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RESEARCH PAPER

Prevalence of vitamin B_{12} deficiency in healthy Indian school-going adolescents from rural and urban localities and its relationship with various anthropometric indices: a cross-sectional study

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Abstract

Background: Micronutrient deficiency is a global health burden, especially among developing countries. The present cross-sectional study aimed to determine the prevalence of vitamin B_{12} deficiency in healthy Indian school-going adolescents, based on area of residence, sex and body mass index (BMI). Furthermore, the relationship of serum B_{12} concentration with dietary vitamin B_{12} intake and anthropometric indices was assessed among adolescents from rural and urban India.

Methods: A total of 2403 school-going adolescents (11–17 years) from National Capital Region and rural areas of Haryana, India were selected. Serum B_{12} concentrations were estimated using an electrochemiluminescence immunoassay. Dietary assessments were conducted on 65% of total participants (n = 1556) by two 24-h diet recalls.

Results: The prevalence of vitamin B_{12} deficiency in the total study population was 32.4% (rural: 43.9% versus urban: 30.1%, $P < 0.001$; male: 34.4% versus female: 31.0%, $P < 0.05$; normal weight: 28.1%, versus overweight: 39.8%, versus obese: 51.2%, $P < 0.001$). More than half (51.2%) of obese adolescents were vitamin B_{12} deficient. On multiple linear regression analysis, serum B_{12} in rural adolescents was associated with age ($\beta = -0.12$, $P < 0.05$). Among urban adolescents, serum B_{12} was associated with BMI ($\beta = -0.08$, $P < 0.05$) and adjusted dietary vitamin B_{12} intake ($\beta = 0.14$, $P < 0.001$). Serum vitamin B_{12} levels were found to be lower in rural females ($\beta = -0.12$, $P = 0.030$) and urban males (β: 0.11, $P < 0.001$) compared to their respective contemporaries.

Conclusions: Vitamin B_{12} deficiency was higher among rural school-going adolescents. Boys had a higher B_{12} deficiency than girls. Inverse associations of serum B_{12} with adiposity indices were observed. Serum B_{12} levels were positively associated with dietary vitamin B_{12} intake.

Introduction

Vitamin B_{12} or cobalamin is a water-soluble vitamin that acts as a coenzyme and is instrumental in building a healthy neural system and formation of red blood corpuscles, thus essential from the early growth period[1]. Humans, similar to most other organisms meet their nutritional requirements for vitamin B_{12} through gut microbial biosynthesis and dietary intake [2]. Chronic vitamin B_{12} deficiency in adults is known to be associated with a wide spectrum of diseases that have serious immunological, haematological, neurological and psychiatric consequences [3]. Furthermore, juvenile deficiency of vitamin B_{12} is considered to be a global health burden as a result of increasing evidence for its role in neural tube development, growth, immunity and cognitive functioning [4–6].

The prevalence of vitamin B_{12} deficiency is common in developing countries, especially in parts of Southeast Asia and Europe. According to national level survey data, the prevalence of vitamin B_{12} deficiency in adults is 8.3% and 14.7% in the UK and Germany, respectively; vitamin B_{12} deficiency is also prevalent among school children in Venezuela (10.9%) [7]. Inadequate dietary intake and malnutrition are known to be key reasons for vitamin B_{12} deficiencies [8]. A substantial number of Indian school-age children are undernourished [9,10] and the consumption of a strict vegetarian diet makes them more susceptible to developing vitamin B_{12} deficiency [11]. Coexisting with the rates of under-nutrition, overweight phenotype and obesity trends have increased tremendously among Indian children [12]. The ongoing socio-economic transition has led to significant changes in lifestyle and dietary habits. With the increasing consumption of processed, nutrient-deficient, high calorie food coupled with irregular meal patterns, micronutrient deficiencies are increasing despite higher energy intakes among overweight and obese children or adolescents [13,14]. The results from recent studies have also reported the association of vitamin B_{12} deficiency with obesity during childhood and adolescence [15,16].

