitive testing (months)	38·8	1·3	38·7	1·2	38·9	1·2	38·9	1·5	0·08
Sex (*n*)									0·75
Male	235	53·1	35	52·2	102	55·1	97	51·3	
Female	208	46·9	33	47·8	83	44·9	92	48·7	

EPDS, Edinburgh Postnatal Depression Scale; STAI, State-Trait Anxiety Inventory; HEI-SGP, Healthy Eating Index for Singapore Pregnant women.
[a] Groups with the same superscript letter in a row indicate no significant difference in *P* values by one-factor ANOVA or Kruskal–Wallis test with Bonferroni *post hoc* analysis.
* Missing data: *n* 2 maternal education, *n* 7 maternal plasma homocysteine, *n* 8 antenatal depression, *n* 7 antenatal anxiety, *n* 36 maternal pre-pregnancy BMI, *n* 14 maternal gestational diabetes mellitus, *n* 3 animal-based protein food intake, *n* 3 dairy product intake.
† *P* values were obtained from the χ^2 test, one-factor ANOVA or Kruskal–Wallis test with Bonferroni *post hoc* analysis.

key confounders (Table 2). This association was not affected by additional adjustment for maternal plasma folate and vitamin B_6 concentrations. Findings were consistent when maternal vitamin B_{12} concentrations were treated as a continuous variable, whereby higher maternal vitamin B_{12} concentrations were associated with higher cognitive scores in infants (online Supplementary Table S2).

No significant associations were observed for maternal vitamin B_{12} status or concentrations with other BSID-III subscales in infants. There were no interactions between maternal vitamin B_{12} and infant sex in relation to each BSID-III subscales (data not shown).

Infants of mothers with high homocysteine concentrations appeared to score lower in most of the BSID-III subscales (four of five subscales) compared with infants of mothers with normal concentrations; but none of these associations reached statistical significance (Table 3).

Combined maternal vitamin B_{12} and folate or vitamin B_6 status with cognitive outcomes in infants

When compared with infants of mothers who were sufficient in both vitamins B_{12} and B_6 (reference group), infants of mothers

Table 2. Associations of maternal plasma vitamin B_{12} status* with infant cognitive development (Bayley Scale of Infant and Toddler Development–III) at 24 months of age in the Growing Up in Singapore Towards healthy Outcomes study (*n* 443)
(*β*-Coefficients and 95 % confidence intervals)

	Cognitive			Receptive language			Expressive language			Fine motor			Gross motor		
	β	95 % CI	*P*	*β*	95 % CI	*P*	*β*	95 % CI	*P*	*β*	95 % CI	*P*	*β*	95 % CI	*P*
Model 1†															
Deficient	−0·49	−0·77, −0·22	<0·001	−0·27	−0·55, 0·001	0·05	−0·19	−0·46, 0·09	0·18	−0·29	−0·57, −0·02	0·033	−0·17	−0·45, 0·10	0·22
Insufficient	−0·12	−0·32, 0·08	0·24	−0·13	−0·33, 0·07	0·21	0·05	−0·16, 0·25	0·65	−0·01	−0·21, 0·19	0·94	0·13	−0·008, 0·33	0·23
Sufficient								Reference							
Model 2‡															
Deficient	−0·42	−0·69, −0·15	0·003	−0·26	−0·54, 0·01	0·06	−0·16	−0·44, 0·12	0·27	−0·26	−0·54, 0·02	0·07	−0·08	−0·36, 0·21	0·59
Insufficient	−0·09	−0·29, 0·10	0·36	−0·12	−0·32, 0·08	0·24	0·05	−0·15, 0·25	0·62	−0·001	−0·20, 0·20	0·99	0·16	−0·04, 0·36	0·13
Sufficient								Reference							
Model 3§															
Deficient	−0·42	−0·70, −0·14	0·003	−0·25	−0·53, 0·03	0·08	−0·15	−0·43, 0·13	0·29	−0·24	−0·52, 0·04	0·10	−0·04	−0·33, 0·24	0·76
Insufficient	−0·09	−0·29, 0·11	0·39	−0·11	−0·31, 0·09	0·29	0·06	−0·15, 0·26	0·59	0·01	−0·19, 0·21	0·91	0·17	−0·03, 0·38	0·09
Sufficient								Reference							

* Vitamin B_{12} status: *n* 89 deficient (<148 pmol/l); *n* 185 insufficient (148 to <221 pmol/l); *n* 189 sufficient (≥221 pmol/l).
† Model 1 – adjusted for infant's age at cognitive testing.
‡ Model 2 – adjusted as for Model 1 and maternal age, ethnicity, education, pre-pregnancy BMI, parity, gestational diabetes status, antenatal depression and anxiety levels.
§ Model 3 – adjusted as for Model 2 and maternal plasma folate and vitamin B_6 concentrations.

