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Plasma vitamin B_{12} concentration is positively associated with cognitive development in healthy Danish 3-year-old children: the SKOT cohort studies

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Abstract

Adequate vitamin B_{12} (B_{12}) and folate concentrations are essential for neural development in early childhood, but studies in well-nourished children are lacking. We investigated the relation between plasma B_{12} and folate at 9 and 36 months and psychomotor development at 36 months in well-nourished Danish children. Subjects from the SKOT cohorts with B_{12} measurement and completed Ages and Stages Questionnaire, 3rd edition (ASQ-3) at 36 months were included (*n* 280). Dietary intake, B_{12} and folate concentrations were collected at 9 and 36 months, and ASQ-3 was assessed at 36 months. Associations between B_{12} and folate at 9 and 36 months and ASQ-3 were analysed using regression models. Associations between diet and B_{12} were also investigated. No children had insufficient B_{12} (<148 pmol/l) at 36 months. B_{12} at 36 month was positively associated with total ASQ-3 corresponding to an increase of 100 pmol/l B_{12} per 1·5 increase in total ASQ-3 score ($P = 0·019$) which remained significant after adjustment for potential confounders including 9 months values. B_{12} at 9 months or folate at any time point was not associated with total ASQ-3. Intake of milk products was associated with B_{12} at 36 months ($P = 0·003$) and showed a trend at 9 months ($P = 0·069$). Intake of meat products was not associated with B_{12}. In conclusion, B_{12} was positively related to psychomotor development at 3 years in well-nourished children, indicating that the impact of having marginally low B_{12} status on psychomotor development in well-nourished children should be examined further.

Key words: Early childhood: Vitamin B_{12} status: Development: Cognition: Folate

The water-soluble micronutrients vitamin B_{12} (cobalamin) and folate are necessary for the rapid growth and development during the early years of life[1]. The relation between these micronutrients and cognitive and motor development is not fully understood, but the shared metabolism suggests that the status mutually affect the metabolism of the other[2]. Both are essential cofactors for RNA and DNA synthesis and required for development of and maintaining the nervous system. Vitamin B_{12} participate in the folate-dependent conversion of homocysteine to methionine and the conversion of methylmalonyl CoA to succinyl CoA[3]. Deficiency leads to increased homocysteine levels and reduced supply of methyl groups and succinyl CoA that affects the production of myelin. Myelin is an essential component of brain development and maturation and is related to psychomotor development. Thus, vitamin B_{12} deficiency may lead to disturbed myelin structure and demyelination of the central nervous system which may affect the process of learning and achieving motor abilities[2,4,5].

Vitamin B_{12} is synthesised by micro-organisms and the primary sources of vitamin B_{12} are animal source foods. Fruit and vegetables are key sources for folate[6]. In low- and middle-income regions with low intake of meat, milk and fish, poor vitamin B_{12} status is widespread, especially among pregnant women and young children[7,8]. Deficiency is less common among children with a typical Western diet, but vegetarians and especially vegans may be at risk[9,10]. In future, this might be an issue to consider due to the increasing interest in sustainable food consumption in high-income countries. This will probably increase the intake of plant-based foods with a concurrent reduction of animal foods[11]. Furthermore, the complementary feeding period with the transition from breast milk to the family diet may affect vitamin B_{12} status. During this period, the need for especially vitamin B_{12} is high, as the content in human breast milk and the child's own depots are sparse[2,12].

Few studies have focused on the impact of vitamin B_{12} status on psychomotor development among well-nourished children in high-income countries[13–15] and not among representative healthy children during the first years of life. In low- and middle-income countries, the association between vitamin B_{12} status and cognitive function has been assessed in more studies[4,10,16–19]. This study aims to address the associations between vitamin B_{12} and folate status in infancy and early childhood and

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Abbreviations: ASQ-3, Ages and Stages Questionnaire, 3rd edition; GLM, general linear models.

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cognitive and motor development in early childhood among healthy well-nourished children. We hypothesised that plasma vitamin B_{12} and folate concentrations would be positively associated with psychomotor development during the first 3 years of life among healthy children. Furthermore, the associations between intake of animal source foods and vitamin B_{12} and psychomotor development were examined as well.

Methods

Study design and participants

The present study used samples from the two comparable prospective observational cohorts SKOT I and SKOT-II. In SKOT-I, infants were randomly selected from the Copenhagen area using the National Danish Civil Registry(20), whereas in SKOT-II, infants were born to obese mother participating in the intervention study 'Treatment of obese pregnant women' (TOP) at Hvidovre Hospital(21). The cohorts followed the same protocol from 9 months making it possible to pool data(22). Both cohorts have been described in detail previously(20,22–25). Briefly, inclusion criteria for both cohorts were healthy singleton full-term infants aged 9 months ± 2 weeks at the first examination and Danish speaking parents. The infants were 36 ± 3 months when they were monitored at the third and last examination. At 9 months, 311 and 166 infants participated in the SKOT-I and SKOT-II cohorts, respectively, whereas at 36 months, the numbers were 266 and 130, respectively. Data collection was carried out from 2007 to 2010 for SKOT-I and from 2011 to 2014 for SKOT-II. Written informed consent was obtained from all parents and legal guardians of the children. SKOT-I (H-KF-2007-0003) and SKOT-II (H-3-2010-122) were approved by The Committees on Biomedical Research Ethics for the Capital Region of Denmark.

Examinations at 9 and 36 months

The examinations were performed at the Department of Nutrition, Exercise and Sports, University of Copenhagen, Denmark, as described in detail previously(22,23,26). Length at 9 months and height at 36 months were calculated as the mean of three measurements and BMI as weight/length^2 or weight/height^2, respectively. Z-scores for weight, length, height and BMI were calculated using WHO growth standards as a reference and the WHO Anthro software(27). From background questionnaires and interviews, information regarding breast-feeding was obtained, and full breast-feeding was defined as only receiving breast milk, vitamins and water. Information regarding mothers' education was also obtained and categorised as basic, short, medium or long.

