DIETARY CORRELATES OF VITAMIN B_{12} STATUS IN PREGNANT WOMEN IN SOUTHERN INDIA

A Thesis

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Master of Science

by

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ABSTRACT

Background: Vitamin B_{12} deficiency during pregnancy has been associated with adverse health outcomes, including maternal anemia, preeclampsia, intrauterine growth restriction, and neural tube defects. The objective of this analysis was to examine the dietary correlates of vitamin B_{12} status in pregnant women participating in a prospective cohort study in Southern India.

Methods: Participants were 400 pregnant women (< 14 weeks gestation) enrolled in a cohort study. Dietary intake was assessed using a validated, interviewer-administered, semi-quantitative food frequency questionnaire, which assessed dietary intake of 108 food items. Serum vitamin B_{12} was measured *via* electrochemiluminescence; vitamin deficiency was defined as vitamin B_{12} concentrations less than 148.0 pmol/L. Linear and binomial regression models were used to examine the associations between dietary variables (i.e., food groups, nutrients) and vitamin B_{12} status.

Results: The median daily vitamin B_{12} intake was 1.7 μg (IQR: 1.1–2.5), and 63.3% of participants were vitamin B_{12} deficient. In food group analyses, consumption of egg-based foods, organ meats, red meat, and grams were associated with higher serum vitamin B_{12} concentrations; consumption of pulses and fruit-based foods were associated with lower vitamin B_{12} concentrations. Similarly, increased consumption of egg-based foods (RR: 0.99, 95% CI: 0.98–0.99, $p = 0.023$), milk products (0.99, 0.99–0.99, $p = 0.038$), and total meat 0.80 (0.69–0.93, $p = 0.004$) were associated with significantly lower risk for vitamin B_{12} deficiency. In nutrient increased intake of vitamin B_{12}; saturated (caprylic, capric, lauric, stearic), monounsaturated

(palmitoleic), and polyunsaturated (linolenic, arachidonic, timnodonic, cervonic) fatty acids; cholesterol; iodine; and most amino acids (tryptophan, threonine, isoleucine, leucine, lysine, methionine, cysteine, tyrosine, valine, alanine, aspartic acid, serine) were associated with higher serum vitamin B_{12} concentrations and lower risk for vitamin B_{12} deficiency ($p < 0.05$).

Conclusion: Vitamin B_{12} intake was low in this population and was associated with risk for vitamin B_{12} deficiency. Low consumption of animal-source foods (i.e., milk, egg, and meat products) was associated with risk for vitamin B_{12} deficiency. Assessing dietary intake during early pregnancy is important to identify risk factors for vitamin B_{12} deficiency and to inform dietary recommendations.

BIOGRAPHICAL SKETCH

Heather M. Guetterman was born and raised in Illinois. She received her Bachelor's of Science in Human Nutrition with a dietetics concentration from the University of Illinois at Champaign-Urbana in May 2016. She came to Cornell University in August 2016 to pursue her Master's degree with Dr. Julia Finkelstein. She looks forward to joining the doctoral program at Cornell University in Fall 2018.

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LIST OF ABBREVIATIONS

CI	95% confidence interval
CV	Coefficient of variability
BMI	Body mass index
BMR	Basal metabolic rate
CDC	Centers for Disease Control and Prevention
EDTA	Ethylenediaminetetraacetic acid
FFQ	Food frequency questionnaire
Hb	Hemoglobin
IQR	Interquartile range
MUAC	Mid-upper arm circumference
NIN	National Institute of Nutrition (India)
PAL	Physical activity level
RBC	Red blood cells
RDA	Recommended dietary allowance
RR	Risk ratio
SE	Standard error
SAS	Statistical Analysis Software
TEE	Total energy expenditure
USDA	U.S. Department of Agriculture
WHO	World Health Organization

INTRODUCTION

Vitamin B_{12} is a water-soluble vitamin required as a cofactor for two enzymes in human physiology: L-methyl-malonyl-coenzyme A mutase and methionine synthase (1). L-methyl-malonyl-coenzyme A is involved in branched chain and odd chain fatty acid catabolism. Methionine synthase is required in folate-mediated one-carbon metabolism to convert 5-methyl-tetrahydrofolate to tetrahydrofolate, and for remethylation of homocysteine to methionine (2) (Figure 1). This process is required for the formation of the methyl donor, S-adenosylmethionine, which is involved in over 100 methylation reactions, including chromatin methylation (3). Due to its role in erythropoiesis, vitamin B_{12} deficiency can lead to megaloblastic anemia. Vitamin B_{12} deficiency can also lead to impairments in cell reproduction, DNA stability, and neurological functions, which may be irreversible (4,5).

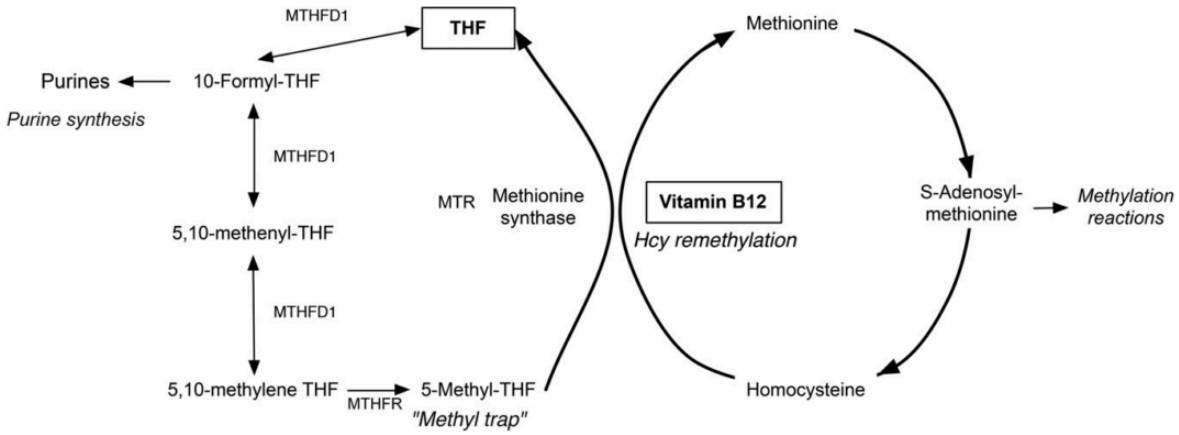


Figure 1. Vitamin B_{12} in one-carbon metabolism. Adapted from Finkelstein JL et al. (5)

Vitamin B_{12} deficiency is a major public health problem, with the highest burden in resource-limited settings and in high-risk populations such as pregnant women and young children. In

India, the prevalence of vitamin B_{12} deficiency in pregnancy is estimated to be the highest globally, with estimates ranging from 32–51% or higher (6,7). Further, vitamin B_{12} deficiency during pregnancy has been associated with increased risk of adverse pregnancy outcomes, including preeclampsia, low birthweight, intrauterine growth restriction, and neural tube defects (5). Infants born to mothers who are vitamin B_{12} deficient are at increased risk for deficiency through decreased placental transfer during gestation and lower hepatic stores in early infancy, as well as low concentrations in feedings of breast milk (8–11). Inadequate vitamin B_{12} status early in life has also been associated with impaired child growth, cognitive function, and psychomotor development, which may be irreversible (5,12).

Vitamin B_{12} is synthesized by microorganisms and is obtained in the diet through consumption of animal-source foods, such as meat, poultry, fish, eggs, and milk. Common causes of vitamin B_{12} deficiency include inadequate dietary intake, bioavailability, and impaired absorption (e.g., gastrointestinal infections, medications that alter gastric acid production, other gastrointestinal abnormalities) (5,10). Several observational studies have reported associations between intake of animal products and vitamin B_{12} status (13–20); however, specific foods identified in observational studies and findings from randomized controlled trials have been heterogeneous (21–25). For example, an observational study among pregnant women in Turkey found that insufficient intake of meat, milk, and eggs was associated with a higher risk for vitamin B_{12} deficiency (18), while a study among pregnant women in India found that consumption of yogurt and fish, but not eggs, organ meat, poultry, or meat, was associated with a lower risk for vitamin B_{12} insufficiency (17). Two randomized controlled trials in women of reproductive age did not find any effects of an intervention including pork (21) or a snack of animal-source foods (22) on

vitamin B_{12} concentrations, compared to control diets. Additionally, a randomized controlled trial among pregnant women in India assigned groups to one of three daily treatments: 500 mL milk and 10 micrograms vitamin B_{12} tablet, 500 mL milk and placebo tablet, placebo tablet. The study did not find significant differences in third-trimester vitamin B_{12} concentrations among the treatment arms. In contrast, a randomized controlled trial among Kenyan school children found that isocaloric snacks of a combined intervention of beans, rice, meat, and milk significantly reduced the prevalence of vitamin B_{12} deficiency, compared to a beans and rice intervention or no intervention (23). Further, studies have also reported differences in bioavailability of vitamin $B_{12,}$ depending on the source and amount consumed, varying losses from cooking method, and a saturation of intrinsic factor–a binding protein required for vitamin B_{12} absorption in the ileum, as vitamin B_{12} intake increases (26,27). However, several gaps exist in the understanding of vitamin B_{12} bioavailability, absorption, mobilization, regulation, and requirements in the context of pregnancy (28).

Assessment of dietary intake during early pregnancy could identify risk factors for vitamin B_{12} deficiency and inform dietary interventions and improve maternal and child health outcomes. Therefore, this study was conducted to examine the dietary correlates of vitamin B_{12} status in pregnant women who were participating in a prospective cohort study in Southern India.

MATERIALS AND METHODS

Study Population

Participants were pregnant women (≤ 14 weeks gestation) enrolled in a prospective perinatal cohort study in Bangalore, India. Inclusion criteria were women who were healthy, with a singleton pregnancy, attending antenatal care, and planning to deliver at St. John's Medical College Hospital. Exclusion criteria were: women anticipating relocating out of the area before delivery; women with a multiple pregnancy, who had history of previous infertility, Caesarian section, infections with hepatitis B, HIV, or syphilis; women taking daily micronutrient supplements (other than standard prenatal iron and folic acid supplements); or those with diabetes mellitus, hypertension, heart disease, thyroid disease, or other pre-existing conditions that required regular or daily medication use. All women received 60 milligrams of elemental iron and 500 micrograms of folic acid per day, as per standard of care. Archived blood samples from a total of 400 mother–infant pairs were selected for analysis of serum vitamin B_{12} concentrations, with a total of 400 participants with available dietary data (FFQ) and serum vitamin B_{12} concentrations. The research protocols and study procedures were approved by the Institutional Ethical Board of St. John's Medical College. Written informed consent was obtained from all study participants.

Data Collection

At the time of enrollment, sociodemographic information (i.e., income, education, employment, age, parity) was assessed *via* interview by trained research assistants. Anthropometric data; vital signs; blood pressure; and obstetric, reproductive, and neurological data were collected during clinical examinations by trained research assistants. Weight was assessed using a digital scale to

the nearest 100 grams; height was recorded using a stadiometer to the nearest 0.1 centimeter; and mid-upper arm circumference, and triceps, biceps, and subscapular skinfold thickness measurements were taken in triplicate. Body mass index (BMI) was calculated as weight in kilograms divided by height in meters squared (kg/m^2). Gestational age in weeks was confirmed on a subset of participants using ultrasonographic measurements within two weeks of enrollment. Physical activity level was calculated as total energy expenditure divided by basal metabolic rate for a 24-hour period (TEE/24/BMR).

