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POPULATION STUDY ARTICLE

Maternal and infant vitamin B12 status during infancy predict linear growth at 5 years

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BACKGROUND: Many children worldwide have poor vitamin B12 status. The objective of this study was to estimate association between maternal and infant vitamin B12 status and long-term growth.
METHODS: We randomly selected 500 Nepali mother–infant pairs and measured maternal intake and infant and maternal vitamin B12 status using plasma cobalamin, total plasma homocysteine, and methylmalonic acid concentrations. We revisited available children when they were 5 years old and measured growth. The associations between intake and maternal and infant markers of vitamin B12 and growth were estimated in multiple linear regression models adjusting for relevant confounders ($n = 331$).
RESULTS: Maternal vitamin B12 intake and status and vitamin B12 status in infancy predicted linear growth at 5 years of age, but not during infancy. Each microgram increase in the vitamin B12 intake of the mother during infancy was associated with an increase in height of 0.4 (0.2, 0.6) height-for-age *z*-scores and 1.7 (0.7, 2.7) cm around the child's fifth birthday.
CONCLUSION: Vitamin B12 status and intake in early life is an important determinant for linear growth at school age. Our findings should be verified in randomized, placebo controlled trials before translated into public health recommendations.

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INTRODUCTION

Vitamin B12 deficiency is often part of general malnutrition, and poor vitamin B12 status may contribute to poor growth and morbidity in children in many low-income and middle-income countries (LMICs).[1–3] Inadequate intake may accordingly be a contributing factor to the estimated 165 million stunted children worldwide.[4]

The best source of vitamin B12 is animal-derived foods, which are expensive and for cultural and religious reasons often not eaten.[1] Vitamin B12 is necessary for DNA and protein synthesis and therefore cell growth and cell differentiation.[5] However, the extent to which suboptimal intake and status have significant consequences for child health and growth is still not clear.

In a factorial, randomized placebo controlled trial (RCT) of folic acid and vitamin B12 in 1000 North Indian children aged 6 to 35 months, vitamin B12 supplementation for 6 months significantly improved linear growth in children who were stunted, wasted, or underweight at baseline.[6] In this previous study, only 16% were considered to be deficient, that is, having plasma cobalamin concentrations <148 pmol/L. Vitamin B12 status at baseline was also significantly and positively associated with linear growth in those who were not given vitamin B12 daily. This association between baseline cobalamin concentration and linear growth was relatively small and absent in the vitamin B12 supplemented children. Important limitations of this cohort study were that we were able to measure the status of only few nutrients, the follow-up period was short, and we had very little information on dietary intake and status of the mother.

In 2008, we undertook a cross-sectional nutritional survey in a random sample of 500 mother–infant dyads in Bhaktapur Municipality in Nepal.[7,8] In this study, we measured the intake and biomarkers of several micronutrients in the mothers and their children. To estimate the association between early vitamin B12 nutrition and later growth, we revisited most of these children around their fifth birthday. The objective of this report is to examine the extent to which vitamin B12 status in lactating mothers and breastfed infants is associated with short-term and long-term growth.

METHODS

Participants

From January 2008 to February 2009, we enrolled 500 lactating women between 15 and 45 years old and their infants 2–11 months of age from the Bhaktapur Municipality in Nepal. We used a two-stage cluster sampling procedure where 66 neighborhoods ("toles") were randomly selected as the primary sampling unit from a total of 160. We listed all women living in these toles, and randomly selected women and infant pairs for inclusion. The inclusion criteria were that mothers and children had no on-going infections, resided in the selected clusters, that household information could be obtained, and that they

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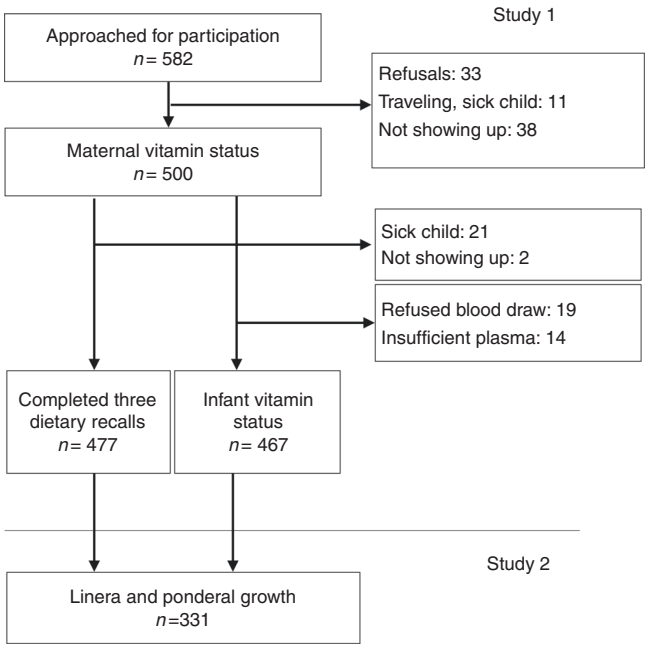