There is a dearth of large-scale global studies (especially in Indian adolescents) regarding the relationship between serum B12 concentration with area of residence, anthropometric indices and dietary vitamin B12 intake [17–19]. In the present study, we hypothesise that serum vitamin B_{12} concentration in Indian school-going adolescents belonging to rural and urban households might be governed by their respective nutritional status. The Indian subcontinent is a cradle of humanity, with one-sixth of the global population. As a developing nation, India is currently under a rapid transitional state as a result of increasing economic growth. Based on the results of the present study, we aim to decipher the impact of this socio-economic reform in a specific group of adolescents aged 11–17 years. This is the largest study conducted by far on the nutritional epidemiology of vitamin B_{12} deficiency in Indian school-going adolescents. The study population comprises of 2403 school-going adolescents of 11–17 years age group from Delhi and National Capital Region (NCR). Study subjects have been chosen from both rural and urban areas to analyse the importance of changing dietary habits; with a focus on serum vitamin B_{12} concentration in Indian school-going adolescents. The present study aims to determine the prevalence of vitamin B_{12} deficiency based on area of residence, sex and body mass index in healthy Indian school-going adolescents. Furthermore, the relationship of serum vitamin B_{12} concentration with dietary vitamin B_{12} intake and anthropometric indices is assessed among Indian school-going adolescents residing in rural and urban areas.

Materials and methods

Ethical approval

The study was approved by the Human Ethics Committees of All India Institute of Medical Sciences, India and Council of Scientific and Industrial Research (CSIR)-Institute of Genomics and Integrative Biology, India. Informed written consent was obtained from the parents/guardians of the study subjects. The study was carried out in accordance with the principles of Helsinki Declaration of 1975 as revised in 1983.

Sample characterisation

The study population was comprised of adolescents purposively collected from various schools among different geographical zones of NCR and rural parts of Haryana (India) [20]. Urban schools were selected to ensure representation of atleast four zones of Delhi, including North, South, West and Central. Rural study subjects were collected from Harsaru and Garhi Harsaru village (Gurugram, Haryana). Data was collected concurrently from both rural and urban schools. Most of the study participants were of Indo-European ethnicity, as described previously [21]. Inclusion criteria were as follows: 11 to 17 years old, not suffering from any known metabolic condition except obesity and free from any acute infection in the past month before inclusion.

Socio-economic and anthropometric measurements

Information on the area of residence was obtained from a parent administered pre-tested questionnaire. Adolescents from rural schools were considered to have lower socio-economic status (LSES), whereas urban school-going adolescents were considered to come from a higher socio-

economic strata (USES), as described previously [22]. Height, weight, waist (WC) and hip (HC) circumference were measured. All anthropometric measurements were conducted by well-trained research personnel. Body weight (to nearest 0.1 kg) was measured using a digital balance (Crown Digital Scales Inc., Karnal, India) and standing height (to nearest 0.1 cm) was measured by an anthropometric rod (Galaxy Scientific, Dombivli, India) against the wall, using standard methodology [23]. HC was measured to the nearest 0.1 cm at the largest posterior extension of buttocks using a non-elastic tape. WC was measured to the nearest 0.1 cm midway between superior border of the iliac crest and lowermost margin of ribs at the end of normal expiration [24]. Body mass index (BMI) and waist-to-hip ratio (WHR) were calculated from measured indices. The obesity status (normal weight, overweight and obese) was assigned to all participants according to internationally acceptable BMI cut-off range for children and adolescents as proposed by Cole *et al.* [25].

Collection and storage of blood samples

Blood samples were drawn by venepuncture after an overnight fast and collected into glass vacutainers. Blood withdrawals from participants were performed by well-trained phlebotomists. For separation of serum, vacutainers were kept tilted over ice for 1 h and then centrifuged at 1000 ***g*** for 15 minutes at 4 °C. Serum was separated from respective vacutainers and stored at −80 °C in aliquots. All vitamin B_{12} measurements were performed with stored serum samples.