Venous blood samples of 5 ml were taken after approximately 2 h fasting in lithium-heparin test tubes. Blood sampling was not successful in all children, so at 9 and 36 months, 401 and 331 blood samples were available, respectively. After preparation, blood samples were stored at −80°C. Vitamin B_{12} and folate were analysed on an Immulite 2000 Xpi System analyser (Siemens Healthcare Diagnostics) and determined by competitive immunoassays using Immulite®2000 vitamin B_{12} and Immulite®2000 Folic Acid kits with intra- and interassay CV of 4·6 and 9·6% for vitamin B_{12} and 4·5 and 5·1 % for folate, respectively. Due to limited plasma volume, some samples had to be diluted twice for analyses, which led to a higher limit of detection for vitamin B_{12} of 221 pmol/l for these samples. Samples below this value were coded as 111 pmol/l. Plasma ferritin was determined on an Immulite 1000 analyzer (Siemens Medical Solutions Diagnostics) using the chemiluminescent immunometric assay Immulite®Ferritin kit (Diagnostic Products Corporation) with an intra- and interassay CV of 3·4 and 3·7 %, respectively.

The definition of vitamin B_{12} deficiency was set to <148 pmol/l(3) and low vitamin B_{12} status to <300 pmol/l(28). Low folate status was defined as <10 nml/l(29).

Diet

Dietary intake was recorded using a validated pre-coded food diary(30). Parents and caregivers recorded for 7 d except for SKOT-II at 36 months where 4 d recording was applied. Household measures and photo booklet for estimation of portion sizes were used as described elsewhere(30). Intake of energy and food items for each child was calculated using the software system GIES (version 1.000d; The National Food Institute). Intake of dairy products was calculated as the combined intake of milk, formula, other milk products and cheese. Human milk was not included, as the intake was not measured. Intake of meat products was calculated as the combined intake of meat, poultry and fish.

Assessment of psychomotor development

At 36 months, motor and cognitive development was assessed by the parents using the Ages and Stages Questionnaire, 3rd edition (ASQ-3) as described previously(24,25). Briefly, the parents were instructed by the project staff at the visit how to complete the questionnaire at home. It was recommended that the parents filled it out when the child was fed and rested. ASQ-3 is a standardised developmental checklist, which for 36 months old children consists of thirty age-appropriate questions divided into five subscale categories: communication, gross motor, fine motor, problem-solving and personal-social skills. The total ASQ-3 score was calculated by adding the scores for the subscales using only cases with completed questionnaires for all subscales. The possible score range for each subscale was 0–60 and hence the range for total ASQ-3 was 0–300.

Statistics

Characteristics are given as means and standard deviations or medians and interquartile ranges (IQR) for continuous variables, and for categorical variables as counts and percentages. Comparison between sex, cohorts or multivitamin supplementation was performed by independent t test or Mann–Whitney for parametric and non-parametric variables. Bivariate correlations between values at 9 and 36 months for vitamin B_{12} and folate were conducted using Pearson's correlation coefficient.

Associations between vitamin B_{12}, folate and ASQ-3 were assessed by multiple linear regression analysis using general linear models (GLM). Vitamin B_{12} and folate at 9 and 36 months were exposures and total ASQ-3 and subscale scores were outcomes. The adjusted models included sex and age at ASQ-3 assessment. Fully adjusted models included adjustment for cohort, maternal education level and vitamin B_{12} or folate value at 9 months for the corresponding 36 months analyses. In addition, models with vitamin B_{12} were adjusted for ferritin and models with folate for BMI at 36 months as vitamin B_{12} was correlated with ferritin and folate with BMI. One model also examined the interaction between sex and vitamin B_{12} including a sex × vitamin B_{12} term, which was removed if not significant. Standardised effect estimates were calculated to compare estimates between the different exposures and outcomes. Vitamin B_{12} quartiles were applied in GLM models with vitamin B_{12} as a categorical variable. As the range for ASQ variables was rather narrow and often left skewed, logistic regression models were also accomplished to assess consistency. The ASQ-3 score variables were categorised at the 25th percentile (≤ 25th percentile *v.* >25th percentile) and logistic regression analyses were performed using the same adjustments as for GLM.

The associations between dietary intake and vitamin B_{12} and total ASQ-3, respectively, were investigated using GLM. Crude and models adjusted for sex, age and cohort were performed. At 9 months, many infants were still breastfed, which may have affected the milk intake. As the intake of human milk was not measured, the analyses of intake of dairy products at 9 months included a model with adjustment for current breast-feeding status as well. At 36 months, adjustment included intake of multivitamin supplements, which may affect plasma vitamin B_{12} concentration. Difference in intake of dairy or meat products across vitamin B_{12} quartiles was assessed by ANOVA.

Model assumptions for regression models and impact of outliers on estimates and significance were checked by residual plots and Cook's distance, respectively. P values <0·05 were considered significant, whereas P values 0·05 to <0·10 as trends. All analyses were performed using IBM SPSS statistics (version 27.0; IBM).

Results

Of the 477 children included in the SKOT cohorts, 280 children had complete ASQ-3 and valid plasma vitamin B_{12} measurements at 36 months. As these variables were of major interest, they defined the sample size. Of these, 278 children had folate measurements at 36 months, and at 9 months, 221 and 226 children had vitamin B_{12} and folate measurements, respectively (online Supplementary Fig. 1). There was no significant difference in characteristics between children with or without ASQ-3 and vitamin B_{12} measurements except that children included in the analyses were about 1 week younger at the 36 months examination than those not included (mean age (SD): 36·4 (1·02) months *v.* 36·7 (1·03) months, $P = 0·028$).

