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Folate, vitamin B6 and vitamin B12 in adolescence: serum concentrations, prevalence of inadequate intakes and sources in food

Josiane Steluti,[1] Lígia A. Martini,[2] Barbara S. E. Peters,[3] Dirce M. L. Marchioni[4]

Abstract

Objective: To investigate serum concentrations and the prevalence of inadequate folate intake and also vitamin B6 and vitamin B12 intakes and to identify those foods that make a major contribution to intake levels of these nutrients.

Methods: This was a cross-sectional, observational study of adolescents of both sexes aged 16 to 19 years from the town of Indaiatuba, SP, Brazil. Data collection was by non-consecutive 3-day dietary record. The samples' habitual diet was estimated by removing intraindividual variability, and the prevalence rates of inadequate intakes were calculated using the estimated average requirement as cutoff points. Biochemical assays for folate, vitamin B6 and vitamin B12 were conducted in accordance with the methods accepted in the literature.

Results: The study sample comprised 99 adolescents, the majority of whom were female (58.6%), with a mean age of 17.6 [standard deviation, (SD) 0.9]. Mean serum concentrations for folate, vitamin B6 and vitamin B12 were 9.2 (SD 3.4) ng/mL, 18.7 (SD 5.1) nmol/L and 397.5 (SD 188.4) pg/mL, respectively; and the prevalence rates of inadequate intake for these vitamins were 15.2, 10.2 and < 1%, respectively. The foods that made a major contribution to vitamin intakes were French bread, pasta and beans for folate; white rice, chicken and beef for vitamin B6; and lean beef, whole milk and fatty beef for vitamin B12.

Conclusions: The prevalence rates of inadequate folate, vitamin B6 and vitamin B12 intakes were low, which is possibly the result of improved access to and availability of foods that are dietary sources of these vitamins. Beans, which are a part of the traditional Brazilian diet, remain one of the primary food items that contribute to folate intake, even after mandatory fortification with folic acid in Brazil.

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Introduction

Much attention has been paid to the B vitamins because of their role as nutrients that are key for the maintenance of good health and prevention of diseases.[1] Folate and the vitamins that are metabolically related to it, including B6 and B12, have been linked to protection against certain types of cancer and to reductions in the concentration of homocysteine in the blood.[2] Elevated levels of homocysteine, in turn, are considered a risk factor for adverse events such

1. Mestre, Departamento de Nutrição, Faculdade de Saúde Pública, Universidade de São Paulo (USP), São Paulo, SP, Brazil.
2. Professora associada, Departamento de Nutrição, Faculdade de Saúde Pública, USP, São Paulo, SP, Brazil.
3. Pós-doutoranda, Ambulatório de Fragilidades Ósseas, Departamento de Medicina, Escola Paulista de Medicina (EPM), Universidade Federal de São Paulo (UNIFESP), São Paulo, SP, Brazil.
4. Doutora. Professora, Departamento de Nutrição, Faculdade de Saúde Pública, USP, São Paulo, SP, Brazil.

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as dementia, Alzheimer's disease, bone fractures, cancers and, especially cardiovascular diseases.[3] Furthermore, epidemiological studies have indicated that 75% of neural tube defects could have been prevented by increasing folate intake.[4]

Because of this, countries such as the United States, Canada and Chile have adopted public policies by which folate fortification of certain foods is mandatory, motivated by the reductions in neural tube defects. Other countries, such as the United Kingdom, Ireland, Portugal, Spain, Austria, Australia and New Zealand, have chosen voluntary fortification of foods.[5]

After the introduction of mandatory fortification the United States, the observed prevalence of inadequate intake among population was 15.7%.[6] One study of adolescents in Brazil, carried out before fortification was made mandatory in that country, found a high prevalence of inadequate folate intake; approximately 89% of the population.[7] Furthermore, other research in Brazil has also detected nutritional imbalances among adolescents. A study using a probabilistic sample from the city of Rio de Janeiro (RJ) found that intake of the following food groups was below the minimum recommended: rice and starchy foods, beans and pulses, vegetable and milk and dairy products.[8] Additionally, other studies of groups of Brazilian adolescents have also indicated unhealthy nutrition[9-11]; in particular under-consumption of fruit and vegetables, which can cause low folate intake.