Measurement of vitamin B_{12} using serum samples

In vitro quantitative determination of vitamin B_{12} in serum samples was performed by electrochemiluminescence immunoassay using Cobas e411 biochemical analyzer (Roche Diagnostics GmbH, Mannheim, Germany). This assay employs a competitive test principle using specific intrinsic factors. Vitamin B_{12} present in sample competes with added biotinylated vitamin B_{12} for the binding sites on ruthenium-labelled intrinsic factor complex. The results were determined by a two-point calibration curve generated by the instrument and a master curve provided via reagent barcode. Vitamin B_{12} deficiency was defined by a universally approved cut-off of serum B_{12} concentration <148 pmol L^{-1} [26].

Dietary assessment

Detailed dietary assessment was conducted on a sample subset of 1556 adolescents (64.8% of our total study population). Dietary intake of adolescents was assessed by a trained diet interviewer, administering two 24-h diet recalls (1 school day and 1 weekend day/holiday) to obtain information on usual consumption pattern of food and beverages on nonconsecutive days. One face-to-face recall to obtain dietary information for school day followed by a telephonic recall for dietary intake on weekend day/holiday was obtained from the study subjects. Furthermore, mothers were also interviewed telephonically to validate the diet information reported by the students. Food items consumed over the entire 24 h of the previous day were recorded with meal timings and occasion. A detailed description of each food item/dish was reported in terms of amount consumed (with respect to household measures), ingredients, each interval and meal time, source (home cooked/outside), brand names and portion size, type and amount of oil consumed, and the consistency, size or greasiness related to the dish. Further probing to recall food items consumed in small quantities was performed. Household measures including bowls, glasses, ladles and measuring spoons were shown to the adolescents to elicit the portion size of the food items consumed. Participants were not interviewed on days when they had been ill or days that fell on a major holiday because this could affect the usual dietary intake. The time difference between face-to-face and subsequent telephonic recall was restricted to 15 days for each participant. To minimise the potential for under-eating during the time frame for 24-h recalls, participants were blinded to telephone recall schedule.

Standardisation of food items/recipes was carried out in terms of household measures shown to study subjects for assessment of dietary data. A market survey was also conducted to obtain information on the nutritional value of processed foods. Information on intake of fortified foods was also obtained from the participants. Energy, macronutrients (including carbohydrate, protein and fat), vitamin B_{12}, total folate from natural diet sources and total dietary fibre were calculated using DIETSOFT version 17.0 (http://dietsoft.in/; Invincible IDeAS, Noida, India), which uses the Indian food composition tables [27]. Information on nutrient supplement consumption was also obtained verbally from the participants.

Statistical analysis

Data did not follow a normal distribution, as indicated by a Kolmogorov–Smirnov test. Hence, data were represented as median with percentiles for continuous variables and as percentages for categorical variables. Differences in anthropometric indices, serum B_{12} concentration and dietary intake among rural and urban school-going adolescents were calculated using Wilcoxon Mann–Whitney test. Differences in the prevalence of vitamin B_{12}

deficiency, based on age categories, area of residence, sex and weight status were assessed using chi-squared test.

The intakes of vitamin B_{12}, total folate and total dietary fibre were adjusted with total energy intake to control for confounding associated with it. Nutrient density was calculated for energy intake adjustment. For macronutrients, the percentage calorie contribution of protein, carbohydrate and fat to the total energy intake was computed. Micronutrient and dietary fibre data were expressed per 4184 kJ to provide values independent of higher energy intake. Furthermore, adolescents can have a variable energy density. Accordingly, to arrive at accurate nutrient density, micronutrients and fibre were expressed in both absolute amounts and per 4184 kJ. Dietary intake data used for adjustment in regression analysis were calculated as the mean of two dietary recalls used to determine the usual intake. Variables did not follow a normal distribution and thus were inverse normalized before conducting the regression analysis. Linear regression analysis was performed using energy adjusted values of micronutrients. Micronutrients were adjusted for energy intake by taking the energy intake as independent variable and absolute micronutrient intake as dependent variable [28]. The association of age, sex, anthropometric variables and energy adjusted vitamin B_{12} intake with serum vitamin B_{12} was determined using multiple linear regression analysis, separately for urban and rural areas, respectively. Potential covariates including total dietary energy intake, protein, carbohydrate, fat, total folate and total dietary fibre were adjusted in the regression analysis predicting serum vitamin B_{12}.