The characteristics of the children are shown in Table 1. No children had vitamin B_{12} deficiency (<148 pmol/l) at 36 months. The number of children with vitamin B_{12} deficiency at 9 months was not possible to evaluate due to dilution of samples with limited amount of material resulting in a higher detection limit of 221 pmol/l. Eight (4 %) children had concentrations below this value. One undiluted sample was <148 pmol (138 pmol/l). There were 37 (17 %) and 6 (2 %) children with low vitamin B_{12} status (<300 pmol/l) at 9 and 36 months, respectively. Furthermore, no children had low folate status (<10 nmol/l) at 9 months and 2 (1 %) had at 36 months. The median (IQR) for total ASQ-3 was 270 (250–280), whereas medians for subscales ranged between 50 (40–60) and 60 (55–60). Girls had higher total ASQ-3 score compared with boys (275 (255–285) *v.* 260 (245–275); $P \leq 0·001$), but there was no sex difference regarding vitamin B_{12} or folate status ($P \geq 0·16$). For vitamin B_{12}, there was a correlation between values at 9 and 36 months ($r = 0·408$, $P \leq 0·001$) but not for folate ($r = 0·052$, $P = 0·44$).

Vitamin B_{12} and ASQ-3

Linear regression analyses showed that vitamin B_{12} at 36 months was positively associated with total ASQ-3 score corresponding to an increase of 100 pmol/l vitamin B_{12} per 1·5 increase in total ASQ-3 score (Table 2). Thus to obtain an increase of about 1 SD in total ASQ-3 score, an increase of approximately 2000 pmol/l vitamin B_{12} would be needed. Adjustment attenuated the significance, but the associations remained significant also after adjustment for vitamin B_{12} at 9 months, which was not significant in any of the models. Although girls had higher total ASQ-3 score than boys, there was no interaction between sex and vitamin B_{12} ($P = 0·12$).

In the ASQ-3 subscale analyses, vitamin B_{12} at 36 months was significantly positively associated with problem-solving score and it remained significant after adjustment. Further, there was a positive trend for an association with the subscales communication and personal-social scores for vitamin B_{12} at 36 months in the crude model, which became significant or vanished after full adjustment, respectively. Standardised effect estimates showed comparable estimates of the total ASQ-3 score and significant subscale scores (standardised estimates (CI)); total ASQ-3:0·17 (0·006; 0·33), problem-solving: 0·18 (0·011; 0·35) and communication: 0·19 (0·027; 0·35), fully adjusted models. There were no associations with the personal-social, gross or fine motor subscales.

Vitamin B_{12} at 9 month was not associated with total ASQ-3 scores or subscale scores except for a positive association with personal-social score in the crude model.

To further investigate the impact of a low vitamin B_{12} status at 36 months on total ASQ-3 score, vitamin B_{12} was divided into quartiles in the GLM analyses. Overall, there was a trend for a difference between vitamin B_{12} quartiles in relation to ASQ-scores ($P = 0·072$). Pairwise comparisons showed that the lowest vitamin B_{12} quartile had lower ASQ score compared with the other quartiles ((first (lowest) *v.* second quartile: $P = 0·036$; first *v.* third quartile: $P = 0·039$ and first *v.* fourth (highest) quartile, $P = 0·022$)) (Fig. 1). When adjusting for sex and age, the overall trend for a difference between vitamin B_{12} quartiles vanished ($P = 0·160$), but total ASQ-3 score in the lowest vitamin B_{12} quartile was still lower compared with the highest quartiles ($P = 0·036$). Equivalent results were obtained after full adjustment.

Logistic models for the total ASQ-3 scores using total ASQ-3 below or above the 25th percentile as outcome showed similar

Table 1. Characteristics of participants*
(Numbers and percentages; Mean values and standard deviations; median values and interquarticle range)

	All cases *n*	*n*	%	Mean	SD	Median	IQR
Sex, boys	280	144	51				
Cohort, SKOT-I	280	197	70				
Mothers education level; basic, short, medium and long	278	51/39/96/92	18/14/35/33				
Birthweight (kg)	275			3·579	0·503		
Weight for age *Z*-score at birth	275			0·54	1·00		
At 9 months							
Age (months)	278			9·08	0·28		
Weight (kg)	278			9·183	1·043		
Length (cm)	278			72·2	2·46		
BMI (kg/m^2)	278			17·59	1·55		
Weight for age *Z*-score	278			0·53	0·92		
Length for age *Z*-score	278			0·44	0·99		
BMI for age *Z*-score	278			0·38	1·00		
Biomarkers							
Vitamin B_{12} (pmol/l)	221			414	174		
Folate (nmol/l)	226			41·2	20·0		
Ferritin 9 months (μg/ml)	240			37·1	28·1		
Feeding mode							
Still breastfed, yes, (*n* (%))	278	130	47				
Duration of full breastfeeding (d)						122	44, 152
At 36 months							
Age (months)	280			36·4	1·02		
Weight (kg)	280			14·84	1·697		
Length (cm)	280			96·1	3·56		
BMI (kg/m^2)	280			16·03	1·26		
Weight for age *Z*-score	280			0·31	0·86		
Length for age *Z*-score	280			0·01	0·89		
BMI for age *Z*-score	280			0·42	0·94		
Biomarkers							
Vitamin B_{12} (pmol/l)	280			653	240		
Folate (nmol/l)	278			28·0	12·4		
Ferritin (μg/ml)	280			31·5	17·0		
ASQ-3 scores							
Total ASQ-3	280					270	250, 280
Subscales							
Communication	280					50	50, 55
Gross motor	280					60	55, 60
Fine motor	280					50	40, 60
Problem-solving	280					55	50, 60
Personal-social	280					55	50, 60

ASQ-3, Ages and Stages Questionnaire, 3rd edition.
* Values are expressed as mean and standard deviation (SD); median and interquartile range (IQR); *n* and %, as appropriate.

results. Per 100 pmol/l increase in vitamin B_{12} concentration, the odds of not being in the lower quartile of total ASQ-3 score was 1·24 ((95 % CI: 1·07, 1·43), $P = 0·004$). Adjustment did not change the associations significantly (OR = 1·28 (95 % CI 1·05, 1·56), $P = 0·016$ for the full adjusted model). Contrary to the linear regression analyses, there were no associations between vitamin B_{12} at 36 months and the subscales scores though a trend was seen for fine motor subscale in the fully adjusted model (OR = 1·21 (95 % CI 0·99, 1·47) for not being in the lower total ASQ-3 quartile per 100 pmol/l increase in vitamin B_{12}, $P = 0·061$). Vitamin B_{12} at 9 months showed no associations with total ASQ-3 scales or any of the subscales.