In Brazil, both the under-consumption and the dietary sources of this vitamin may have changed since fortification of corn and wheat flour was made obligatory in 2004.[12] The objectives of this study are as follows: to report serum concentrations of these nutrients; to describe the prevalence of inadequate folate intake and also of inadequate vitamin B6 and vitamin B12 intakes, since they are interrelated in the homocysteine metabolic pathway; and to identify those foods that make a major contribution to intake levels of these nutrients.

Methods

The study population consisted of adolescents of both sexes who were aged 16 to 19 in 2006 and were enrolled at the Fundação Indaiatubana de Educação e Cultura (FIEC) in the city of Indaiatuba, SP, Brazil. All of the data analyzed in this paper come from the study, "Vitamin D status in obese and healthy weight adolescents."[13] A total of 330 students met the inclusion criteria (free from chronic diseases such as diabetes mellitus, arterial hypertension, chronic renal failure and heart failure; not taking corticosteroids or anti-inflammatories; not pregnant or breastfeeding at the time of data collection). Of these, 205 of agreed to take part in the study and provided a free and informed consent form signed by their guardians, 163 completed the dietary recall protocol and 132 provided blood samples. There was not enough serum for analysis in 33 cases and so the final sample comprised 99 adolescents.

Dietary intake was measured using food diaries kept for 3 non-consecutive days. Foods and preparations listed in the diaries were converted into grams using specific tables and manuals. These food intakes were then converted into energy and nutrient intakes using the software program Nutrition Data System for Research 2007 (NDS, Minneapolis, USA), which is based on data from tables published by the United States Department for Agriculture (USDA). The tables list folate levels as dietary folate equivalents (DFE), differentiating between the bioavailability of folate that is contained naturally in foods and the synthetic folate (folic acid) that is added to fortified products. Synthetic folate levels, and as a result DFE levels, were corrected to account for the level of fortification of corn and wheat flour that has been mandatory in Brazil since 2004. There is a difference between the quantities of folic acid added to fortified foods in Brazil (150 mcg) and in the USA (140 mcg).

For the biochemical analyses, 20 mL of blood was collected after 12 hours' fasting. These samples were centrifuged at 2,000 rpm for 10 minutes at room temperature, and serum was separated and stored at -80 ºC. Serum folate and vitamin B12 were assayed using the Elecsys® 2010 Rack Version (Roche, Switzerland) automated electrochemiluminescence immunoassay, using Folate II and Vitamin B12 test kits (Elecsys and cobas analyzers, Roche Diagnostics). Serum vitamin B6 concentrations were analyzed by an ImmunDiagnostik AG® HPLC-Analytik system using high performance liquid chromatography (HPLC) with fluorometric detection.

With regard to analyses of the dietary data, empirical estimates were made of habitual folate, vitamin B6 and vitamin B12 intakes after adjusting intake distributions for intraindividual variability, using the method proposed by the Iowa State University (ISU),[14] as implemented in the software program PC-SIDE version 1.0 (ISU, Ames, USA).

The prevalence rates of inadequate intake levels among the adolescents were estimated for each nutrient using the estimated average requirement (EAR) as cutoff points after weighting for population size and are defined as the proportion of individuals whose intake is below the EAR defined for each nutrient. The equivalent procedure was adopted to define the proportion whose intakes exceeded the tolerable upper intake level (UL) for each nutrient.[15]

The method of residuals was used to adjust the dietary intake levels for each nutrient by energy intake. Methodology that has been described by Block et al.[16] was used to calculate the contribution to nutrient intakes made by each food. The foods were then put in descending order of the quantity of each nutrient contained in 100 g and in a portion, based on the mean quantity (in grams) eaten by the study population.

Normality was verified using the skewness-kurtosis test. Data that you did not exhibit normal distribution were natural log transformed. Dietary vitamin intake data were described as geometric means, 95% confidence interval and percentiles. As a result of the lack of a consensus definition for the cutoff points for identifying vitamin deficiencies among adolescents, the distributions of serum concentrations of the vitamins studied are presented as arithmetic means, standard deviations and percentiles. Student's *t* test was used to analyze differences between mean nutrient intakes. All tests were performed using Stata® version 10.0 (College Station, Texas, USA). The significance level was set at 5%.

