

Prevalence of mild serum vitamin B₁₂ deficiency in rural and urban Costa Rican young adults

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Suggested citation

Holst-Schumacher I, Monge-Rojas R, Barrantes-Santamaría M. Prevalence of mild serum vitamin B₁₂ deficiency in rural and urban Costa Rican young adults. *Rev Panam Salud Publica*. 2007;22(6):396–401.

ABSTRACT

Objective. Recent studies have shown an increase of mild serum cobalamin (vitamin B₁₂) deficiency in some Latin American countries; however, no data are available from Costa Rica. The purpose of this work was to establish the prevalence of serum vitamin B₁₂ deficiency among Costa Rican young adults and to study some factors that may help explain the serum cobalamin concentrations.

Methods. A cross-sectional survey was conducted among 400 adults aged 20 to 40 years old from urban and rural areas of the central valley of Costa Rica to determine serum vitamin B₁₂ levels. Additionally, cobalamin dietary intake and the detection of *Helicobacter pylori* IgG antibodies were studied as possible determinants of the serum vitamin B₁₂ concentrations.

Results. The mean serum concentration of vitamin B₁₂ was 268 ± 125 pmol/L, and no significant differences were found by gender or area. Study data indicate an overall prevalence of inadequate serum cobalamin levels of 42.4% (11.2% deficient and 31.2% marginal); more than 50% but less than 75% of individuals had an intake of vitamin B₁₂ below the U.S. Estimated Average Requirement (EAR) and 61.2% had IgG antibodies to *H. pylori*.

Conclusions. The prevalence of mild serum vitamin B₁₂ deficiency in young Costa Rican subjects is as high as in other Latin American countries. More investigation should be done to elucidate the etiological factors that are generating deficient and marginal serum cobalamin levels in Costa Rican adults in order to define appropriate public health actions.

Key words

Vitamin B 12 deficiency, *Helicobacter pylori*, Costa Rica.

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Vitamin B₁₂ or cobalamin deficiency has been identified frequently in older people and vegetarians (1–3). Nevertheless, recent studies in Latin America have shown a high prevalence of mild serum vitamin B₁₂ deficiency in

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younger, omnivorous subjects (4, 5). In 1995, a Cuban study reported a prevalence of deficient serum vitamin B₁₂ values ranging from 52 to 82% and marginal values ranging from 13 to 36%, respectively, among a healthy population of middle-aged men (5). In another study, about 45% of fertile women in a rural Mexican commu-

nity were identified with deficient and marginal serum vitamin B₁₂ concentrations (4). In Costa Rica, cobalamin status has not been assessed among the middle-aged population.

Low concentrations of vitamin B₁₂ have been associated with megaloblastic anemia (3), neuropsychiatric manifestations (6), and hyperhomocysteinemia, an important cardiovascular risk factor (7).

Cobalamin deficiency has been attributed to low vitamin B₁₂ intake or protein-bound vitamin B₁₂ malabsorption syndromes (8). The low intake of foods with a high content of animal protein—main dietary sources of vitamin B₁₂—markedly limits the intake of this micronutrient (9, 10), particularly in developing countries where availability of these products is low and their cost is high.

In several studies, food-cobalamin malabsorption (FCM) has been associated with atrophic gastritis induced by a bacterium known as *Helicobacter pylori* (11, 12). Lack of or inadequate secretion of intrinsic factor (due to pernicious anemia), gastric acid, and pepsin, which are needed to release and absorb protein-bound cobalamin in food, can cause vitamin B₁₂ malabsorption (13). Likewise, hypochlorhydria and bacterial overgrowth in the mucosa can play an important role in the malabsorption process (8, 12). Moderate and high alcohol consumption has also been associated with vitamin B₁₂ deficiency (14, 15).

The purpose of this study was to determine the prevalence of serum cobalamin deficiency among Costa Rican young adults and to examine whether dietary intake of vitamin B₁₂ and the presence of *H. pylori* help to explain serum vitamin B₁₂ concentrations.

MATERIALS AND METHODS

Study population

A total of 400 adults from the central valley of Costa Rica, aged 20 to 40 years, were recruited for this study. Subjects were asked to participate through a written circular sent home

with about 700 randomly selected boys and girls from 20 representative rural and urban schools. A total of 490 adults offered to collaborate, but only 400 of them fulfilled all the requirements for participation in the study. Inclusion criteria included being of Costa Rican nationality and being between 20 and 40 years old. Exclusion criteria included conditions such as vegetarianism, alcoholism, gastrointestinal disorders, and chronic diarrhea. Also excluded were people taking vitamin supplements.

