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Contribution of food sources to the vitamin B₁₂ status of South Indian children from a birth cohort recruited in the city of Mysore

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Abstract

Objective: There is evidence that subclinical vitamin B₁₂ (B₁₂) deficiency is common in India. Vegetarianism is prevalent and therefore meat consumption is low. Our objective was to explore the contribution of B₁₂-source foods and maternal B₁₂ status during pregnancy to plasma B₁₂ concentrations.

Design: Maternal plasma B₁₂ concentrations were measured during pregnancy. Children's dietary intakes and plasma B₁₂ concentrations were measured at age 9.5 years; B₁₂ and total energy intakes were calculated using food composition databases. We used linear regression to examine associations between maternal B₁₂ status and children's intakes of B₁₂ and B₁₂-source foods, and children's plasma B₁₂ concentrations.

Setting: South Indian city of Mysore and surrounding rural areas.

Subjects: Children from the Mysore Parthenon Birth Cohort (*n* 512, 47.1% male).

Results: Three per cent of children were B₁₂ deficient (<150 pmol/l). A further 14% had 'marginal' B₁₂ concentrations (150–221 pmol/l). Children's total daily B₁₂ intake and consumption frequencies of meat and fish, and micronutrient-enriched beverages were positively associated with plasma B₁₂ concentrations (*P*=0.006, *P*=0.01 and *P*=0.04, respectively, adjusted for socio-economic indicators and maternal B₁₂ status). Maternal pregnancy plasma B₁₂ was associated with children's plasma B₁₂ concentrations, independent of current B₁₂ intakes (*P*<0.001). Milk and curd (yoghurt) intakes were unrelated to B₁₂ status.

Conclusions: Meat and fish are important B₁₂ sources in this population. Micronutrient-enriched beverages appear to be important sources in our cohort, but their high sugar content necessitates care in their recommendation. Improving maternal B₁₂ status in pregnancy may improve Indian children's status.

Keywords
India
Vitamin B₁₂
Child
Source

Vitamin B₁₂ (B₁₂), or cobalamin, plays a key role in cellular metabolism and DNA synthesis⁽¹⁾. It is produced in nature only by microbial synthesis and animal products are the principal dietary sources for man. Uncooked plant-based foods contaminated with B₁₂-synthesising bacteria, and fermented foods, may also be important sources^(2,3). In India, vegetarian diets have been associated with an increased risk of B₁₂ deficiency and a high prevalence of B₁₂ deficiency has been attributed to low meat intakes for religious or economic reasons^(2,4). Prevalence rates of deficiency of 47–71% have been reported among adults^(4–9). There is no standard

definition of deficiency among children and there is a paucity of data on B₁₂ status among Indian children. However, recent studies using adult cut-offs have reported that 2–44% of infants and school-age children are deficient^(10–14). A study in Pune, India showed normal B₁₂ absorption in the majority (90%) of individuals studied⁽¹⁵⁾.

Severe B₁₂ deficiency, as seen in pernicious anaemia, is characterised by megaloblastic anaemia and/or neurological dysfunction. However, subclinical cobalamin deficiency, currently defined as asymptomatic, mild metabolic abnormalities, may be of greater public health importance⁽¹⁶⁾. Recent research has related low B₁₂ status with an increased risk of adiposity and gestational diabetes in India⁽¹⁷⁾. Low status among Indian and Nepalese mothers during pregnancy was associated with increased insulin resistance in

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their children^(5,18). B₁₂ deficiency is associated with raised plasma homocysteine levels, which is a risk factor for CVD⁽¹⁹⁾. There is also evidence of low B₁₂ status being related to increased cognitive decline in elderly people in the USA⁽²⁰⁾.

Several studies in low- and middle-income countries have identified significant correlations between dietary intakes of B₁₂ or consumption frequency of B₁₂-rich foods and plasma B₁₂ in early childhood^(13,21–26). The association between diet and plasma B₁₂ concentration among older children remains under-studied. There is also a need to identify affordable and acceptable sources of B₁₂ for the Indian population.

In the current study, the diets of children aged 9.5 years from the Parthenon Birth Cohort, Mysore, India, were assessed using a semi-quantitative FFQ from which daily B₁₂ intakes were calculated. We aimed to investigate associations between children's B₁₂ intake and frequency of consumption of potential B₁₂-source foods, with biochemical measures of B₁₂. We hypothesised that higher B₁₂ intakes, and more frequent consumption of non-vegetarian foods (meat, fish and eggs) and dairy products (milk, butter and yoghurt), would be associated with higher B₁₂ status. We also examined whether intakes of traditional Indian fermented foods (whose preparation involves microbial activity) and of raw vegetables (that may be contaminated with bacteria) were associated with higher B₁₂ concentrations. Maternal B₁₂ status is correlated with neonatal B₁₂ status^(19,27,28). We therefore assessed whether maternal B₁₂ concentrations in plasma samples collected during the pregnancy of each child were related to children's B₁₂ concentrations at 9.5 years. The primary outcome in all analyses was children's plasma B₁₂ concentration.

Methods

Participants and setting

Details of the Mysore Parthenon Birth Cohort have been published elsewhere^(29,30). In brief, between June 1997 and August 1998, pregnant women attending the antenatal clinic of Holdsworth Memorial Hospital (HMH) and living in the city of Mysore or surrounding rural areas were recruited to the study if they fulfilled the following criteria: non-diabetic prior to pregnancy; <32 weeks' gestation at time of recruitment; and planning to deliver at HMH (Fig. 1). A total of 1233 women were eligible for the study and 830 (67%) agreed to participate, of whom 663 delivered live, singleton babies without major congenital abnormalities at HMH. In 2007, 539 (81%) children attended for follow-up at 9.5 years (fifty-six refused, eight were not traced, twenty-six moved away, twenty-five died and nine were withdrawn from the study because of severe chronic medical conditions). Dietary data were available for 538 children, and of these, plasma B₁₂ concentrations were available for 527.

The study was conducted according to the guidelines laid down in the Declaration of Helsinki and all procedures were approved by the HMH Research Ethics Committee. Written informed consent was obtained from parents and assent from the children.