Results

Descriptive characteristics of the study population

The study population comprised a total of 2403 school-going adolescents from rural and urban schools in India. Median with percentiles for continuous variables and percentages for categorical variables are shown in Table 1. The distribution of adolescents according to sex was comparable between urban and rural areas. Median age was same in rural and urban study subjects, although rural adolescents had a higher distribution of age in comparison. Higher values of anthropometric indices including BMI, WC, HC and WHR were observed among urban subjects, as compared to rural adolescents. A distinctly higher prevalence of adiposity was found among urban compared to rural adolescents (ie, prevalence of overweight and obesity was 23.3% and 7.0%, respectively), without a single obese adolescent among the rural study subjects. Dietary intake data showed higher intake of energy, macronutrients (carbohydrate, protein and fat), vitamin B_{12}, total folate and dietary fibre among urban study participants, in comparison to rural adolescents.

Assessment of vitamin B_{12} deficiency prevalence across different groups in the study population

The prevalence of vitamin B_{12} deficiency was assessed in all study subjects according to their age, locality of residence, sex and weight status (Table 2). For the total study population, the prevalence of vitamin B_{12} deficiency was 32.4%. On age-wise categorisation, we did not find a significant difference in serum B_{12} level across adolescents of different ages. When categorised based on area of residence, 43.9% of rural adolescents were found to have vitamin B_{12} deficiency compared to 30.1% of urban adolescents ($P < 0.001$). A significant difference was also obtained for vitamin B_{12} deficiency between sexes with males having a higher prevalence compared to females (34.4% versus 31.0%, $P = 0.043$). Based on weight status, a significantly higher prevalence of vitamin B_{12} deficiency was observed among overweight (39.8%) and obese (51.2%) compared to normal weight (28.1%) adolescents, respectively ($P < 0.001$). Our data were compared with the Indian recommended dietary allowances (RDA) for vitamin B_{12} intake [29]. For the total study population, 15.6% were not meeting the RDA of vitamin B_{12}. Based on area of residence, 32.8% rural adolescents were not meeting the RDA of vitamin B_{12} compared to 9.5% urban adolescents ($P = 0.015$). No significant difference was obtained between sexes (males: 17.7% versus females: 13.2%) and among various weight categories (normal weight: 16.8% versus overweight: 10.2% versus obese: 16.1%).

Relationship of serum vitamin B_{12} concentration with various anthropometric indices and dietary B_{12} intake

The relationship of serum vitamin B_{12} levels with anthropometric and dietary factors were analysed in both rural and urban study subjects. Table 3 shows the multiple linear regression analysis for rural adolescents having an inverse normalized serum vitamin B_{12} concentration as the dependent variable and age, sex, BMI, WHR and energy adjusted dietary vitamin B_{12} as the independent variables. Among rural adolescents, age (β: −0.11, $P = 0.038$) and WHR (β: −0.10, $P = 0.049$) were significantly associated with serum vitamin B_{12} concentration. Serum vitamin B_{12} levels were found to be lower in rural females compared to rural males (β: −0.12, $P = 0.030$).