Ethical procedures

Written, informed consent was obtained from all participants. The study protocol was approved by the Ethics Committees of the Costa Rican Institute for Research and Education on Nutrition and Health (INCIENSA) and the University of Costa Rica.

Socio-demographic and dietary variables

Information about age, gender, area, economic level, and vitamin consumption were collected through validated instruments. Madrigal's method was used to establish the socioeconomic status of study participants based on their possession of various material goods and comforts in their homes (16). Dietary intake of vitamin B₁₂ was determined through the use of semi-quantitative 24-hour recall questionnaires. A series of photographs of food commonly consumed in Costa Rica and three-dimensional (3-D) food models were used to estimate portion size. The Food Processor, for Windows version 6.0 (ESHA Research, Salem, Oregon, USA) was used to perform nutrient calculations from dietary data (17). A comparison with the U.S. Estimated Average Requirement (EAR) for vitamin B₁₂ was made to evaluate micronutrient intake, and the recommended dietary allowance of 2.4 µg for vitamin B₁₂ was considered. The EAR cut-point method (counting the

number of individuals with normal intake below fixed EAR-based cut-off points) was used to assess the prevalence of inadequate intake of the vitamin (18).

Biochemical variables

Blood was drawn after 8–12 hours of fasting by antecubital venipuncture according to the procedures of the Clinical and Laboratory Standards Institute (CLSI; formerly the National Committee for Clinical Laboratory Standards or NCCLS) (19) and collected into a plain tube for serum vitamin B₁₂ and immunological analyses. Serum was separated immediately from the blood cells and stored at –20°C until the tests were performed.

Vitamin B₁₂ serum concentration was determined with a commercial immunoassay using the Abbott IMx® System (Abbott Laboratories Diagnostics Division, Abbott Park, IL, USA), a fully automated immunoassay analyzer, and the intra-assay coefficient of variation was <6%. Internal quality control with deficient, normal, and high serum concentrations of vitamin B₁₂ was performed to control variability in the measurements. In the cases in which the samples exceeded ± 2 standard deviations (SDs) of the average control value, the samples were analyzed again. Vitamin B₁₂ levels were classified as deficient (<150 pmol/L), marginal (150–221 pmol/L), and adequate (≥222 pmol/L) according to the recommendations of the U.S. National Academy of Sciences Institute of Medicine (20).

Qualitative detection of IgG antibodies to *Helicobacter pylori* was performed using a commercial enzyme-linked immunoassay (ELISA) kit (Panbio, Brisbane, Queensland, Australia). This test was limited to a subsample of study participants (139 individuals), however, due to its high cost. A result was considered negative when the test indicated <9 Panbio units, equivocal when between 9–11 Panbio units, and positive when >11 Panbio units (21).

Data analysis

Statistical analysis was performed using the Statistical Package for Social Sciences (SPSS), version 10.0 for Windows (SPSS Inc., Chicago, IL, USA, 1999). Continuous variables are summarized using means \pm SD, and categorical variables are presented as frequency distributions. In order to study the behavior of the variables as age increases, the adult population was divided into two groups: 20–29 years and 30–40 years. Student's *t*-test was used to compare the means of the continuous variables and chi-square tests were used to compare categorical data. Partial Spearman correlation coefficients were calculated to determine association between variables.

RESULTS

The study sample consisted of 187 men and 213 women with a mean age of 32.5 ± 5.6 years; 76.8% of the individuals came from urban areas and 23.2% from rural areas (Table 1). The total mean serum vitamin B₁₂ concentration of the study population was

268 ± 125 pmol/L, and no significant differences were found in the means of this micronutrient by gender, area, presence or absence of IgG antibodies for *H. pylori*, age group (20–29 years or 30–40 years), or socioeconomic level of the subjects (data not shown).

The overall prevalence of IgG antibodies to *H. pylori* in the studied population was 61.2%, with a significantly higher proportion of seropositive individuals in urban areas (48.9% vs. 12.2%, respectively; $p < 0.001$) (Table 1). No differences between male and female subjects were found in the prevalence of *H. pylori* antibodies, and the proportion of seropositive individuals in the 30–40-year age group (56 out of 85) was higher than in the 20–29-year age group (29 out of 85), although the difference was not statistically significant ($p = 0.121$) (data not shown). Vitamin B₁₂ average intake was higher than 50% but lower than 75% of the EAR for this micronutrient.