Sociodemographic factors

Maternal education and socio-economic status were recorded, the latter using the Standard of Living Index (SLI) questionnaire developed for the second Indian National Family Health Survey (NFHS-2)⁽³¹⁾. This uses information on household possessions, house type, drinking-water source and sanitation facilities to derive an SLI score. A higher score denotes higher socio-economic status. The NFHS-2 categorised SLI scores of ≥ 25 as 'high' standard of living⁽³¹⁾. Children were classified as 'urban' or 'rural' based on their address at 9.5 years; towns with a population > 100 000 were defined as urban⁽³²⁾.

Anthropometry

Height was measured to the nearest 0.1 cm using a wall-mounted stadiometer (Microtoise; CMS Instruments, London, UK). Weight was measured to the nearest 100 g using digital scales (Salter, Kent, UK). Standard deviation scores for height-for-age and BMI-for-age were calculated relative to the WHO Growth Reference Data⁽³³⁾. BMI-for-age Z-scores were used to identify underweight, overweight or obese children (< -2 , $> +1$ and $\leq +2$ or $> +2$, respectively)⁽³³⁾.

Biochemical measurements

Fasting venous blood samples were collected at 28.6 weeks (25th, 75th percentile (P25, P75): 27.9, 30.6 weeks; min, max: 23.7, 35.1 weeks) of gestation from mothers and stored for 8 years at -80°C ⁽³⁴⁾. Children's samples were collected at 9.5 years. Blood was separated within 2 h of venesection and plasma aliquots were stored at -80°C before transfer to the laboratory. B₁₂ concentrations were measured using a microbiological assay at the Diabetes Unit, KEM Hospital Research Centre, Pune, India^(17,23). Intra- and inter-assay CV were $< 8\%$. Children's Hb concentrations were measured on the day of blood collection using a cell counter (Haematology analyser MEK 6420; Nihon Kohden, Japan) at the HMH Laboratory, Mysore.

B₁₂ deficiency for mothers and children was defined using WHO plasma concentration cut-offs for adults (< 150 pmol/l)⁽¹⁹⁾. Given the lack of consensus cut-offs for use in children, we also used a definition of 'marginal' B₁₂ status (150–221 pmol/l)^(21,22,35). Anaemia was defined as Hb concentration < 11.5 g/dl⁽³⁶⁾.

Dietary assessment

A 136-item semi-quantitative interviewer-administered FFQ was developed to characterise food and micronutrient intakes in this cohort of children⁽²³⁾. The FFQ was administered by one of three trained nutritionists, to both the child and one parent (usually the mother). The reference

period was a typical month. Consumption frequency was recorded as number of times eaten daily, weekly or monthly. Portion size was quantified using common household utensils. In cases where units or frequency data were missing (<2%), a nutritionist assigned the most frequently reported response. Current medication use, including micronutrient supplements, was also recorded.

Foods on the FFQ that were considered potential sources of B₁₂ were grouped into the following categories: (i) flesh foods (meat and fish); (ii) eggs; (iii) curd (yoghurt) foods; (iv) dairy foods; (v) micronutrient-enriched beverages; (vi) fermented foods; and (vii) raw vegetables. These categories were not necessarily mutually exclusive.

Calculation of nutrient intakes

Daily energy, protein and B₁₂ intakes were calculated using published databases. The nutrient content of raw or 'non-prepared foods' (e.g. apple, almond, milk) was assigned based on values published by the Indian National Institute of Nutrition⁽³⁷⁾. If data were not available for a particular food, we obtained values from UK and US databases, scrutinised in that order^(38,39). We used weighed records to calculate the nutrient content of approximately 60% of the prepared foods. For each food item, three different homes were visited by the research team. Raw ingredients and the cooked food were weighed. Calculations were then performed using the nutrient content of the raw ingredients, with the databases' recommended conversion factors for cooking losses (e.g. 20% loss of B₁₂ when cooking meat)⁽³⁸⁾. Mean values across the three households were used. For shop-bought ready-prepared foods (40% of prepared foods), published databases were used to identify a food as close as possible in nutritional content to the item on the FFQ and the corresponding values were used (e.g. packaged skimmed milk)⁽³⁸⁻⁴⁰⁾.

The units used in portion-size estimates (spoonful, bowlful, etc.) of each food were weighed, to give grams per portion. The nutrient content per portion size was then derived. Daily nutrient intakes were calculated by multiplying the number of units consumed per day by the nutrient content per unit. Nutrient density was also calculated to give a standardised B₁₂ intake estimate in µg/4184 kJ (1000 kcal).

Data analysis

Analyses were performed using the STATA statistical software package version 12. Descriptive characteristics (maternal height and B₁₂ status in pregnancy, urban/rural residence, gender ratio, birth weight, child's weight and height at 5 years) were compared between children included in the analysis and those excluded or not followed up, using Wilcoxon rank-sum or *t* tests and Pearson's χ^2 tests. Associations between sociodemographic factors (religion, SLI score, urban/rural residence and maternal education) and physiological factors (children's gender and anthropometry) with dietary characteristics at 9.5 years were examined using

Spearman's correlation, Wilcoxon rank-sum, Kruskal-Wallis equality-of-populations rank or Pearson's χ^2 tests. Crude associations between dietary characteristics, Hb concentration or maternal plasma B₁₂ during pregnancy and children's plasma B₁₂ concentrations were examined using Spearman's correlation or Kruskal-Wallis equality-of-populations rank tests. A Kruskal-Wallis test was used to compare plasma B₁₂ concentrations of children with low B₁₂ intakes by thirds of maternal B₁₂.

Linear regression analyses, with corresponding likelihood ratio tests, were used to examine associations between exposures (B₁₂ intake, B₁₂ dietary density and frequencies of food consumption) and outcomes. Non-parametric variables were transformed for analysis. Covariates considered were child's gender, age, height, BMI, urban/rural residence, religion, SLI score and maternal education. It was decided *a priori* to include children's gender, age, height and BMI in all regression models, as these factors influence total daily food intake. SLI score was included because multivariate analysis showed evidence of an association with plasma B₁₂ concentration independent of diet. We did not adjust for religion or urban/rural residence in the models we present because it was likely that diet was the principal mechanism whereby these factors influence B₁₂ status (e.g. the Hindu religion advocates vegetarianism). However, we did run models including religion and residence as predictor variables. It was decided *a priori* to adjust for other B₁₂ food groups in food consumption analyses. Additional models and likelihood ratio tests were used to examine diet associations independent of maternal B₁₂ concentration in pregnancy and maternal B₁₂ associations independent of diet. To quantify effects of diet on B₁₂ status (categories: low (≤ 221 pmol/l) *v.* 'normal' (> 221 pmol/l)), odds ratios, corresponding 95% confidence intervals and Wald test significance values were derived for each B₁₂ intake/density or food consumption third using logistic regression.