Fig. 1 Flow chart in a cross-sectional study (Study 1) and in a follow-up study (Study 2) in nepalese mother–infant pairs

consented to participate. We approached 582 women and enrolled 500 (Fig. 1). Details of the selection criteria and the recruitment of the study subjects have been published elsewhere.[7] The study is registered at clinicaltrials.gov (NCT03285399). The study obtained ethical clearance from the institutional review board at the Institute of Medicine in Kathmandu, Nepal, Harvard TH Chan School of Public Health, and the Regional Research Ethical Committee in Norway. In 2012 and 2013, approximately 5 years after the first inclusions, we approached 331 children of the initial 500 enrolled women–child pairs and measured growth. We collected a new written consent form for these follow-up assessments.

Anthropometry and biochemical markers. Mothers were asked to bring their children to Siddhi Memorial Hospital in Bhaktapur for the administration of the household questionnaire, physical examination, anthropometric measurement, and for blood collection. We measured infant length with locally made wooden boards that were periodically calibrated. A trained phlebotomist drew approximately 3 mL of whole blood from the cubital vein into polypropylene tubes with lithium heparin (Sarstedt, Germany). We measured hemoglobin using the HemoCue 201 system (HemoCue, Vedbæk, Denmark) before the blood was spun down and the plasma was separated from the blood pellet. We calibrated the HemoCue on a regular basis according to the recommendations of the manufacturer. The samples were centrifuged at $760 \times g$ for 10 min at room temperature, and the plasma was transferred into polypropylene vials (Eppendorf, Hinz, Germany). Samples were stored at −20 °C at the field site laboratory and brought to the central laboratory with an ice pack at the end of each day. There, samples were stored at −80 °C until transported on dry ice to Norway. Blood samples were analyzed at the Bevital Laboratory (Bergen, Norway) (www.bevital.no). Plasma vitamin B12 concentration were determined by a microbiological assay based on growth support of *Lactobacillus leichmannii*.[9] Folate concentration was determined using a microbiological assay, using a chloramphenicol-resistant strain of *Lactobacillus casei*.[10] These assays were adapted to a microtiter plate format and carried out by a robotic workstation; the within-day coefficients of variation were 5%. Methylmalonic acid (MMA) and total plasma homocysteine (tHcy) were analyzed using a gas chromatography-mass spectrometry based on methylchloroformate derivatization.[11] A detailed description of these procedures, the population, the data collection, the laboratory and technical equipment used, and treatment have been presented elsewhere.[8]

Maternal dietary intake. Information on maternal dietary intake during infancy was collected through three repetitive 24-h recalls on different weekdays approximately 1 week apart. Usual estimated energy and nutrient intake was calculated by the multiple source method,[12] and Black's adaptation of the Goldberg approach was used to identify under-reporters and over-reporters.[13] A thorough description of the dietary methods has been published elsewhere.[14]