More than 42% (42.4%) of the study participants presented either deficient (11.2%) or marginal levels (31.2%) of serum vitamin B₁₂ (Table 2). The proportion of adults ≥ 30 years old with marginal concentrations of vita-

min B₁₂ was significantly higher than the proportion of subjects <30 years old (35.9% vs. 24.5%, respectively; $p = 0.021$) (data not shown). Likewise, the proportion of adults with deficient levels of vitamin B₁₂ with a low socioeconomic profile was significantly higher than the proportion of individuals with deficient vitamin B₁₂ concentrations and a high socioeconomic condition (14.8% vs. 3.9%; $p = 0.028$). Weak associations were found between the presence of IgG antibodies for *H. pylori* and the age group ($r = 0.122$) and area ($r = 0.110$) of the subjects. Likewise, vitamin B₁₂ concentrations levels correlated weakly with study participants' socioeconomic level ($r = 0.114$).

DISCUSSION

For decades, it has been assumed that vitamin B₁₂ deficiency is unlikely to occur except under special circumstances, such as the presence of pernicious anemia, vegetarianism, malabsorption syndromes, bacterial overgrowth, and other clinical conditions in which the function of the

TABLE 1. Study sample distribution by age group, area, socioeconomic status, serum cobalamin level, vitamin B₁₂ intake, and presence of *Helicobacter pylori* antibodies, Costa Rica, 2003

Variable	Gender		<i>p</i> value	Area		<i>p</i> value	Total (<i>n</i> = 400)
	Male (<i>n</i> ^a = 187)	Female (<i>n</i> = 213)		Urban (<i>n</i> = 307)	Rural (<i>n</i> = 93)		
Age group (years)							
20–29	68 (36.4%)	96 (45.1%)	0.100	121 (39.4%)	43 (46.2%)	0.295	164 (41.0%)
30–40	119 (63.6%)	117 (54.9%)	0.065	186 (60.6%)	50 (53.8%)	0.295	236 (59.0%)
Socioeconomic level							
Low	42 (22.4%)	86 (40.4%)	$< 0.001^b$	68 (22.1%)	60 (64.5%)	$< 0.001^b$	128 (32.0%)
Medium	106 (56.7%)	90 (42.2%)	0.005 ^b	164 (53.4%)	32 (34.4%)	0.002 ^b	196 (49.0%)
High	39 (20.9%)	37 (17.4%)	0.446	75 (24.4%)	1 (1.1%)	$< 0.001^b$	76 (19.0%)
Serum vitamin B ₁₂ level (pmol/L ^c \pm SD ^d)	267 \pm 122	268 \pm 129	0.937	270 \pm 124	261 \pm 131	0.545	268 \pm 125
Vitamin B ₁₂ intake <EAR ^e	>50% but <75%	>50% but <75%	...	>50% but <75%	>50% but <75%	...	>50% but <75%
IgG antibodies to <i>H. pylori</i> ^f	43 (31.0%)	42 (30.2%)	0.949	68 (48.9%)	17 (12.2%)	$< 0.001^b$	85 (61.2%)

^a Number of subjects.

^b Significant difference at < 0.05 using Student's *t*-test.

^c Picomole/L; to convert pmol/L vitamin B₁₂ to pg/mL, divide pmol/L by 0.738.

^d Standard deviation.

^e Estimated Average Requirement.

^f This test was limited to a sub-sample of study participants ($n = 139$) due to its high cost.

TABLE 2. Distribution of serum vitamin B₁₂ status among 400 Costa Rican adults by selected characteristics, Costa Rica, 2003

Variable	Vitamin B ₁₂ status			p ^c value
	Deficient (<150 pmol/L) (n ^b = 45)	Marginal (150–221 pmol/L) (n = 125)	Adequate (≥ 222 pmol/L) (n = 230)	
Gender				
Male (n = 187)	20 (10.7%)	53 (28.3%)	114 (61.0%)	0.411
Female (n = 213)	25 (11.7%)	72 (33.8%)	116 (54.5%)	
Area				
Urban (n = 307)	31 (10.1%)	94 (30.6%)	182 (59.3%)	0.293
Rural (n = 93)	14 (15.1%)	31 (33.3%)	48 (51.6%)	
Age group				
20–29 years (n = 164)	19 (11.6%)	40 (24.4%)	105 (64.0%)	0.052 ^d
30–40 years (n = 236)	26 (11.0%)	85 (36.0%)	125 (53.0%)	
Socioeconomic level				
Low (n = 128)	19 (14.8%)	42 (32.8%)	67 (52.3%)	0.128
Medium (n = 196)	23 (11.7%)	61 (31.1%)	112 (57.1%)	
High (n = 76)	3 (3.9%)	22 (28.9%)	51 (67.1%)	
IgG antibodies to <i>H. pylori</i>^e				
Positive (n = 85)	24 (28.2%)	30 (35.3%)	31 (36.5%)	0.674
Negative (n = 54)	19 (35.2%)	18 (33.3%)	17 (31.5%)	

^a Picomole/L; to convert pmol/L vitamin B₁₂ to pg/mL, divide pmol/L by 0.738.