Dietary Data

Dietary intake was assessed using a semi-quantitative, interviewer-administered food frequency questionnaire (FFQ), which was developed and validated for the study population using two 24-hour recalls and biomarkers (29). For vitamin $B_{12,}$ the validation study noted positive correlations of the FFQ dietary intake with the 24-hour recall data and plasma vitamin B_{12} concentrations (29). Trained research assistants assessed intake of 108 individual food items during the past 3 months, and recorded 4 frequency categories (i.e., daily, weekly, monthly, yearly) and amount consumed using standard measuring utensils.

Nutritional content of individual food items were calculated using a food composition database, which was developed based on the National Institute of Nutrition (NIN) Indian Food Composition Tables (30) and the U.S. Department of Agriculture (USDA) (31). This method used recipes tested in the laboratory, with weights taken for raw ingredients and volume-to-weight ratios calculated for cooked foods; nutrient composition was derived from the NIN Indian Food Composition Tables (30), and if not available, the USDA food composition database

(31,32). For further analysis, food items were then grouped into 30 food groups based on food group values assigned to each food item.

Daily intake of nutrients (i.e., energy, macronutrients, amino acids, fatty acids, and 17 micronutrients) were calculated for each participant by multiplying the nutrient composition of the standard portion size of each food item (i.e., using data from the NIN or USDA food composition database) by the reported frequency of consumption, and summing nutrient data for all reported food items consumed. Self-reported iron and folic acid supplement intakes were recorded separately.

Blood Collection and Laboratory Analyses

Approximately 10 milliliters of venous blood was collected from participants in ethylenediaminetetraacetic acid (EDTA) (Becton Dickenson, Franklin Lakes, NJ, US) vacutainers. Plasma and red blood cells (RBC) were separated from whole blood using a refrigerated centrifuge at 4°C and stored at less than –80°C until analysis. Hemoglobin (Hb) concentrations were assessed using an automated cyanmethemoglobin technique (ABX Pentra 60 C+ Hematology Analyzer, Horiba ABX Diagnostics). Serum vitamin B_{12} concentrations were measured using electrochemiluminescence (Elecsys 2010, Roche Diagnostics Mannheim, Germany), with intra-day and inter-day assay coefficients of variability (CVs) of 1.5% and 4.5%, respectively.

Definition of Variables

Individual foods were categorized into 30 food groups (i.e., grams per day), including: cereals (i.e., whole and processed grains), pulses (total pulses including lentils, peas, and grams—i.e., beans), total meat (i.e., fish, chicken, red meat, organ meat), egg-based foods, milk products, tea, coffee, total vegetable-based foods (i.e., raw vegetable-based, leafy vegetable-based, root vegetable, carrot-based, other vegetable-based), fruit-based foods, nut-based foods, fiber from cereals, fiber from fruits, fiber from vegetables, ghee, beer, wine, and spirits.

Animal-source food consumption was categorized as vegan (i.e., no fish, meat, poultry, eggs, or milk), lacto-ovo vegetarian (i.e., no fish, meat, or poultry), lacto-vegetarian (i.e., no fish, meat, poultry, or eggs), ovo-vegetarian (i.e., no fish, meat, poultry, or milk), vegetarian (i.e., no fish, meat, or poultry), and pescatarian (i.e., no meat or poultry). Food groups were also categorized as consumed and not consumed, and analyses for continuous variables were restricted to individuals who consumed those food items.

Vitamin B_{12} deficiency was defined as serum vitamin B_{12} concentrations less than 148.0 pmol/L (or < 150.0 pmol/L), and vitamin B_{12} insufficiency was defined as less than 221.0 pmol/L (or < 200.0 pmol/L), based on Centers for Disease Control and Prevention (CDC) (33) or World Health Organization (34) criteria, respectively.

Maternal age was categorized as less than 20 years, 20 to < 25 years, 25 to < 30 years, 30 to < 35 years, 35 to < 40 years, and ≥ 40 years; parity was categorized as 1, 2, or > 2; BMI was categorized as underweight (< 18.5 kg/m^2), normal (18.5 to < 25.0 kg/m^2), overweight (25.0 to <

30.0 kg/m^2), and obese (≥ 30.0 kg/m^2), and overweight or obese (≥ 25.0 kg/m^2); and physical activity levels (TEE/24/BMR) were defined as extremely inactive (< 1.4 TEE/24/BMR), sedentary (1.4 to < 1.7 TEE/24/BMR), moderately active (1.7 to < 2.0 TEE/24/BMR), vigorously active (2.0 to 2.4 TEE/24/BMR), or extremely active (> 2.4 TEE/24/BMR) (35). Maternal anemia was defined as Hb less than 11.0 g/dL and severe anemia as less than 7.0 g/dL, in accordance with WHO criteria (36). The poverty line was defined as ≤ 1,089 rupees monthly per capita, using the government of India's criteria for urban Karnataka (37).

Statistical Analyses

Linear and binomial regression models were used to examine the associations between dietary variables (i.e., intake of food groups, nutrients) with continuous and categorical vitamin B_{12} status, respectively. Analyses were conducted separately for food groups and nutrients. Serum vitamin B_{12} was assessed for normality using Q–Q plots and the Kolmogorov–Smirnov test, and was natural logarithmically transformed to ensure normality prior to analyses. Descriptive statistics were conducted for biochemical, anthropometric, sociodemographic, and dietary variables. Continuous data were reported as median and interquartile range (IQR), and categorical data were reported as number and percent (%).

Binomial regression models were used to obtain risk ratio (RR) estimates for dichotomous variables; the Poisson distribution was used when models using the binomial distribution failed to converge. Dietary intake of micronutrients and macronutrients were energy-adjusted using the residual method (38): i.e., regressing natural logarithmically transformed nutrient intake with the

natural logarithm of energy intake, and extracting residuals from these regression models to add to mean energy-centered nutrient intakes for inclusion in the energy-adjusted model:

$$\text{Vitamin } B_{12} \text{ concentrations} = \beta_1 \text{ nutrient residual} + \beta_2 \text{ calories} \quad (38).$$

Potential predictors were examined as continuous and categorical variables. Variables with univariate *p*-values of less than 0.20 were eligible for inclusion in multivariate regression models; covariates were evaluated using forward stepwise regression and retained in the final model if their *p*-values were less than 0.05.

Final linear models were checked for normality using the Kolmogrov–Smirnof test, collinearity using variance inflation factors, and homoscedasticity (using residual vs. predicted plots). Goodness of fit tests were used to assess the final binomial regression models. Statistical analyses were conducted using SAS software, version 9.4 (SAS Institute, Cary, NC)

RESULTS

Baseline Characteristics

The characteristics of participants in this study are presented in **Table 1**, and the flow diagram of study participants is presented in **Figure 1**. Baseline characteristics of participants in the current analysis (n = 400 with available FFQ and serum vitamin B_{12} data) and the total perinatal cohort were similar, in terms of maternal age, gestational age at enrollment, socioeconomic status, and nutritional indicators.

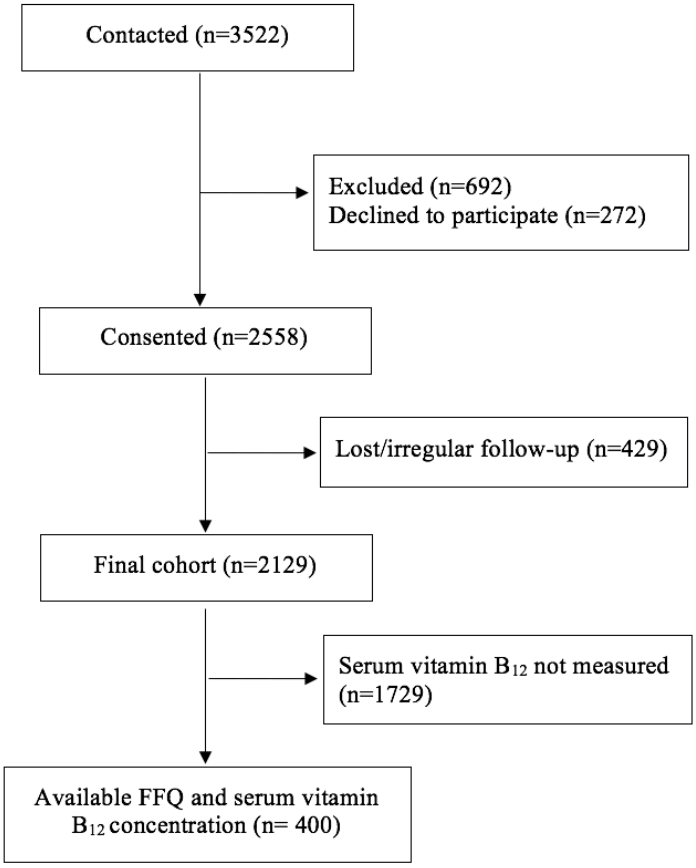


Figure 2. Participant flow diagram

The median maternal age was 24.0 (IQR: 21.0–26.0) years, and median gestational age was 12.0 (IQR: 9.6–13.3) weeks. A total of 55.5% of women were primiparous, and 44.5% were multiparous. Most women had at least high school education (94.5%) and were not formally employed (80.3%). No participants reported tobacco use, but 18.3% reported the presence of people smoking at home or at work. The median BMI was 20.6 (IQR: 18.6–23.3); 23.5% of women were underweight (BMI < 18.5 kg/m^2), and 14.3% were overweight or obese (BMI (≥ 25.0 kg/m^2). In terms of physical activity level (PAL), most participants (55.5%) were classified as sedentary. Most individuals (65.8%) reported experiencing symptoms in the first trimester, including vomiting (51.0%).

A total of 21.0% of women were anemic (Hb < 11.0 g/dL), and 0.8% were severely anemic (Hb < 7.0 g/dL) at baseline, with median Hb concentrations of 12.0 (IQR: 11.2–12.7) g/dL.

Median vitamin B_{12} intake was 1.7 μg (IQR: 1.1–2.5) per day (Table 1), with 77.5% and 29.3% of individuals below the RDA for vitamin B_{12} for pregnant women for the US (2.6 μg/day) and India (1.2 μg/day), respectively. No individuals were classified as vegan; however, consumption of animal-source foods was low, with 17.8% of individuals who did not consume any meat, poultry, or fish.

The prevalence of vitamin B_{12} deficiency was high in this population: 63.3% of participants had vitamin B_{12} deficiency (< 148.0 pmol/L) and 87.3% had vitamin B_{12} insufficiency (< 221 pmol/L). Median serum vitamin B_{12} concentrations were 126.9 (IQR: 89.6–172.2) pmol/L.