Folate and ASQ-3

Folate was not associated with total ASQ-3 score or subscales at any time points in any of the models except for an association between folate at 9 months and communication subscale in the fully adjusted logistic regression model (OR = 1·02 (95 % CI 1·00, 1·03)) for not being in the lower total ASQ-3 quartile, per nmol/l increase in folate, $P = 0·040$).

Dietary intake and vitamin B_{12}

At 9 and 36 months, dietary intake was available for 272 and 262 children, respectively. The energy intake and intake of milk- and meat-related products are shown in Table 3. At 9 months, no infants were fully breastfed, while almost half of the children were still breastfed (47 %) (Table 1). Multivitamin supplements were only given to one child (0·4 %) at 9 month, whereas 226 children (81%) had received multivitamin supplements at 36 months. The vitamin B_{12} concentration was significantly higher in the children receiving multivitamin supplement than those not receiving supplement (680 (250) pmol/l *v.* 540 (141) pmol/l, $P \leq 0·001$.)

At both 9 and 36 months, the intake of cheese was minimal compared with the milk intake, which included formula (Table 3). Likewise was the intake of poultry and fish low

Table 2. Associations between vitamin B_{12} and folate and ASQ-3 scores by linear regressions analyses*
(Coeffients values and 95 % confidence intervals)

	Vitamin B_{12} 9 months (100 pmol/l) (*n* 221)			Vitamin B_{12} 36 months (100 pmol/l) (*n* 280)			Folate 9 months (nmol/l) (*n* 226)			Folate 36 months (nmol/l) (*n* 278)		
	β	95 % CI	*P*	β	95 % CI	*P*	β	95 % CI	*P*	β	95 % CI	*P*
Total ASQ-3												
Crude	0·60	−1·41, 2·62	0·56	1·54	0·26, 2·83	0·019	−0·006	−0·180, 0·168	0·95	0·105	−0·147, 0·357	0·41
Model 1†	0·26	−1·74, 2·26	0·80	1·43	0·16, 2·67	0·027	−0·034	−0·205, 0·138	0·70	0·068	−0·178, 0·314	0·59
Model 2‡,§	1·06	−1·04, 3·16	0·32	1·84	0·61, 3·62	0·043	0·079	−0·111, 0·268	0·41	0·047	−0·223, 0·317	0·73
Communication												
Crude	−0·007	−0·42, 0·41	0·97	0·23	−0·041, 0·50	0·096	0·007	−0·029, 0·042	0·71	0·006	−0·046, 0·059	0·81
Model 1†	−0·045	−0·46, 0·38	0·83	0·22	−0·06, 0·049	0·12	0·004	−0·032, 0·040	0·83	0·003	−0·050, 0·056	0·91
Model 2‡,§	0·081	−0·38, 0·52	0·72	0·43	0·06, 0·80	0·023	0·020	−0·019, 0·060	0·32	−0·003	−0·059, 0·053	0·90
Gross motor												
Crude	0·002	−0·56, 0·56	0·99	0·14	−0·19, 0·48	0·40	−0·008	−0·055, 0·040	0·76	0·042	−0·023, 0·107	0·20
Model 1†	−0·036	−0·60, 0·53	0·90	0·12	−0·22, 0·46	0·50	−0·010	−0·058, 0·038	0·68	0·043	−0,022, 0·109	0·19
Model 2‡,§	0·13	−0·47, 0·73	0·68	0·11	−0·41, 0·62	0·69	−0·0001	−0·054, 0·054	1·00	0·041	−0·035, 0·117	0·29
Fine motor												
Crude	0·30	−0·60, 1·21	0·51	0·38	−0·21, 0·97	0·21	−0·011	−0·089, 0·066	0·77	0·016	−0·099, 0·130	0·79
Model 1†	0·19	−0·70, 1·08	0·68	0·36	−0·22, 0·94	0·22	−0·047	−0·097, 0·056	0·60	−0·006	−0·118, 0·105	0·91
Model 2‡,§	0·48	−0·46, 1·41	0·32	0·45	−0·35, 1·25	0·27	0·023	−0·061, 0·107	0·60	−0·014	−0·134, 0·106	0·82
Problem-solving												
Crude	−0·12	−0·83, 0·59	0·74	0·52	0·06, 0·97	0·025	0·014	−0·048, 0·075	0·67	0·016	−0·072, 0·105	0·72
Model 1†	−0·14	−0·86, 0·58	0·70	0·51	0·05, 0·96	0·030	0·011	−0·051, 0·073	0·73	0·014	−0·075, 0·103	0·76
Model 2‡,§	0·08	−0·68, 0·84	0·84	0·69	0·04, 1·34	0·037	0·046	−0·23, 0·116	0·19	−0·001	−0·100, 0·098	0·99
Personal-social												
Crude	0·43	0·01, 0·84	0·043	0·28	−0·01, 0·56	0·055	−0·007	−0·043, 0·028	0·69	0·025	−0·030, 0·080	0·37
Model 1†	0·29	−0·10, 0·68	0·14	0·23	−0·04, 0·49	0·093	−0·018	−0·051, 0·015	0·29	0·014	−0·037, 0·065	0·60
Model 2‡,§	0·29	−0·12, 0·70	0·16	0·16	−0·19, 0·51	0·37	−0·011	−0·048, 0·027	0·57	0·024	−0·029, 0·077	0·37

ASQ-3, Ages and Stages Questionnaire, 3rd edition.