^b Number of subjects.

^c p < 0.05 (chi-square test).

^d Significant difference.

^e This test was limited to a sub-sample of study participants (n = 139) due to its high cost.

ileum is impaired (2, 3). It has also been established that vitamin B₁₂ deficiency is common among the elderly, even in industrialized countries. Nevertheless, reports of cobalamin deficiency status in Latin American children and young adults have been increasing over the past decade (5, 22, 23), and Allen (24) has recently shown that at least 40% of individuals in the Americas had deficient or marginal levels of serum vitamin B₁₂.

This is the first report indicating deficient serum vitamin B₁₂ concentrations among Costa Ricans. Report results suggest a significant problem, with almost 43% of the population presenting marginal or deficient serum levels of cobalamin (≤ 221 pmol/L). Given that the study participants were young and apparently healthy, this level of vitamin B₁₂ deficiency, which is common among the elderly (largely due to gastric atrophy), is striking. A significantly higher proportion of individuals with marginal levels of vitamin B₁₂ was observed in the older age group (30–40-

year), clearly suggesting that this deficiency becomes worse as age increases. Study data also suggest that the assumption of low prevalence of vitamin B₁₂ deficiency among young adults need to be re-examined, particularly because vitamin B₁₂ deficiency has been associated with increased risk of cardiovascular disease and serious neurological manifestations, including the development of Alzheimer's disease and dementia (25).

Unlike Costa Rica and other Latin American countries (24), which have a prevalence of deficient and marginal levels of vitamin B₁₂ of more than 40%, developed countries such as United States have a prevalence of less than 3% (26).

Vitamin B₁₂ deficiency can occur in individuals with dietary patterns that exclude animal or fortified foods, and in adults who are unable to absorb vitamin B₁₂ from food (8). In developing countries, low serum vitamin B₁₂ concentrations could be a consequence of reduced consumption of animal protein due to its high cost. Vitamin B₁₂ is

provided in foods of animal origin, including dairy products and eggs, and is not a normal component of plant-based foods.

In several Latin American countries the diet is characterized by a predominance of vegetables, so intake of dietary vitamin B₁₂ is quite low (9, 10). According to the Food and Agricultural Organization of the United Nations (FAO), per capita intake of animal products in developing countries constitutes less than one-third of the registered intake in industrialized countries (27).

Although this study found no significant correlation between serum vitamin B₁₂ concentrations and socioeconomic level, a higher prevalence of young adults with deficient levels of cobalamin was observed in those from low socioeconomic levels versus those with a higher socioeconomic condition.

H. pylori infection has also been associated with FCM (food-cobalamin malabsorption), in some studies (11, 12, 28), but not in others (29, 30). *H. py-*

lori infection induces a reduction in the production of gastric acid and thus limits the release of vitamin B₁₂ from the chymus (31).

The presence of *H. pylori* IgG antibodies in serum is highly indicative of *H. pylori* infection (8) and can therefore be used as a non-invasive means of screening large groups of people. Although, in this study, the prevalence of *H. pylori* antibodies was high (61.2%), an association could not be ascertained between the presence of this bacterium and deficient or marginal levels of serum vitamin B₁₂. This situation is consistent with the one reported by Van Asselt et al. (8), who determined that *H. pylori* infection was related to atrophic gastritis but could not determine an association between the infection and mild vitamin B₁₂ deficiency. However, Carmel et al. (28) found the presence of IgG antibodies to *H. pylori* in 75% of patients with severe FCM, and Serin et al. (32) provided strong evidence of association between *H. pylori* infection and vitamin B₁₂ deficiency, even in patients with minimal or no gastric atrophy.

This study could not find an association between the presence of the bacteria and age, probably because the study population was too young (mean age: 32.5 years). It is well known that the prevalence of both *H. pylori* antibodies and serum cobalamin deficiency increases with age (8).