Table 1. Characteristics of the study population

	Median (IQR) or n (%)
Sociodemographic	
Age, years	24.0 (21.0–26.0)
< 20	37 (9.3)
20 to < 25	209 (52.2)
25 to < 30	115 (28.8)
30 to < 35	35 (8.8)
35 to < 40	2 (0.5)
≥ 40	2 (0.5)
Gestational age, weeks	12.0 (9.6–13.3)
Parity	0 (0–1)
1	222 (55.5)
2	162 (40.5)
> 2	16 (4.0)
Education	
No formal education	5 (1.3)
Primary school	4 (1.0)
Middle school	13 (3.3)
High school	101 (25.3)
Higher education	134 (33.5)
Graduates	118 (29.5)
Professional course	25 (6.3)
Employment	
No formal employment	321 (80.3)
Unskilled worker	4 (1.0)
Skilled worker	13 (3.3)
Secretarial staff	17 (4.3)
Semi-professional	20 (5.0)
Professional	25 (6.3)
Household income, INR[1]	14,000 (10,000–25,000)
Household income per capita, INR[1]	3,464 (2,000–6,000)
< 1,089	33 (8.3)
Exposure to smoking[2]	
People smoking in presence	73 (18.3)
Type of cooking fuel[3,4]	
Firewood	11 (2.8)
Coal	114 (28.6)
Kerosene	7 (1.8)
Liquid petroleum gas	267 (66.9)
Dietary Intake of Vitamin B_{12}	
Vitamin B_{12} intake, µg/day	1.7 (1.1–2.5)
< 1.2	117 (29.3)
< 2.6	310 (77.5)

Table 1 (Continued)

Dietary Pattern	
Vegan (i.e., no fish, meat, poultry, eggs, milk)	0 (0.0)
Vegetarian (i.e., no fish, meat, or poultry)	71 (17.8)
Lacto-ovo vegetarian (i.e., no fish, meat, poultry)	17 (4.3)
Lacto vegetarian (i.e., no fish, meat, poultry, eggs)	54 (13.5)
Ovo-vegetarian (i.e., no fish, meat, poultry, milk)	0 (0.0)
Pescatarian (i.e., no meat, poultry)	4 (1.0)
Anthropometric and Health	
Height, cm	156.2 (152.0–160.0)
Weight, kg	50.2 (44.9–56.2)
MUAC, cm	23.5 (21.7–25.7)
BMI, kg/m^2	20.6 (18.6–23.3)
Underweight (< 18.5)	94 (23.5)
Normal (≥ 18.5 and < 25.0)	249 (62.3)
Overweight (≥ 25.0 and < 30.0)	52 (13.0)
Obese (≥ 30.0)	5 (1.3)
Physical activity level, TEE/24/BMR [5]	1.4 (1.4–1.6)
Inactive (< 1.4)	154 (38.5)
Sedentary (1.4 to < 1.7)	222 (55.5)
Moderately active (1.7 to < 2.0)	24 (6.0)
Morbidity	
Acute diseases	263 (65.8)
Loose stool	4 (1.0)
Vomiting	204 (51.0)
Giddiness	105 (26.3)
Fever	20 (5.0)
Cough/cold	68 (17.0)
Other illness[6]	3 (0.8)
Chronic disease	35 (8.8)
Biochemical	
Serum Vitamin B_{12}, pmol/L	126.9 (89.6–172.2)
< 148.0	253 (63.3)
< 150.0	257 (64.3)
< 200.0	333 (83.3)
< 221.0	349 (87.3)
Hemoglobin, g/dL	12.0 (11.2–12.7)
< 11.0 g/dL	84 (21.0)
< 7.0 g/dL	3 (0.8)

[1]100 INR was equivalent to approximately 2 USD at the time the study was conducted
[2]Tobacco consumption not displayed due to 0.0% prevalence
[3]n = 399
[4]Gobar gas, electricity, LPG + Wood, LPG + kerosene are not displayed due to 0.0% prevalence
[5] Extremely inactive, vigorously active, and extremely active are not displayed due to 0.0% prevalence
[6]n = 383
Abbreviations: IQR, interquartile range; INR, Indian rupees; RDA, Recommended Dietary Allowance; MUAC, Mid-upper arm circumference; BMI, body mass index; WHO, World Health Organization; CDC, Centers for Disease Control and Prevention

Dietary Intake

Participant dietary intake of food groups and nutrients are presented in **Table 2** and **Table 3**, respectively. The median total meat consumption was 14.6 grams (IQR: 4.5–27.7) per day: 0.0 grams fish (IQR: 0.0–2.7), 6.8 grams chicken (IQR:0.0–14.8), 2.8 grams red meat (IQR: 0.0–9.0), and 0.0 grams of organ meats (IQR: 0.0–0.0) per day. For other animal-source foods, the median intake was 7.7 grams egg-based foods (IQR: 0.0–16.0), and 194.1 grams of milk (IQR: 64.3–364.4) per day. This is equivalent to approximately one-fifth of a large egg and three-fourths of a cup of milk, respectively (32). Median energy intake was 1,794.1 kCal (IQR: 1,500.1–2,097.1) and median protein intake was 51.8 grams (IQR: 42.4–-61.1) per day.

Table 2. Food groups consumed per day by pregnant women enrolled in a perinatal cohort

Food groups (g/day)	Median (IQR)
Grains	
Whole grain	56.4 (24.4–94.7)
Refined grains-based food	160.8 (122.3–207.5)
Cereal-based food	231.3 (176.6–279.1)
Pulses	15.3 (8.8–28.1)
Grams	2.2 (1.1–3.4)
Animal-source Foods	
Total meat [1]	14.6 (4.5–27.7)
Fish	0.0 (0.0–2.7)
Chicken	6.8 (0.0–14.8)
Red meat	2.8 (0.0–9.0)
Organ meats	0.0 (0.0–0.0)
Egg-based food	7.7 (0.0–16.0)
Milk	194.1 (64.3–364.4)
Beverages	
Tea	0.0 (0.0–120.0)
Coffee	0.0 (0.0–60.0)
Fruits and Vegetables	
Total vegetable-based food	84.1 (55.4–121.6)
Raw vegetable-based food	3.2 (0.0–8.5)
Leafy vegetable-based food	19.3 (11.5–31.1)
Root vegetable-based food	10.5 (4.9–17.5)
Carrot-based food	4.9 (0.0–14.2)
Other vegetable-based food	33.1 (16.5–54.7)
Total fruit-based food	88.0 (52.3–138.4)
Other fruit-based food	21.6 (11.5–37.6)
Nut-based food	3.2 (0.0–6.4)
Fiber from cereals	2.5 (1.5–3.8)
Fiber from fruits	1.0 (0.6–1.7)
Fiber from vegetables	1.0 (0.7–1.4)
Other	
Ghee	0.0 (0.0–0.9)

[1]Total meat = fish + poultry + red meat + organ meats
Beer, wine, and spirits are not displayed due to 0.0% prevalence
Abbreviation: IQR, interquartile range

Table 3. Nutrients consumed per day by pregnant women enrolled in a perinatal cohort

Nutrients	**Median (IQR)**	**US RDA**(39,40)	**India RDA**(41,42)
Energy (kCal)	1794.1 (1500.1–2097.1)	1600[2]	1925[3]
Protein (g)	51.8 (42.4–61.1)	40-140[4] (10-35)	48-72(10-15)[5]
Fat (g)	47.1 (37.8–57.9)	36-62[6]	42-64(20-30)[7]
Carbohydrate (g)	290.8 (241.7–346.4)	180-260 (45-65)[8]	240-289(50-60%)[9]
Fiber (g)	8.0 (6.2–10.3)	22[1, 10]	Not available
Saturated fats (g)	16.0 (11.9–21.0)		
Monounsaturated fat (g)	11.5 (9.1–14.5)		
Polyunsaturated fat (g)	13.7 (10.8–17.3)		
Cholesterol (mg)	109.8 (68.0–156.5)		
Micronutrients (mg)			
Vitamin B_{12} (µg)	1.7 (1.1–2.5)	2.6	1.2
Vitamin E	11.2 (8.8–14.0)	15	8–10
Vitamin C	102.3 (73.8–150.5)	85	60
Thiamin	1.2 (0.9–1.4)	1.4	1.2
Riboflavin	1.3 (1.0–1.8)	1.4	1.4
Niacin	11.2 (9.1–13.6)	18	14
Pantothenic acid	5.7 (4.7–6.6)	6[1]	Not available
Vitamin B_6	1.6 (1.3–2.0)	1.9	2.5
Folate (µg)	253.4 (206.7–308.9)	600	500
Calcium	914.0 (663.6–1152.7)	1,000	1,200
Phosphorus	1,241.8 (998.8–1,500.7)	700	1,200
Iron	13.1 (10.4–16.1)	27	35
Magnesium	438.1 (365.1–518.5)	350–360	310
Sodium	2,569.2 (2,057.1–3,226.9)	1,500[1]	2,500
Potassium	2,267.9 (1,790.4–2,920.1)	4,700[1]	3,225
Copper	1.8 (1.5–2.3)	1.0	2.0
Manganese	5.1 (3.6–7.7)	2.0[1]	2.0–5.0
Zinc	7.8 (6.4–9.2)	11	12
Selenium (µg)	57.6 (45.0–72.6)	60	40
Iodine (µg)	97.7 (73.4–124.9)	220	150

[1]Adequate intakes (AI)
[2]Based on 50 kg women aged 24 years with height 1.56 meters, and low activity; and recommendation to not increase energy intake in first trimester
[3]Based on 50 kg women with sedentary activity and recommendation to increase energy intake by 85 kcal in first trimester
[4]10-35% of total energy
[5]10-15% of total energy
[6]20-35% of total energy
[7]20-30% of total energy
[8]45-65% of total energy
[9]50-60% of total energy
[10]14 g fiber/1,000 kcal/day
Abbreviation: IQR, interquartile range

Food Group Analyses

The associations between food group variables and serum vitamin B_{12} concentrations (natural logarithmically transformed) are presented in **Table 4**. In univariate analyses, consumption of refined grains-based foods (β: 0.001, SE: 0.00), grams (β: 0.02, SE: 0.01), total meat (β: 0.01, SE: 0.00), chicken (β: 0.01, SE: 0.00), red meat (β: 0.01, SE: 0.00), egg-based-foods (β: 0.01, SE: 0.00), and organ meats (β: 0.04, SE: 0.01) were associated with higher vitamin B_{12} concentrations ($p < 0.05$). In contrast, greater consumption of other fruit-based foods was associated with significantly lower vitamin B_{12} concentrations (β: –0.002, SE: 0.00; $p < 0.05$). Consumption of any fish (β: 0.16, SE: 0.06), chicken (β: 0.22, SE: 0.06), total red meat (β: 0.28, SE: 0.06), egg-based foods (β: 0.12, SE: 0.06), organ meats (β: 0.26, SE: 0.10), or total meat (β: 0.31, SE: 0.07) were associated with higher vitamin B_{12} concentrations ($p < 0.05$).

In multivariate analyses, greater consumption of organ meats (β: 0.03, SE: 0.01), egg-based foods (β: 0.01, SE: 0.00), grams (β: 0.03, SE: 0.01), and any red meat (β: 0.24, SE: 0.06) were associated with significantly higher serum vitamin B_{12} concentrations, while higher consumption of pulses (β: –0.004, SE: 0.00) and other fruit-based foods (β: –0.003, SE: 0.00) were associated with significantly lower vitamin B_{12} concentrations ($p < 0.05$).