Table 3. Associations of maternal plasma homocysteine status* with infant cognitive development (Bayley Scale of Infant and Toddler Development–III) at 24 months of age in the Growing Up in Singapore Towards healthy Outcomes study (*n* 436)
(*β*-Coefficients and 95 % confidence intervals)

	Cognitive			Receptive language			Expressive language			Fine motor			Gross motor		
	β	95 % CI	*P*	*β*	95 % CI	*P*	*β*	95 % CI	*P*	*β*	95 % CI	*P*	*β*	95 % CI	*P*
Model 1†															
High	−0·10	−0·32, 0·12	0·373	−0·19	−0·40, 0·03	0·091	−0·08	−0·30, 0·14	0·472	−0·04	−0·26, 0·17	0·685	0·05	−0·17, 0·27	0·661
Normal								Reference							
Model 2‡															
High	−0·04	−0·25, 0·17	0·709	−0·10	−0·32, 0·11	0·334	−0·02	−0·24, 0·19	0·838	−0·03	−0·24, 0·19	0·803	0·05	−0·.17, 0·27	0·659
Normal								Reference							
Model 3§															
High	−0·02	−0·23, 0·19	0·840	−0·10	−0·31, 0·12	0·377	−0·01	−0·23, 0·20	0·902	−0·02	−0·24, 0·20	0·851	0·06	−0·16, 0·28	0·598
Normal								Reference							

* Homocysteine status: *n* 117 high (≥75th percentile – ≥5·5 μmol/l;); *n* 326 normal (<75th percentile – <5·5 μmol/l).
† Model 1 – adjusted for infant's age at cognitive testing.
‡ Model 2 – adjusted as for Model 1 and maternal age, ethnicity, education, pre-pregnancy BMI, parity, gestational diabetes status, antenatal depression and anxiety levels.
§ Model 3 – adjusted as for Model 2 and maternal plasma vitamin B_{12}, folate and vitamin B_6 concentrations.

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Table 4. Associations of combined maternal plasma vitamins B_{12} and vitamin B_6 or folate status* with infant cognitive development (Bayley Scale of Infant and Toddler Development–III) at 24 months of age in the Growing Up in Singapore Towards healthy Outcomes study (*n* 443)†
(*β*-Coefficients and 95 % confidence intervals)

		Cognitive			Receptive language			Expressive language			Fine motor			Gross motor		
	n	*β*	95 % CI	*P*	*β*	95 % CI	*P*	*β*	95 % CI	*P*	*β*	95 % CI	*P*	*β*	95 % CI	*P*
B_{12}–B_6‡																
Insufficient B_{12} and B_6	49	−0·37	−0·69, −0·06	0·019	−0·30	−0·61, 0·02	0·06	−0·14	−0·46, 0·18	0·40	−0·14	−0·45, 0·18	0·40	−0·06	−0·38, 0·27	0·74
Insufficient B_{12}, sufficient B_6	205	−0·13	−0·33, 0·07	0·19	−0·10	−0·30, 0·10	0·34	0·05	−0·15, 0·26	0·60	−0·03	−0·23, 0·17	0·76	0·15	−0·06, 0·36	0·15
Sufficient B_{12} and B_6	172								Reference							
B_{12}–folate§																
Insufficient B_{12} and folate	39	−0·36	−0·73, 0·003	0·06	0·04	−0·33, 0·40	0·85	−0·19	−0·56, 0·19	0·32	−0·16	−0·53, 0·21	0·39	0·01	−0·36, 0·39	0·94
Insufficient B_{12}, sufficient folate	215	−0·14	−0·33, 0·06	0·17	−0·16	−0·36, 0·03	0·10	0·03	−0·17, 0·23	0·78	−0·05	−0·24, 0·15	0·65	0·10	−0·10, 0·30	0·31
Sufficient B_{12} and folate	182								Reference							

* Vitamin B_{12} status: insufficient (<221 pmol/l including deficient), sufficient (≥221 pmol/l); vitamin B_6 status: insufficient (<20 nmol/l), sufficient (>20 nmol/l); folate status: insufficient (<13·6 nmol/l), sufficient (≥13·6 nmol/l).
† Models adjusted for infant's age at testing; maternal age, ethnicity, education, pre-pregnancy BMI, parity, gestational diabetes status, antenatal depression and anxiety levels, and maternal plasma ‡folate or §vitamin B_6 concentrations.

who were insufficient in both vitamins B_{12} and B_6 had 0·37 (95 % CI −0·69, −0·06) SD lower cognitive score, while nostatistical significant association was observed for infants of mothers with insufficient B_{12} but sufficient B_6 (Table 4).