Data management and statistics. We manually checked all forms for inconsistencies and the data were continuously double entered into a relational database. Infant's weight-for-age (WAZ), length-for-age (LAZ), height-for-age (HAZ), weight-for-length (WLZ), and weight-for-height (WHZ) *z*-scores were calculated based on WHO Child Growth Standards.[15] We calculated a combined indicator of vitamin B12 status (3cB-12) based on the three biomarkers (plasma cobalamin, MMA, and tHcy) according to the method suggested by Fedosov et al.,[16] where age and folate status also is taken into account.[16] The maternal and child baseline features are shown in Table 1 for the total sample and for those that were included in the 5-year follow-up. We identified predictors for LAZ and WLZ in infancy and for HAZ and WHZ at 5 years of age using a manual stepwise modeling approach[17]; all variables that are listed in Table 1 and estimated intake of nutrients from the 24-h dietary recalls were included in this process. Correlations between variables are presented as Spearman's rank-order correlation coefficients. We used multiple linear regression models to estimate the association between vitamin B12 intake and status of the mother and status in infancy and growth in infancy and at 5 years. In these analyses, we used the estimated mean daily intake of cobalamin (µg/day), the log (base2) cobalamin (pmol/L), tHcy (µmol/L), and MMA (nmol/L) concentrations and the 3cB-12[16] as the main exposure variables in the different regression models. We adjusted for the predictors that were significantly associated with the various outcomes; both crude and adjusted estimates are presented. We also used a backward stepwise automatic variable selection procedure where we included the potential confounders and the main exposure variables (intake, tHcy, MMA, cobalamin, and 3cB-12). These main exposure variables were not included in the models simultaneously because they were assumed to be strongly correlated and/or in the causal pathway of each other. We also adjusted the models for other nutrient biomarkers (retinol, folate, vitamin D, niacin, vitamin A, ferritin) and intake of other nutrients (vitamins B1, B2, B3, B6, folate, beta-carotene, zinc, iron, total energy, protein, fat, and fiber). The results of the automatic variable selection process and the adjustments for the status or intake of the other nutrients are not shown as it did not alter our observed associations.

We used STATA version 15 (Stata Corporation, College Station, TX, USA) for most statistical analyses. We also used generalized additive models in the statistical software R version 2.0. (The R Foundation for Statistical Computing) to explore nonlinear associations between intake of vitamin B12 and markers of vitamin B12 status.[18]

RESULTS

Baseline features

Maternal and child features are shown in Table 1 where we include information about the total sample and of the families that we included in the follow-up. The features of the total sample

Table 1. Background characteristics of the total study population and the subsample where growth was measured at 5 years

	Subsample			Total sample		
	N	Mean (%)	SD	*N*	Mean (%)	SD
Child characteristics at baseline						
Total	331			500		
Boys	180	54.4		277	55.7	
Age (months)	331	7.0	2.9	500	6.9	3.0
Birth weight (g)	326	2872	475.8	485	2891.5	491.8
Exclusively breastfed at enrollment	45	13.6		72	14.4	
Growth status in infancy						
WAZ	331	−0.29	1.0	500	−0.3	1.0
WLZ	331	0.03	1.0	500	0.0	1.1
LAZ	331	−0.46	1.3	500	−0.5	1.3
Plasma concentrations of nutritional markers at age 2 to 12 months						
Cobalamin (pmol/L)	317	264.0	133.2	466	261.2	131.4
<148 pmol/L	48	15.1		74	15.9	
Homocysteine (μmol/L)	317	11.9	5.0	466	12.5	5.5
>10 μmol/L	182	57.4		289	62.0	
Methylmalonic acid (μmol/L)	317	0.77	0.73	466	0.84	0.84
>0.28 μmol/L	248	78.2		384	82.0	
Composite score (units)	317	-0.65	0.48	466	-0.68	0.47
<−0.5 U	205	64.7		316	67.80	
Folate (nmol/L)	317	71.9	33.8	466	73.0	35.3
<10 nmol/L	0	0		0	0	
Iron status						
Plasma ferritin (μmol/L)	304	54.0	70.41	449	57.9	94
Hemoglobin concentration	322	10.7	1.2	474	10.7	1.3
(g/L) <11	192	59.6		278	58.6	
Family situation at baseline						
Maternal characteristics						
Age	331	26.1	4.2	500	25.8	4.2
Less than grade 10	147	44.4		243	48.6	
10th grade and more	158	47.7		220	44.0	
Mothers who work	80	24.2		122	24.4	
Paternal characteristics						
Less than grade 10	94	28.4		165	33.0	
10th grade and more	216	65.3		298	59.6	
Fathers who work	281	84.9		430	86.0	
Household characteristics						
Joint family	170	51.4		250	50.0	
Own land	182	55.0		270	54.0	
Plasma concentrations of nutritional biomarkers in the mothers						
Cobalamin (pmol/L)	331	288.9	114.8	500	300.2	121.8
<148 pmol/L	16	4.8		22	4.4	
Total homocysteine (μmol/L)	331	10.1	5.9	500	10.2	6.4
>10 μmol/L	103	31.1		163	32.6	
Methylmalonic acid (μmol/L)	331	0.53	0.46	500	0.53	0.45
>0.28μmol/L	221	66.8		353	70.6	
Cobalamin composite score (units)	331	−0.34	0.66	500	−0.32	0.67
<−0.5 U	138	41.7		193	38.6	
Plasma folate (nmol/L)	331	20.1	15.1	500	20.6	16.2
<10 nmol/L	78	23.6		118	23.6	
Plasma ferritin (μmol/L)	331	73.4	46.0	500	68.8	45.9
Hemoglobin concentration (g/L)	331	13.2	1.2	500	13.1	1.3
<11 g/L	11	3.3		27	5.4	
Mean daily intake of cobalamin (μg/day)	316	0.83	0.62	465	0.81	0.62
<2.4	3	99.0		460	98.9	