Results

Exclusions and comparison of children studied and not studied

Of the 539 children who attended for investigations at 9.5 years, 512 were included in the final analyses where maternal plasma B₁₂ during pregnancy was not considered (Fig. 1). Maternal plasma vitamin B₁₂ measures were available for 506. Children taking supplements or tonics containing B₁₂ (*n* 8) had higher median plasma B₁₂ concentrations than other children (476 (P25, P75: 247, 638) pmol/l *v.* 311 (P25, P75: 250, 401) pmol/l) and were excluded from the analysis. Children with plasma B₁₂ measures > 1000 pmol/l (*n* 3) were also excluded. Children with calculated energy intakes $\geq 20\,920$ kJ/d (≥ 5000 kcal/d; *n* 4) were excluded. The 512 children studied did not differ from the rest of the cohort in terms of religion, gender ratio, height and BMI at 5 years, or their mothers' height

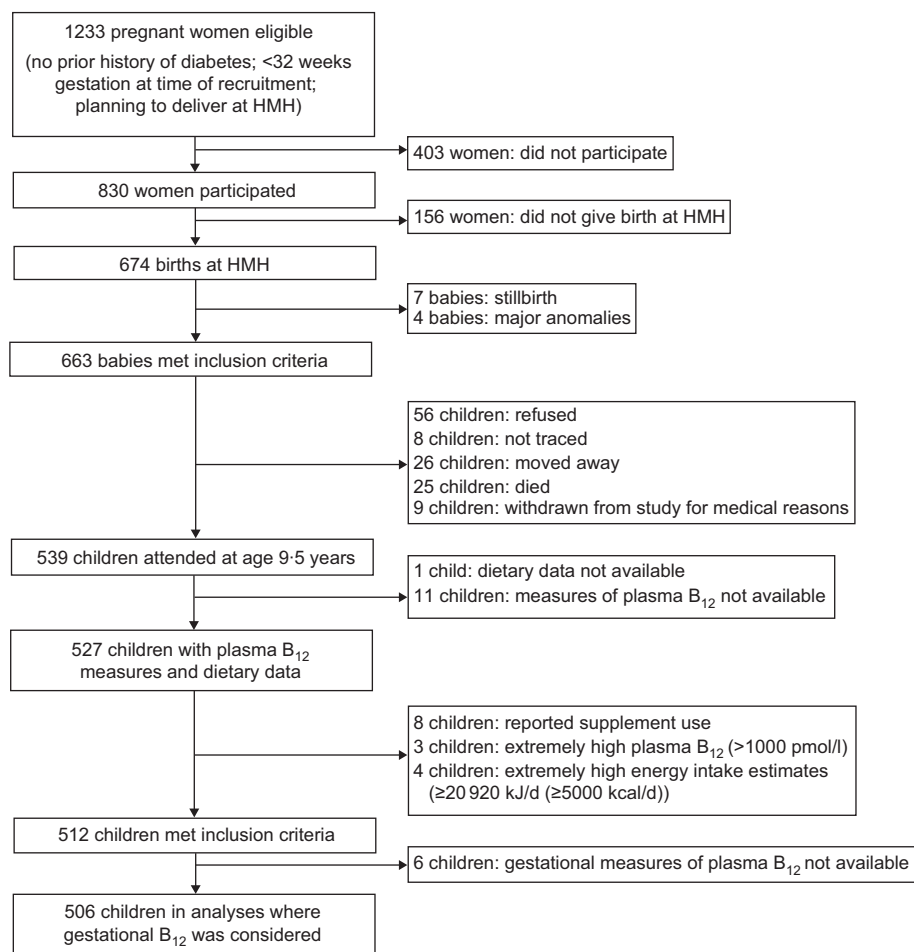


Fig. 1 The Mysore Parthenon Birth Cohort (HMH, Holdsworth Memorial Hospital; B₁₂, vitamin B₁₂)

(all $P > 0.05$). They were more likely to be urban dwelling (72.5% *v.* 63.8%; $P=0.009$), have a heavier birth weight (2.87 kg *v.* 2.75 kg; $P=0.007$) and a lower weight at 5 years (15.8 kg *v.* 16.4 kg; $P=0.028$) on average compared with the remainder of the cohort.

Description of the cohort

Table 1 summarises the characteristics of the children included in the analysis. Mean BMI-for-age Z-score was -1.20 (SD 1.25). One hundred and twenty-five (24.4%) children were underweight, eighteen (3.5%) were overweight and five (1.0%) were obese⁽³³⁾. Median plasma B₁₂ concentration was 312 (P₂₅, P₇₅: 251, 400) pmol/l. The prevalence of B₁₂ deficiency (<150 pmol/l) was 2.5%. A further 14.3% had marginal B₁₂ deficiency (150–221 pmol/l). Eighty-nine per cent of children had a normal Hb concentration. There was no association between plasma B₁₂ and Hb concentrations ($P=0.541$).

Dietary intakes

The children's calculated energy intakes ranged from 2674 to 20 217 kJ/d (639 to 4832 kcal/d) with a median of 9832 (P₂₅, P₇₅: 8025, 11 912) kJ/d (2350 (P₂₅, P₇₅: 1918, 2847) kcal/d; Table 1). The median calculated B₁₂ intake was 2.9

(P₂₅, P₇₅: 1.6, 4.4) µg/d, and ranged from 0.1 to 10.0 µg/d. Approximately 23% of children had B₁₂ intakes below the WHO Estimated Average Requirement for their age group (1.5 µg/d)⁽¹⁾.

Few children (7%) reported no consumption of meat, fish or eggs in a typical month (Table 2). Most consumed meat or fish at least once weekly, but less than every day. Dairy foods (including milk, butter and yoghurt) were consumed at least several times weekly by 86% of the cohort. More than half the children reported consuming milk-based micronutrient-enriched beverages (such as Horlicks or Complan) and generally consumed them at least several times weekly. Most children reported consumption of traditional fermented foods (*idli* and *dosa*) and raw vegetables. Micronutrient-enriched beverages made the largest contribution to B₁₂ intakes in the cohort (Table 2).

