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Folate and Vitamin B_{12} Status in the Americas

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There is growing interest in the potential for folic acid fortification in the Americas and recognition of the high prevalence of low plasma vitamin B_{12} concentrations reported in various studies. This review summarized available data on plasma vitamin B_{12} and folate concentrations in the Americas. At least 40% of individuals had deficient or marginal plasma vitamin B_{12} concentrations in almost all locations and across age groups. Low plasma folate concentrations were less common. It is hypothesized that vitamin B_{12} deficiency may result from a low intake of animal source foods, while a higher intake of refined flour may result in low plasma folate.

Key words: folate, vitamin B_{12}, Latin America

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Background

It is generally assumed that, due to efficient enterohepatic recycling of the vitamin, B_{12} deficiency is unlikely to occur except in special circumstances. These include long-term consumption of a diet that is strictly vegetarian, pernicious anemia, malabsorption syndromes including tropical sprue and bacterial overgrowth, and other clinical conditions in which the function of the ileum (where vitamin B_{12} absorption occurs) is impaired. In the past decade we have also become aware that vitamin B_{12} deficiency occurs commonly in the elderly—even in industrialized countries—due to gastric atrophy.

Our assumption that there is a low prevalence of vitamin B_{12} deficiency during childhood through adult years needs to be re-examined. Since we discovered a consistently high prevalence of low plasma vitamin B_{12} concentrations in rural Mexico and peri-urban Guatemala City during the past decade, in adults as well as infants and children, we have become interested in understanding the causes and consequences of this deficiency. Thus this opportunity to review the existing data on the prevalence of vitamin B_{12} deficiency in The Americas is very relevant to this objective.

Because plasma folate is typically measured in the same assay as plasma vitamin B_{12}, our laboratory has made simultaneous measures of plasma vitamin B_{12} and folate in Guatemala, Mexico, and other countries. In recent years, countries have obtained data on folate status because of their interest in the prevention of neural tube defects by folic acid fortification of the food supply. In some cases, vitamin B_{12} was analyzed in the same samples.

Approach Taken

The prevalence data presented here were obtained by searching Index Medicus since 1980 for the terms "vitamin B_{12}," "cobalamin," "folate," and "folic acid," and asking PAHO and other contacts for names of investigators who might have relevant data. Data were only collected after 1980 because of doubts about the validity of some of the earlier assay methods for vitamin B_{12}.

A major problem when comparing prevalence of low levels across studies is the variability of cut-offs used to denote deficiency. Thus, in some cases we asked investigators to recalculate their prevalence data based on our proposed cut-points. These were $<$150 pmol/L ($<$200 μg/mL) for vitamin B_{12} deficiency ("deficient" plasma levels) and $<$150–221 pmol/L (200–299 μg/mL) for marginal vitamin B_{12} status ("marginal" plasma levels). For plasma folate a cut-point of 7 μmol/L (3 ng/mL) was used. These cut-points are recommended by the Institute of Medicine.[1] Any data on plasma or urinary methylmalonic acid concentrations (which are elevated in vitamin B_{12} deficiency), and erythrocyte or whole blood folate, are included for the few cases in which these were available. One risk of using the same cut-points across projects is that the assays used among the investigators could have slightly different normal reference ranges. However, the common cut-point strategy provides the most useful information for the current exercise.

Results

The data are presented and discussed at the country level, first for vitamin B_{12} and then for folate in each case.

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Except in the case of the Mexican National Nutrition Survey, few of the data were obtained from nationally representative samples, so the characteristics of each sample population are described briefly when available.