Baseline features in a study on the association between maternal intake and maternal and infant status of vitamin B12 and growth in infancy and at 5 years of age

and those who also participated in the follow-up were very similar with regard to all baseline features including socioeconomic status, age, breastfeeding habits, and nutritional and micronutritional status. The mean (SD) age of the children at enrollment was 7 months (3.0), and the mean maternal age when maternal vitamin status was measured was 25.8 years. All children were breastfed but only 14% exclusively so. At enrollment mean WHZ was −0.3 and mean LAZ −0.46, and only 3.1 % were wasted (WHZ < −2Z) and 9.9 % stunted (LAZ < −2Z). When the children were 5 years, the mean WHZ was −1.2 and 1.8% were wasted, the mean HAZ was −1.6 and 39.0% were stunted.

Vitamin B12 and folate status

The results of the biochemical analyses are shown in Table 1. All of the biomarkers were within the limits of detection. Fifteen percent of the children had cobalamin <148 pmol/L, and most of the infants had elevated tHcy and MMA. Five percent of the women had cobalamin <148 pmol/L, tHcy was elevated in one-third and MMA in approximately two-thirds of the women. 3cB-12 was lower than −0.5, which is the cut-off for mild deficiency,[16] in 68% of the children and 39% of the mothers. It should be noted that this cut-off has not been validated in children and lactating women. The folate concentrations ranged from 10.2 to 193.0 nmol/L in the infants and 2.0 to 138.9 in their mothers. In women, but not children, folate concentration was negatively associated with tHcy (data not shown). None of the children were folate deficient, as all children had plasma folate concentrations ≥10 nmol/L. In contrast, 24% of the women had a plasma folate concentration <10 nmol/L. Almost 60% of the children and only around 5% of the women were anemic. The mean maternal daily intake of energy was 2087 (SD: 318) kcal, the mean daily intake was 0.8 (SD 0.62) μg for vitamin B12, and 186 (SD: 70.6) μg for folate. Only 5 out of the 466 women of whom we had 3, 24-h dietary recalls had a mean estimated intake that was over the RDA of 2.4 μg/day.[19]

Associations between intake and biomarkers

Pairwise Spearman's correlation coefficients between maternal intake of vitamin B12 and maternal and infant markers of cobalamin status are shown in Table 2. Maternal intake of vitamin B12 was significantly associated with maternal plasma cobalamin, MMA concentration, and maternal 3cB-12 score as well as the child's cobalamin, tHcy, MMA concentrations, and 3cB-12 scores. Maternal intake of vitamin B12, however, was not significantly associated with the maternal tHcy concentration.

Associations between vitamin B12 and growth

The associations between intake of vitamin B12 and the various markers of vitamin B12 status and growth are shown in Tables 3 and 4. Except for a borderline significant association between MMA concentration and linear growth, neither maternal intake of vitamin B12 nor any of the vitamin B12 biomarkers were associated with growth during infancy. At 5 years of age, however, maternal vitamin B12 intake and all maternal and infant biomarkers reflecting B12 status, except for maternal tHcy, were significantly associated with length and HAZ, but not WHZ. Each increase in the vitamin B12 intake of 1 μg/day of the mother during infancy was associated with an increase in the height of 0.4 (0.2, 0.6) HAZ and 1.7 (0.7, 2.6) cm. The association between maternal vitamin B12 intake and HAZ (Fig. 2) and B12 biomarker concentrations and HAZ (data not shown) were approximately linear throughout the range of observed values.