Sociodemographic factors

Fifty-seven per cent of children were Hindu and 35% were Muslim; 75% were urban dwelling (Table 1). Higher socioeconomic status and urban residence were associated with higher B₁₂ intakes (Table 3, $P < 0.001$ for both) and higher consumption frequency of meat and fish, and micronutrient-enriched beverages. Boys had higher B₁₂ intakes ($P=0.059$),

Table 1 Cohort characteristics: the Mysore Parthenon Birth Cohort, South India

	<i>n</i>	Mean or median	sd or P25, P75	%
Children's characteristics at 9.5-year follow-up				
Gender (% male)	512			46.3
Age (years)	512	9.36	0.11	
Height (cm)	512	130.7	5.7	
Height-for-age Z-score*	512	-0.62	0.92	
BMI (kg/m ²)	512	14.3	13.4, 15.5	
BMI-for-age Z-score*	512	-1.20	1.25	
<-2	125			24.4
≥-2 to ≤+1	364			71.1
>+1 to ≤+2	18			3.5
>+2	5			1.0
Hb (g/dl)	507	12.8	1.3	
Hb category				
<8.0 g/dl	1			0.2
8.0-10.9 g/dl	32			6.3
11.0-11.4 g/dl	22			4.3
≥11.5 g/dl	452			89.2
Plasma B ₁₂ concentration (pmol/l)	512	312	251, 400	
Plasma B ₁₂ category				
<150 pmol/l	13			2.5
150-221 pmol/l	73			14.3
>221 pmol/l	426			83.2
Children's nutrient intakes at 9.5-year follow-up				
Total energy (kJ/d)	512	9832	8025, 11 912	
Total energy (kcal/d)	512	2350	1918, 2847	
Protein (g/d)	512	55.2	44.4, 67.6	
B ₁₂ (µg/d)	512	2.9	1.6, 4.4	
B ₁₂ nutrient density (µg/4184 kJ (1000 kcal))	512	1.2	0.7, 1.8	
Dietary B ₁₂ intake < EAR (1.5 µg)†	119			23.2
Pregnancy measures				
Maternal plasma B ₁₂ (pmol/l)	506	166	125, 224	
Family demographic characteristics				
SLI score‡	512	36.2	8.2	
Religion				
Hindu	293			57.2
Muslim	180			35.2
Other	39			7.6
Current residence				
Rural	130			25.4
Urban	382			74.6

P25, 25th percentile; P75, 75th percentile; B₁₂, vitamin B₁₂.

*As compared with WHO Growth Reference Data⁽³³⁾.

†Age-specific Estimated Average Requirement (EAR; WHO)⁽¹⁾.

‡Standard of Living Index (SLI) calculated using the National Family Health Survey (NFHS-2) algorithm⁽³¹⁾.

but B₁₂ density in the diet did not differ by gender (Table 3, $P=0.717$). Although there was no evidence that total B₁₂ intakes differed with religion ($P=0.348$), meat and fish intakes were lower among Hindu children than children of other faiths and micronutrient-enriched beverage intakes were lowest among Muslim children (Table 3, $P<0.001$ for both). Socio-economic status was positively associated with plasma B₁₂ concentrations ($P=0.006$). Hindu children had lower plasma B₁₂ concentrations than children of other religions ($P<0.001$). There was no evidence that plasma B₁₂ concentrations differed by urban/rural residence ($P=0.124$) or by gender ($P=0.465$).

Diet and plasma B₁₂ concentrations

Nutrient intakes

Children with lower B₁₂ intakes or those consuming diets with a lower B₁₂ density had lower plasma B₁₂ concentrations

(Table 4), although there was considerable overlap in the plasma B₁₂ ranges when children were divided into groups by B₁₂ intake. The odds ratio for low B₁₂ status (deficiency or marginal deficiency, ≤ 221 pmol/l) was 1.60 (95% CI 0.84, 3.04) in the lowest third of B₁₂ intake relative to the highest third. Respective values for B₁₂ dietary density were 3.42 (95% CI 1.69, 6.93).

Food intakes

Consumption frequencies of non-vegetarian foods (meat, fish and eggs) or flesh foods (meat and fish) were positively associated with plasma B₁₂ concentrations (all $P<0.01$, Table 4). Children in the lowest group of non-vegetarian and flesh food intakes had 50-60% increased odds of low B₁₂ status (adjusted OR = 1.62 (95% CI 0.89, 2.96) and 1.49 (0.78, 2.85), respectively). Micronutrient-enriched beverages were also positively associated with plasma B₁₂ concentrations but this association did not

Table 2 Consumption frequency of B₁₂-source foods/food groups and their median percentage contribution to total daily B₁₂ intake: Mysore Parthenon Birth Cohort, South India