Among urban adolescents (Table 4), multiple linear regression analysis with potential confounder adjustment showed that BMI (β: −0.08, $P = 0.022$) and adjusted dietary vitamin B_{12} intake (β: 0.14, $P < 0.001$) were significantly associated with serum vitamin B_{12} concentrations. Urban males had significantly lower vitamin B_{12}

Table 1 Anthropometric indices and serum vitamin B_{12} concentration of the study population by school type

	Total		Rural		Urban		
	Median or *n*	P25, P75 or %	Median or *n*	P25, P75 or %	Median or *n*	P25, P75 or %	*P*-value[†]
Total samples (*n*)	2403		403		2000		–
Male (*n* and %)	1024	42.6	187	46.4	837	41.9	–
Age (years)	14	12, 15	14	13, 16	14	12, 15	<0.001***
BMI (kg m^{-2})	19.3	16.4, 23.4	16.1	14.5, 17.7	20.4	17.2, 24.1	<0.001***
% overweight	560	23.3	10	2.5	550	27.5	–
% obese	168	7.0	0	0.0	168	8.4	–
Waist circumference (cm)	71.0	62.9, 81.0	58.0	55.0, 65.0	74.5	66.0, 83.5	<0.001***
Hip circumference (cm)	85.0	78.0, 93.0	78.0	70.0, 83.0	87.0	79.0, 94.0	<0.001***
WHR	0.85	0.79, 0.90	0.77	0.73, 0.83	0.86	0.81, 0.91	<0.001***
Serum vitamin B_{12} (pmol L^{-1})	186.57	132.18, 263.69	157.78	116.90, 210.92	194.39	136.60, 273.65	<0.001***
Dietary information on a sample subset							
Total samples (*n*)	1556		403		1153		–
Male (*n* and %)	827	53.1	182	45.2	645	55.9	–
Energy (kJ day^{-1})	8161	6608, 10039	6428	5243, 7867	8818	7314, 10578	<0.001***
Protein (g day^{-1})	55.4	43.6, 68.9	41.4	32.1, 54.0	59.6	48.7, 72.5	<0.001***
% calories from protein	11.4	11.0, 11.5	10.8	10.2, 11.5	11.3	11.1, 11.5	–
Carbohydrates (g day^{-1})	273.5	216.8, 331.1	220.4	175.6, 275.3	289.9	241.0, 345.5	<0.001***
% calories from carbohydrate	56.1	54.9, 55.2	57.4	56.1, 58.6	55.0	55.1, 54.7	–
Fat (g day^{-1})	69.0	52.9, 89.7	51.2	39.0, 63.4	75.7	59.1, 97.4	<0.001***
% calories from fat	31.8	30.1, 33.6	30.0	28.0, 30.3	32.3	30.4, 34.7	–
Vitamin B_{12} (μg day^{-1})	0.7	0.4, 1.1	0.4	0.2, 0.6	0.8	0.4, 1.3	<0.001***
Vitamin B_{12} (μg 4184 kJ^{-1})	0.3	0.2, 0.5	0.2	0.1, 0.3	0.4	0.2, 0.6	<0.001***
Total folate (μg day^{-1})	154	113, 206	123	85, 178	164	125, 216	<0.001***
Total folate (μg 4184 kJ^{-1})	77	60, 99	77	60, 103	77	61, 98	0.894
Total dietary fibre (g day^{-1})	9.1	6.0, 12.6	7.2	4.2, 10.0	9.7	6.7, 13.4	<0.001***
Total dietary fibre (g 4184 kJ^{-1})	4.6	3.2, 6.2	4.4	2.9, 6.0	4.7	3.3, 6.3	0.059

P25, 25th percentile; P75, 75th percentile; WHR, waist-to-hip ratio.

[†]Wilcoxon Mann–Whitney test was used to test for difference between rural and urban adolescents. Significant values: ***$P < 0.001$.

concentrations compared to their female contemporaries (β: 0.11, $P < 0.001$).

Discussion

There is limited global information available on vitamin B_{12} dietary intake among school-going adolescents. Also, there are few large-scale studies reporting a deficiency of vitamin B_{12} in an apparently healthy population. India is unique with respect to its enormous population size and ethnic diversities. As a developing nation, India is presently undergoing a rapid economic reform that has already occurred in developed countries. This socio-economic transitional state has a huge impact on lifestyle, thereby affecting the nutritional status of individuals. Based on the results of the present study, we aim to decipher the impact of socio-economic status on vitamin B_{12} deficiency in healthy Indian adolescents of school-going age. This is by far the largest study on vitamin B_{12} status in Indian school-going adolescents from rural and urban areas and thus comparable to global large-scale studies in terms of sample size.