This project was approved by the Ethics Committee at the Faculty of Public Health, University of Sao Paulo. The adolescents were enrolled on the study after providing free and informed consent forms signed by themselves or their guardians (for those under 18).

Results

The study sample comprised 99 adolescents, 58.6% of whom were female. Mean age was 17.6 years [standard deviation (SD) 0.9]. The majority described themselves as white. Nutritional status assessment classified 21.2% of the adolescents as overweight.

Mean serum concentrations of folate, vitamin B6, and vitamin B12 were 9.2 (SD 3.4) ng/mL, 18.7 (SD 5.1) nmol/L and 397.5 (SD 188.4) pg/mL respectively (Table 1). Boys had higher serum vitamin B6 levels than girls (20.9 nmol/L vs. 17.3 nmol/L, p = 0.001).

Mean habitual energy intake was 2,335.8 (SD 576.1) kcal, with 2,633 (652.8) kcal for the boys and 2,125.8 (403.8) kcal for the girls. The prevalence of inadequate folate intake was 15.2; with 10.2% of inadequate vitamin B6 intake; and < 1% inadequate vitamin B12 intake (Table 2). The synthetic form of folate (folic acid) added to fortified foods accounted for 59.1% of folate intake. Folate intake was different for boys and girls (p = 0.03), according to the deattenuaded and energy-adjusted data.

The adolescents' diets included a total of 438 different food items, 400, 382 and 246 of which contained folate, vitamin B6 and vitamin B12, respectively. The foods that made the greatest contribution to vitamin intakes were French bread, pasta and beans for folate; white rice, chicken and beef for vitamin B6; and lean beef, whole milk and fatty beef for vitamin B12 (Tables 3 and 4). Although these foods were the principal sources of these vitamins in this population, the majority of them were not high on the ranking when classified according to the quantity of each vitamin in 100 g or in the average portion. First place in the 100 g ranking for both folate and B6 was taken by breakfast cereal, and first place for B12 was taken by beef liver. However, these foods contributed less than 1% of each nutrient, with the exception of liver, which contributed 5.3% (data not shown in these tables).

Discussion

This is the first study to be conducted in Brazil estimating the prevalence of inadequate folate, vitamin B6 and vitamin B12 intakes whilst at the same time providing data on serum concentrations and describing which foods make a major contribution to intakes of these vitamins in a sample

Table 1 - Means, standard deviations and percentiles for serum folate, vitamin B6 and vitamin B12 concentrations, by sex (Indaiatuba, Brazil, 2006)

			Percentile					
Serum concentrations/ sex	**n**	**Mean (SD)**	**5**	**25**	**50**	**75**	**95**	**p***
Folate (ng/mL)†								
Males	36	9.5 (4.1)	4.0	6.8	8.8	11.4	17.7	0.886
Females	53	9.0 (2.9)	5.0	7.1	8.9	10.3	15.8	
Vitamin B6 (nmol/L)								
Males	32	20.9 (5.9)	11.9	17.1	19.9	25.3	29.7	0.001‡
Females	46	17.2 (3.9)	11.2	13.9	17.2	20.0	23.2	
Vitamin B12 (pg/mL)†								
Males	39	417.9 (214.9)	132	232	377	530	840	0.647
Females	56	383.3 (168.1)	142	262	359	473	704	

SD = standard deviation.
* Student's *t* test for the difference between males and females.
† Data natural log transformed before applying Student's *t* test.
‡ Significant (p < 0.05).