Food group/food	B ₁₂ content/portion (µg)	Number (%) of children consuming food/food group at different frequencies														
		% contribution to total daily B ₁₂ intake		Frequency per week												
		Median	P25, P75	<1/month		<1		1-3		4-7		8-14		>14		
				n	%	n	%	n	%	n	%	n	%	n	%	
1	Keema* ball	0.520	0.6	0, 3.7	247	48.2	161	31.5	104	20.3	0	0.0	0	0.0	0	0.0
2	Keema curry	0.070	0.0	0, 0.2	318	62.1	120	23.4	74	14.5	0	0.0	0	0.0	0	0.0
3	Fish (curry, fried, biriyani†)	0.540	0.6	0, 2.5	239	46.7	175	34.2	95	18.6	3	0.6	0	0.0	0	0.0
4	Chicken (curry, biriyani)	0.160, 3.690	0.0	0, 6.6	98	19.1	99	19.3	307	60.0	8	1.6	0	0.0	0	0.0
5	Mutton (curry, biriyani)	0.500, 0.849	0.0	0, 2.6	140	27.3	92	18.0	267	52.2	13	2.5	0	0.0	0	0.0
1-5	Meat and fish (total of foods 1-5)	-	6.6	0, 19.4	58	11.3	33	6.5	226	44.1	168	32.8	27	5.3	0	0.0
6	Egg (boiled, fried, omelette, biriyani)	0.572, 1.080, 1.080, 1.476	10.2	4.5, 20.4	61	11.9	30	5.9	296	57.8	111	21.7	12	2.3	2	0.4
1-6	Non-vegetarian foods (total of foods 1-6)	-	21.4	8.7, 42.1	35	6.8	8	1.6	109	21.3	238	46.5	112	21.9	10	2.0
7	Buttermilk	1.062	0.0	0.0, 0.0	493	96.3	4	0.8	11	2.2	4	0.8	0	0.0	0	0.0
8	Curd‡, plain	0.060	0.0	0.0, 1.2	290	56.6	11	2.2	141	27.5	63	12.3	6	1.2	1	0.2
9	Curd‡, rice	0.004	0.0	0.0, 0.1	129	25.2	41	8.0	207	40.4	127	24.8	8	1.6	0	0.0
10	Raitha§	0.140	0.0	0.0, 0.0	423	82.6	19	3.7	70	13.7	0	0.0	0	0.0	0	0.0
7-10	Curd‡ foods (total of foods 7-10)	-	0.3	0, 1.8	96	18.8	29	5.7	180	35.2	93	18.2	95	18.6	19	3.7
11	Fresh cow's milk	1.062	0.0	0.0, 0.0	395	77.2	1	0.2	24	4.7	64	12.5	28	5.5	0	0.0
12	Skimmed packaged cow's milk	0.944	0.0	0.0, 0.0	488	95.3	2	0.4	14	2.7	7	1.4	1	0.2	0	0.0
13	Butter	0.033	0.0	0.0, 0.0	467	91.2	12	2.3	24	4.7	8	1.6	1	0.2	0	0.0
7-13	Dairy foods (total of foods 7-13)	-	1.2	0.0, 25.2	73	14.3	19	3.7	134	26.2	94	18.4	123	24.0	69	12.5
14	Micronutrient-enriched beverages (e.g. Horlicks, badam milk)¶	2.311, 1.962	44.1	0.0, 78.0	229	44.7	4	0.8	21	4.1	135	26.4	112	21.9	11	2.2
15	Dosa¶¶	<0.001	0.0	0.0, 0.0	8	1.6	31	6.1	452	88.3	20	3.9	1	0.2	0	0.0
16	Idli**	<0.001	0.0	0.0, 0.0	22	4.3	101	19.7	381	74.4	7	1.4	1	0.2	0	0.0
15-16	Traditional fermented foods (total of foods 15-16)	-	-	-	5	1.0	13	2.5	397	77.5	89	17.4	7	1.4	1	0.2
17	Raw vegetables††	<0.001	0.0	0.0, 0.0	10	2.0	17	3.3	180	35.2	226	44.1	67	13.1	12	2.3
1-17	All potential sources (total of foods 1-17)	-	-	-	0	0.0	0	0.0	0	0.0	10	2.0	56	10.9	446	87.1

B₁₂, vitamin B₁₂; P25, 25th percentile; P75, 75th percentile.

*Minced meat.

†Rice dish.

‡Yoghurt.

§Yoghurt with a small amount of salad (e.g. onion, tomato, cucumber).

¶Milk-based drinks fortified with vitamins and minerals, popularly given to children in India.

¶¶Pancake made from fermented rice and pulses.

**Steamed dumpling made from fermented rice and pulses.

††Cucumber, tomato, onion, carrot, radish, French bean, cabbage, beetroot.

Table 3 Sociodemographic and physiological correlates of selected food group consumption, total dietary B₁₂ intake/density and plasma B₁₂ concentration: Mysore Parthenon Birth Cohort, South India

	<i>n</i>	% reporting highest third of consumption				Total B ₁₂ intake (µg/d)			B ₁₂ nutrient density (µg/4184 kJ (1000 kcal))			Plasma B ₁₂ (pmol/l)			
		Meat, fish		Enriched beverages		Median	P25, P75	<i>P</i> †	Median	P25, P75	<i>P</i> †	Median	P25, P75	<i>P</i> †	<i>P</i> ‡
		%	<i>P</i> *	%	<i>P</i> *										
Religion			<0.001		<0.001			0.348			0.365			0.001	0.001
Hindu	293	12.3		34.1		2.8	1.4, 4.7		1.3	0.6, 1.8		293	241, 366		
Muslim	180	58.3		6.7		2.9	1.8, 3.8		1.2	0.8, 1.6		330	266, 424		
Other	39	41.0		28.2		3.3	1.6, 5.4		1.4	0.6, 2.0		344	270, 436		
SLI tertile			0.073		<0.001			<0.001			<0.001			0.006	0.034
1 (lowest)	186	34.4		11.3		2.4	1.2, 3.4		1.0	0.5, 1.5		292	243, 398		
2	178	30.9		25.8		3.0	1.5, 4.2		1.3	0.7, 1.8		313	247, 385		
3 (highest)	148	25.7		37.8		3.8	2.4, 5.2		1.5	1.1, 2.1		322	271, 412		
Residence			<0.001		<0.001			<0.001			<0.001			0.124	0.600
Urban	382	35.1		25.9		3.1	1.8, 4.6		1.3	0.8, 1.8		318	241, 374		
Rural	130	17.7		18.5		2.3	0.9, 3.3		0.9	0.4, 1.5		294	255, 407		
Maternal education			0.287		<0.001			<0.001			<0.001			0.004	0.032
Illiterate/primary school	43	34.9		14.0		2.0	0.6, 4.3		1.0	0.4, 1.6		318	265, 443		
Middle school	149	26.2		16.8		2.4	1.3, 3.6		1.0	0.5, 1.5		287	230, 366		
Secondary school	161	35.4		23.0		2.8	1.7, 4.2		1.2	0.7, 1.6		320	247, 404		
Higher secondary/above	159	28.9		34.6		3.6	2.3, 5.2		1.6	1.1, 2.1		322	271, 416		
Gender			0.454		0.618			0.059			0.717			0.465	0.433
Male	237	32.5		24.5		3.1	1.8, 4.7		1.3	0.7, 1.8		301	248, 393		
Female	275	29.1		23.6		2.8	1.5, 4.3		1.2	0.7, 1.7		314	251, 412		
BMI tertile			0.681		0.858			0.100			0.077			0.223	0.132
1 (lowest)	171	32.2		24.0		2.9	1.5, 4.2		1.2	0.6, 1.7		317	256, 423		
2	171	32.8		23.4		2.8	1.4, 4.5		1.1	0.6, 1.7		305	254, 374		
3 (highest)	170	27.1		24.7		3.0	1.8, 4.7		1.3	0.8, 1.8		304	247, 401		

B₁₂, vitamin B₁₂; P25, 25th percentile; P75, 75th percentile; SLI, Standard of Living Index.*Pearson's χ^2 test of proportions.†Spearman's correlation coefficient *P* values (for continuous variables: SLI and BMI) or Wilcoxon rank-sum test (for comparison of two categories: residence, gender) or Kruskal–Wallis equality-of-populations rank test (for examination across more than two categories: religion, maternal education).‡Multivariable linear regression likelihood ratio test (effectively, H₀: there is no association between sociodemographic/physiological factor *X* and log(plasma B₁₂ concentration) when children's daily dietary B₁₂ intake is controlled for; H_a: there is a linear association between factor *X* and log(plasma B₁₂ concentration) independent of dietary B₁₂).

