Public Health Nutrition: 18(4), 596–609 doi:10.1017/S1368980014000974

Contribution of food sources to the vitamin B_{12} status of South Indian children from a birth cohort recruited in the city of Mysore

Anna M Christian[1,*,†], Ghattu V Krishnaveni[2], Sarah H Kehoe[3], Sargoor R Veena[2], Rumana Khanum[2], Ella Marley-Zagar[3], Phil Edwards[1], Barrie M Margetts[4] and Caroline HD Fall[3]

[1]Faculty of Epidemiology and Population Health, London School of Hygiene & Tropical Medicine, London, UK: [2]Epidemiology Research Unit, CSI Holdsworth Memorial Hospital, Mysore, India: [3]Medical Research Council Lifecourse Epidemiology Unit, University of Southampton, Southampton, UK: [4]Faculty of Medicine, Primary Care and Population Sciences, University of Southampton, Southampton, UK

Submitted 27 August 2013: Final revision received 21 March 2014: Accepted 23 April 2014: First published online 27 May 2014

Abstract

Objective: There is evidence that subclinical vitamin B_{12} (B_{12}) deficiency is common in India. Vegetarianism is prevalent and therefore meat consumption is low. Our objective was to explore the contribution of B_{12}-source foods and maternal B_{12} status during pregnancy to plasma B_{12} concentrations.

Design: Maternal plasma B_{12} concentrations were measured during pregnancy. Children's dietary intakes and plasma B_{12} concentrations were measured at age 9·5 years; B_{12} and total energy intakes were calculated using food composition databases. We used linear regression to examine associations between maternal B_{12} status and children's intakes of B_{12} and B_{12}-source foods, and children's plasma B_{12} 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 B_{12} deficient (<150 pmol/l). A further 14 % had 'marginal' B_{12} concentrations (150–221 pmol/l). Children's total daily B_{12} intake and consumption frequencies of meat and fish, and micronutrient-enriched beverages were positively associated with plasma B_{12} concentrations ($P=0·006$, $P=0·01$ and $P=0·04$, respectively, adjusted for socio-economic indicators and maternal B_{12} status). Maternal pregnancy plasma B_{12} was associated with children's plasma B_{12} concentrations, independent of current B_{12} intakes ($P<0·001$). Milk and curd (yoghurt) intakes were unrelated to B_{12} status.

Conclusions: Meat and fish are important B_{12} 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 B_{12} status in pregnancy may improve Indian children's status.

Keywords
India
Vitamin B_{12}
Child
Source

Vitamin B_{12} (B_{12}), 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 B_{12}-synthesising bacteria, and fermented foods, may also be important sources[2,3]. In India, vegetarian diets have been associated with an increased risk of B_{12} deficiency and a high prevalence of B_{12} 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 B_{12} 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 B_{12} absorption in the majority (90 %) of individuals studied[15].

Severe B_{12} 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 B_{12} 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

† Current address: Nuffield Department of Primary Care Health Sciences, University of Oxford, Radcliffe Observatory Quarter, Woodstock Road, Oxford OX2 6GG, UK.

*Corresponding author: Email annamaychristian@hotmail.com

their children[5,18]. B_{12} deficiency is associated with raised plasma homocysteine levels, which is a risk factor for CVD[19]. There is also evidence of low B_{12} 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 B_{12} or consumption frequency of B_{12}-rich foods and plasma B_{12} in early childhood[13,21–26]. The association between diet and plasma B_{12} concentration among older children remains under-studied. There is also a need to identify affordable and acceptable sources of B_{12} 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 B_{12} intakes were calculated. We aimed to investigate associations between children's B_{12} intake and frequency of consumption of potential B_{12}-source foods, with biochemical measures of B_{12}. We hypothesised that higher B_{12} 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 B_{12} 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 B_{12} concentrations. Maternal B_{12} status is correlated with neonatal B_{12} status[19,27,28]. We therefore assessed whether maternal B_{12} concentrations in plasma samples collected during the pregnancy of each child were related to children's B_{12} concentrations at 9·5 years. The primary outcome in all analyses was children's plasma B_{12} 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 B_{12} 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 ≤ +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. B_{12} 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.