Mexico

Our own research on vitamin B_{12} deficiency was stimulated by the observation that erythrocyte mean cell volumes in blood samples from a poor region of rural Mexico, 170 km northwest of Mexico City, tended to be large, although not actually macrocytic. We had assumed that the probable cause of the large erythrocytes was folate deficiency, possibly aggravated in adults by consumption of *pulque,* a local alcoholic beverage. Surprisingly however, we found a high prevalence of low serum vitamin B_{12} concentrations, but no low serum folate values.[2] The prevalence of deficient and marginal plasma B_{12} values respectively was 41% and 16% in preschoolers (ages 18–36 months), 22% and 25% in schoolers (7–10 years), 19% and 19% in non-pregnant women, 19% and 43% in pregnant women, 30% and 25% in lactating women, and 27% and 15% in adult men. The cut-point for vitamin B_{12} deficiency was <103 pmol/L (deficient) and 103–148 pmol/L (marginal), and in pregnancy, <74 pmol/L (deficient), and 74–148 pmol/L (marginal), based on the manufacturer's recommended normal values for this assay. No individual had a serum folate concentration <6.1 nmol/L. In addition, we reported that 62% of rural women ($n = 50$) had a breast milk vitamin B_{12} concentration below 362 pmol/L at 204 ± 30 days of lactation. This cut-point was based on the fact that urinary MMA concentration increased in infants of vegan mothers whose breast milk vitamin B_{12} concentration was less than this value.[3] In a small subsample of the Mexican women on whom both plasma and milk vitamin B_{12} data were available, these values were significantly correlated ($r = 0.48$, $P = 0.06$, $n = 16$).

In a subsequent study designed to assess the benefits of iron and/or zinc supplements on preschoolers in the same communities, we again measured plasma vitamin B_{12}, holoTC II, and folate. A total of 219 children was enrolled, almost all of those between 18 and 36 months of age in the communities.[4] Plasma samples were analyzed at baseline and 6 and 12 months later. The prevalence of marginal and deficient plasma B_{12} concentrations, respectively, was eight and 33% at baseline , three and 22% six months later, and seven and 29% twelve months after baseline. Low holoTC II concentrations averaged 18–40% across the periods. The intra-individual correlation in plasma B_{12} across periods was strong; $r = 0.58–0.73$ ($P<0.001$). This suggests consistency of plasma vitamin B_{12} in individual children during the year. Plasma vitamin B_{12} and holoTC II concentrations were strongly correlated for the group as a whole ($r =$ 0.56–0.83 across the three periods) but not within individual children. As in the previous study, no child had a low plasma folate concentration (<6.1 nmol/L).

Oral supplemental vitamin B_{12}, 1 mg, three times a week, for three months, was provided to 128 preschoolers, ages 18 to 36 months, during another study in the same communities (Allen et al., unpublished). The children were part of a multiple micronutrient intervention trial, and all were selected for having low hemoglobin concentrations (80 g/L to <115 g/L). At baseline, 29% of the children had marginal or deficient plasma vitamin B_{12}. In all except one child, whose plasma B_{12} concentration was >1000 pg/mL at baseline, there was a substantial increase in plasma levels of the vitamin after supplementation. No children had low plasma concentrations of the vitamin after supplementation. In spite of a high prevalence of elevated MCV and MCH levels at baseline, vitamin B_{12} supplementation did not significantly affect these values. Only 2% of these children had a plasma folate concentration <6 pmol/L at baseline.

The Mexico National Survey

Mexico is the only country in Latin America that has assessed folate and vitamin B_{12} status in a probabilistic sample of the population.[5] The 1999 National Survey of Nutrition included measures of "total blood folate." Dried blood spots were collected on filter paper, the folate later extracted and assayed with *Lactobacillus caseii.* It was then assumed that all the folate was in erythrocytes so that this value could be entered in a regression equation to estimate total blood folate. While this value is more difficult to interpret, the prevalence of low (<57 ng/mL) total blood folate was around 10% in children below four years of age, 5% between four and 11 years of age, and 5% in rural and urban women. We had the opportunity to measure plasma vitamin B_{12} and folate in some of these samples. Preliminary data show that for children under age five and for women of fertile age respectively, approximately 30% and 25% had deficient serum B_{12} concentrations and an additional 12% of children and 20% of women had marginal concentrations. Serum folate was low in approximately 5% of the children and 25% of the women.