Table 4 Associations between dietary B₁₂ intakes, food consumption frequency and maternal plasma B₁₂ during pregnancy with children's plasma B₁₂ concentration at 9.5 years: Mysore Parthenon Birth Cohort, South India

B ₁₂ intake	n	Plasma B ₁₂ (pmol/l)		Unadjusted P‡	Adjusted*			Further adjusted for maternal plasma B ₁₂ †		
		Median	P25, P75		β	95 % CI	P§	β	95 % CI	P§
Total intake (min-max µg/d)				< 0.001	0.084	0.023, 0.143	0.006	0.081	0.023, 0.140	0.006
Low (0.06–2.15)	171	287	233, 348							
Medium (2.16–3.73)	171	314	250, 418							
High (3.74–10.01)	170	329	270, 409							
Dietary density (min-max µg/4184 kJ (1000 kcal))				< 0.001	0.098	0.048, 0.147	< 0.001	0.102	0.054, 0.150	< 0.001
Low (0.03–0.90)	171	282	220, 337							
Medium (0.91–1.55)	171	317	250, 425							
High (1.56–3.75)	170	336	280, 400							
Food group** consumption frequency (min-max/month)	n	Plasma B ₁₂ (pmol/l)		Unadjusted P††	Adjusted‡‡			Further adjusted for maternal plasma B ₁₂ ‡‡		
		Median	P25, P75		β	95 % CI	P§	β	95 % CI	P§
Keema				0.430			0.905			0.952
Low (0)	236	300	244, 295		base			base		
Medium (1–3)	115	302	256, 407		– 0.001	– 0.093, 0.091		0.012	– 0.078, 0.102	
High (4–20)	161	320	259, 408		0.018	– 0.072, 0.107		0.011	– 0.077, 0.100	
Fish				0.203			0.484			0.407
Low (0)	239	296	251, 392		base			base		
Medium (1)	105	309	249, 425		– 0.002	– 0.095, 0.091		0.005	– 0.087, 0.096	
High (2–28)	168	321	265, 414		0.045	– 0.039, 0.129		0.052	– 0.031, 0.134	
Chicken				0.106			0.509			0.671
Low (0–2)	188	295	251, 392		base			base		
Medium (3–4)	228	313	255, 400		– 0.035	– 0.129, 0.058		– 0.036	– 0.128, 0.056	
High (5–28)	96	328	271, 411		0.018	– 0.093, 0.128		– 0.003	– 0.112, 0.105	
Mutton				0.004			0.048			0.165
Low (0–2)	226	292	239, 385		base			base		
Medium (3–4)	199	314	256, 415		0.072	– 0.019, 0.163		0.047	– 0.043, 0.137	
High (5–28)	87	333	280, 443		0.131	0.022, 0.240		0.101	– 0.007, 0.209	

Table 4 Continued

Food group** consumption frequency (min–max/month)	n	Plasma B ₁₂ (pmol/l)		Unadjusted P††	Adjusted‡‡			Further adjusted for maternal plasma B ₁₂ §§		
		Median	P25, P75		β	95 % CI	P§	β	95 % CI	P§
Meat and fish				0.002				0.001		0.012
Low (0–7)	181	286	239, 392		base			base		
Medium (8–14)	174	314	256, 374		0.071	–0.010, 0.152		0.045	–0.035, 0.126	
High (15–52)	157	331	275, 428		0.159	0.073, 0.246		0.126	0.041, 0.212	
Eggs				0.246				0.153		0.140
Low (0–7)	174	300	245, 392		base			base		
Medium (8–12)	213	311	258, 433		0.048	–0.030, 0.125		0.048	–0.028, 0.124	
High (13–96)	125	317	246, 374		–0.031	–0.123, 0.062		–0.031	–0.122, 0.061	
Non-vegetarian (meat, fish, eggs)				0.005				0.001		0.006
Low (0–14)	176	287	233, 377		base			base		
Medium (15–25)	167	317	259, 415		0.114	0.034, 0.195		0.089	0.010, 0.169	
High (26–112)	169	323	265, 416		0.150	0.069, 0.231		0.124	0.044, 0.203	
Curd (yoghurt) foods				0.368				0.155		
Low (0–4)	178	318	258, 422		base			base		
Medium (5–16)	171	306	256, 373		–0.034	–0.114, 0.045		–0.040	–0.119, 0.038	
High (17–120)	163	301	239, 400		–0.081	–0.164, 0.003		–0.075	–0.157, 0.007	
Milk/dairy				0.262				0.642		0.775
Low (0–8)	182	323	258, 423		base			base		
Medium (9–32)	163	302	256, 385		–0.031	–0.113, 0.051		–0.011	–0.091, 0.070	
High (33–148)	167	301	232, 392		–0.036	–0.119, 0.048		–0.029	–0.110, 0.053	
Micronutrient-enriched beverages				< 0.001				0.060		0.042
Low (0)	229	289	232, 358		base			base		
Medium (1–28)	160	315	251, 408		0.046	–0.035, 0.128		0.056	–0.024, 0.136	
High (29–84)	123	337	280, 417		0.108	0.017, 0.199		0.112	0.023, 0.201	
Traditional fermented foods				0.031				0.095		0.075
Low (0–8)	254	319	258, 427		base			base		
Medium (9–11)	90	292	236, 358		–0.087	–0.180, 0.065		–0.090	–0.090, 0.181	
High (12–63)	168	305	243, 381		–0.062	–0.136, 0.013		–0.063	–0.136, 0.011	
Raw vegetables				0.338				0.037		0.087
Low (0–12)	207	314	251, 416		base			base		
Medium (13–20)	155	317	258, 415		–0.048	–0.127, 0.031		–0.048	–0.126, 0.029	
High (21–109)	150	305	244, 374		–0.106	–0.188, –0.024		–0.088	–0.169, –0.007	