Table 4. Food group analyses: Association between food groups and natural logarithmically transformed

	Univariate[1]		Multivariate[2,3]	
Food Group Intake (g)	**β (SE)**	***p*-value**	**β (SE)**	***p*-value**
Whole grain	–0.00 (0.00)	0.116		
Refined grains-based food	0.001 (0.00)	0.044		
Pulses	–0.00 (0.00)	0.107	–0.004 (0.00)	0.013
Grams	0.02 (0.01)	0.042	0.03 (0.01)	0.015
Total meat [4]	0.01 (0.00)	0.0001		
Consumed[5]	0.31 (0.07)	< 0.0001		
Fish	0.01 (0.00)	0.092		
Consumed[6]	0.16 (0.06)	0.006		
Chicken	0.01 (0.00)	0.026		
Consumed[7]	0.22 (0.06)	0.0004		
Red meat	0.01 (0.00)	0.002		
Consumed[8]	0.28 (0.06)	< 0.0001	0.24 (0.06)	< 0.0001
Organ meats	0.04 (0.01)	0.003	0.03 (0.01)	0.025
Consumed[9]	0.26 (0.10)	0.010		
Egg based food	0.01 (0.00)	0.0002	0.007 (0.00)	0.005
Consumed[10]	0.12 (0.06)	0.048		
Leafy vegetable-based food	0.00 (0.00)	0.135		
Consumed[11]	0.01 (0.11)	0.923		
Other fruit-based food	–0.002 (0.00)	0.047	–0.003 (0.00)	0.024
Consumed[12]	–0.41 (0.21)	0.045		

[1]All $p < 0.20$ presented
[2]All $p < 0.05$ presented
[3] Multivariate linear regression model with pulses (continuous), grams (continuous), total red meat (categorical), organ meats (continuous), egg-based food (continuous), other fruit-based food (continuous)
[4]Total meat = fish + poultry + red meat + organ meats
[5]n = 329; [6]n =177; [7]n = 282; [8]n = 255; [9]n = 35; [10]n = 280; [11]n = 369; [12]n = 392 that consumed each food item as a dichotomous variable
Abbreviations: SE, standard error

The associations between food group variables and risk of vitamin B_{12} deficiency are presented in **Table 5**. In univariate analyses, total meat consumption (RR: 0.99, 95% CI: 0.99–0.99, $p < 0.05$) and the consumption of organ meats (RR: 0.93, 95% CI: 0.86–0.99), egg-based food (RR: 0.99, 95% CI: 0.98–0.96), and milk (RR: 0.99, 95% CI: 0.99–0.99) were associated with a significantly lower risk for vitamin B_{12} deficiency ($p < 0.05$). Similarly, any consumption of fish (RR: 0.80, 95% CI: 0.68–0.93), chicken (RR: 0.83, 95% CI: 0.71–0.96), red meat (RR: 0.82, 95% CI:0.70–0.94), and total meat (RR: 0.74, 95% CI: 0.64–0.86) were associated with lower risk for vitamin B_{12} deficiency ($p < 0.05$). In contrast, higher whole grain consumption was

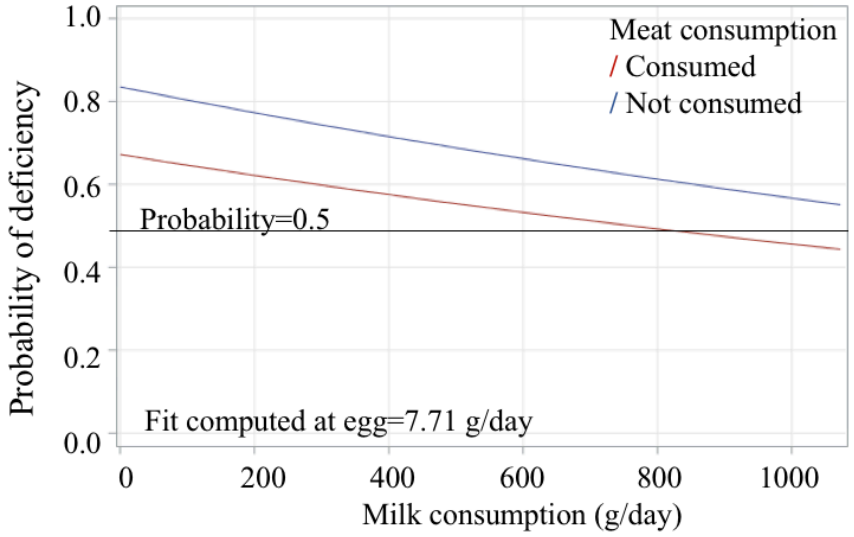
associated with increased risk for vitamin B_{12} deficiency (RR: 1.001, 95% CI: 1.00–1.002, $p < 0.05$). In multivariate analyses, increased consumption of egg-based foods (RR: 0.99, 95% CI: 0.98–0.99, *p*-value: 0.023) and milk products (RR: 0.99, 95% CI: 0.99–0.99, *p*-value: 0.038) was associated with a lower risk for vitamin B_{12} deficiency; any consumption of total meat was also associated with a 0.80 (95% CI: 0.69–0.93, *p*-value: 0.004) times lower risk for vitamin B_{12} deficiency. Findings from multivariate analyses are highlighted in **Figure 3**. The graphs depict the probability of vitamin B_{12} deficiency as milk (Figure 3a) and egg (Figure 3b) consumption increase, respectively, among participants who did not consume meat (blue line) and among those who did consume meat (red line), with the median intake of the third variable fixed for interpretation purposes.

Table 5. Food group analyses: Association between food groups and risk for vitamin B_{12} deficiency (< 148.0 pmol/L) among pregnant women enrolled in a perinatal cohort study

	Univariate[1]		Multivariate[2,3]	
Food Group Intake (g)	**RR (95% CI)**	***p*-value**	**RR (95% CI)**	***p*-value**
Whole grains	1.00 (1.00–1.00)	0.047		
Refined grains-based food	0.99 (0.99–1.00)	0.145		
Grams	0.96 (0.93–1.00)	0.052		
Total meat [4]	0.99 (0.99–0.99)	0.005		
Consumed[5]	0.74 (0.64–0.86)	< 0.0001	0.80 (0.69–0.93)	0.004
Fish	0.98 (0.96–1.00)	0.101		
Consumed[6]	0.80 (0.68–0.93)	0.005		
Chicken	0.99 (0.98–1.00)	0.166		
Consumed[7]	0.83 (0.71–0.96)	0.012		
Red meat	0.99 (0.98–1.00)	0.050		
Consumed[8]	0.81 (0.70–0.94)	0.006		
Organ meat	0.93 (0.86–0.99)	0.046		
Consumed[9]	0.70 (0.49–1.02)	0.062		
Egg based food	0.99 (0.98–0.99)	0.002	0.99 (0.98–0.99)	0.023
Consumed[10]	0.88 (0.75–1.02)	0.093		
Milk	0.99 (0.99–0.99)	0.033	0.99 (0.99–0.99)	0.038
Consumed[11]	0.79 (0.62–1.01)	0.061		
Raw vegetable-based food	0.99 (0.99–1.00)	0.159		
Leafy vegetable-based food	0.99 (0.99–1.00)	0.064		
Other fruit-based food	1.00 (1.00–1.01)	0.163		
Fiber from cereals	1.02 (0.99–1.05)	0.110		

[1]All $p < 0.20$ presented
[2]All $p < 0.05$ presented
[3]Multivariate binomial regression model with total meat (categorical), egg-based food (continuous), and milk (continuous)
[4]Total meat = fish + poultry + red meat + organ meat
[5] n = 329; [6] n = 177; [7]n = 282; [8]n =35; [9]n = 280; [10]n = 381 that consumed each food item as a dichotomous variable
Abbreviations: RR, risk ratio; 95% CI, 95% confidence interval

a)



b)

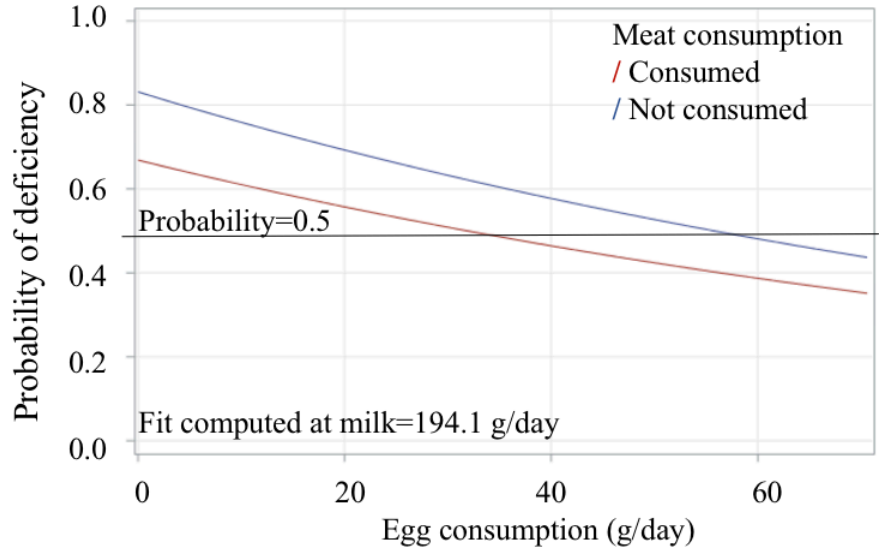


Figure 3. Food group analyses: Multivariate model of association between food groups and risk for vitamin B_{12} deficiency (< 148.0). The graphs depict the probability of vitamin B_{12} deficiency as milk (Figure 3a) and egg (Figure 3b) consumption increase, respectively, among participants who did not consume meat (blue line) and among those who did consume meat (red line).

Nutrient Analyses

The associations between dietary intake of nutrients and logarithmically transformed serum vitamin B_{12} concentrations are presented in **Table 6**. In univariate analyses, dietary intake of vitamin B_{12} (μg) (β: 0.05, SE: 0.01, $p < 0.0001$) and iodine (μg) (β: 0.002, SE: 0.00, $p < 0.05$) was associated with significantly increased vitamin B_{12} concentrations. No other B–vitamins were significantly associated with serum vitamin B_{12} concentrations in the univariate models. Consumption of saturated fats (i.e., total saturated fats—caprylic acid, capric acid, lauric acid, myristic acid, stearic acid), polyunsaturated fats (i.e., linolenic acid, arachidonic acid, timnodonic acid, and cervonic acid), and cholesterol was associated with higher vitamin B_{12} concentrations. Palmitoleic acid was the only monounsaturated fatty acid associated with significantly higher vitamin B_{12} concentrations. Although protein was not significantly associated with vitamin B_{12} concentrations, most of the amino acids were associated with higher vitamin B_{12} concentrations, including tryptophan, threonine, isoleucine, leucine, lysine, methionine, cysteine, tyrosine, valine, alanine, aspartic acid, and serine.

After adjusting for energy, dietary intake of vitamin B_{12} (μg) (β: 0.01, SE: 0.003, $p < 0.0001$), riboflavin, pantothenic acid, and iodine were associated with higher serum vitamin B_{12} concentrations. In contrast, dietary intake of iron, magnesium, and copper were associated with significantly lower vitamin B_{12} concentrations, in analyses adjusting for energy. Consumption of protein, fat, cholesterol, and saturated, monounsaturated, and polyunsaturated fatty acids were associated with significantly higher vitamin B_{12} concentrations, while consumption of carbohydrates and fiber was associated with lower vitamin B_{12} concentrations. Similarly, the

amino acids that were reported in univariate models were significantly associated with higher vitamin B_{12} concentrations after energy adjustment.