No significant associations were observed for combinations of maternal vitamin B_{12} and folate status with all BSID-III subscales in infants.

Independent of vitamin B_{12}, however, there were no significant associations between maternal folate and vitamin B_6 concentrations or status with each BSID-III subscales in infants (online Supplementary Table S3).

Discussion

Our study found infants of mothers deficient in vitamin B_{12} deficiency to perform less well in the cognitive domain compared with infants of mothers who were sufficient in vitamin B_{12}. In addition, infants performed less well in the cognitive domain if their mothers had co-occurrence of vitamins B_{12} and B_6 insufficiencies/deficiencies during pregnancy, but not if the mothers were sufficient in vitamin B_6 although also insufficient/deficient in vitamin B_{12}.

Our finding regarding the role of maternal vitamin B_{12} on infant's BSID-III cognitive domain is in line with two previous birth cohort studies examining maternal vitamin B_{12} and cognitive development in 1–2-year-old infants measured with BSID-II or -III[7,8]. Another cohort study in Canada, however, showed no significant associations between maternal vitamin B_{12} concentrations and BSID-III outcomes in their offspring at 18 months[9], which may be due to a small sample size of 154 mother–infant pairs or insufficient variation in maternal vitamin B_{12} status given the low prevalence of deficient/insufficient vitamin B_{12} in their participants. One randomised controlled trial did not find significant effects of maternal B_{12} supplementation during pregnancy on cognitive development (also measured with BSID-III) in infants at 9 months[10]. The lack of effect could be due to the young age at cognitive assessment which may have affected the reliability of the results.

The effect estimate of maternal vitamin B_{12} and infant cognitive score association in our study appears to be fairly similar to studies reporting significant associations. Previous studies found children born to vitamin B_{12}-deficient mothers to score 1·6–3 points lower in BSID-II mental development index compared with children born to vitamin B_{12}-sufficient mothers[7,8]. Our study found infants of vitamin B_{12}-deficient mothers to score two points (0·42 SD) lower in BSID-III cognitive subscale compared with infants of vitamin B_{12}-sufficient mothers, although the differences in BSID editions, vitamin B_{12} measurement methods and statistical methods meant that results may not be directly comparable. The clinical significance of this effect estimate is unclear, but it is important to note that the effect size is similar to that of the association between maternal education and infant cognitive scores in our study (0·41 SD lower comparing infants of mothers with the lowest *v.* the highest education level), which has been identified to be a strong predictor of child's cognition in the literature[32].

Similar to two other studies reporting a lack of associations between maternal vitamin B_{12} concentration or intakes and offspring psychomotor development[7,8], we too did not observe any association between maternal vitamin B_{12} concentrations and the gross motor subscale in our infants. Studies examining vitamin B_{12} concentrations or intakes in children with motor development also reported similar findings[29,33,34]. These studies, on the other hand, found significant associations with mental development and several cognitive aspects, which is consistent with our findings. Interestingly, one randomised controlled trial found vitamin B_{12} supplementation in infants to improve gross motor development, although the effect was attenuated after accounting for baseline differences of important confounders (e.g. sex, age, family income and physical growth)[35].

We did not find maternal vitamin B_{12} to be associated with offspring language development. The literature relating vitamin B_{12} to language development in children is inconsistent. Two studies reported inverse associations between maternal vitamin B_{12} and offspring receptive language[13] and verbal fluency[14], while another study found no significant association between maternal vitamin B_{12} and offspring verbal intelligence[11]. Likewise, vitamin B_{12} supplementation in infants appears to have no effect on communication ability[35]. Direct comparison of these study findings is not possible, as there is no current consensus in the instruments used to assess language development[36].

The association between maternal B_{12} deficiency and lower cognitive scores appears to be more evident among mother–offspring pairs where the mothers were also vitamin B_6 insufficient during pregnancy. Vitamins B_{12} and B_6 are the sources of coenzymes which participate in one-carbon metabolism shown to play a role in neurodevelopment[37]; the lack of both nutrients may thus have an additive negative effect on cognitive function. Being insufficient in vitamin B_6 may also contribute to malabsorption of vitamin B_{12}[17], and further contribute to impairing neurocognitive development. This is supported by our observation that mothers who were vitamin B_{12} deficient were also more likely to have the lowest concentrations of vitamin B_6, and a greater proportion of them to have insufficient vitamin B_6, indicating that these two B vitamins mutually influence the synthesis of each other. Note, however, that the group who were insufficient in both vitamins B_{12} and B_6 were much smaller in comparison with the other two groups (sufficient in vitamins B_{12} and B_6, and insufficient vitamin B_{12} but sufficient vitamin B_6); the effect estimate may be biased by underpowered analysis.