B_{12} 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' B_{12} 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 B_{12} 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 B_{12} 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 B_{12} 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 B_{12} 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 B_{12} 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 B_{12} during pregnancy and children's plasma B_{12} concentrations were examined using Spearman's correlation or Kruskal–Wallis equality-of-populations rank tests. A Kruskal–Wallis test was used to compare plasma B_{12} concentrations of children with low B_{12} intakes by thirds of maternal B_{12}.

Linear regression analyses, with corresponding likelihood ratio tests, were used to examine associations between exposures (B_{12} intake, B_{12} 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 B_{12} 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 B_{12} 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 B_{12} food groups in food consumption analyses. Additional models and likelihood ratio tests were used to examine diet associations independent of maternal B_{12} concentration in pregnancy and maternal B_{12} associations independent of diet. To quantify effects of diet on B_{12} 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 B_{12} 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 B_{12} during pregnancy was not considered (Fig. 1). Maternal plasma vitamin B_{12} measures were available for 506. Children taking supplements or tonics containing B_{12} (*n* 8) had higher median plasma B_{12} 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 B_{12} measures > 1000 pmol/l (*n* 3) were also excluded. Children with calculated energy intakes ≥ 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

https://doi.org/10.1017/S1368980014000974 Published online by Cambridge University Press

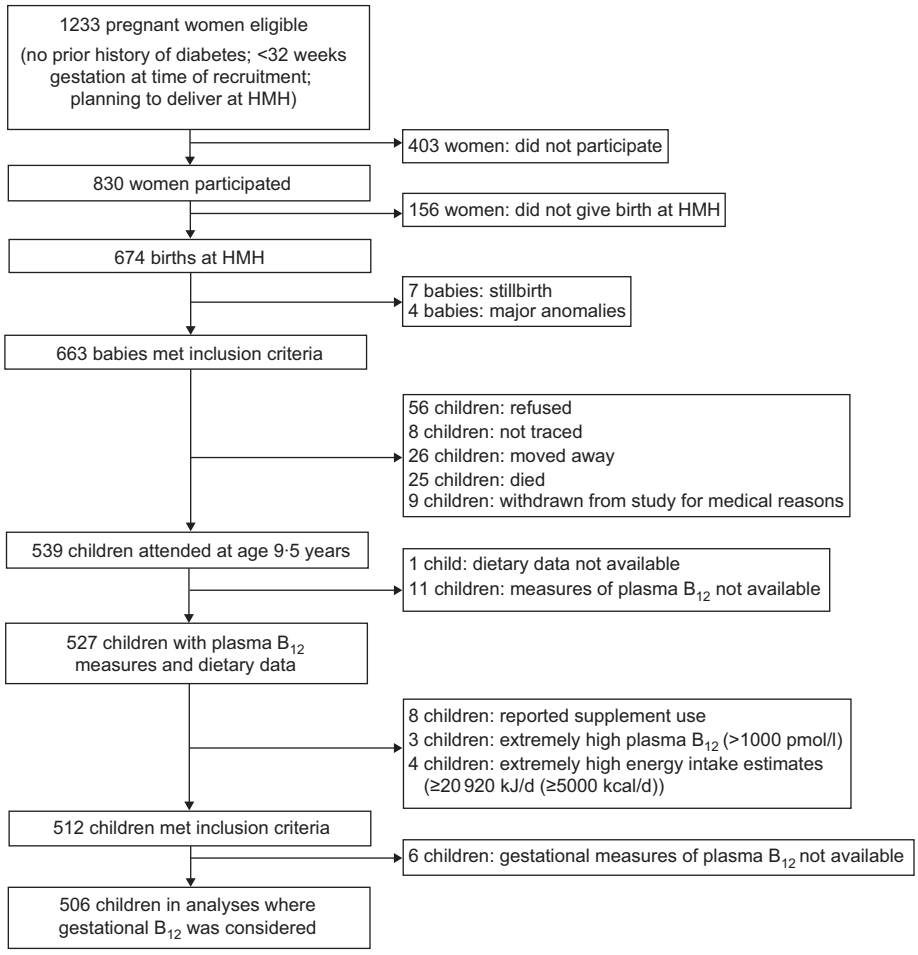

Fig. 1 The Mysore Parthenon Birth Cohort (HMH, Holdsworth Memorial Hospital; B_{12}, vitamin B_{12})