In conclusion, the data from Mexico indicate a very high prevalence of vitamin B_{12} deficiency in all age and physiological status groups, but a lower prevalence of low serum folate.

Guatemala

We have also conducted several studies of the prevalence and consequences of vitamin B_{12} and folate deficiency in low-income districts of peri-urban Guatemala City. The first study was conducted in a group of school-aged children, all of whom were selected on the basis of

having Giardia (unpublished data). The 109 children were aged 6–12 years. Of this group, 13% had a deficient plasma vitamin B_{12} concentration at baseline, and an additional 34% had a marginal value. None of the children had low plasma folate, only 7% had iron deficiency and 10% had anemia. The second study explored the prevalence of vitamin B_{12} deficiency in lactating women, and the association between maternal vitamin B_{12} status, human milk concentrations of the vitamin, and the vitamin B_{12} status of the infants.[6] Participants were 113 women and their infants at 3 months of lactation. Plasma vitamin B_{12} was deficient or marginal in 47% of the mothers, and concentrations in breast milk were low in 31%. Urinary MMA was elevated in 12% of the infants, and inversely correlated with levels of vitamin B_{12} in breast milk ($r = -0.22$). Plasma folate levels were low in 9% of the mothers.

In a third Guatemalan study, plasma vitamin B_{12} and folate were measured in 128 infants aged 7 to 12 months, from a similar poor, peri-urban location in Guatemala City.[7] All of these infants were at least partially breastfed (at least three times per day) and <-3 Z for length-for-age, and <-2 Z for weight-for-age. Plasma vitamin B_{12} indicated deficiency in 25%, and was marginal in an additional 36%. None had low plasma folate.

In the same school district as the first study, the prevalence, predictors and consequences of vitamin B_{12} deficiency were investigated in schoolers. Children aged 8–12 years were screened to identify 60 children with plasma vitamin B_{12} levels indicating deficiency, then these were matched by age, gender and school grade with similar numbers of children who had marginal and normal plasma B_{12} concentrations (final $n = 180$). Of the total of 554 children screened, 11% had deficient levels of plasma B_{12} and 22% had marginal values.[8] Mean plasma B_{12} concentrations fell with age across the age period. No child had serum folate <6.8 nmol/L, and only 1% had a value less than 13.4 nmol/L. Two percent had low serum ferritin and 5% were anemic. The prevalence of elevated serum MMA and plasma Hcy was significantly higher in the deficient and marginal groups than in those with normal plasma vitamin B_{12} concentrations; in the two inadequate groups about one third had elevated levels but in the normal group, only 7% had elevated values. Mean serum MMA was high in all groups compared to values reported in other populations. In spite of the normal plasma folate levels, plasma Hcy was more strongly inversely correlated with plasma folate ($r = -0.371$, $P<0.0001$) than with cobalamin ($r = -0.201$, $P<0.01$).

In this Guatemalan study predictors of the children's vitamin B_{12} status were evaluated.[9] The prevalence of *Helicobacter pylori* and bacterial overgrowth was not different across the B_{12} status groups. However, there was a significant positive correlation between serum MMA and serum gastrin suggesting that gastric inflammation due to *Helicobacter pylori* may play some role. The average daily intake of dietary vitamin B_{12} was 5.5 ± 5.2 ug/day, but 23% had intakes <1.8 ug/d. B_{12} intake was not significantly different among the plasma cobalamin groups, but it was significantly correlated with plasma vitamin B_{12} ($r = 0.167$, $P<0.03$).

Evaluation of the neurobehavioral function of the schoolers across the three vitamin B_{12} status groups revealed that lower plasma vitamin B_{12} predicted slower reaction times on perception and memory tasks, and less accurate reasoning.[10] There were no differences in motor skills or attention. In multiple regression models, both MMA and Hcy predicted the same functional deficits as low plasma B_{12}. Academic performance, and interest in working and learning, were negatively associated with plasma MMA, but not Hcy.[11]

In conclusion, there are no representative data for Guatemala. In the peri-urban region of Guatemala City, the prevalence of vitamin B_{12} deficiency in women, infants and children is very high, while almost no cases of low plasma folate were reported across the five studies. The strongest predictor of low plasma B_{12} in schoolers was their dietary intake of the vitamin. Low plasma and breast milk vitamin B_{12} concentrations in the group of lactating women may explain the fact that the majority of infants aged 7 to 12 months in a later study were deficient in the vitamin.