* Values are β-coefficients, 95 % CI, *P*-values.

† Model 1 is adjusted for sex and age at 36 months.

‡ Model 2 is adjusted for sex, age at 36 months, cohort, maternal education and value at 9 months for analyses at 36 months. Furthermore, vitamin B_{12} analyses were adjusted for ferritin and folate analyses for BMI at 36 months.

§ *n* 221 and 224 for vitamin B_{12} and folate both at 36 months, respectively.

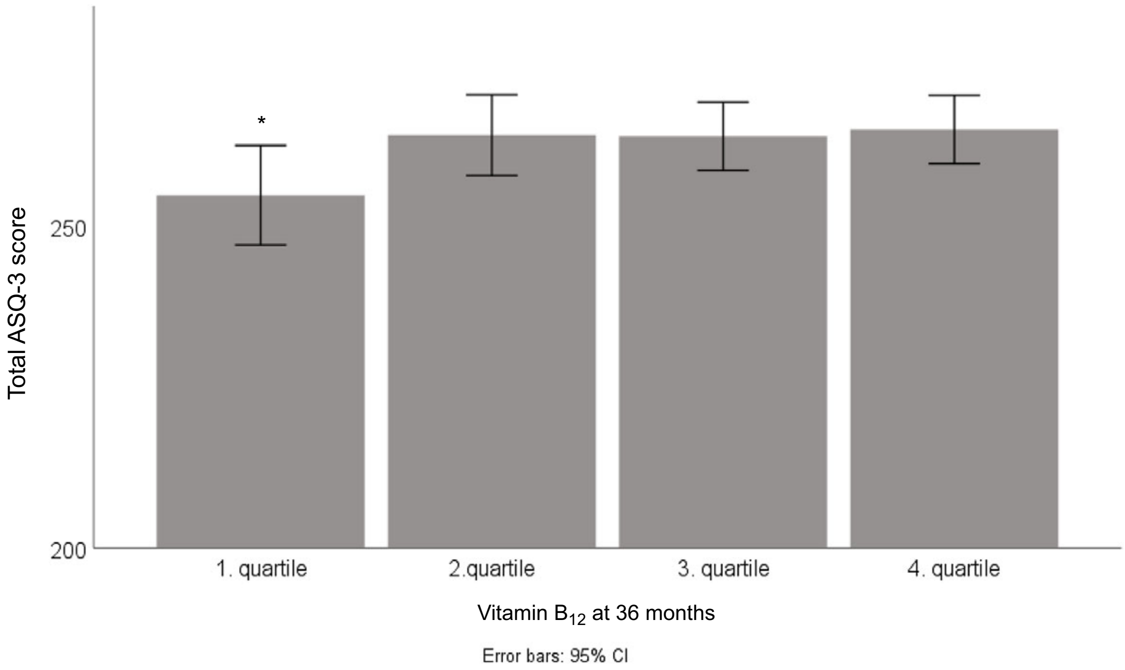

Fig. 1. Total ASQ-3 score according to vitamin B_{12} quartiles at 36 months (means, 95 % CI). *Significant different from other quartiles ($P \leq 0·039$). ASQ-3, Ages and Stages Questionnaire, 3rd edition.

Table 3. Energy, dairy- and meat-related products intake in the children at 9 and 36 months
(Mean values and standard deviations; median values and interquartile range)

	Mean	SD	Median	IQR
At 9 months (*n* 272)				
Energy (kJ/d)	3326	1041		
Milk (g/d)			122	44, 221
Cheese (g/d)			2·9	0·3, 8·2
Meat (g/d)	17·7	15·4		
Fish (g/d)	6·7	7·2		
Poultry (g/d)	3·50	5·1		
At 36 months (*n* 262)				
Energy (kJ/d)	5161	1032		
Milk (g/d)	378	155		
Cheese (g/d)	13·2	12·0		
Meat (g/d)	44·0	22·8		
Fish (g/d)	12·2	11·9		
Poultry (g/d)	7·2	9·9		

compared with the intake of meat. However, to investigate the associations between dietary intake and serum vitamin B_{12} concentration, dairy products included all relevant milk products and meat products included all meat-related products. Intake of dairy products was positively associated with current vitamin B_{12} concentration at 36 months. A 100 g increase in dairy products per d was associated with 30 pmol/l higher vitamin B_{12} concentration ((95 % CI 0·11, 0·48), $P = 0{\cdot}002$) (online Supplementary Table 1), equivalent to an increase of about 5 %. Adjustment for sex, age, intake of multivitamin supplements and cohort did not change the result significantly ($P = 0{\cdot}003$). At 9 months, there was a trend for an association (β (95 % CI): 0·16 pmol/l per g/d (-0·02, 0·33), $P = 0{\cdot}082$), but adjustment attenuated the association ($P = 0{\cdot}13$) also when adjusting for breast-feeding status at 9 months ($P = 0{\cdot}18$). Intake of meat products was not associated with vitamin B_{12} at any time point ($P \geq 0{\cdot}57$). The intake of milk products across the vitamin B_{12} quartiles was different ($P = 0{\cdot}011$). The lowest vitamin B_{12} quartile had lower intake of milk products than third and the fourth quartile ((mean (SD); *P*) 348 (141) g/d *v.* 421 (156) g/d, $P = 0{\cdot}007$ and 348 (141) g/d *v.* 424 (140) g/d, $P = 0{\cdot}005$, respectively). The intake of meat products did not differ across the vitamin B_{12} quartiles ($P = 0{\cdot}64$).