DISCUSSION

Except for maternal tHcy concentration, all markers of vitamin B12 status during infancy, as well as maternal dietary intake, predicted linear growth at 5 years of age. None of these variables were associated with linear growth during infancy or with ponderal growth (weight for height) at any time point. Our observed associations remained stable and significant also after adjusting for relevant confounders, including socioeconomic

Table 2. Correlation matrix between maternal vitamin B12 intake and plasma markers of infant and maternal B12 status

	Mother					Infant		
	B12 intake	3c-B12	MMA	tHcy	Cobalamin	3c-B12	MMA	tHcy
Mother								
3c-B12	0.15	1						
	0.002							
MMA	−0.12	−0.86	1					
	0.013	<0.001						
tHcy	−0.07	−0.60	0.31	1				
	0.123	<0.001	<0.001					
Cobalamin	0.15	0.67	−0.41	−0.26	1			
	0.001	<0.001	<0.001	<0.001				
Infant								
3c-B12	0.20	0.44	−0.39	−0.26	0.33	1		
	<0.001	<0.001	<0.001	<0.001	<0.001			
MMA	−0.14	−0.35	0.36	0.21	−0.20	−0.85	1	
	0.003	<0.001	<0.001	<0.001	<0.001	<0.001		
tHcy	−0.20	−0.41	0.32	0.30	−0.28	−0.74	0.46	1
	<0.001	<0.001	<0.001	<0.001	<0.001	<0.001	<0.001	
Cobalamin	0.19	0.32	−0.23	−0.17	0.38	0.73	−0.37	−0.53
	<0.001	<0.001	<0.001	0.002	<0.001	<0.001	<0.001	<0.001

Pairwise Spearman's correlation coefficients (upper figure) and corresponding *P* values (lower figure)
3c-B12 composite vitamin B12 status indicator, *MMA* methylmalonic acid, *tHcy* total plasma homocysteine

status, intake of energy, and the intake and plasma concentrations of other nutrients. The associations with child length were linear throughout the range of observed vitamin B12 intake and maternal and infant status. Thus, our results do not suggest any cut-offs indicating when intake or status was adequate and indicate that most of the participants would benefit from increasing their intake of vitamin B12. This is supported by the intake data showing that almost all of the mothers had vitamin B12 intakes below the recommended daily intake. Vitamin B12 and folate interact and the effect of either depends on the other. In our population, where folic acid supplementation or fortification is not mandatory but common practice,[20] poor folate status is rare, and the intake of folate from food is high.[21] This is reflected in the findings from our study where none of the infants and <10% of the mothers had concentrations <7.5 nmol/L. This left us with an opportunity to study the associations between vitamin B12 status and growth in a folate replete population.

Markers of vitamin B12 status

When using the cut-off of 148 pmol/L plasma cobalamin concentration to define deficiency, only 5% of the children and 15% of the women were deficient. These prevalences suggest that vitamin B12 deficiency is not a significant public health problem in this population. However, this approach to define deficiency may lack sufficient sensitivity and accordingly underestimate the true prevalence. The functional markers tHcy and MMA are considered to be more sensitive in detecting mild B12 deficiency.[22] Homocysteine remethylation to methionine requires vitamin B12, and poor vitamin B12 status leads to elevated plasma tHcy levels. Vitamin B12 also functions as a co-factor in the enzyme methylmalonyl-CoA mutase, which explains the increased levels of MMA during suboptimal B12 status and deficiency.[23] An increase in these markers may indicate a reduced intracellular B12 availability which has negative consequences for cell functioning and growth. In other studies, which included women and children in South Asia, we found that these metabolites start to increase when the plasma cobalamin concentrations fall below 250–300 pmol/L,[3,20,21,24] which also suggests that the cut-off of 148 pmol/L is not sufficiently sensitive to define deficiency.

The composite biomarker (here: 3cB-12) includes the concentrations of cobalamin, MMA, and tHcy.[16] According to this indicator, 39% of the women and 68% of the children were deficient. It should be noted, however, that the combined vitamin B12 indicator has not been validated in pediatric populations. It should also be noted that MMA is usually higher during infancy which limits the validity of the 3cB-12 indicator during this period of life.[25,26] However, the associations described in Tables 2 and 3 suggest that MMA is a useful marker for both infant and maternal vitamin B12 status. Furthermore, the fact that vitamin B12 intake predicted 3cB-12 in both women and children well, and that 3cB-12 in both women and infants was a strong predictor for growth lends validity to this biomarker.