The prevalence of vitamin B_{12} deficiency was 32.4% for the total study population. Furthermore, 43.9% rural adolescents were vitamin B_{12} deficient, which was significantly higher compared to their urban counterparts. The power of the study to detect the difference in prevalence of vitamin B_{12} deficiency among rural and urban adolescents was found to be 99.9%. In line with this, dietary vitamin B_{12} intake was also lower among rural subjects, as compared to urban adolescents. A significantly higher proportion of rural adolescents did not meet the RDA of vitamin B_{12}, in comparison to their urban contemporaries. This might be explained by the huge proportion of Indian rural adolescents suffering from undernutrition with inadequate micronutrient intakes [30,31]. Socio-economic factors are important attributes of dietary habits that contribute to nutrient intake and level of different micronutrients in the body [30–32]. Hence, our data reaffirm the contribution of area of residence with respect to determining the nutritional health in adolescents. Global studies on rural Mexican population, Kenyan and European population also yielded findings that are similar to our results [33–35].

Table 2 Prevalence of vitamin B_{12} deficiency across different groups in study population

Serum vitamin B_{12} concentration[†]	*n*	Serum vitamin B_{12} < 148 pmol L^{-1}[‡]	Serum vitamin $B_{12} \geq 148$ pmol L^{-1}[‡]	*P*-value[§]
Total Sample	2403	32.4	67.6	–
Age (years)				
11–<12	285	31.2	68.8	0.065
12–<13	366	27.6	72.4	
13–<14	452	36.3	63.7	
14–<15	395	32.4	67.6	
15–<16	363	35.0	65.0	
16–<17	323	28.5	71.5	
17–<18	219	36.5	63.5	
Rural	403	43.9	56.1	<0.001***
Urban	2000	30.1	69.9	
Male	1024	34.4	65.6	0.043*
Female	1379	31.0	69.0	
Normal weight	1675	28.1	71.9	<0.001***
Overweight	560	39.8	60.2	
Obese	168	51.2	48.8	

[†]Universally accepted cut-off was used to assess the prevalence of vitamin B12 deficiency [26].

[‡]Values represent percentage of children.

[§]Chi-squared test applied to test for difference among various groups. Significant values: ***$P < 0.001$, *$P < 0.05$.

Vitamin B_{12} deficiency was significantly higher among males compared to females in the present study. Studies on European adolescents and school-children from South American countries (Guatemala and Columbia) have also reported a higher circulatory vitamin B_{12} concentration in female school-children compared to their male contemporaries [35–37]. A higher prevalence of vitamin B_{12} deficiency among boys may be explained by a higher requirement of micronutrients among them to sustain rapid muscular growth during adolescence, as compared to girls. Furthermore, dietary vitamin B_{12} intake was also lower among males compared to females.

The prevalence of vitamin B_{12} deficiency in more than half of the sampled obese adolescents was another important finding of the present study and may be explained in a number of ways. A sedentary lifestyle and the consumption of a nutrient-deficit energy-rich diet might be key players implicated in this outcome [38]. Vitamin B_{12} deficiency in a paediatric obese population results from insufficient intake as a result of nutrient-deficit high caloric diets, repeated short-term restrictive diets, and/or higher nutrient requirements attributed to growth and body size [15]. Moreover, obesity is known to be associated with chronic tissue-grade inflammation in humans [39]. One of our earlier studies has reported an association of inflammatory marker genes such as *IL6* with adiposity in Indian children [20]. The higher circulatory concentration of inflammatory cytokines in the case of overweight/obese adolescents might have an inhibitory effect on vitamin B_{12} biosynthesis and absorption [40,41]. A number of global studies have also shown an inverse association of serum vitamin B_{12} with obesity [17,42–44].