Dietary intake and ASQ

None of the diet variables were associated with total ASQ-3 at any time point ($P \geq 0{\cdot}11$). However, duration of full breast-feeding and breast-feeding status at 9 months were associated with total ASQ-3 score ($P = 0{\cdot}021$, $P = 0{\cdot}033$, respectively), but it disappeared after adjustment for sex, age, cohort and maternal education ($P \geq 0{\cdot}11$).

Discussion

In this study, including healthy Danish children with adequate vitamin B_{12} concentrations, we found that plasma vitamin B_{12} was positively associated with psychomotor development measured as total ASQ-3 at 36 month also after adjustment for essential confounders. The relation was consistent using logistic regression or vitamin B_{12} concentrations categorised into quartiles. Furthermore, the total ASQ-3 score for children in the lowest vitamin B_{12} quartile was about 10 scores lower compared the other quartiles. This could indicate that even though these children were not vitamin B_{12}-deficient, attention should be paid to children with low vitamin B_{12} concentration. Though the effect size was relatively small, it is interesting that a positive association could be observed in this sample of well-nourished children which has not been reported previously. For the ASQ-3 subscales, positive associations were observed for the communication and problem-solving scores in the linear regression analyses but not in the logistic regression analyses. This may be due to the low range and variability of the responses and risk of ceiling effect in the subscales.

Although there was a highly significant correlation between vitamin B_{12} at 9 and 36 months, vitamin B_{12} status at 9 months was not associated with ASQ-3 at 36 months. Hence, in this study, only concurrent B_{12} values were associated with psychomotor development though tracking from 9 months cannot be excluded.

Several studies have investigated the association between vitamin B_{12} status and psychomotor development in children and adolescents but mainly in low- and middle-income countries(4,10,16–19) or in special populations with risk of low vitamin B_{12} status due to diet or medical issues in high-income countries(13–15). In line with our results, a higher vitamin B_{12} status was associated with improved cognitive and motoric development in many studies, but studies have also shown no associations(18,31). A Dutch observational study measured cognitive functions in adolescents who had been on a macrobiotic diet, which is close to a vegan diet and low in vitamin B_{12}, for the first 6 years of life(32). In early childhood, the children had reduced plasma vitamin B_{12} concentration, impaired growth and psychomotor development compared with children on omnivorous diets. Follow-up examinations in adolescence revealed that these children underperformed in cognitive tests compared with adolescents fed on an omnivorous diet(13). In a larger observational study from the USA (NHANES III), plasma vitamin B_{12} was not associated with cognitive tests scores in children aged 6–16 years(31). Compared with our study the children were older, the age range broader and tests for cognitive function were different, which may contribute to the different results.

Two Norwegian randomised studies investigated the effect of vitamin B_{12} injection to high-risk groups with impaired vitamin B_{12} function in infancy(14,15). After 1 month, infants treated with vitamin B_{12} showed improved motor function. In our study, we did not observe any associations of motor development ASQ-3 subscales and vitamin B_{12}, but the difference in age might be important as associations between nutrition and motor development might be difficult to observe in later childhood(17).

In a Nepalese observational study, vitamin B_{12} status in infancy (2–12 months) was associated with cognitive development 5 years later(17). ASQ-3 scores were assessed and in accordance with our results, the strongest association was found for total ASQ-3 score but also associations for problem-solving scores were found. In contrast, we did not find any long-term association between vitamin B_{12} in infancy at 9 months and

ASQ-3 scores at 36 months. Contrary to our study, different assessments of vitamin B_{12} status were applied and Kvested *et al.* found most associations for other markers, that is, total homocysteine and methylmalonic acid, than total vitamin B_{12} concentration. Total homocysteine and methylmalonic acid have been recognised as sensitive markers of low levels and mild deficiency reflecting status for metabolic function(3,33). In infancy, differences in breast-feeding might influence the vitamin B_{12} status as some studies have showed lower vitamin B_{12} status in breastfed infants(7,17,34,35). Studies from populations with a low vitamin B_{12} status have shown associations between vitamin B_{12} concentration and cognitive development among 12–18 months old children(16,19).

In addition, a few randomised trials have investigated the effect of vitamin B_{12} on psychomotor development showing inconsistent results. A group of 6–30 months old children receiving vitamin B_{12} supplementation for 6 months showed improved gross motor scores compared with placebo but, contrary to our study, there was no effect on total ASQ-3(4). However, in the same study, where one of the four randomised groups received both folate and vitamin B_{12}, an effect was observed for gross motor and problem-solving and a trend for total ASQ-3 compared with placebo(4). Contrary, no effect on psychomotor development of vitamin B_{12} supplementation of 6–11 months old infants for 1 year was found in a Nepalese study(18). However, the study group consisted of mildly stunted children who might suffer from insufficient levels of other nutrients and the highly selected group might reduce the generalisability of the results to populations with lower prevalence of micronutrient deficiencies.

Generally, the studies are challenging to compare as they vary in study design, motor and cognitive development tests, B_{12} status assessment, age, sample size and setting of participants. Different specific developmental domains vary between tests and may not be directly comparable. In addition, the rapid development of the brain with different timing of regional brain growth spurts complicate comparison of results on developmental domains across ages.

We found no associations between folate and total ASQ-3 score. The association in the fully adjusted logistic model for the communication subscale might be a chance finding as no associations were noticed for the other models or for total ASQ-3, which has a broader range and variability, which increase the ability to detect associations as observed for the vitamin B_{12} analyses. In the literature, divergent results have been reported. In the study by Kvestad *et al.*, supplementation with folate alone did not improve total ASQ-3 and subscale scores(4). However, a positive association between folate and cognitive scores in a cross-sectional study in 6–16 years old children from the USA was observed(31) as well as in 12–18 months old Indian children provided that their vitamin B_{12} concentration was above the 25th percentile(16).