It is interesting to observe that, in contrast to the significant association between maternal B12 intake and infant tHcy concentration, maternal tHcy was not associated with vitamin B12 intake. This observation is in line with findings from a recent RCT in pregnant South Indian women. In this study, vitamin B12 supplementation during the last two trimesters improved all indices of maternal cobalamin status except the tHcy concentration.[27] In contrast, maternal vitamin B12 supplementation significantly and substantially reduced the tHcy concentration in the infants born to these mothers. The findings from this RCT and the current study indicate that plasma tHcy concentration is not a good marker of vitamin B12 status during pregnancy and lactation.[27]

Strengths and limitations

To the best of our knowledge, this study is the first to investigate the associations between infant and maternal vitamin B12 status with growth until school age. The strengths of our study include

Table 3. Crude and adjusted associations between vitamin B12 intake and status and linear attained growth in centimetres, LAZ, and HAZ

	Infancy			5 years		
	Length (cm)			Height (cm)		
	Coeff		*P*	Coeff		*P*
Maternal B12 intake (µg/day)						
Crude	−0.04	(−0.77–0.68)	0.906	1.63	(0.60–2.66)	0.002
Adjusted	−0.15	(−0.63–0.33)	0.548	1.68	(0.65–2.70)	0.001
	LAZ			HAZ		
	Coeff		*P*	Coeff		*P*
Maternal B12 intake (µg/day)						
Crude	0.08	(−0.12–0.28)	0.410	0.38	(0.20–0.55)	<0.001
Adjusted	−0.09	(−0.30–0.12)	0.385	0.40	(0.22–0.58)	<0.001
Maternal B12 status						
3cB-12 (units)						
Crude	0.09	(−0.09–0.27)	0.333	0.35	(0.09–0.41)	0.002
Adjusted	0.06	(−0.11–0.23)	0.498	0.30	(0.19–0.51)	<0.001
MMA (log 2 µmol/L)						
Crude	−0.06	(−0.30–0.19)	0.649	−0.28	(−0.49–0.06)	0.012
Adjusted	−0.09	(−0.32–0.14)	0.453	−0.38	(−0.59–0.16)	0.001
tHcy (log 2 µmol/L)						
Crude	−0.03	(−0.18–0.12)	0.684	−0.04	(−0.18–0.09)	0.512
Adjusted	0.05	(−0.09–0.19)	0.466	−0.08	(−0.21–0.05)	0.237
Cobalamin (log 2 pmol /L)						
Crude	0.09	(−0.07–0.25)	0.257	0.22	(0.08–0.35)	0.002
Adjusted	0.08	(−0.07–0.23)	0.302	0.26	(0.12–0.39)	0.000
Infant B12 status						
3cB-12 (units)						
Crude	0.05	(−0.10–0.19)	0.536	0.23	(0.09–0.36)	0.001
Adjusted	0.06	(−0.08–0.20)	0.365	0.21	(0.08–0.34)	0.002
MMA (log 2 µmol/L)						
Crude	−0.12	(−0.22–0.01)	0.026	−0.16	(−0.26–0.07)	0.001
Adjusted	−0.11	(−0.20–0.01)	0.034	−0.15	(−0.24–0.06)	0.001
tHcy (log 2 µmol/L)						
Crude	0.20	(−0.01–0.41)	0.068	−0.22	(−0.41–0.02)	0.030
Adjusted	0.09	(−0.12–0.30)	0.381	−0.15	(−0.35–0.05)	0.136
Cobalamin (log 2 pmol /L)						
Crude	−0.03	(−0.18–0.13)	0.719	0.13	(−0.01–0.27)	0.060
Adjusted	−0.02	(−0.16–0.13)	0.819	0.14	(0.01–0.27)	0.041

All multiple models were adjusted for age and sex. In the models where length or LAZ during infancy is the main outcome, we also adjusted for parity, maternal body mass index, maternal intake of energy, intake of fiber, place of birth, and paternal education, as well as maternal length. Where length or HAZ at 5 years is the main outcome, we also adjusted for maternal education, intake of energy, and fiber, and place of birth, as well as maternal length. We also adjusted for the design effect by adjusting the CI and *P* values for clustering according to the cluster sampling procedure.
Coeff regression coefficients from crude and adjusted regression models, *3cB-12* composite vitamin B12 status indicator (15), *MMA* methylmalonic acid, *tHcy* total plasma homocysteine, *LAZ* length-for-age *z*-score, *HAZ* height-for-age *z*-score