Table 2 - Geometric mean, 95% confidence interval and distribution percentiles for folate, vitamin B6 and vitamin B12 intakes, by sex (Indaiatuba, Brazil, 2006)

Nutrients*	n	Geometric mean (95%CI)	p†	Percentile 5	25	50	75	95	PI < EAR	> UL
Folate (µg)‡										
Males	41	512.23 (476.90-550.18)	0.031§	338.29	446.09	535.95	600.55	690.49	15.15	0
Females	58	415.19 (394.32-437.17)		279.20	375.71	425.23	464.57	561.66		
Total	99	452.92 (432.39-474.43)		299.48	395.89	451.32	535.95	673.73		
Vitamin B6 (mg)										
Males	41	1.56 (1.45-1.67)	0.085	1.07	1.36	1.54	1.80	2.18	10.19	0
Females	58	1.43 (1.37-1.50)		0.99	1.29	1.43	1.63	1.93		
Total	99	1.48 (1.42-1.54)		1.01	1.30	1.48	1.69	2.11		
Vitamin B12 (µg)										
Males	41	4.66 (4.36-4.99)	0.628	3.41	4.04	4.55	5.40	6.40	0.92	NE
Females	58	4.31 (4.10-4.53)		2.96	4.00	4.36	4.98	5.58		
Total	99	4.45 (4.28-4.64)		2.99	4.01	4.41	5.14	6.38		

95%CI = 95% confidence interval; EAR = estimated average requirement; NE = not established; PI = prevalence of inadequate; UL = tolerable upper intake level.
* Based on figures that had been deattenuated to remove intraindividual variability.
† Student's *t* test for differences between sexes.
‡ Given as dietary folate equivalents (DFE). 1 DFE = 1 µg natural folate = 0.6 µg of the synthetic folate added to fortified foods and used in dietary supplements.
§ Significant ($p < 0.05$).

of healthy adolescents after the introduction of mandatory fortification of flour with folic acid. The prevalence rates of inadequate folate, vitamin B6 and vitamin B12 intakes were 15.2, 10.2 and < 1%, respectively, and the mean serum concentrations of these vitamins were 9.2 (SD 3.4) ng/mL, 18.7 (SD 5.1) nmol/L and 397.5 (SD 188.4) pg/mL, respectively.

The serum concentrations observed in these adolescents were mildly inferior to levels observed recently among adolescents in the United States, where serum folate was 11.0 ng/mL and serum vitamin B12 was 504 pg/mL, among adolescents aged 12 to 19,[17] and where serum vitamin B6 was 37 nmol/L in a population aged 13 to 20.[18]

The elevated serum concentrations of vitamins observed in this and other studies,[17,19,20] are possibly the result of fortification of foods. This hypothesis is supported by the elevated proportion of synthetic folate in the groups' total folate intake. Even so, there are still individuals within the population with reduced serum levels, which is of relevance if we consider the association between low serum concentrations of these vitamins and elevated plasma homocysteine in adolescents.[21]

Verly Júnior[22] conducted a study in Brazil using the same method as described here (using the EAR as cutoff) investigating a representative sample of adolescents from the city of São Paulo, SP, and found that 21% of the male and 12% of the female population had inadequate vitamin B6 intakes, and 33 and 11% respectively had inadequate vitamin B12 intakes. Data on the folate intake of people aged 14 to 18 collected by the Health and Nutrition Examination Survey (NHANES) between 2003 and 2006 found that mean intake (standard deviation) in DFE was 674 (SD 19) µg for males and 496 (SD 14) µg for females. The survey also provides a figure of 15.7% for the sex-weighted prevalence of inadequate intake.[6] The majority of these results are compatible with those found in our study, with the exception of mean folate intake among the boys, which was lower in the present study (512.2 µg).

In the only Brazilian study that was conducted before fortification, mean intake in DFE was 145 (SD 177) µg and there was an inadequate intake rate of 89%.[7] This difference is the result of the fact that our study was conducted after adoption of the resolution making fortification of flour with folic acid obligatory in Brazil. While there are no studies that have assessed the impact of mandatory fortification in Brazil, there are many studies, primarily conducted in the United States, that have shown the positive trends in both intakes and serum levels of this vitamin from the pre-fortification to post-fortification periods.[20,23]

Despite the apparent success of mandatory fortification of foods with folate, the scientific community is not unanimous in its approval of policies on fortification and

Table 3 - Contribution of food items to folate intake, plus contribution of natural and synthetic folates, for adolescents from Indaiatuba, Brazil, in 2006