The etiology of mild vitamin B₁₂ deficiency in young Costa Rican subjects is unclear, and its clinical manifestations could be misdiagnosed if accompanied by high intake of folic acid. Several studies have shown that folic acid deficiency is not a nutritional problem in the adult Costa Rican population (33), probably due to the ongoing policy of fortifying several foods with synthetic folic acid established by the Costa Rican Ministry of Health in 1997. Although high folic acid intake in the presence of vitamin B₁₂ deficiency can delay the clinical diagnosis of vitamin B₁₂ deficiency-induced megaloblastic anemia, it does not affect the irreversible progression of neurological dysfunction and cognitive decline that results from cobalamin deficiency (6).

More investigation should be done to elucidate the etiological factors that are generating deficient and marginal levels of serum vitamin B₁₂ among Costa Rican young adults in order to define appropriate public health actions. Synthetic cobalamin food fortification could be a key strategy for public health in Costa Rica as well as other countries in the Americas where food is fortified with folic acid and vitamin B₁₂ deficiency is suspected to be common. The Pan American Health Organization has indicated that the daily intake of 1 µg of synthetic vitamin B₁₂ from fortified foods is a reliable way to

optimize the status of this vitamin in the Americas (34).

The results of this study regarding intake of vitamin B₁₂ should be interpreted with caution, as in some cases the 24-hour recall questionnaire may have only been applied once among the adult population (as opposed to the three-time intervention considered necessary for adequate evaluation nutrient intake). Taking this limitation into account, it should be noted that a higher prevalence of adults with deficient concentrations of cobalamin was observed at low socioeconomic levels versus higher socioeconomic levels.

The results of this research indicated a high prevalence of deficient and marginal levels of serum vitamin B₁₂ among Costa Rican young adults. However, no significant association was found among the presence of *H. pylori*, dietary intake of vitamin B₁₂, and serum levels of cobalamin.

Acknowledgements. The authors are grateful to Minor Soto Carranza and Roberto Chaves Vega for their help with blood sample collection. This work was supported by grants from three sources at the University of Costa Rica: (1) Office of Postgraduate Studies, (2) Office of Vice-president for Research (Project 807-A2-308), and (3) Fundación de la Universidad de Costa Rica para la Investigación (Project 217).

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Manuscript received on 5 February 2007. Revised version accepted for publication on 7 November 2007.

ABSTRACT

Prevalencia de la deficiencia moderada de vitamina B₁₂ sérica en jóvenes adultos de zonas rurales y urbanas de Costa Rica

Objetivo. Aunque estudios recientes han demostrado un incremento en el déficit moderado de cobalamina (vitamina B₁₂) sérica en algunos países de América Latina, no hay datos de Costa Rica. El propósito de este trabajo fue determinar la prevalencia de la deficiencia de vitamina B₁₂ sérica en jóvenes adultos de Costa Rica y estudiar algunos factores que pueden ayudar a explicar las concentraciones séricas de cobalamina.

Métodos. Se realizó un estudio transversal para determinar los niveles de vitamina B₁₂ en el suero de 400 adultos de 20 a 40 años de edad de zonas urbanas y rurales del valle central de Costa Rica. Adicionalmente, se estudió la ingesta de cobalamina en la dieta y la presencia de anticuerpos IgG contra *Helicobacter pylori*, como posibles factores determinantes de las concentraciones séricas de vitamina B₁₂.

Resultados. La concentración media de vitamina B₁₂ en el suero fue de 268 ± 125 pmol/L; no se encontraron diferencias significativas entre los sexos ni entre las áreas de residencia. Estos datos demostraron una prevalencia general de niveles insuficientes de cobalamina sérica de 42,4% (11,2% con déficit y 31,2% con concentraciones marginales); de las personas estudiadas más de 50% —aunque menos de 75%— tenían una ingesta de vitamina B₁₂ por debajo del valor de las necesidades promedio estimadas para los Estados Unidos de América y 61,2% tenía anticuerpos contra *H. pylori*.

Conclusiones. La prevalencia de deficiencia moderada de vitamina B₁₂ sérica en jóvenes costarricenses es tan elevada como en otros países latinoamericanos. Se necesitan más investigaciones para aclarar los factores etiológicos que producen niveles insuficientes y marginales de cobalamina sérica en los adultos de Costa Rica para poder definir intervenciones sanitarias apropiadas.

Key words

Deficiencia de vitamina B₁₂, *Helicobacter pylori*, Costa Rica.