the prospective design, large sample size, assessment of vitamin B12 status using several biomarkers, and that we could estimate maternal intake of vitamin B12 using 3, 24-h dietary recalls. Maternal vitamin B12 intake, maternal vitamin B12 status, and infant vitamin B12 status were significantly correlated lending validity to these measures. We originally designed the study as a cross-sectional survey, and we did not restrict participation to those who planned to reside in the study area permanently or who would consent re-inclusion later. We were accordingly only able to revisit 331 children of the original sample after 5 years. This is an important limitation. Our follow-up subsample did not, however, substantially differ from the initial sample regarding the main demographic, biochemical, or clinical characteristics (Table 1). It should also be noted that the study was powered to estimate prevalences, and not associations. We measured intake over a short period and status only once during infancy. An underlying assumption in our analyses is that our measured exposure variables reflect a longer period of the child's early life. This assumption also implies a cumulative effect of vitamin B12 over the first few months or years of life.

Significance of our findings

The findings from this observational study are important because the suggested cumulative effect may, for practical reasons, be

Table 4. Crude and adjusted associations between vitamin B12 intake and status and ponderal growth in kilograms and WLZ and WHZ

	Weight (kg)			Weight (kg)		
	Coeff		*P*	Coeff		*P*
Maternal B12 intake (µg/day)						
Crude	0.11	(−0.07–0.30)	0.235	0.76	(0.38–1.14)	0.001
Adjusted	0.05	(−0.10–0.19)	0.523	0.46	(−0.01–0.93)	0.053
	WLZ			WHZ		
	Coeff		*P*	Coeff		*P*
Maternal B12 intake (µg/day)						
Crude	0.20	(0.04–0.36)	0.016	0.20	(0.04–0.36)	0.012
Adjusted	0.10	(−0.07–0.28)	0.253	0.11	(−0.10–0.31)	0.306
Maternal B12 status						
3cB-12 (units)						
Crude	0.14	(−0.01–0.28)	0.068	−0.06	(−0.20–0.08)	0.397
Adjusted	0.12	(−0.03–0.27)	0.119	−0.10	(−0.25–0.05)	0.173
MMA (log 2 µmol/L)						
Crude	−0.12	(−0.32–0.08)	0.229	0.09	(−0.10–0.27)	0.354
Adjusted	−0.12	(−0.32–0.08)	0.229	0.12	(−0.06–0.33)	0.179
tHcy (log 2 µmol/L)						
Crude	−0.12	(−0.24–0.00)	0.050	0.01	(−0.10–0.12)	0.826
Adjusted	−0.09	(−0.21–0.03)	0.156	0.02	(−0.09–0.15)	0.626
Cobalamin (log 2 pmol/L)						
Crude	0.06	(−0.07–0.18)	0.378	−0.03	(−0.15–0.09)	0.589
Adjusted	0.07	(−0.07–0.19)	0.377	−0.06	(−0.18–0.07)	0.378
Infant B12 status						
3cB-12 (units)						
Crude	0.01	(−0.11–0.13)	0.894	−0.05	(−0.16–0.07)	0.403
Adjusted	−0.02	(−0.14–0.11)	0.780	−0.07	(−0.19–0.05)	0.270
MMA (log 2 µmol/L)						
Crude	0.00	(−0.09–0.08)	0.931	0.05	(−0.03–0.13)	0.220
Adjusted	0.02	(−0.07–0.11)	0.702	0.07	(−0.02–0.15)	0.108
tHcy (log 2 µmol/L)						
Crude	−0.08	(−0.25–0.10)	0.392	0.10	(−0.07–0.26)	0.267
Adjusted	−0.07	(−0.25–0.12)	0.463	0.12	(−0.06–0.30)	0.186
Cobalamin (log 2 pmol/L)						
Crude	−0.03	(−0.15–0.10)	0.674	0.03	(−0.09–0.14)	0.659
Adjusted	−0.04	(−0.17–0.09)	0.506	0.03	(−0.09–0.15)	0.649