Table 6. Nutrient analyses: Association between nutrients consumed and natural logarithmically transformed vitamin B_{12} concentrations in pregnant women enrolled in a perinatal cohort study

	Univariate		Energy-Adjusted	
Nutrients	**β (SE)**	***p*-value**	**β (SE)**	***p*-value**
Energy (kCal)	0.00 (0.00)	0.297		
Protein (g)	0.003 (0.00)	0.053	0.02 (0.01)	0.0003
Fat (g)	0.003 (0.00)	0.092	0.007 (0.00)	0.044
Carbohydrate (g)	0.00 (0.00)	0.677	–0.003 (0.00)	0.014
Fiber (g)	–0.01 (0.01)	0.323	–0.07 (0.02)	0.001
Saturated fat (g)	0.009 (0.00)	0.034	0.01 (0.00)	0.019
Butyric acid	0.15 (0.10)	0.135	0.15 (0.10)	0.157
Caproic acid	0.27 (0.17)	0.107	0.30 (0.20)	0.132
Caprylic acid	0.39 (0.16)	0.013	0.56 (0.21)	0.008
Capric acid	0.25 (0.11)	0.024	0.32 (0.14)	0.021
Lauric acid	0.07 (0.03)	0.014	0.10 (0.04)	0.011
Myristic acid	0.05 (0.02)	0.041	0.09 (0.04)	0.023
Palmitic acid	0.02 (0.01)	0.081	0.03 (0.01)	0.076
Stearic acid	0.05 (0.02)	0.020	0.09 (0.03)	0.006
Monounsaturated fat (g)	0.01 (0.01)	0.118	0.02 (0.01)	0.049
Palmitoleic acid	0.38 (0.13)	0.004	0.50 (0.15)	0.001
Oleic acid	0.01 (0.01)	0.127	0.03 (0.01)	0.057
Gadeolic acid	–0.02 (0.53)	0.976	–0.10 (0.80)	0.905
Euric acid	–0.11 (0.36)	0.769	–0.60 (0.52)	0.248
Polyunsaturated fat (g)	0.00 (0.01)	0.444	–0.00 (0.01)	0.943
Linoleic acid	0.00 (0.01)	0.491	–0.00 (0.01)	0.855
Linolenic acid	0.34 (0.16)	0.039	0.67 (0.29)	0.022
Parinaric acid	19.33 (12.56)	0.125	31.94 (36.84)	0.387
Arachidonic acid	5.02 (1.04)	< 0.0001	3.14 (0.61)	< 0.0001
Timnodonic acid	7.76 (3.69)	0.036	17.65 (8.69)	0.043
Clupanodonic acid	27.26 (14.12)	0.054	56.23 (27.33)	0.040
Cervonic acid	5.09 (1.82)	0.005	8.45 (2.93)	0.004
Cholesterol (mg)	0.002 (0.00)	< 0.0001	0.002 (0.00)	< 0.0001
Amino Acids (g)				
Tryptophan	0.31 (0.15)	0.043	1.67 (0.48)	0.0005
Threonine	0.11 (0.05)	0.021	0.54 (0.13)	< 0.0001
Isoleucine	0.09 (0.04)	0.018	0.43 (0.11)	< 0.0001
Leucine	0.05 (0.02)	0.030	0.26 (0.07)	0.0002
Lysine	0.08 (0.03)	0.011	0.25 (0.07)	0.0001
Methionine	0.24 (0.08)	0.006	1.05 (0.21)	< 0.0001
Cysteine	0.29 (0.13)	0.029	1.44 (0.38)	0.0002
Phenylalanine	0.05 (0.04)	0.138	0.18 (0.11)	0.105
Tyrosine	0.12 (0.05)	0.027	0.51 (0.14)	0.0002
Valine	0.08 (0.03)	0.028	0.40 (0.10)	< 0.0001
Arginine	0.05 (0.03)	0.071	0.25 (0.09)	0.007
Histidine	0.13 (0.07)	0.059	0.59 (0.21)	0.005

Table 6 (Continued)

Alanine	0.09 (0.04)	0.027	0.45 (0.12)	0.0001
Aspartic acid	0.04 (0.02)	0.046	0.22 (0.07)	0.0008
Glutamic acid	0.01 (0.01)	0.147	0.05 (0.03)	0.095
Glycine	0.09 (0.05)	0.059	0.40 (0.14)	0.005
Proline	0.05 (0.03)	0.080	0.15 (0.07)	0.032
Serine	0.08 (0.04)	0.040	0.45 (0.12)	0.0003
Micronutrients (mg)				
Vitamin B_{12} (µg)	0.05 (0.01)	< 0.0001	0.01 (0.00)	< 0.0001
Vitamin E	0.00 (0.01)	0.563	–0.00 (0.01)	0.673
Vitamin C	0.00 (0.00)	0.984	0.00 (0.00)	0.489
Thiamin	0.00 (0.07)	0.957	–0.36 (0.19)	0.059
Riboflavin	0.10 (0.05)	0.058	0.16 (0.08)	0.043
Niacin	0.01 (0.01)	0.188	0.02 (0.01)	0.243
Pantothenic acid	0.03 (0.02)	0.111	0.12 (0.06)	0.042
Vitamin B_6	0.01 (0.05)	0.870	–0.20 (0.12)	0.092
Folate (µg)	0.00 (0.00)	0.287	0.00 (0.00)	0.784
Calcium	0.00 (0.00)	0.452	0.00 (0.00)	0.816
Phosphorus	0.00 (0.00)	0.308	0.00 (0.00)	0.662
Iron	–0.00 (0.01)	0.519	–0.04 (0.01)	0.003
Magnesium	0.00 (0.00)	0.917	–0.002 (0.00)	0.020
Sodium	0.00 (0.00)	0.622	0.00 (0.00)	0.450
Potassium	0.00 (0.00)	0.617	0.00 (0.00)	0.614
Copper	–0.00 (0.04)	0.932	–0.15 (0.07)	0.027
Manganese	–0.01 (0.01)	0.564	–0.02 (0.02)	0.134
Zinc	0.02 (0.01)	0.143	0.07 (0.04)	0.080
Selenium (µg)	0.00 (0.00)	0.067	0.00 (0.00)	0.081
Iodine (µg)	0.002 (0.00)	0.009	0.002 (0.00)	0.008

Abbreviations: SE, standard error

The associations between dietary intake of nutrients and risk for vitamin B_{12} deficiency are presented in **Table 7**. In univariate analyses, increased vitamin B_{12} intake was associated with a significantly lower risk (RR: 0.88, 95% CI: 0.82–0.94, $p < 0.05$) of vitamin B_{12} deficiency, with a 12.0% lower risk per microgram increase in vitamin B_{12} intake. Other micronutrients, riboflavin, selenium, and iodine, were also associated with a lower risk for vitamin B_{12} deficiency. Similarly, increased consumption of protein and fat, and most saturated fats (i.e., total saturated fats—butyric acid, caproid acid, caprylic acid, capric acid, lauric acid, palmitic acid, stearic acid), monounsaturated fats (i.e., total monounsaturated fat—palmitoleic acid, oleic acid), polyunsaturated fats (i.e., linolenic acid, arachidonic acid, timnodonic acid, cervonic acid), cholesterol, and most amino acids (i.e., tryptophan, threonine, isoleucine, leucine, lysine, methionine, cysteine, tyrosine, valine, arginine, alanine, aspartic acid, serine) were associated with a lower risk for vitamin B_{12} deficiency.

After energy adjustment, increased vitamin B_{12} intake (μg) was associated with a significantly lower risk (RR: 0.97, 95% CI: 0.96–0.99, $p < 0.0001$) for vitamin B_{12} deficiency. Dietary intake of magnesium, copper, and manganese were associated with increased risk for vitamin B_{12} deficiency after energy adjustment, while riboflavin, selenium, and iodine were associated with a lower risk of deficiency. After energy adjustment, carbohydrates and fiber were associated with a increased risk for vitamin B_{12} deficiency. Consumption of protein, fat, cholesterol, and all saturated, monounsaturated, and polyunsaturated fatty acids, and all amino acids that were associated with a lower risk for vitamin B_{12} deficiency in univariate models were associated with significantly lower risk for vitamin B_{12} deficiency in energy-adjusted models with the addition of myristic acid and glutamic acid.

Table 7. Nutrient analyses: Association between nutrients consumed and risk for vitamin B_{12} deficiency (< 148.0 pmol/L) among pregnant women enrolled in a perinatal cohort study

	Univariate		Energy-Adjusted	
Nutrient	**RR (95% CI)**	***p*-value**	**RR (95% CI)**	***p*-value**
Energy (kCal)	1.00 (1.00–1.00)	0.214		
Protein (g)	0.99 (0.90–0.99)	0.024	0.98 (0.96–0.99)	0.0003
Fat (g)	0.99 (0.99–0.99)	0.029	0.98 (0.98–0.99)	0.005
Carbohydrate (g)	1.00 (0.99–1.00)	0.648	1.004 (1.002–1.008)	0.002
Fiber (g)[1]	1.02 (0.99–1.04)	0.173	1.10 (1.01–1.20)	0.023
Saturated fat (g)	0.98 (0.97–0.99)	0.0008	0.97 (0.96–0.99)	0.0001
Butyric acid	0.63 (0.46–0.86)	0.004	0.63 (0.48–0.84)	0.002
Caproic acid	0.44 (0.26–0.74)	0.002	0.40 (0.23–0.70)	0.001
Caprylic acid	0.44 (0.26–0.74)	0.002	0.38 (0.22–0.66)	0.0007
Capric acid	0.55 (0.40–0.77)	0.0004	0.51 (0.36–0.72)	0.0002
Lauric acid	0.88 (0.79–0.97)	0.013	0.87 (0.78–0.97)	0.011
Myristic acid	0.95 (0.89–1.01)	0.087	0.84 (0.76–0.93)	0.0004
Palmitic acid	0.95 (0.93–0.98)	0.002	0.93 (0.90–0.97)	0.0003
Stearic acid	0.90 (0.84–0.95)	0.0006	0.86 (0.80–0.93)	< 0.0001
Monounsaturated fat (g)	0.98 (0.96–0.99)	0.034	0.96 (0.93–0.99)	0.006
Palmitoleic acid	0.45 (0.31–0.65)	< 0.0001	0.43 (0.30–0.62)	< 0.0001
Oleic acid	0.98 (0.96–0.99)	0.043	0.96 (0.92–0.99)	0.010
Gadeolic acid	1.47 (0.28–7.87)	0.653	2.01 (0.23–17.49)	0.526
Erucic acid	0.82 (0.31–2.22)	0.702	1.17 (0.30–4.59)	0.823
Polyunsaturated fat	0.99 (0.98–1.01)	0.726	1.01 (0.99–1.03)	0.477
Linoleic acid	0.99 (0.98–1.01)	0.828	1.01 (0.99–1.03)	0.368
Linolenic acid	0.42 (0.26–0.68)	0.0004	0.22 (0.12–0.42)	< 0.0001
Parinaric acid	0.00 (0.00–1.18e6)	0.125	0.00 (0.00–8.48e39)	0.428
Arachidonic acid	0.00 (0.00–0.07)	< 0.0001	0.03 (0.00–0.20)	0.0003
Timnodonic acid	0.00 (0.00–0.41)	0.041	0.00 (0.00–0.13)	0.039
Clupanodonic acid	0.00 (0.00–105.71)	0.067	0.00 (0.00–1.59)	0.051
Cervonic acid	0.00 (0.00–0.06)	0.003	0.00 (0.00–0.03)	0.011
Cholesterol (mg)	0.99 (0.99–0.99)	< 0.0001	0.99 (0.99–0.99)	< 0.0001
Amino Acids (g)				
Tryptophan	0.55 (0.35–0.88)	0.013	0.14 (0.05–0.38)	0.0002
Threonine	0.82 (0.71–0.95)	0.007	0.54 (0.40–0.73)	< 0.0001
Isoleucine	0.84 (0.75–0.95)	0.005	0.60 (0.48–0.76)	< 0.0001
Leucine	0.91 (0.85–0.98)	0.010	0.73 (0.63–0.85)	< 0.0001
Lysine	0.87 (0.79–0.95)	0.002	0.73 (0.63–0.84)	< 0.0001
Methionine[1]	0.66 (0.51–0.87)	0.003	0.27 (0.10–0.71)	0.008
Cysteine	0.64 (0.43–0.95)	0.026	0.27 (0.11–0.66)	0.004
Phenylalanine	0.90 (0.81–1.01)	0.068	0.76 (0.57–1.00)	0.052
Tyrosine	0.81 (0.69–0.94)	0.007	0.53 (0.40–0.72)	< 0.0001
Valine[1]	0.87 (0.79–0.97)	0.010	0.58 (0.37–0.91)	0.018
Arginine	0.93 (0.85–1.02)	0.131	0.87 (0.69–1.10)	0.241
Histidine	0.79 (0.64–0.97)	0.024	0.47 (0.29–0.76)	0.002