Nutrients (μg)/food items	Contribution (%)*	Portion			In 100 g	
		Mean†	Quantity‡	Ranking§	Quantity‖	Ranking¶
Folate** (n = 400)						
French bread	23.08	67.14	163.9	28	244.11	7
Pasta	9.74	187.81	276.56	7	147.25	25
Beans	8.15	56.09	45.43	129	81.0	75
Baked pastries	3.54	93.12	86.07	71	92.43	68
Sliced bread	2.78	88.37	212.34	18	240.29	8
Biscuits with filling	2.59	51.63	93.77	65	181.64	11
Other††	< 2	117.17‡‡	45.11‡‡	–	49.01‡‡	–
Natural folate (n = 400)						
Beans	21.33	56.09	45.43	44	81.0	14
French bread	7.67	67.14	20.81	108	31.0	68
Whole milk	4.12	230.44	11.52	186	5.0	327
Natural orange juice	3.26	286.92	51.65	36	18.0	124
White rice	3.11	171.45	5.14	268	3.0	360
Baked pastries	2.35	129.9	86.71	7	66.75	22
Other††	< 2	116.16‡‡	18.06‡‡	–	20.74‡‡	–
Synthetic folate (n = 132)						
French bread	32.61	67.14	84.17	23	125.36	5
Pasta	15.01	187.81	154.95	5	82.5	17
Biscuits with filling	5.43	93.12	47.89	47	51.43	45
Baked pastries	3.96	88.37	109.83	13	124.29	7
Sliced bread	3.61	51.63	47.57	48	92.14	11
Savory pies and quiches	2.57	306.15	193.19	3	63.1	30
Other††	< 2	141.49‡‡	47.75‡‡	–	51.38‡‡	–

* Percentage of each nutrient contributed by each food type as a proportion of the total quantity of that nutrient consumed by the study population.
† Calculated from the mean quantity (in grams) consumed by the study population.
‡ Quantity of nutrient in question in a single portion of the food in question.
§ Position after classification in descending order of the quantity of the nutrient in question in a single portion.
‖ Quantity of the nutrient in question in 100 g of the food in question.
¶ Position after classification in descending order of the quantity of the nutrient in question in 100 g.
** Given as dietary folate equivalents (DFE). 1 DFE = 1 μg natural folate = 0.6 μg of the synthetic folate added to fortified foods and used in dietary supplements.
†† Food items that contribute less than 1% of total intake of nutrient in question.
‡‡ Mean for food items that contribute less than 1% of total intake of nutrient in question.

supplements, since there are still discussions within the scientific literature.[24] Some researchers criticize the need to expose the whole population to high doses of the vitamin and point out that overexposure to this micronutrient may be associated with adverse effects, including increased incidence of colorectal cancer[25] and masking of vitamin B12 deficiency anemia.[26] In the present study, however, none of the subjects had an intake of any of the vitamins that was over its UL.

Observing the position of each food when ranked in order of the quantity of each vitamin contained in 100 g, it will be noted that the foods that make a major contribution to folate and vitamin B6, in particular, were not those foods that contained major quantities in 100 g – the known dietary sources – reinforcing the hypotheses that it is necessary to investigate which foods are part of the population's diet, including those foods that contribute nutrients after fortification. This study has shown the relevant contribution of foods in which wheat flour is one of the basic ingredients, such as bread, pasta and biscuits.

Brazilian surveys that have analyzed developments the range of foods available in Brazilian households have reported a tendency towards increased consumption of meat, milk and dairy products and processed foods. In contrast, they have also observed a reduction in consumption of cereals, pulses and fruit and vegetables, which corroborates our results if one considers that these make an important contribution to intakes of the nutrients in question.[27] It should also be pointed out that no other study conducted in recent years has attempted to determine which foods

Table 4 - Contribution of food items to dietary intakes of vitamins B6 and B12 of adolescents in Indaiatuba, SP, Brazil, in 2006