After adjustment with potential covariates, serum vitamin B_{12} concentration in rural adolescents was found to be related to age and sex among rural adolescents. However for urban adolescents, serum B_{12} level was found to be linked with sex, BMI and energy-adjusted dietary vitamin B_{12} intake. Vitamin B_{12} acts as a cofactor for the synthesis of methionine from homocysteine [45]. A deficiency in vitamin B_{12} leading to hyper-homocysteinaemia is shown to predict cardiovascular morbidity and mortality [46,47]. Both WHR (rural area) and BMI (urban area) were found to be inversely related to serum vitamin B_{12} levels. These data are suggestive of overweight/obese adolescents being at a potential risk for several late-onset cardiometabolic disorders. Along the same lines, we have shown earlier that polymorphisms in AMD1 comprise one of the key homocysteine metabolism pathway genes associated with obesity and plasma leptin concentrations in Indian children [48]. The positive association of dietary vitamin B_{12} intake with serum B_{12} concentration observed in the present study has been previously confirmed by other studies across the globe [49–51].

Table 3 Linear regression analysis of serum vitamin B_{12} concentration with selected parameters for rural school-going adolescents

Parameters (*n* = 403)	B	β[†]	95% CI[†]	*P*-value	B	β[‡]	95% CI[‡]	*P*-value
Age (years)	−13.51	−0.11	−26.29, −0.74	0.038*	−14.20	−0.12	−27.27, −1.14	0.033*
Gender	−57.0	−0.12	−108.59, −5.40	0.030*	−65.13	−0.14	−123.66, −6.60	0.029*
BMI (kg m^{-2})	−5.92	−0.07	−14.51, 2.67	0.176	−4.98	−0.06	−13.87, 3.92	0.272
WHR	−284.97	−0.10	−581.29, 11.35	0.049*	−270.43	−0.10	−569.96, 29.11	0.077
Vitamin B_{12} (μg 4184 kJ^{-1})	73.60	0.09	−8.05, 155.26	0.077	65.90	0.08	−31.36, 163.7	0.184
Constant	825.59				869.88			

WHR, waist-to-hip ratio.

[†]Values are coefficient regression β [95% confidence interval (CI)] by multiple linear regression analysis. Categorical variables: male as reference for sex. Serum and dietary vitamin B_{12} concentrations were inverse normalized.

[‡]Adjusted for total energy intake, carbohydrate, fat, protein, total folate and total dietary fibre.

Significant values: *$P < 0.05$.

Table 4 Linear regression analysis of serum vitamin B_{12} concentration with selected parameters for urban school-going adolescents

Parameters (n = 1153)	B	β†	95% CI†	*P*-value	B	β‡	95% CI‡	*P*-value
Age (years)	−1.60	−0.01	−8.20, 5.01	0.636	−1.10	−0.01	−7.71, 5.50	0.743
Gender	57.17	0.13	32.41, 81.93	<0.001***	47.83	0.11	21.70, 73.96	<0.001***
BMI (kg m^{-2})	−3.90	−0.08	−6.97, −0.82	0.013*	−3.80	−0.08	−7.04, −0.56	0.022*
WHR	−27.95	−0.01	−208.97, 153.06	0.762	−6.81	0.00	−186.88, 173.26	0.941
Vitamin B_{12} (μg 4184 kJ^{-1})	103.11	0.17	67.62, 138.60	<0.001***	86.09	0.14	46.93, 125.24	<0.001***
Constant	330.42				418.00			

WHR, waist-to-hip ratio.

†Values are coefficient regression β [95% confidence interval (CI)] by multiple linear regression analysis. Categorical variables: male as reference for sex. Serum and dietary vitamin B_{12} concentrations were inverse normalized.

‡Adjusted for total energy intake, carbohydrate, fat, protein, total folate and total dietary fibre.

Significant values: ***$P < 0.001$, *$P < 0.05$.