(all $P > 0{\cdot}05$). They were more likely to be urban dwelling (72·5 % *v.* 63·8 %; $P = 0{\cdot}009$), have a heavier birth weight (2·87 kg *v.* 2·75 kg; $P = 0{\cdot}007$) and a lower weight at 5 years (15·8 kg *v.* 16·4 kg; $P = 0{\cdot}028$) 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 B_{12} concentration was 312 (P25, P75: 251, 400) pmol/l. The prevalence of B_{12} deficiency (<150 pmol/l) was 2·5 %. A further 14·3 % had marginal B_{12} deficiency (150–221 pmol/l). Eighty-nine per cent of children had a normal Hb concentration. There was no association between plasma B_{12} and Hb concentrations ($P = 0{\cdot}541$).

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 B_{12} 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 B_{12} 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 B_{12} 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 B_{12} intakes (Table 3, $P < 0{\cdot}001$ for both) and higher consumption frequency of meat and fish, and micronutrient-enriched beverages. Boys had higher B_{12} intakes ($P = 0{\cdot}059$),

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

	n	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; B_{12}, vitamin B_{12}.
*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 B_{12} density in the diet did not differ by gender (Table 3, $P=0·717$). Although there was no evidence that total B_{12} 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 B_{12} concentrations ($P=0·006$). Hindu children had lower plasma B_{12} concentrations than children of other religions ($P<0·001$). There was no evidence that plasma B_{12} concentrations differed by urban/rural residence ($P=0·124$) or by gender ($P=0·465$).

Diet and plasma B_{12} concentrations

Nutrient intakes

Children with lower B_{12} intakes or those consuming diets with a lower B_{12} density had lower plasma B_{12} concentrations (Table 4), although there was considerable overlap in the plasma B_{12} ranges when children were divided into groups by B_{12} intake. The odds ratio for low B_{12} status (deficiency or marginal deficiency, ≤221 pmol/l) was 1·60 (95 % CI 0·84, 3·04) in the lowest third of B_{12} intake relative to the highest third. Respective values for B_{12} 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 B_{12} 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 B_{12} 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 B_{12} concentrations but this association did not

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Table 2 Consumption frequency of B_{12}-source foods/food groups and their median percentage contribution to total daily B_{12} intake: Mysore Parthenon Birth Cohort, South India

			% contribution to total daily B_{12} intake		Number (%) of children consuming food/food group at different frequencies											
							Frequency per week									
					<1/month		<1		1–3		4–7		8–14		>14	
	Food group/food	B_{12} content/portion (μg)	Median	P25, P75	*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

B_{12}, vitamin B_{12}; 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 B_{12} intake/density and plasma B_{12} concentration: Mysore Parthenon Birth Cohort, South India

		% reporting highest third of consumption													
		Meat, fish		Enriched beverages		Total B_{12} intake (μg/d)			B_{12} nutrient density (μg/4184 kJ (1000 kcal))			Plasma B_{12} (pmol/l)			
	n	%	*P**	%	*P**	Median	P25, P75	*P*†	Median	P25, P75	*P*†	Median	P25, P75	*P*†	*P*‡
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		

B_{12}, vitamin B_{12}; 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, H_0: there is no association between sociodemographic/physiological factor *X* and log(plasma B_{12} concentration) when children's daily dietary B_{12} intake is controlled for; H_a: there is a linear association between factor *X* and log(plasma B_{12} concentration) independent of dietary B_{12}).