We found a positive association between the intake of dairy products and vitamin B_{12} at 36 months but no association with meat intake. Though the intake of dairy products in the lowest vitamin B_{12} quartile was around the recommended intake of 350 g/d(36), it was still possible to observe a difference in intake across the B_{12} quartiles. The association was independent of intake of multivitamin supplements. This may indicate that dairy products especially milk, which constituted the main part of the dairy intake, probably is the key contributor to vitamin B_{12} intake. This also conforms to the higher intake of dairy products compared with intake of meat products. At 9 months, many infants were still breastfed, which may underlie the missing association between milk intake and vitamin B_{12} at this age. In many studies, animal source food and plasma vitamin B_{12} concentrations were positively correlated(3,37–39), but in a study among healthy Norwegian 4–6 years old children, no associations between dietary intake and biomarkers for vitamin B_{12} were found(6). In accordance with our results, dairy products but not meat products were correlated with plasma vitamin B_{12} concentration among 2 years old Norwegian children(39). The difference in results between dairy products and meat products may be that dairy products are the principal source of vitamin B_{12} and that meat intake is low at this age. Furthermore, the bioavailability of vitamin B_{12} from dairy products might also be higher as previously suggested in a study in adults(37).

A strength of the study is that the SKOT cohorts are healthy well-nourished children with detailed information on cognitive development and dietary intake of the first years of life. A limitation of the study is that only plasma vitamin B_{12} (cobalamin) was measured as biomarker of vitamin B_{12} status due to limited amount of material. It reflects long-term status and is not affected by recent intake. Additional biomarkers would have increased the sensitivity and specificity of vitamin B_{12} status and increased the validity of the results. Another limitation is the reduced sample size as not all children had complete ASQ-3 assessment or blood samples. Nevertheless, as the only difference in baseline characteristics between children with or without valid B_{12} measurements and total ASQ-3 at 36 months was a difference in age at 36 months of 7 d. It is unlikely that this has affected the results. Furthermore, we did not have measurement of intake of human milk at 9 months. There is a risk of residual confounding as this is an observational study and no causative conclusions can be made as well as associations should be interpreted with carefulness. We did not adjust for multiplicity as this was an explorative study and the risk of chance findings may occur. The results should therefore be confirmed in other studies preferentially including other biomarkers for vitamin B_{12} status.

In summary, this study indicates that even in well-nourished children, a low vitamin B_{12} concentration is associated with a lower score for psychomotor development. The growing concern for sustainable food production might increase the interest for plant-based diet and reduced intake of animal source foods, which may compromise the vitamin B_{12} status. The current study indicates that special attention should be paid to the association between vitamin B_{12} status and psychomotor development. Future studies should address how a sufficient vitamin B_{12} status can be achieved, so optimal psychomotor development is secured.

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Supplementary material

For supplementary material/s referred to in this article, please visit https://doi.org/10.1017/S0007114521004888

