High prevalence of cobalamin deficiency in Guatemalan schoolchildren: associations with low plasma holotranscobalamin II and elevated serum methylmalonic acid and plasma homocysteine concentrations[1–3]

Lisa M Rogers, Erick Boy, Joshua W Miller, Ralph Green, Jennifer Casterline Sabel, and Lindsay H Allen

ABSTRACT

Background: Studies conducted in Guatemala, Mexico, and Venezuela have found high prevalences of low plasma cobalamin (vitamin B-12) concentrations in infants and children. It is not known whether these low cobalamin concentrations are accompanied by altered metabolic functions.

Objective: We sought to assess the prevalence of cobalamin deficiency in Guatemalan children by using sensitive and specific markers of deficiency.

Design: Children ($n = 553$) were screened for low plasma cobalamin. Those with low plasma cobalamin (< 162 pmol/L) were matched by age, grade, and sex to those with marginal (162–221 pmol/L) and adequate (> 221 pmol/L) concentrations. In this matched subset ($n = 180$), additional biochemical indicators of cobalamin deficiency were measured.

Results: Of the 553 children screened, 11% had low plasma cobalamin and an additional 22% had marginal concentrations. The prevalences of elevated serum methylmalonic acid (MMA), plasma homocysteine, or both were significantly higher in children with low and marginal plasma cobalamin than in children with adequate plasma cobalamin. Mean serum MMA was high in all groups compared with values reported in other populations. Mean plasma holotranscobalamin II concentrations were significantly lower in children with low rather than marginal or adequate plasma cobalamin. However, holotranscobalamin II was a less sensitive indicator of cobalamin depletion than was MMA.

Conclusion: Biochemical markers of cobalamin deficiency confirmed that the cobalamin status of children with low and marginal plasma cobalamin is inadequate to support normal metabolic function.   *Am J Clin Nutr* 2003;77:433–40.

INTRODUCTION

Cobalamin (vitamin B-12) deficiency is uncommon in children in industrialized countries, occurring only in exclusively breast-fed infants born to mothers with poor cobalamin status (1, 2), children consuming vegan-type diets (3, 4), and children with inborn errors of cobalamin metabolism (5, 6). In contrast, cobalamin deficiency may be highly prevalent in children in developing countries. Studies in Mexico (7) and Venezuela (8) found that 33–52% of children had low plasma cobalamin concentrations. The significance of low plasma cobalamin concentrations in these populations remains unknown, but potential functional consequences of cobalamin deficiency include poor growth, megaloblastic anemia, and neurologic manifestations including changes in mood and altered sensory, motor, and cognitive functions (9–11). Less severe deficiency may cause only elevated serum or urinary methylmalonic acid (MMA) and plasma homocysteine, and lower plasma holotranscobalamin II. A study in Guatemala found that 12% of breast-fed infants had elevated urinary MMA (12). Holotranscobalamin II has been proposed as an early indicator of cobalamin deficiency (13, 14), but has only been compared with plasma cobalamin, MMA, and homocysteine concentrations in one study of adult patients who were attending cardiology clinics in India (15). No population-based surveys measuring all of these variables in adults or children have been reported.

The present study was designed to evaluate the prevalence, causes, and consequences of cobalamin deficiency in a sample of schoolchildren residing in a poor, peri-urban area of Guatemala. In this article, we report information on the prevalence of low plasma cobalamin and associated metabolic changes. In an initial screening phase, plasma cobalamin concentrations were measured, and samples from children with low values were matched to those from children with marginal and adequate cobalamin concentrations. In the second phase, which will be referred to as the metabolite substudy, the matched samples were analyzed for serum MMA, plasma homocysteine, and plasma holotranscobalamin II. Our objective was to determine whether low plasma

cobalamin concentrations were associated with abnormal metabolism, and if so, to identify the cutoff cobalamin values below which metabolic abnormalities occurred. In addition, the prevalence of low plasma folate concentrations was assessed to account for any contribution of folate deficiency to elevated plasma homocysteine. Hemoglobin and hematocrit were measured to determine whether cobalamin deficiency was associated with anemia.