Table 4 Associations between dietary B_{12} intakes, food consumption frequency and maternal plasma B_{12} during pregnancy with children's plasma B_{12} concentration at 9·5 years: Mysore Parthenon Birth Cohort, South India

		Plasma B_{12} (pmol/l)		Unadjusted	Adjusted*			Further adjusted for maternal plasma B_{12}†		
B_{12} intake	*n*	Median	P25, P75	*P*‡	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							
		Plasma B_{12} (pmol/l)		Unadjusted	Adjusted‖			Further adjusted for maternal plasma B_{12}¶		
Food group** consumption frequency (min–max/month)	*n*	Median	P25, P75	*P*††	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	Adjusted‖			Further adjusted for maternal plasma B_{12}¶		
		Median	P25, P75	*P*††	*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}	*n*	Plasma B_{12} (pmol/l) Median	P25, P75	Unadjusted *P*‡	Adjusted* *β*	95 % CI	*P*§	Further adjusted for dietary B_{12}‡‡ *β*	95 % CI	*P*§
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							

B_{12}, vitamin B_{12}; 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 B_{12} concentration.
‡Spearman's correlation coefficient *P* values.
§Likelihood ratio test (effectively, H_0: there is no association between B_{12} intake or food consumption or maternal plasma B_{12} and children's plasma B_{12} concentration when factors listed in *, †, ||, ¶ or ‡‡ are controlled for; H_a: there is a linear association between B_{12} intake/food consumption/maternal plasma B_{12} and children's plasma B_{12} 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 B_{12} 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 B_{12}.

reach statistical significance ($P = 0{\cdot}060$). Children reporting no consumption of these beverages had a threefold increased odds of having low B_{12} 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 B_{12} concentration in fully adjusted analyses. Religion was found to be an independent predictor of B_{12} concentration whereas residence was not associated with B_{12} status (data not shown).

Maternal B_{12} concentrations during pregnancy

Median maternal B_{12} concentration was 166 (P25, P75: 125, 224) pmol/l. Forty-two per cent of mothers had levels indicating B_{12} deficiency (<150 pmol/l). Maternal plasma B_{12} concentration was positively associated with B_{12} concentration in the children (Table 4, $P < 0{\cdot}001$). A 1 % increase in maternal B_{12} concentration was associated with a 0·22 % increase in the child's B_{12} concentration. There was no association between maternal B_{12} concentration and the children's B_{12} intake ($P = 0{\cdot}870$). The association between maternal and child B_{12} concentrations remained after adjustment for the children's dietary intake (Table 4, $P < 0{\cdot}001$). Among children with low B_{12} intakes, those born to non-deficient mothers had higher median plasma B_{12} concentrations (311 (P25, P75: 259, 436) pmol/l) than those born to mothers with the lowest B_{12} status (269 (P25, P75: 217, 302) pmol/l; $P = 0{\cdot}003$). The associations described above of B_{12} intakes, flesh and non-vegetarian foods and micronutrient-enriched beverage consumption with children's B_{12} concentrations were statistically significant after adjusting for maternal B_{12} status (Table 4).

Discussion

Summary of main findings

We have studied the relationship of dietary vitamin B_{12} intakes and B_{12}-source foods with plasma B_{12} concentrations in a large sample of healthy 9·5-year-old South Indian children. There are currently no standard criteria for defining B_{12} 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 B_{12} concentrations were positively related to dietary B_{12} 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 B_{12} concentration during pregnancy, which itself was positively associated with plasma B_{12} concentrations in the children.

https://doi.org/10.1017/S1368980014000974 Published online by Cambridge University Press

Nutrient intakes

The strong positive association between plasma B_{12} concentration and B_{12} intake supports dietary B_{12} as a key determinant of B_{12} status in this population. The relationship between diet and biochemical measures was clearer when dietary B_{12} 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 B_{12} 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 B_{12} correlated with plasma B_{12} concentration in Guatemalan schoolchildren[43]. B_{12} intake from complementary foods was recently identified as an important determinant of plasma B_{12} 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 B_{12} concentrations suggests that these foods are important contributors to children's B_{12} 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 B_{12} 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 B_{12} concentrations[25]. The dose-related association between micronutrient-enriched beverage consumption and plasma B_{12} in our study is highly plausible given their high B_{12} content (approximately 2 μg per serving). No association was seen with dairy food consumption. Although the B_{12} content of the widely consumed 'curd' (yoghurt) is thought to be relatively low, less frequent yoghurt intake was a predictor of lower B_{12} 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 B_{12} concentrations[23].