Table 4 Continued

Maternal B ₁₂	Plasma B ₁₂ (pmol/l)			Unadjusted			Adjusted*			Further adjusted for dietary B ₁₂ ††		
	n	Median	P25, P75	P‡	β	95% CI	P§	β	95% CI	P§	β	95% CI
Plasma B ₁₂ during pregnancy (min–max pmol/l)				<0.001	0.221	0.142, 0.299	<0.001	0.222	0.144, 0.300	<0.001		
Low (69–135)	170	286	229, 355									
Medium (136–202)	170	312	259, 398									
High (203–1366)	166	336	276, 438									

B₁₂, vitamin B₁₂; P25, 25th percentile; P75, 75th percentile; SLI, Standard of Living Index.
 *Multivariable linear regression adjusting for age, gender, BMI, height, SLI score and maternal education.
 †Multivariable linear regression adjusting for factors listed in * and pregnancy plasma B₁₂ concentration.
 ‡Spearman's correlation coefficient, P values.
 §Likelihood ratio test (effectively, H₀: there is no association between B₁₂ intake or food consumption or maternal plasma B₁₂ and children's plasma B₁₂ concentration when factors listed in *, †, ††, ††† or †††† are controlled for; H₁: there is a linear association between B₁₂ intake/food consumption/maternal plasma B₁₂ and children's plasma B₁₂ concentration under these conditions).
 ††Multivariable linear regression adjusting for age, gender, BMI, height, SLI score, maternal education and all other food groups in the table except traditional fermented foods and raw vegetables (i.e. meat, fish, eggs, dairy foods and micronutrient-enriched beverages).
 †††Multivariable linear regression adjusting for factors listed in †† and pregnancy plasma B₁₂ concentration.
 ††††Food groups listed here are not necessarily mutually exclusive (see Table 2 for details of included foods).
 †††††Kruskal–Wallis equality-of-populations rank test.
 †††††Multivariable linear regression adjusting for factors listed in * and dietary intakes of B₁₂.

reach statistical significance ($P=0.060$). Children reporting no consumption of these beverages had a threefold increased odds of having low B₁₂ status (adjusted OR = 2.83 (95% CI 1.31, 6.10)). There were no associations between the consumption frequency of dairy foods, traditional fermented foods or raw vegetables and plasma B₁₂ concentration in fully adjusted analyses. Religion was found to be an independent predictor of B₁₂ concentration whereas residence was not associated with B₁₂ status (data not shown).

Maternal B₁₂ concentrations during pregnancy

Median maternal B₁₂ concentration was 166 (P25, P75: 125, 224) pmol/l. Forty-two per cent of mothers had levels indicating B₁₂ deficiency (<150 pmol/l). Maternal plasma B₁₂ concentration was positively associated with B₁₂ concentration in the children (Table 4, $P<0.001$). A 1% increase in maternal B₁₂ concentration was associated with a 0.22% increase in the child's B₁₂ concentration. There was no association between maternal B₁₂ concentration and the children's B₁₂ intake ($P=0.870$). The association between maternal and child B₁₂ concentrations remained after adjustment for the children's dietary intake (Table 4, $P<0.001$). Among children with low B₁₂ intakes, those born to non-deficient mothers had higher median plasma B₁₂ concentrations (311 (P25, P75: 259, 436) pmol/l) than those born to mothers with the lowest B₁₂ status (269 (P25, P75: 217, 302) pmol/l; $P=0.003$). The associations described above of B₁₂ intakes, flesh and non-vegetarian foods and micronutrient-enriched beverage consumption with children's B₁₂ concentrations were statistically significant after adjusting for maternal B₁₂ status (Table 4).

Discussion

Summary of main findings

We have studied the relationship of dietary vitamin B₁₂ intakes and B₁₂-source foods with plasma B₁₂ concentrations in a large sample of healthy 9.5-year-old South Indian children. There are currently no standard criteria for defining B₁₂ deficiency in children, but 2.5% were deficient according to the adult definition and a further 14% had 'marginal' status. Given that rates of deficiency in India have been measured at 1–44%, the prevalence in our cohort could be considered low^(10–14). However the 150 pmol/l cut-off was derived for adults and may not be a valid measure of deficiency for children. The children's plasma B₁₂ concentrations were positively related to dietary B₁₂ intakes and to consumption frequency of meat, fish and micronutrient-enriched beverages, but not dairy or fermented foods. These associations between diet and plasma concentrations were independent of maternal plasma B₁₂ concentration during pregnancy, which itself was positively associated with plasma B₁₂ concentrations in the children.

Nutrient intakes

The strong positive association between plasma B₁₂ concentration and B₁₂ intake supports dietary B₁₂ as a key determinant of B₁₂ status in this population. The relationship between diet and biochemical measures was clearer when dietary B₁₂ density, rather than absolute intakes, was examined. This is not surprising given variability in reported energy intakes which, if not fully accounted for by adjustment for age and body size, can obscure nutrient intake relationships⁽⁴¹⁾. The possibility of reverse causality (i.e. biochemical status affecting intake) seems unlikely in children; this has been suggested in the elderly, among whom B-vitamin deficiency-associated cognitive decline could have an adverse impact on diet⁽²⁰⁾. Our data support FFQ as suitable tools for measuring relative B₁₂ intakes within a population, although bias towards reporting consumption of foods associated with affluence must be considered⁽⁴²⁾. Our findings are consistent with other observational studies^(13,22,43). Estimated dietary B₁₂ correlated with plasma B₁₂ concentration in Guatemalan schoolchildren⁽⁴³⁾. B₁₂ intake from complementary foods was recently identified as an important determinant of plasma B₁₂ concentrations in toddlers in rural India, as well as in Guatemala^(13,22).