Table 7 (Continued)

Alanine	0.88 (0.78–0.99)	0.038	0.69 (0.52–0.92)	0.010
Aspartic acid	0.93 (0.88–0.99)	0.039	0.80 (0.68–0.94)	0.005
Glutamic acid	0.97 (0.95–1.00)	0.057	0.92 (0.85–0.99)	0.021
Glycine	0.89 (0.77–1.03)	0.105	0.78 (0.55–1.11)	0.164
Proline	0.89 (0.82–0.97)	0.008	0.75 (0.64–0.88)	0.0004
Serine	0.87 (0.77–0.97)	0.015	0.60 (0.46–0.78)	0.0002
Micronutrients (mg)				
Vitamin B_{12} (µg)	0.88 (0.82–0.94)	0.0001	0.97 (0.96–0.99)	< 0.0001
Vitamin E	0.99 (0.98–1.02)	0.790	1.01 (0.99–1.04)	0.408
Vitamin C	1.00 (0.99–1.00)	0.541	1.00 (0.99–1.00)	0.727
Thiamine	0.98 (0.81–1.18)	0.821	1.65 (0.98–2.76)	0.058
Riboflavin	0.79 (0.68–0.92)	0.002	0.71 (0.58–0.86)	0.0004
Niacin	0.98 (0.96–1.01)	0.152	0.98 (0.94–1.02)	0.332
Pantothenic acid	0.96 (0.91–1.01)	0.117	0.91 (0.77–1.07)	0.247
Vitamin B_6	0.96 (0.83–1.10)	0.537	1.18 (0.87–1.58)	0.287
Folate (µg)	0.99 (0.99–1.00)	0.220	1.00 (0.99–1.00)	0.760
Calcium	0.99 (0.99–1.00)	0.132	0.99 (0.99–1.00)	0.261
Phosphorus	0.99 (0.99–1.00)	0.089	0.99 (0.99–1.03)	0.086
Iron[1]	1.00 (0.99–1.02)	0.898	1.04 (0.99–1.09)	0.172
Magnesium	1.00 (0.99–1.00)	0.918	1.002 (1.0008–1.004)	0.004
Sodium	1.00 (1.00–1.00)	0.360	1.00 (1.00–1.00)	0.624
Potassium	1.00 (1.00–1.00)	0.238	1.00 (1.00–1.00)	0.744
Copper	0.99 (0.89–1.12)	0.941	1.19 (1.02–1.39)	0.029
Manganese	1.01 (0.99–1.03)	0.236	1.05 (1.01–1.08)	0.007
Zinc	0.98 (0.94–1.01)	0.207	0.97 (0.88–1.09)	0.639
Selenium (µg)	0.99 (0.99–0.99)	0.006	0.99 (0.99–0.99)	0.004
Iodine (µg)[1]	0.99 (0.99–0.99)	0.0001	0.99 (0.99–0.99)	0.039

[1]Energy-adjusted Poisson distribution used due to failed Hessian convergence in energy-adjusted logistic model
Abbreviations: RR, risk ratio; 95% CI, 95% confidence interval

DISCUSSION

Dietary intake of vitamin B_{12} was low in this population and was associated with a high prevalence of vitamin B_{12} deficiency. The median intake of vitamin B_{12} was 1.7 μg (IQR: 1.1–2.5) per day, with 29.3% and 77.5% of women consuming less than recommended intake for pregnant women in India (1.2 μg/day) and the United States (2.6 μg/day), respectively. A total of 63.3% of women had vitamin B_{12} deficiency (< 148.0 pmol/L), and 87.3% had vitamin B_{12} insufficiency (< 221.0 pmol/L). Although only 17.5% of participants were classified as vegan or vegetarian, the daily intake of animal-source foods in this population was low.

In this analysis, increased consumption of animal-source foods (i.e., milk, egg, and meat products), vitamin B_{12}, and nutrients related to these foods were associated with lower risk of vitamin B_{12} deficiency. However, consumption of other animal products (i.e., fish or chicken) was not associated with vitamin B_{12} concentrations or risk of vitamin B_{12} deficiency. Other observational studies have noted heterogeneous findings in pregnant women (17,18) and women of reproductive age (17–29 years) (20) with a similar prevalence of vitamin B_{12} deficiency Similar to our findings, in a study among pregnant women in their third trimester in Turkey, sufficient consumption (i.e., 2–3 times per week or more) of meat, milk, dairy products, and eggs were each higher among women with vitamin B_{12} concentrations greater than 160 pg/mL (i.e., approximately 118 pmol/L) (18). In a study among women of reproductive age in Nepal, eating meat one time per week was associated with higher plasma vitamin B_{12} concentrations (20). However, neither of these studies assessed or reported the effects of other animal-source foods. In contrast to our findings, a study among pregnant women in Bangalore, India, found that intake

of yogurt and fish (but not eggs, organ meats, poultry, and meat) was associated with significantly lower risk for impaired vitamin B_{12} status (vitamin B_{12} < 150 pmol/L, MMA > 0.26) (17).

Findings from previous studies in other populations (i.e., not pregnant individuals, with a low prevalence of vitamin B_{12} deficiency) on the association between animal products and vitamin B_{12} status have varied (13,14). Our findings contrast with a study among women of reproductive age in Norway, which noted significant associations between vitamin B_{12} intake from dairy products and fish (but not meat or eggs) and increased vitamin B_{12} concentrations (13). However, a study among adults in the Framingham Offspring Study (United States) found that dairy products, enriched cereals, and meat (i.e., meat, poultry, fish) were associated with vitamin B_{12} concentrations (14). Combining meat with poultry and fish in their analysis may have attenuated the effect of meat consumption on vitamin B_{12} concentrations, as meat and milk from ruminants are considered to be improved sources of vitamin B_{12} (compared to monogastric animals, such as chickens and pigs) due to microbes that synthesize cobalamin in the rumen (43). In contrast, other studies have noted a significant association between fish intake and improved vitamin B_{12} status. The low range of fish consumption in the current study (median intake 0.0 g, IQR: 0.0–2.7 g) may explain the lack of association found in this analysis. These observational studies also varied in terms of method of dietary assessment (e.g., FFQ, 24-hour recalls); categorization of food groups (e.g., no categories, by frequency, by median), method of analysis (e.g., multivariate analysis with or without non-dietary covariates, univariate analysis), and cultural food consumption patterns, which may explain the heterogeneity in findings.

In the current study, consumption of any red meat and total meat consumed, rather than continuous variables, was significantly associated with higher vitamin B_{12} concentrations and lower risk for vitamin B_{12} deficiency, respectively, in multivariate models. This may be due to differences in bioavailability as intake of vitamin B_{12} increases (26,27): during absorption, intrinsic factor receptors become saturated at approximately 1.5 μg per meal (26). Red meat is a rich source of vitamin B_{12}, but a typical serving of goat meat contains approximately 4.0 μg (32), which could saturate intrinsic factors and limit absorption of vitamin B_{12} from a single meal. In contrast, more frequent consumption of a lower dose of vitamin B_{12} from foods such as milk and eggs may be absorbed at a higher rate and more closely reflect patterns of intake (27).

In addition to animal-source foods, intake of grams were associated with increased serum vitamin B_{12} concentrations, while consumption of pulses and other fruit-based foods were associated with lower serum vitamin B_{12} concentrations in multivariate models. These findings are related to results from previous studies: a study among women in France noted that consumption of sugary drinks, sugar, honey, jam, and syrups was associated with lower vitamin B_{12} concentrations (16). The observed association between increased intake of pulses and fruit-based foods with lower vitamin B_{12} concentrations could be due to an overall food pattern in which this intake displaces animal products or are not typically eaten with animal products. These foods may also be differentially associated with income or socioeconomic status. For example, those in a lower socioeconomic status may consume more pulses and less animal-source foods due to cost. It is also possible that these foods inhibit vitamin B_{12} absorption and modify the effect of animal-source food consumption on serum vitamin B_{12} concentrations.

As expected, intake of vitamin B_{12} was associated with significantly higher vitamin B_{12} concentrations and a lower risk for vitamin B_{12} deficiency. Most findings from observational studies among adults and pregnant women have noted significant associations between vitamin B_{12} intake and status (13,15,16,20,29,44–47). However, in a systematic review of 13 studies among adult men and women in European countries, only one study reported a significant association between vitamin B_{12} intake and status (48); this could be due to differences in background dietary patterns, low variation in vitamin B_{12} intake or status in the populations, or low prevalence of vitamin B_{12} deficiency (10).

Findings from nutrient analyses are consistent with food group analyses. As expected, intake of nutrients typically found in animal-source foods were significantly associations with improved vitamin B_{12} status. For example, among macronutrients, intake of protein and fat were associated with higher vitamin B_{12} concentrations and lower risk for vitamin B_{12} deficiency, while carbohydrates and fiber intake were associated with lower vitamin B_{12} concentrations and a greater risk for deficiency. Findings for individual fatty acids and amino acids were consistent with associations noted for fats and protein: most fatty acids and amino acids were associated with vitamin B_{12} status, and the fatty acids that were not associated with vitamin B_{12} concentration or deficiency, such as parinaric acid and linoleic acid, are typically found in plant sources (or in both plant and animal sources) (49). In terms of micronutrients, the observed associations of vitamin B_{12}, riboflavin, iodine, and selenium intake with increased vitamin B_{12} status was consistent with food group analyses, as dairy products are high in both vitamin B_{12} and riboflavin, and iodine and selenium are found in higher concentrations in animal-source foods (50, 51). In contrast, other B-vitamins found in both animal- and plant-based foods (i.e.,

thiamin, niacin, pantothenic acid, vitamin B_6) or exclusively in plant-based foods (i.e., folate), could explain the lack of associations noted with vitamin B_{12} status for these nutrients (50). Minerals associated with lower vitamin B_{12} concentrations or higher risk of vitamin B_{12} deficiency, including copper, manganese, magnesium, and iron, were also consistent with food group analyses. Magnesium is typically found in legumes, whole grains, and vegetables (52), while copper and iron can be found in both animals and plants (53, 54). Thus, findings may be attributable to nutrient intake of commonly consumed animal-source foods.

The residual energy adjustment method was used to evaluate the association between nutrients and vitamin B_{12} status, independent of energy intake (38). For example, higher intake of vitamin B_{12} may be due to increased consumption of foods in general. Additionally, this method helps to reduce random error in nutrient measurements. Other methods could have been used include density, standard, and energy partition. The density method involves dividing the nutrient intake by total energy intake, but this method can introduce variation related to energy intake (38). The standard method includes nutrient and energy intakes as terms in a multivariate model. However, this method can introduce collinearity into a model (38). Lastly, the energy partition model does not apply to non-energy nutrients (i.e., micronutrients).

Limitations

Selection bias

In this study, archived blood samples from a total of 400 mother–infant pairs were selected for analysis of serum vitamin B_{12} concentrations. This subset of 400 participants was sampled among women who gave birth to live infants as part of maternal–infant dyads with available

archived blood sample for analysis. Although participants in this sub-study and the overall cohort were similar in terms of maternal age, gestational age at enrollment, socioeconomic status, and nutritional indicators, there may be differences in other unmeasured variables. Women who experienced a pregnancy loss or who were lost to follow-up would not be eligible for sampling for this analysis. For example, if participants in this analysis were healthier than the overall cohort on other unmeasured variables, this could introduce selection bias that would bias the association between vitamin B_{12} intake and status away from the null.