We examined the relationship between frequency of consumption of raw vegetables and B_{12} 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 B_{12} in some contexts[44]. We found no convincing association between raw vegetable consumption level and B_{12} status. Foods made of fermented rice or lentils, such as *dosa* and *idli*, were also of interest as potential B_{12} sources through microbial B_{12} production during fermentation[3]. Our data suggest that the content and/or bioavailability of B_{12} 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 B_{12} concentrations to greater adiposity, glucose intolerance and adult cognitive decline, and lower maternal B_{12} 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 B_{12} in a rural Indian population produced a marked fall in plasma homocysteine, suggesting functional benefit[47]. B_{12}-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 B_{12} status that are suitable for India's vegetarians. Although micronutrient-enriched beverages are apparently a rich B_{12} 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 B_{12} intakes in India, especially among vegetarians. These may include food fortification[16].

Maternal B_{12} status

B_{12} concentrations in our children showed a strong positive association with maternal concentrations during pregnancy. B_{12} is actively transported across the placenta from mother to fetus; newborn B_{12} concentration is related to maternal status[1,53]. The importance of gestational B_{12} exposure in later childhood can only be studied in a birth cohort context like ours, and to the best of our knowledge B_{12} 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 B_{12} status is that children eat the same foods as their parents. However, in our study, maternal B_{12} status was not related to children's B_{12} intakes and the association between maternal and children's plasma B_{12} 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 B_{12} status if they are born to mothers with low B_{12} status[2]. We also

found that among children with low current B_{12} intakes, B_{12} concentrations were lower if their mothers had lower B_{12} status in pregnancy. Improving maternal status, by adding B_{12} to the routine pregnancy Fe and folate supplements, for example, could improve long-term B_{12} 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 B_{12} intakes being comprehensively measured, a limitation may be that we did not measure maternal B_{12} intake. An important limitation of our study was the absence of measures of relevant biomarkers (e.g. methylmalonic acid) to triangulate with B_{12} concentration to assess B_{12} status more accurately[55].

Conclusions

Flesh food (meat and fish) consumption is an important determinant of plasma vitamin B_{12} 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 B_{12} sources for Indians who consume these foods infrequently for economic or religious reasons. Micronutrient-enriched beverages are an important source of vitamin B_{12} in our cohort, but they should be promoted with care due to their high sugar content. Maternal B_{12} concentrations during pregnancy remain strongly associated with children's B_{12} concentrations well into childhood, independent of B_{12} intakes at 9·5 years; improving maternal B_{12} status in pregnancy may improve B_{12} status in Indian children.

Acknowledgements

Acknowledgements: The authors thank the study participants for their time and interest; Dr S.C. Karat (Medical Director of HMH); the research staff at the Epidemiology Research Unit, HMH; the laboratory staff at the KEM Hospital Diabetes Unit in Pune who performed the biochemical analyses; and SNEHA India for its support. *Financial support:* This study was funded by the Parthenon Trust, Wellcome Trust, Medical Research Council and UK Department for International Development. The funders had no role in the design, analysis or writing of this article. *Conflict of interest:* None. *Authorship:* The study was conceived and designed by A.M.C., C.H.D.F. and S.H.K. G.V.K. and S.R.V. led the research team and supervised data collection, B.M.M. and S.H.K. designed the FFQ, R.K. collected the nutritional data. E.M.-Z., P.E. and A.M.C. conducted the statistical analysis and A.M.C., C.H.D.F. and S.H.K. drafted the manuscript. All authors contributed intellectually to the final version. *Ethics of human subject participation:* Ethical approval for the study was granted by the HMH Research Ethics Committee.

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