Costa Rica

Data on serum folate concentrations (but not on serum vitamin B_{12}) are available from the 1996 National Survey, and are described in more detail by Tacsan et al. elsewhere in this symposium. The Costa Rican data, using a representative national sample, are expressed in terms of severe (<6.8 nmol/L, which corresponds to the concentration used to define deficiency in this manuscript), moderate (6.8–13.9 nmol/L), normal (14–45 nmol/L), and high (>45 nmol/L) serum folate concentrations. In nonpregnant, nonlactating women of fertile age the prevalence of these values was 4, 11, 68, and 8%, respectively, and in children <6 y it was 2, 9, 66, and 22%, respectively. This survey indicates that there was a very low risk of inadequate folate intakes in Costa Rica.

Cuba

One study of serum vitamin B_{12} and folate concentrations was conducted in Cuba, two years after the neuropathy epidemic of 1993.[12] Volunteers were 141 healthy middle-aged men (mean age 39 y) from health centers in Havana, who gave blood samples every three months for one year. The prevalence of deficient plasma vitamin B_{12} values ranged from 52 to 82% across seasons, and the prevalence of marginal values ranged from

13 to 36%. Folate status of these men was also poor; 64% to 89% had low serum folate across the seasons, and about one third consumed less than 133 ug/day.

Chile

Chile collected information on serum folate as part of their assessment of the impact of folic acid fortification of wheat flour, which started in 2000. More information on the impact of the fortification is provided by Hertrampf elsewhere in this symposium. Prior to folate fortification there was a very high prevalence of both folate and vitamin B_{12} deficiency. Low serum folate was found in 25%, and low erythrocyte folate, in 65%. In the 598 women studied, serum vitamin B_{12} was <150 pmol/L (deficient) in 10% and marginal (150–221 pmol/L) in an additional 30%.

In a smaller study of free-living, low-middle socioeconomic status, elderly Chileans recruited from an outpatient clinic in Santiago, serum folate and vitamin B_{12} were also measured.[13] The 93 men and 181 women had a mean age of 70.1 ± 6.7 y (range 60–89 y). The prevalence of low serum folate was 33% in women and 50% in men. Serum vitamin B_{12} <150 pmol/L was found in 31% of women and 50% of men, and an additional 20% of women and 19% of men had marginal values. Only 4–5% of the total group had anemia and 1–2% had macrocytosis. Based on consumption of less than two thirds of the RDA, 23% of women and 14% of men consumed an inadequate amount of folate, and 45 and 34% respectively consumed insufficient vitamin B_{12}. The authors suggested that the low folate intake might be due to a low consumption of vegetables and fresh fruits and the custom of over-cooking foods. The poor B_{12} status was assumed to be due to atrophic gastritis.

A recent study of serum folate in Chilean elderly was conducted to evaluate the effect of flour fortification with folic acid.[14] A total of 208 people were recruited from public outpatient clinics in Santiago. Serum folate, B_{12}, and homocysteine were assessed at baseline and six months later. At baseline, vitamin B_{12} deficiency (in this study, <165 pmol/L) was found in 27.6%, low serum folate in 1.8% and hyperhomocysteinemia (>14 umol/L) in 31%. Six months later serum folate had increased from 16.2 ± 6.2 nmol/L at baseline to 32.7 ± 7.1 nmol/L (P<0.001) and mean homocysteine had fallen from 12.9 ± 3.7 to 11.4 ± 7.1 nmol/L (P<0.001). Vitamin B_{12} levels were unchanged.