Exposure information bias

Dietary records over an extended period of time and food-weighing methods are considered gold standard for dietary assessment, but FFQs are considered an appropriate methods for assessing average intake over a longer period of time (38). However, FFQs are subject to measurement error due to over- or under-reporting, and the close-ended nature of the questionnaire may lead to errors of omission for dietary intake. The FFQ used in this study was validated for this population (29), which is a strength of the analysis. In the validation study, the FFQ overestimated vitamin B_{12} intake by 33%, compared to the average of two 24-hour recalls administered in the first trimester of pregnancy (29). This observation is consistent with a limitation of FFQs which may overestimate dietary intake (55, 56). In the same validation study, using Bland-Altman plots, there was systematic bias observed for all nutrients in terms of underreporting as actual intake increased; however, vitamin B_{12} intake reported during the first trimester of pregnancy had the lowest limit of agreement, and no other examined covariates (e.g., education) significantly predicted observed differences between FFQ and 24-hour recalls for vitamin B_{12} intake (29). Based on these finding, participants with higher energy intake may have

underreported vitamin B_{12} intake; however, energy-adjusted models were conducted to address this potential limitation.

The databases used to calculate nutrients in self-reported foods may have introduced measurement error in this analysis. Although most food items reported were in the National Institute of Nutrition Indian Food Composition Tables, values from the USDA Food and Nutrient Database was extracted for items which were not available; these items may not reflect the environmental and genetic differences of nutrient composition (31). Additionally, the nutrient databases used did not take into account nutrient losses from cooking. Calculated vitamin B_{12} intakes may therefore be overestimated for foods that are usually cooked (i.e., meat, poultry, fish), but not for other sources of vitamin B_{12} (e.g., milk), which may have overestimated total vitamin B_{12} intake and attenuated the observed association between vitamin B_{12} intake and status.

Further, our analysis was limited to the food groups into which the food items from the FFQ were categorized. For example, specific fermented foods (i.e., dosa, curd) may contribute more to serum vitamin B_{12} concentrations than the food groups in which they were categorized (i.e., whole grain-based foods, milk products). Future analyses could be conducted to examine individually reported food items from the FFQ or dietary pattern analyses which would place less restrictions on clustering of food items in analyses. Additionally, dietary data was not collected or analyzed in a matter that provided information on timing or amount of vitamin B_{12} consumed at each meal; as a result, it was not possible to evaluate variation in vitamin B_{12} absorption. It could be helpful to use a combination of 24-hour recalls and an FFQ to examine patterns of

vitamin B_{12} intake and other foods consumed which may influence bioavailability and absorption of vitamin B_{12}.

Outcome information bias

In biochemical analyses, assessment of vitamin B_{12} status was limited to a single biomarker of total serum vitamin B_{12} concentrations. Assessment of functional markers (i.e., methylmalonic acid) and other circulating biomarkers such as holo-transcobalamin is recommended for optimal assessment of vitamin B_{12} status (5, 57). This may be particularly true during pregnancy when increased plasma volume and hemodilution can lead to decreased total vitamin B_{12} concentrations during gestation while holo-transcobalamin concentrations remain relatively constant (5). Additionally, vitamin B_{12} status was assessed at a single timepoint during early pregnancy, which may not reflect periconceptional vitamin B_{12} status or changes in vitamin B_{12} status during pregnancy. Although vitamin B_{12} was assessed early in pregnancy (≤14 weeks) when the effects of hemodilution on circulating biomarkers is limited, the single time point assessed did not allow for examining temporal associations between dietary variables and vitamin B_{12} concentrations or deficiency.

Confounding

Other variables related to vitamin B_{12} status were not considered in this analysis, including medications, gastrointestinal infections, and genetic polymorphisms, or other environmental factors. Unmeasured confounders and residual confounding may have influenced observed associations of food groups and nutrients with vitamin B_{12} status. For example, socioeconomic status may be an important potential confounder in this analysis: participants with a higher

income or socioeconomic status may be able to purchase and consume more animal-source foods; and individuals with higher socioeconomic status may also have improved nutritional status and gut integrity and subsequent nutrient absorption, and bias the observed associations away from the null. Future analyses of the association between dietary intake and vitamin B_{12} status should include a thorough assessment of potential confounders and consideration of residual confounding in the interpretation of findings (58).

In conclusion, dietary intake of vitamin B_{12} was low in this population and was associated with risk of vitamin B_{12} deficiency. Consumption of milk, egg, and meat products was associated with lower risk for vitamin B_{12} deficiency. Assessing dietary intake during early pregnancy can help to identify individuals at higher risk for vitamin B_{12} deficiency and inform screening and tailored interventions. Further research is needed to understand the burden and risk factors for vitamin B_{12} deficiency periconceptionally, to inform interventions to improve vitamin B_{12} status and maternal and child health outcomes.

APPENDIX

Table A.1. Characteristics of all the study population with available vitamin B_{12} status (n = 400) and all pregnant women (n = 2,129) enrolled to date in a perinatal cohort study

	Sub-study (n = 400)		Total (N = 2,129)	
	n	**Median (IQR) or n (%)**	**N**	**Median (IQR) or n (%)**
Sociodemographic				
Age, years	400	24 (21–26)	2,129	24 (22–27)
< 20	400	37 (9.25)	2,129	185 (8.7)
20–24	400	209 (52.25)	2,129	958 (45)
25–29	400	115 (28.75)	2,129	759 (35.7)
30–34	400	35 (8.75	2,129	198 (9.3)
35–39	400	2 (0.50)	2,129	24 (1.1)
> 39	400	2 (0.50)	2,129	5 (0.2)
Gestational age, weeks	400	12.0 (9.6–13.3)	2,129	12.0 (9.5–13.5)
Parity	400	0 (0–1)	2,129	0 (0–1)
1	400	222 (55.5)	2,129	1250 (58.7)
2	400	162 (40.5)	2,129	793 (37.3)
> 2	400	16 (4.0)	2,129	86 (4.0)
Education				
No formal education	400	5 (1.3)	2,129	30 (1.4)
Primary school	400	4 (1.0)	2,129	20 (0.9)
Middle school	400	13 (3.3)	2,129	96 (4.5)
High school	400	101 (25.3)	2,129	585 (27.5)
Higher education	400	134 (33.5)	2,129	563 (26.4)
Graduates	400	118 (29.5)	2,129	581 (27.3)
Professional course	400	25 (6.3)	2,129	254 (11.9)
Employment				
Unemployed	400	321 (80.3)	2,129	1542 (72.4)
Unskilled worker	400	4 (1.0)	2,129	46 (2.2)
Skilled worker	400	13 (3.3)	2,129	86 (4.0)
Shop owner	400	0 (0.0)	2,129	9 (0.4)
Secretarial staff	400	17 (4.3)	2,129	78 (3.7)
Semi-professional	400	20 (5.0)	2,129	174 (8.2)
Professional	400	25 (6.3)	2,129	194 (9.1)
Houeshold income, INR	400	14,000 (10,000–25,000)	2,094	10,000 (6,000–22,000)
Exposure to smoking				
Consume tobacco	400	0 (0.0)	2,093	3 (0.1)
People smoking in presence	400	73 (18.3)	2,094	319 (15.2)
Type of cooking fuel				
Firewood	399	11 (2.8)	2,093	41 (2.0)
Coal	399	114 (28.6)	2,093	125 (6.0)
Kerosene	399	7 (1.8)	2093	90 (4.3)
Liquid petroleum gas	399	267 (66.9)	2093	1816 (86.8)

Table A.1 (Continued)

Gobar gas	399	0 (0.0)	2093	4 (0.2)
Electricity	399	0 (0.0)	2093	1 (0.1)
LPG + Wood	399	0 (0.0)	2093	5 (0.2)
LPG + kerosene	399	0 (0.0)	2093	11 (0.5)
Dietary				
Energy, kCal	400	1794.1 (1500.1–2097.1)	2107	1863.0 (1577.7–2229.8)
Protein, g/day	400	51.8 (42.4–61.1)	2107	53.7 (44.4–64.9)
Fat, g/day	400	47.1 (37.8–57.9)	2107	49.0 (38.9–60.9)
Carbohydrate, g/day	400	290.8 (241.7–346.4)	2107	302.2 (255.8–357.0)
Vitamin B_{12}, µg/day	400	1.7 (1.1–2.5)	2107	1.8 (1.2-2.6)
<2.6	400	310 (77.5)	2107	1585 (75.2)
<1.2	400	117 (29.3)	2107	537 (25.5)
Dietary Pattern				
Vegan (i.e. no fish, meat, poultry, eggs, milk)	400	0 (0.0)	2107	9 (0.4)
Vegetarian (i.e., no fish, meat, poultry)	400	71 (17.8)	2107	407 (19.3)
Lacto-ovo vegetarian (i.e., no fish, meat, poultry)	400	17 (4.3)	2107	100 (4.8)
Lacto vegetarian (i.e., no fish, meat, poultry, eggs)	400	54 (13.5)	2107	294 (14.0)
Ovo-vegetarian (i.e., no fish, meat, poultry, eggs, milk)	400	0 (0.0)	2107	4 (0.2)
Pescatarian (i.e., no meat, poultry)	400	4 (1.0)	2107	21 (1.0)
Anthropometric and health				
Height, cm	400	156.2 (152.0–160.0)	2129	155.0 (152.0–160.0)
Weight, kg	400	50.15 (44.9–56.2)	2129	51.5 (45.8–58.1)
MUAC, cm	400	23.5 (21.7–25.7)	2105	24.0 (22.0–26.0)
BMI, kg/m^2	400	20.6 (18.6–23.3)	2129	21.2 (19.0–24.0)
Underweight (< 18.5)	400	94 (23.5)	2129	411 (19.3)
Normal range (≥ 18.5 and < 25.0)	400	249 (62.3)	2129	1316 (61.8)
Overweight (≥ 25.0 and < 30.0)	400	52 (13.0)	2129	346 (16.3)
Obese (≥ 30.0)	400	5 (1.3)	2129	56 (2.6)
Physical activity level, TEE/24/BMR [2]	400	1.4 (1.4–1.6)	2106	1.4 (1.4–1.6)
Inactive (< 1.4)	400	154 (38.5)	2106	755 (35.9)
Sedentary (1.4 to < 1.7)	400	222 (55.5)	2106	1172 (55.7)
Moderately active (1.7 to < 2.0)	400	24 (6.0)	2106	177 (8.4)
Biochemical				
Hemoglobin, g/dL	400	12.0 (11.2–12.7)	2067	12.00 (11.1–12.7)
<11.0 g/dL	400	84 (21.0)	2067	452 (21.9)
<7.0 g/dL	400	3 (0.8)	2067	9 (0.4)

[1] 100 INR was equivalent to approximately 2 USD at the time the study was conducted

[2] Extremely inactive, vigorously active, and extremely active are not displayed due to 0.0% prevalence

Abbreviations: IQR, interquartile range; INR, Indian rupees; RDA, Recommended Dietary Allowance; LPG, Liquid petroleum gas; MUAC, Mid-upper arm circumference; BMI, body mass index

Table A.2. Descriptive statistics of food group consumption by vitamin B_{12} status