Food consumption

The observation that meat and fish consumption was positively related to plasma B₁₂ concentrations suggests that these foods are important contributors to children's B₁₂ status. It is consistent with data from Colombian schoolchildren showing that a diet pattern that included frequent beef, chicken and dairy consumption was positively associated with plasma B₁₂ concentration⁽²⁴⁾. A small study of Dutch adolescents showed that frequency of consumption of animal-derived foods (dairy foods, meat, eggs and fish) explained nearly half the variance in serum B₁₂ concentrations⁽²⁵⁾. The dose-related association between micronutrient-enriched beverage consumption and plasma B₁₂ in our study is highly plausible given their high B₁₂ content (approximately 2 µg per serving). No association was seen with dairy food consumption. Although the B₁₂ content of the widely consumed 'curd' (yoghurt) is thought to be relatively low, less frequent yoghurt intake was a predictor of lower B₁₂ status in a recent study of South Indian women⁽⁶⁾. It is likely that our findings reflect adherence to the lacto-vegetarian dietary pattern (characterised by frequent yoghurt consumption and a low frequency of meat consumption) previously described in our cohort⁽²³⁾. Lacto-vegetarian diet pattern scores were negatively correlated with plasma B₁₂ concentrations⁽²³⁾.

We examined the relationship between frequency of consumption of raw vegetables and B₁₂ status because of the suggestion that microbial contamination of uncooked vegetables (e.g. due to contamination by animal faeces) may be an important source of B₁₂ in some contexts⁽⁴⁴⁾. We found no convincing association between raw vegetable consumption level and B₁₂ status. Foods made of fermented rice or lentils, such as *dosa* and *idli*, were also of interest as

potential B₁₂ sources through microbial B₁₂ production during fermentation⁽³⁾. Our data suggest that the content and/or bioavailability of B₁₂ from these foods is negligible⁽³⁾.

Implications of the dietary findings

It remains to be established whether subclinical (asymptomatic) cobalamin deficiency is meaningful in terms of functional and health outcomes⁽⁴⁵⁾. Data linking lower B₁₂ concentrations to greater adiposity, glucose intolerance and adult cognitive decline, and lower maternal B₁₂ status to insulin resistance and poorer cognitive function in children, come mainly from associations in observational studies^(5,18,20,30,46). However, supplementation with physiological doses of B₁₂ in a rural Indian population produced a marked fall in plasma homocysteine, suggesting functional benefit⁽⁴⁷⁾. B₁₂-containing supplements also improved cognitive function among elderly people with mild cognitive impairment and high homocysteine levels in the UK⁽⁴⁸⁾. We did not identify foods contributing to B₁₂ status that are suitable for India's vegetarians. Although micronutrient-enriched beverages are apparently a rich B₁₂ source, they are high in sugar (4–8 teaspoons/serving, with extra sugar usually added). The drinks should be promoted with caution due to the associated risk of dental caries and, given increasing childhood obesity in India, the effect on overall energy intake^(49–51). Although a greater proportion of our cohort was underweight (24.4%), overweight or obesity was present in 4.5%. In a recent sample of 23 000 children in Mysore, overweight or obesity was found in 11.9% of children⁽⁵²⁾. If subclinical cobalamin deficiency is shown to be an important problem, other affordable approaches will need to be found to improve vitamin B₁₂ intakes in India, especially among vegetarians. These may include food fortification⁽¹⁶⁾.

Maternal B₁₂ status

B₁₂ concentrations in our children showed a strong positive association with maternal concentrations during pregnancy. B₁₂ is actively transported across the placenta from mother to fetus; newborn B₁₂ concentration is related to maternal status^(1,53). The importance of gestational B₁₂ exposure in later childhood can only be studied in a birth cohort context like ours, and to the best of our knowledge B₁₂ concentrations in children of this age in relation to maternal status during pregnancy have not previously been reported. The most obvious explanation for the correlation between maternal and children's B₁₂ status is that children eat the same foods as their parents. However, in our study, maternal B₁₂ status was not related to children's B₁₂ intakes and the association between maternal and children's plasma B₁₂ was independent of children's dietary measures. Thus, although we did not measure maternal diet directly, our results suggest that the association was not due to similarities in the diets of mother and child. Children with low dietary intakes are likely to be at increased risk of low B₁₂ status if they are born to mothers with low B₁₂ status⁽²⁾. We also

found that among children with low current B₁₂ intakes, B₁₂ concentrations were lower if their mothers had lower B₁₂ status in pregnancy. Improving maternal status, by adding B₁₂ to the routine pregnancy Fe and folate supplements, for example, could improve long-term B₁₂ status among children.

Strengths and limitations of the study

Our cohort is likely to differ in some key characteristics from India nationally: it is predominantly urban; there is a greater proportion of Muslim families in the cohort than in the nation as a whole (35% *v.* 12.5%); and the range of standard of living is narrower within our cohort⁽⁵⁴⁾.

Assessment of nutrient intakes using an FFQ and nutrient content tables inevitably introduces imprecision. Calculated energy intakes were high for children of this age and size. Although our FFQ has not been validated in terms of energy, food patterns derived from the FFQ have shown expected associations with blood micronutrient markers⁽²³⁾. Nutrient losses during cooking had to be estimated because Indian nutrient composition data were available only for raw foods. Given these limitations, the avoidance of a fully reductionist approach (i.e. only examining diet in terms of calculated nutrient intakes) was a strength. Although supplementation is not thought to be common in this cohort, we were able to avoid distortion of our results by excluding children who reported use of multivitamin supplements. In spite of children's B₁₂ intakes being comprehensively measured, a limitation may be that we did not measure maternal B₁₂ intake. An important limitation of our study was the absence of measures of relevant biomarkers (e.g. methylmalonic acid) to triangulate with B₁₂ concentration to assess B₁₂ status more accurately⁽⁵⁵⁾.

Conclusions

Flesh food (meat and fish) consumption is an important determinant of plasma vitamin B₁₂ level in this population of South Indian children. Although there may be a secular trend towards serving children meat and fish, new approaches are needed to identify and/or develop appropriate dietary B₁₂ sources for Indians who consume these foods infrequently for economic or religious reasons. Micronutrient-enriched beverages are an important source of vitamin B₁₂ in our cohort, but they should be promoted with care due to their high sugar content. Maternal B₁₂ concentrations during pregnancy remain strongly associated with children's B₁₂ concentrations well into childhood, independent of B₁₂ intakes at 9.5 years; improving maternal B₁₂ status in pregnancy may improve B₁₂ status in Indian children.

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