Conclusion

Based on the prevalence data described above and summarized in Figure 1, the following conclusions can be

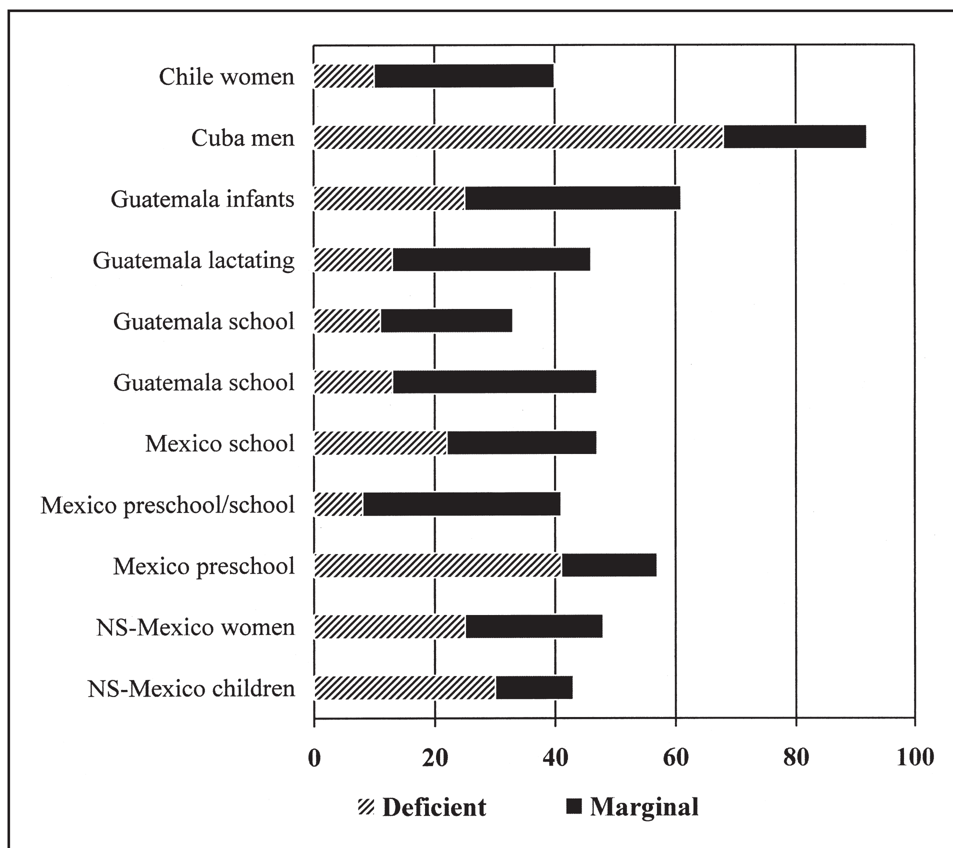

Figure 1. Prevalence (%) of deficient and marginal plasma B_{12} concentrations in Latin America. NS = National Nutrition Survey.

drawn:

1. There is a high prevalence of vitamin B_{12} deficiency (at least 40%) in all populations studied. This deficiency affected individuals from infancy through adult years. It is expected to be even more prevalent in the elderly but there are few data on this age group in Latin America.
2. Vitamin B_{12} concentrations in breast milk were low in rural Mexican and peri-urban Guatemalan women. This suggests that women and their infants may be at particularly high risk of deficiency, and its potential consequences, during the perinatal period.
3. The extraordinarily high prevalence of vitamin B_{12} deficiency implies that the vitamin be added as a fortificant to dietary staples. This may be especially important if folic acid is used as a fortificant.[1]
4. The prevalence of low plasma folate concentrations is highly variable. Folate deficiency does not seem to be a serious problem in Mexico, Guatemala, or Costa Rica. The highest prevalence of low serum folate was found in Cuban men and Chilean women.
5. Possible reasons for these differences in nutritional status among populations include variability in vitamin B_{12} intake from animal source foods, especially meats, and variability in folate intake from legumes (high in folate) vs. refined cereals such as white wheat flour (low in folate).
6. It remains to be determined whether there are any functional consequences of vitamin B_{12} deficiency in these populations.

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