The present study has several strengths. Our study population of Indian school-going adolescents is important and relevant for assessing the effects of recent socio-economic transitions in this rapidly urbanising country. Foremost, the strength of the present study was the large number of study subjects, mostly of Indo-European ethnicity but from urban and rural areas. Most of the studies concerning the prevalence of vitamin B_{12} deficiency across the globe are reported on a limited sample size [7,52]. Hence, the present study adds to the limited pool of literature on vitamin B12 deficiency, by use of a larger study group. In addition, we included information about a wide range of potential confounding factors that were adjusted for when assessing the relationships between serum vitamin B_{12} concentration with anthropometric variables and dietary vitamin B_{12} intake among urban and rural adolescents. The use of repeated 24-h diet recalls and nutritional biomarker information added to the strength of the present study because the repeated 24-h recall method is known to have less of a bias compared to other recall methods. The inclusion of a nutrient biomarker reduces the error associated with the dietary methods. Furthermore, serum vitamin B_{12} concentration was analysed in a single laboratory and inter-interviewer bias associated with dietary data collection and analysis was controlled by having a single trained diet interviewer, which added to the reliability of the results obtained.

However, the present study is not without limitations. The data were observational, with dietary data being self-reported. To counteract shortcomings associated with the 24-h recall method, a number of strategies (such as-use of household measures to estimate portion sizes, data collection within 2 weeks, blinding the participant to the recall schedule, and telephonic conversation with the mother) were taken to validate the information reported by the adolescent. Furthermore, all of the nutrients were adjusted for energy intake. Twenty-four hour recall data were not used in association with a food frequency questionnaire to arrive at usual intakes. Another limitation of the present study was the non-availability of detailed dietary supplement intake information that acts as a covariate between socio-economic factors and vitamin B_{12} status. However, at the time of sample collection, all study subjects were asked about their daily intake of vitamin supplements. Moreover, none of the schools from where we collected our samples were under any nutrient supplementation programme. Furthermore, the gut microbiome composition should be analysed in adolescents from various socio-economic strata to add more information to our findings. Nutrient intake estimations were based on Indian food composition tables that may have introduced some bias in nutrient calculations. The cross-sectional design of the study also limits the results with respect to obtaining causality. Only serum vitamin B_{12} levels were assessed as a biomarker to detect vitamin B_{12} deficiency.

In conclusion, the present study comprehensively provides information on the status of vitamin B_{12} in Indian school-going adolescents from urban and rural areas. The prevalence of vitamin B_{12} deficiency was higher among rural study subjects, as compared to urban adolescents. Males from our study population were found to be more prone to vitamin B_{12} deficiency with respect to their female contemporaries. We observed a significant inverse relationship of obesity and serum B_{12} level in Indian adolescents. Furthermore, serum vitamin B_{12} levels were positively associated with dietary vitamin B_{12} intake in urban school-going study subjects. However, well-planned statewise studies on various anthropometric, biochemical and dietary parameters would be required to produce a correct evaluation on the nutritional status of school-going adolescents in India. To reduce the prevalence of vitamin B_{12} deficiency among adolescents, the increased consumption of animal food products must be encouraged. Thus, the higher prevalence of vegetarianism in India can become a potential problem in this case. Food fortification hence becomes a feasible, cost-effective, reliable and practical approach for combating or reducing vitamin B_{12} deficiency and associated complications. Government

guidelines are available for the fortification of cereals, especially wheat flour; however, this is not mandatory at present and the outreach through various channels is difficult and miniscule. Our children are the nation's most valuable assets; hence, government should give importance to food fortification and conduct health camps that aim to build awareness among people about meeting the nutritional requirements of children and adolescents.

Transparency declaration

The lead author affirms that this manuscript is an honest, accurate and transparent account of the study being reported. The reporting of this work is compliant with STROBE guidelines. The lead author affirms that no important aspects of the study have been omitted and that any discrepancies from the study as planned (and registered with) have been explained.

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Conflict of interests, source of funding and authorship

The authors declare that they have no conflicts of interest.
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SC, MC, KM, NSS and AS designed the research. SC and MC conducted the research and analysed data. SC, MC, AKG and PB wrote the paper. NT and DB formulated research questions and had primary responsibility for the final content of the manuscript submitted for publication. All authors critically reviewed the manuscript and approved the final version submitted for publication.

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