References

1. Obeid R & Herrmann W (2005) Homocysteine, folic acid and vitamin B_{12} in relation to pre- and postnatal health aspects. *Clin Chem Lab Med* **43**, 1052–1057.
2. Black MM (2008) Effects of vitamin B_{12} and folate deficiency on brain development in children. *Food Nutr Bull* **29**, S126–S131.
3. Allen LH, Miller JW, de Groot L, *et al.* (2018) Biomarkers of nutrition for development (BOND): vitamin B_{12} review. *J Nutr* **148**, 1995S–2027S.
4. Kvestad I, Taneja S, Kumar T, *et al.* (2015) Vitamin B_{12} and folic acid improve gross motor and problem-solving skills in young North Indian children: a randomized placebo-controlled trial. *PLoS One* **10**, e0129915.
5. Dror DK & Allen LH (2008) Effect of vitamin B_{12} deficiency on neurodevelopment in infants: current knowledge and possible mechanisms. *Nutr Rev* **66**, 250–255.
6. Solvik BS, Strand TA, Kvestad I, *et al.* (2020) Dietary intake and biomarkers of folate and cobalamin status in Norwegian preschool children: the FINS-KIDS study. *J Nutr* **150**, 1852–1858.
7. Taneja S, Bhandari N, Strand TA, *et al.* (2007) Cobalamin and folate status in infants and young children in a low-to-middle income community in India. *Am J Clin Nutr* **86**, 1302–1309.
8. Finkelstein JL, Layden AJ & Stover PJ (2015) Vitamin B_{12} and perinatal health. *Adv Nutr* **6**, 552–563.
9. Pawlak R, Lester SE & Babatunde T (2014) The prevalence of cobalamin deficiency among vegetarians assessed by serum vitamin B_{12}: a review of literature. *Eur J Clin Nutr* **68**, 541–548.
10. Venkatramanan S, Armata IE, Strupp BJ, *et al.* (2016) Vitamin B_{12} and cognition in children. *Adv Nutr* **7**, 879–888.
11. Aschemann-Witzel J, Gantriis RF, Fraga P, *et al.* (2021) Plant-based food and protein trend from a business perspective: markets, consumers, and the challenges and opportunities in the future. *Crit Rev Food Sci Nutr* **61**, 3119–3128.
12. Greibe E, Lildballe DL, Streym S, *et al.* (2013) Cobalamin and haptocorrin in human milk and cobalamin-related variables in mother and child: a 9-months longitudinal study. *Am J Clin Nutr* **98**, 389–395.
13. Louwman MW, van Dusseldorp M, van de Vijver FJ, *et al.* (2000) Signs of impaired cognitive function in adolescents with marginal cobalamin status. *Am J Clin Nutr* **72**, 762–769.
14. Torsvik I, Ueland PM, Markestad T, *et al.* (2013) Cobalamin supplementation improves motor development and regurgitations in infants: results from a randomized intervention study. *Am J Clin Nutr* **98**, 1233–1240.
15. Torsvik IK, Ueland PM, Markestad T, *et al.* (2015) Motor development related to duration of exclusive breastfeeding, B vitamin status and B_{12} supplementation in infants with a birth weight between 2000–3000 g, results from a randomized intervention trial. *BMC Pediatr* **15**, 218.
16. Strand TA, Taneja S, Ueland PM, *et al.* (2013) Cobalamin and folate status predicts mental development scores in North Indian children 12–18 months of age. *Am J Clin Nutr* **97**, 310–317.
17. Kvestad I, Hysing M, Shrestha M, *et al.* (2017) Vitamin B_{12} status in infancy is positively associated with development and cognitive functioning 5 year later in Nepalese children. *Am J Clin Nutr* **105**, 1122–1131.
18. Strand TA, Ulak M, Hysing M, *et al.* (2020) Effects of vitamin B_{12} supplementation on neurodevelopment and growth in Nepalese infants: a randomized controlled trial. *PLoS Med* **17**, e1003430.
19. Sheng X, Wang J, Li F, *et al.* (2019) Effects of dietary intervention on vitamin B_{12} status and cognitive level of 18-month-old toddlers in high-poverty areas: a cluster-randomized controlled trial. *BMC Pediatr* **19**, 334.
20. Madsen AL, Schack-Nielsen L, Larnkjaer A, *et al.* (2010) Determinants of blood glucose and insulin in healthy 9-month-old term Danish infants; the SKOT cohort. *Diabet Med* **27**, 1350–1357.
21. Renault KM, Nørgaard K, Nilas L, *et al.* (2014) The treatment of obese pregnant women (TOP) study: a randomized controlled trial of the effect of physical activity intervention assessed by pedometer with or without dietary intervention in obese pregnant women. *Am J Obstet Gynecol* **210**, 134.e1–134.e9.
22. Andersen LBB, Pipper CB, Trolle E, *et al.* (2015) Maternal obesity and offspring dietary patterns at 9 months of age. *Eur J Clin Nutr* **69**, 668–675.
23. Ejlerskov KT, Larnkjaer A, Pedersen D, *et al.* (2014) IGF-I at 9 and 36 months of age – relations with body composition and diet at 3 years – the SKOT cohort. *Growth Horm IGF Res* **24**, 239–244.
24. Andersen KR, Harsløf LBS, Schnurr TM, *et al.* (2017) A study of associations between early DHA status and fatty acid desaturase (FADS) SNP and developmental outcomes in children of obese mothers. *Br J Nutr* **117**, 278–286.
25. Engel S, Tronhjem KMH, Hellgren LI, *et al.* (2013) Docosahexaenoic acid status at 9 months is inversely associated with communicative skills in 3-year-old girls. *Matern Child Nutr* **9**, 499–510.
26. Laursen MF, Andersen LBB, Michaelsen KF, *et al.* (2016) Infant gut microbiota development is driven by transition to family foods independent of maternal obesity. *mSphere* **1**, e00069–e00115.
27. The WHO Child Growth Standards WHO Anthro Survey Analyser and Other Tools (2006) https://www.who.int/tools/child-growth-standards/software (accessed November 2021).
28. Federal Commission for Nutrition (2018) *Vegan Diets: Review of Nutritional and Health Benefits and Risks (2018)*. Bern: Federal Food Safety and Veterinary Office.

29. Bailey LB, Stover PJ, McNulty H, *et al.* (2015) Biomarkers of nutrition for development-folate review. *J Nutr* **145**, 1636S–1680S.
30. Gondolf UH, Tetens I, Hills AP, *et al.* (2012) Validation of a pre-coded food record for infants and young children. *Eur J Clin Nutr* **66**, 91–96.
31. Nguyen CT, Gracely EJ & Lee BK (2013) Serum folate but not vitamin B_{12} concentrations are positively associated with cognitive test scores in children aged 6–16 years. *J Nutr* **143**, 500–504.
32. Dagnelie PC & van Staveren WA (1994) Macrobiotic nutrition and child health: results of a population-based, mixed-longitudinal cohort study in the Netherlands. *Am J Clin Nutr* **59**, 1187S–1196S.
33. Bjørke Monsen AL & Ueland PM (2003) Homocysteine and methylmalonic acid in diagnosis and risk assessment from infancy to adolescence. *Am J Clin Nutr* **78**, 7–21.
34. Bjørke-Monsen A-L, Torsvik I, Saetran H, *et al.* (2008) Common metabolic profile in infants indicating impaired cobalamin status responds to cobalamin supplementation. *Pediatrics* **122**, 83–91.
35. Hay G, Johnston C, Whitelaw A, *et al.* (2008) Folate and cobalamin status in relation to breastfeeding and weaning in healthy infants. *Am J Clin Nutr* **88**, 105–114.
36. Danish Health Authority (2019) Nutrition for Infants and Young Children – A Manual for Healthcare Professional. https://www.sst.dk/da/udgivelser/2019/ernaering-til-spaedboern-og-smaaboern—en-haandbog-for-sundhedspersonale (accessed August 2021).
37. Vogiatzoglou A, Smith AD, Nurk E, *et al.* (2009) Dietary sources of vitamin B_{12} and their association with plasma vitamin B_{12} concentrations in the general population: the hordaland homocysteine study. *Am J Clin Nutr* **89**, 1078–1087.
38. McLean ED, Allen LH, Neumann CG, *et al.* (2007) Low plasma vitamin B_{12} in Kenyan school children is highly prevalent and improved by supplemental animal source foods. *J Nutr* **137**, 676–682.
39. Hay G, Trygg K, Whitelaw A, *et al.* (2011) Folate and cobalamin status in relation to diet in healthy 2-years-old children. *Am J Clin Nutr* **93**, 727–735.