		Portion			In 100 g	
Nutrients/food items	**Contribution (%)***	**Mean†**	**Quantity‡**	**Ranking§**	**Quantity‖**	**Ranking¶**
Vitamin B6 (mg) (n = 382)						
White rice	9.93	171.45	0.16	115	0.09	200
Chicken	5.67	67.46	0.40	37	0.60	12
Lean beef	4.20	149.26	0.42	34	0.28	67
Milk-based drinks	3.88	27.49	0.25	74	0.91	11
Whole milk	3.01	230.44	0.08	182	0.04	334
Fatty beef	2.75	145.71	0.37	44	0.26	75
French bread	2.54	67.14	0.07	206	0.10	177
Beans	2.50	56.09	0.05	227	0.09	202
Ground beef	2.08	61.01	0.21	86	0.34	42
Other**	< 2	122.86††	0.19††	–	0.18††	–
Vitamin B12 (μg) (n = 246)						
Lean beef	16.42	149.26	4.03	9	2.70	13
Whole milk	15.01	230.44	1.01	52	0.44	103
Fatty beef	12.29	145.71	4.12	7	2.83	11
Ground beef	6.12	61.01	1.53	31	2.50	16
Beef liver	5.27	100.0	70.58	1	70.58	1
Hamburgers	4.39	60.57	1.78	27	2.94	10
Other**	< 2	129.88††	0.67††	–	0.66††	–

* Percentage of each nutrient contributed by each food type as a proportion of the total quantity of that nutrient consumed by the study population.
† Calculated from the mean quantity (in grams) consumed by the study population.
‡ Quantity of nutrient in question in a single portion of the food in question.
§ Position after classification in descending order of the quantity of the nutrient in question in a single portion.
‖ Quantity of the nutrient in question in 100 g of the food in question.
¶ Position after classification in descending order of the quantity of the nutrient in question in 100 g.
** Food items that contribute less than 1% of total intake of nutrient in question.
†† Mean for food items that contribute less than 1% of total intake of nutrient in question.

are the major contributors of vitamin B12. One study investigating the young people in Korea showed that pork and rice were the primary sources of vitamin B6.[28] With regard to folate, orange juice, bread, biscuits, beans and green vegetables were the principal sources for intake of this vitamin in the United States at the end of the 1970s, in the pre-fortification era.[29] After mandatory fortification it was observed that bread and biscuits were the primary sources.[30] In Brazil little is known about the dietary sources after introduction of fortification of flour with folic acid, and this is one contribution made by the present study.

It is worth pointing out that beans remain one of the primary sources of this vitamin, together with fortified foods. This scenario shows that irrespective of fortification, stimulating consumption of foods known to be dietary sources and which are a part of the habitual diet of the Brazilian population may contribute to reducing the prevalence of inadequate micronutrient intakes.

This study suffers from certain limitations. The cross-sectional design means that it is not possible to investigate relationships of causality between the events investigated. There was no information available on this population's use of vitamin supplements, which could lead to overestimation of the prevalence of inadequate vitamin intakes. Notwithstanding, dietary supplement usage is rare in adolescence, increasing in prevalence as age increases and making a significant contribution to vitamin intake among adults and the elderly.[21] Furthermore, this is not a representative sample of the adolescents from Indaiatuba, and as such does not allow for generalizations or inferences to be extrapolated to other populations of adolescents in the same city or in other locations, which, in turn, underscores the need for further studies which can corroborate these findings. Notwithstanding, this study has shown a significant change in the scenario of the rate of inadequate intake and the serum concentrations of these vitamins, especially folate.

This study has provided information on the prevalence rates of inadequate intake and serum concentrations of folate, vitamin B6 and vitamin B12 among adolescents. The prevalence rates of inadequate intake were low, possibly as a result of improvements in access to and availability of foods that are dietary sources of these vitamins, including of processed foods whose ingredients include flours fortified with folic acid. Of note is the fact that beans, which are a part of the traditional Brazilian diet, remain one of the primary food items that contribute to folate intake, even after mandatory fortification with the vitamin in this country.

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Correspondence:
Dirce Maria Lobo Marchioni
Avenida Dr. Arnaldo, 715 – Cerqueira César
CEP 01246-904, – São Paulo, SP – Brazil
Tel.: +55 (11) 3061.7856
Fax: +55 (11) 3061.7705
E-mail: marchioni@usp.br