	Overall (n = 400)	**Vitamin B_{12} Deficient (< 148.0 pmol/L) (n = 253)**	**Not Vitamin B_{12} Deficient (≥ 148.0 pmol/L) (n = 147)**
Food groups (g/day)	**Median (IQR)**	**Median (IQR)**	**Median (IQR)**
Whole grain	56.4 (24.4–94.7)	60.5 (27.3–101.9)	42.6 (18.9–83.6)
Refined grains-based food	160.8 (122.3–207.5)	157.9 (121.6–204.2)	164.2 (124.4–209.0)
Cereal-based food	231.3 (176.6–279.1)	231.5 (180.8–281.7)	226.1 (167.8–267.6)
Pulses	15.3 (8.8–28.1)	15.3 (8.3–28.4)	15.2 (10.0–27.1)
Grams	2.2 (1.1–3.4)	2.2 (1.1–3.1)	2.2 (1.1–4.5)
Total meat[1]	14.6 (4.5–27.7)	12.8 (1.6–25.6)	19.1 (7.9–29.3)
Fish	0.0 (0.0–2.7)	0.0 (0.0–2.2)	1.1 (0.0–3.4)
Chicken	6.8 (0.0–14.8)	6.1 (0.0–14.5)	8.5 (1.6–15.7)
Red meat	2.8 (0.0–9.0)	1.4 (0.0–8.3)	4.9 (0.0–10.1)
Organ meats	0.0 (0.0–0.0)	0.0 (0.0–0.0)	0.0 (0.0–0.0)
Egg-based food	7.7 (0.0–16.0)	7.5 (0.0–15.5)	8.3 (0.0–18.2)
Milk	194.1 (64.3–364.4)	158.1 (52.5–349.5)	240.0 (120.0–446.6)
Tea	0.0 (0.0–120.0)	0.0 (0.0–120.0)	0.0 (0.0–120.0)
Coffee	0.0 (0.0–60.0)	0.0 (0.0–60.0)	0.0 (0.0–68.6)
Total vegetable-based food	84.1 (55.4–121.6)	82.6 (53.1–120.0)	88.4 (58.8–128.0)
Raw vegetable-based food	3.2 (0.0–8.5)	3.0 (0.0–7.9)	4.5 (0.0–9.5)
Leafy vegetable-based food	19.3 (11.5–31.1)	18.8 (10.0–28.1)	21.5 (12.3–37.3)
Root vegetable-based food	10.5 (4.9–17.5)	10.5 (4.9–15.8)	10.6 (4.9–20.3)
Carrot-based food	4.9 (0.0–14.2)	4.9 (0.0–14.2)	6.1 (0.0–14.6)
Other vegetable-based food	33.1 (16.5–54.7)	31.0 (16.5–54.4)	35.9 (16.4–55.1)
Total fruit-based food	88.0 (52.3–138.4)	86.1 (50.4–141.0)	95.7 (58.5–130.1)
Other fruit-based food	21.6 (11.5–37.6)	20.4 (10.4–37.6)	22.7 (12.6–38.0)
Nut-based food	3.2 (0.0–6.4)	3.2 (0.0–6.4)	3.2 (0.0–6.5)
Fiber from cereals	2.5 (1.5–3.8)	2.8 (1.6–3.9)	2.1 (1.4–3.6)
Fiber from fruits	1.0 (0.6–1.7)	1.0 (0.6–1.8)	1.0 (0.7–1.7)
Fiber from vegetables	1.0 (0.7–1.4)	1.0 (0.7–1.4)	1.0 (0.7–1.5)
Ghee	0.0 (0.0–0.9)	0.0 (0.0–0.9)	0.0 (0.0–0.9)

[1]Total meat = fish + poultry + red meat + organ meats
Abbreviation: IQR, interquartile range

Table A.3. Descriptive statistics of nutrient consumption by vitamin B_{12} status

	Overall (n = 400)	**Deficient (< 148.0 pmol/L) (n = 253)**	**Not deficient (≥ 148.0 pmol/L) (n = 147)**
Nutrients	**Median (IQR)**	**Median (IQR)**	**Median (IQR)**
Energy (kCal)	1,794.1 (1,500.1–2,097.1)	1,785.0 (1,468.8–2,085.3)	1,835.4 (1,554.6–2,133.0)
Protein (g)	51.8 (42.4–61.1)	51.1 (41.6–59.2)	53.7 (45.3–64.9)
Fat (g)	47.1 (37.8–57.9)	46.1 (36.0–54.8)	49.6 (40.3–60.8)
Carbohydrate (g)	290.8 (241.7–346.4)	290.2 (239.9–348.0)	290.9 (242.9–346.1)
Fiber (g)	8.0 (6.2–10.3)	8.3 (6.3–10.5)	7.7 (5.9–10.2)
Saturated fat (g)	16.0 (11.9–2)	15.2 (11.0–20.1)	17.7 (14.1–22.0)
Butyric acid	0.5 (0.3–0.7)	0.4 (0.3–0.6)	0.5 (0.4–0.7)
Caproic acid	0.3 (0.2–0.4)	0.3 (0.2–0.4)	0.3 (0.2–0.4)
Caprylic acid	0.4 (0.3–0.5)	0.3 (0.2–0.4)	0.4 (0.3–0.5)
Capric acid	0.5 (0.4–0.7)	0.5 (0.3–0.7)	0.6 (0.4–0.8)
Lauric acid	1.6 (1.2–2.0)	1.5 (1.1–2.0)	1.7 (1.3–2.3)
Myristic acid	1.7 (0.8–2.6)	1.5 (0.8–2.4)	1.9 (1.0–2.8)
Palmitic acid	6.7 (4.9–8.6)	6.3 (4.5–8.3)	7.4 (5.9–8.9)
Stearic acid	3.1 (2.3–4.0)	2.8 (2.1–3.8)	3.3 (2.6–4.2)
Monounsaturated fat (g)	11.5 (9.1–14.5)	11.1 (8.5–13.9)	12.2 (9.8–15.0)
Palmitoleic acid	0.4 (0.3–0.6)	0.4 (0.3–0.5)	0.5 (0.4–0.6)
Oleic acid	10.2 (8.1–13.0)	9.8 (7.6–12.6)	10.9 (8.9–13.2)
Gadeolic acid	0.1 (0.1–0.1)	0.1 (0.2–0.1)	0.1 (0.1–0.1)
Erucic acid	0.2 (0.1–0.2)	0.2 (0.1–0.2)	0.2 (0.1–0.2)
Polyunsaturated fat (g)	13.7 (10.8–17.3)	13.7 (10.7–17.1)	14.0 (11.0–17.8)
Linoleic acid	13.2 (10.2–16.6)	13.0 (10.2–16.5)	13.3 (10.4–16.8)
Linolenic acid	0.5 (0.4–0.6)	0.4 (0.3–0.6)	0.5 (0.4–0.6)
Parinaric acid	0.0 (0.0–0.0)	0.0 (0.0–0.0)	0.0 (0.0–0.0)
Arachidonic acid	0.0 (0.0–0.0)	0.0 (0.0–0.0)	0.0 (0.0–0.1)
Timnodonic acid	0.0 (0.0–0.0)	0.0 (0.0–0.0)	0.0 (0.0–0.0)
Clupanodonic acid	0.0 (0.0–0.0)	0.0 (0.0–0.0)	0.0 (0.0–0.0)
Cervonic acid	0.0 (0.0–0.0)	0.0 (0.0–0.0)	0.0 (0.0–0.0)
Cholesterol (mg)	109.8 (68.0–156.5)	101.8 (61.9–146.0)	124.4 (89.7–173.5)
Amino Acids (g)			
Tryptophan	0.6 (0.5–0.7)	0.6 (0.5–0.7)	0.6 (0.5–0.8)
Threonine	1.9 (1.5–2.2)	1.8 (1.5–2.2)	2.0 (1.7–2.4)
Isoleucine	2.3 (1.8–2.7)	2.2 (1.8–2.6)	2.4 (2.0–2.9)
Leucine	4.0 (3.2–4.7)	3.8 (3.1–4.5)	4.1 (3.5–5.0)
Lysine	2.8 (2.2–3.4)	2.7 (2.1–3.3)	2.9 (2.5–3.7)
Methionine	1.0 (0.8–1.2)	1.0 (0.8–1.2)	1.1 (0.9–1.3)
Cysteine	0.7 (0.6–0.9)	0.7 (0.6–0.8)	0.7 (0.6–0.9)
Phenylalanine	2.5 (2.1–3.1)	2.5 (2.0–3.0)	2.6 (2.2–3.2)
Tyrosine	1.7 (1.4–2.1)	1.7 (1.3–2.0)	1.8 (1.5–2.2)
Valine	2.7 (2.2–3.2)	2.7 (2.1–3.1)	2.8 (2.4–3.4)
Arginine	3.0 (2.5–3.6)	3.0 (2.5–3.5)	3.1 (2.6–3.7)

Table A.3 (Continued)

Histidine	1.3 (1.1–1.6)	1.3 (1.0–1.5)	1.4 (1.1–1.7)
Alanine	2.2 (1.8–2.7)	2.2 (1.8–2.6)	2.3 (2.0–2.8)
Aspartic acid	4.4 (3.6–5.2)	4.2 (3.5–5.0)	4.4 (3.8–5.4)
Glutamic acid	9.9 (8.3–12.0)	9.8 (8.0–12.0)	10.1 (8.8–12.5)
Glycine	1.8 (1.5–2.3)	1.8 (1.5–2.2)	1.9 (1.6–2.3)
Proline	3.1 (2.4–3.7)	3.0 (2.3–3.7)	3.2 (2.70–3.9)
Serine	2.5 (2.0–2.9)	2.4 (1.9–2.9)	2.5 (2.15–3.1)
Micronutrients (mg)			
Vitamin B_{12} (µg)	1.7 (1.1–2.5)	1.6 (1.0–2.3)	2.0 (1.5–2.9)
Vitamin E	11.2 (8.8–14.0)	11.2 (8.5–13.8)	11.2 (9.1–14.3)
Vitamin C	102.3 (73.8–150.5)	102.0 (74.8–139.4)	103.1 (71.8–162.9)
Thiamine	1.2 (0.9–1.4)	1.2 (0.9–1.5)	1.2 (0.9–1.4)
Riboflavin	1.3 (1.0–1.8)	1.3 (1.0–1.7)	1.5 (1.2–1.9)
Niacin	11.2 (9.1–13.6)	11.1 (8.7–13.1)	11.2 (9.5–14.3)
Pantothenic acid	5.7 (4.7–6.6)	5.6 (4.5–6.6)	5.7 (4.8–6.7)
Vitamin B_6	1.6 (1.3–2.0)	1.6 (1.3–2.0)	1.6 (1.3–2.0)
Folate (µg)	253.4 (206.7–308.9)	249.5 (203.6–307.3)	257.8 (216.1–313.8)
Calcium	914.0 (663.6–1152.7)	872.1 (633.2–1138.6)	955.2 (718.5–1221.1)
Phosphorus	1,241.8 (998.8–1500.7)	1,233.6 (964.4–1456.3)	1,295.2 (1058.8–1558.0)
Iron	13.1 (10.4–16.1)	13.1 (10.4–16.0)	12.9 (10.3–16.1)
Magnesium	438.1 (365.1–518.5)	438.2 (361.2–521.4)	429.2 (366.8–510.9)
Sodium	2,569.2 (2,057.2–3,226.9)	2,577.8 (2,036.0–3,197.6)	2,558.8 (2,108.5–3,291.3)
Potassium	2,267.9 (1,790.4–2,920.1)	2,216.4 (1,765.2–2,866.6)	2,391.6 (1,887.0–3,002.3)
Copper	1.8 (1.5–2.3)	1.8 (1.4–2.3)	1.8 (1.5–2.4)
Manganese	5.1 (3.6–7.7)	5.2 (3.7–8.1)	4.7 (3.4–7.3)
Zinc	7.8 (6.4–9.2)	7.7 (6.2–9.1)	8.0 (6.6–9.4)
Selenium (µg)	57.6 (45.0–72.6)	56.4 (44.2–70.0)	61.1 (46.7–77.6)
Iodine (µg)	97.7 (73.4–124.9)	90.2 (65.8–118.9)	108.3 (84.3–136.3)

Abbreviations: IQR, interquartile range

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