All multiple models were adjusted for age and sex. In the models where weight or WLZ during infancy is the main outcome, we also adjusted for maternal body mass index, paternal education, maternal hemoglobin concentration, place of birth, and intake of energy. Where weight or WHZ at 5 years is the main outcome, we adjusted for maternal body mass index, place of birth, daily mean intake of energy, and daily mean intake of fat. We also adjusted for the design effect by adjusting the CI and *P* values for clustering according to the cluster sampling procedure
WLZ weight-for-length *z*-scores, *WHZ* weight-for-height *z*-scores, *coeff* regression coefficients from crude and adjusted regression models, *3cB-12* composite vitamin B12 status indicator (15), *MMA* methylmalonic acid, *tHcy* total plasma homocysteine

difficult to capture in an RCT design. Another limitation of RCTs is that the effects of the intervention will be heterogeneous and probably none existing in those who are not deficient and grow according to their potential. Targeting those with preexisting deficiency may not be possible due ethical reasons and because of challenges in defining status. Our findings are also important because poor linear growth is the most common feature reflecting undernutrition globally and linked to increased morbidity and impaired neurodevelopment.[4] In a recent publication from this cohort, we also demonstrated that vitamin B12 status in infancy was significantly associated with several domains of neurodevelopment when these children were 5 years old.[28] This suggests that inadequate vitamin B12 nutrition can be a common cause of both poor growth and poor neurodevelopment in young children.

The etiology of stunting is complex and multifaceted and includes exposure to pathogens, inflammation, and low energy and micronutrient intake.[29] The findings from this study suggest a role for vitamin B12. We need to measure the effect of vitamin B12 supplementation in randomized, placebo controlled trials in populations where stunting and poor vitamin B12 status is common. Important aspects of studying are timing and duration as well as dose and whether vitamin B12 status interacts with other nutritional and environmental factors such as the gut microbiome, inflammation, other micronutrients, and total energy intake.

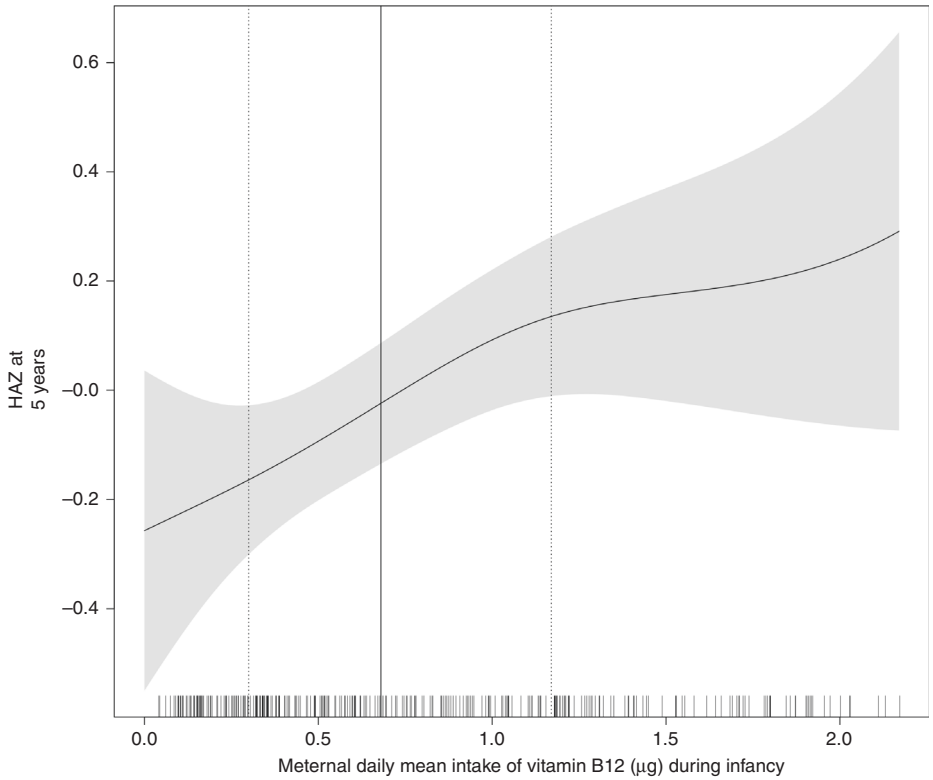

Fig. 2 The adjusted association between maternal daily intake of vitamin B12 and height-for-age *z*-scores (HAZ) of their children at 5 years of age. The graph was generated using generalized additive models in R and adjusting for relevant confounders (age, sex, maternal education, intake of energy, intake of fiber, place of birth, and maternal length). The vertical lines represent the 25th, 50th, and 75th percentiles of vitamin B12 intake. The shaded area is the 95% confidence interval of the smoothed regression line

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ADDITIONAL INFORMATION

Competing interests: The authors declare no competing interests.

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