Vitamin B_{12} is an essential nutrient not synthesised by the human body and can be obtained only through the consumption of meat and animal products or foods fortified with vitamin B_{12}[28]. This may explain our observation of mothers deficient or insufficient in vitamin B_{12} having significantly lower intake of animal-based protein foods and dairy products but did not differ in diet quality, as vitamin B_{12} concentrations are more reflective of meat and animal product intakes rather than an overall healthier diet. Concordantly, we found mothers with deficiency or insufficiency vitamin B_{12} tended to belong to the Indian ethnic group, and a higher proportion of them in our cohort were adopting a vegetarian diet during pregnancy (7·9 % *v.* 1·4 % Chinese and 2 % Malay).

The interpretation of maternal vitamin B_{12} status during pregnancy is complicated by haemodilution and complex physiological changes and may not be a true reflection of inadequate dietary intake. As such, we also measured maternal plasma homocysteine, a functional biomarker of vitamin B_{12} status. We found vitamin B_{12}-deficient mothers to have significantly higher plasma homocysteine concentrations, suggestive of a vitamin B_{12} deficiency, although the concentrations in our sample did not reach the level necessary for hyperhomocysteinemia (>10). Given that plasma homocysteine reduced by 36 % of non-pregnant values during mid-pregnancy[38], and that homocysteine is affected by the availability of other B vitamins (e.g. folate, vitamins B_6 and B2), thus may not be a specific biomarker of vitamin B_{12}[39], helps explain the disproportionate prevalences of vitamin B_{12} deficiency to hyperhomocysteinemia in our sample. This observation is also supported by two other studies reporting a much higher prevalence of vitamin B_{12} deficiency compared with the prevalence of hyperhomocysteinemia (Veena *et al.*[14]: 42·5 % *v* 3·4 %; Bhate *et al.*[15]: 65 % *v.* 35 %). The lack of association between maternal plasma homocysteine and offspring BSID-III outcomes in our study may be explained by the absence of neurotoxic effect arising from hyperhomocysteinemia.

The present study has several strengths. First, the use of plasma B-vitamin concentrations are independent of self-reported bias and would be fairly more accurate than conventional methods of dietary assessments such as food frequency questionnaires and 24-hour recalls, which have the potential for over- or underestimation[40]. We also considered the contribution of the other B vitamins involved in the one-carbon cycle to determine their level of influence on the development of cognition. Our results are robust, as they remained significant even after adjusting for several key confounders such as socio-economic status (using maternal education as proxy) and maternal mental health.

Some limitations of our study include the fact that the study is an observational study, thus no causative relationships can be drawn from the results. The analysis was performed on a subset of infants who have completed BSID-III and may lead to selection bias; comparison of participant characteristics showed that non-participants were similar in profile for a number of key determinants. Vitamin B_{12} concentrations were not measured in children; hence a better (or poorer) performance in neurocognitive assessments may be a reflection of better (or poorer) nutritional status in children rather than of their mothers, although there is evidence to suggest that dietary patterns of the offspring are very similar to those of their mothers[41,42]. A number of important contributors to early cognitive development such as maternal intelligence and home stimulation were not measured in the cohort, but our statistical models adjusted for maternal education which is often used as a proxy. Our study could benefit from having measured methymalonic acid which is a more specific functional biomarker of vitamin B_{12} compared with homocysteine, to provide a more comprehensive aspect of whether vitamin B_{12} deficiency is truly present in our population of pregnant women. Finally, study findings may be biased by how well infant's cognitive performance is captured, but much efforts have been put in place to ensure information collected is

reliable in terms of training of research coordinators, requesting for minimal distractions during administration and ensuring that infant's performance in BSID-III is minimally influenced by cultural and language bias[24].

In conclusion, maternal vitamin B_{12} deficiency was associated with poorer cognitive function in 2-year-old infants. Further studies on circulating and functional biomarkers of vitamin B_{12} to comprehensively assess vitamin B_{12} status and inclusion of multiple measures of cognitive outcomes at later time points are needed to clearly elucidate the associations between maternal vitamin B_{12} and cognition in children. It is also essential that the associations observed are tested in well-designed randomised controlled trials before recommending vitamin B_{12} during pregnancy for improved offspring cognitive development. Nevertheless, there is still a need to advise pregnant women on optimal diets to ensure adequate vitamin B_{12} especially those with low consumption of animal-based protein foods and dairy products, in view of the high prevalence of B_{12} deficiency and insufficiency (57·5 %) in our cohort.

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Supplementary material

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