SUBJECTS AND METHODS

Subjects

The study was conducted between June and October 1998 in 3 public primary schools located in a low socioeconomic status, peri-urban area of Guatemala City called El Mezquital. Informed consent was obtained from the parents of all children who were reportedly in good health, between the ages of 8 and 12 y, and enrolled in grades 2 through 6. The children were then screened with the goal of identifying 60 children with low plasma cobalamin concentrations (< 162 pmol/L). At the point when 60 children with low plasma cobalamin had been identified, 553 children had been recruited. For the metabolite substudy, all 60 children in the low-cobalamin group were matched to 60 children in each of the marginal-cobalamin (162–221 pmol/L) and adequate-cobalamin (> 221 pmol/L) groups, on the basis of age, sex, and grade in school. The sample size, $n = 60$ per group, was selected to detect differences in cognitive and psychoeducational scores among the cobalamin-status groups; these findings will be reported elsewhere.

The protocol was approved by the Human Subjects Research Committees at the University of California, Davis and the Instituto de Nutrición de Centro América y Panamá (INCAP), Guatemala City.

Height and weight

The weight and height of each child in the metabolite substudy was measured to the nearest 0.1 kg and 0.1 cm, respectively, with a digital scale and a wall-mounted stadiometer. Measurements were taken without shoes, with the child wearing only socks, shorts, and an undershirt.

Blood sampling and laboratory analysis

Blood samples were collected by venipuncture after an overnight fast and were immediately placed in a cooler with ice packs. Within ≈4 h after collection, whole blood was analyzed for hemoglobin (HemoCue, Mission Viejo, CA) and hematocrit (microhematocrit centrifuge) and was then centrifuged at 1500 $\times$ g for 20 min at 4 °C. Serum and EDTA-stabilized plasma samples were aliquoted and stored at −20 °C.

Plasma samples were analyzed for cobalamin and folate within 2 wk after collection with the MAGIC Vitamin B-12/Folate Radioassay, using ligands 1 and 3 for quality control (Chiron Diagnostics, Norwood, MA).

Additional analyses were performed on samples collected from the 180 children in the metabolite substudy. Serum MMA concentration was determined with gas chromatography–mass spectrometry (16) with one modification: MMA values were calculated by using a 2-point external calibration curve instead of by isotope dilution. We used d3-MMA as an internal standard. In our laboratory, the assay is linear to at least 50 000 nmol/L and has a sensitivity as low as 50 nmol/L. Intraassay CVs for normal (199 nmol/L) and high (5135 nmol/L) serum-based quality

control specimens are 7.0% and 3.0%, respectively ($n = 7$ for each). Interassay CVs for the same normal and high serum quality controls are 5.8% and 3.3%, respectively ($n = 20$ for each), and reference intervals (mean ± 2 SD) have been established for men (59–359 nmol/L; $n = 21$) and women (49–333 nmol/L; $n = 27$). In addition, the serum MMA analyses in this study were performed immediately after our participation in a national round-robin study of MMA assays in which values generated in our laboratory were found to be comparable with, and within range, of those from other laboratories (17).

Plasma total homocysteine was determined by using HPLC with fluorescence detection (18). In our laboratory, the assay is linear to at least 100 μmol/L and has a sensitivity of 1 μmol/L. Intraassay CVs for normal (7.5 μmol/L) and high (27.8 μmol/L) plasma-based quality control specimens are 1.3% and 1.1%, respectively ($n = 10$ for each). Interassay CVs for the same normal and high quality controls are 4.2% and 3.0%, respectively ($n = 23$ for each), and reference intervals (mean ± 2 SD) have been established for men (4.2–10.6 μmol/L; $n = 30$) and women (3.1–9.9 μmol/L; $n = 30$).

The amount of total plasma cobalamin bound to the carrier protein transcobalamin II was determined with an indirect assay that uses anti-transcobalamin II antibodies (19). For this assay, activated Sepharose beads were coupled with a polyclonal antibody against transcobalamin II produced in goats inoculated with rabbit transcobalamin II protein that had been purified by photodissociative affinity chromatography (20). The polyclonal antibody shows immunologic cross-reactivity with human holotranscobalamin II (21). Goat anti-rabbit transcobalamin II antibody-coated Sepharose beads were washed and resuspended as a 5% mixture in phosphate-buffered saline. One-mL aliquots of the washed beads were then pipetted into microfuge tubes and centrifuged at 2940 $\times$ g for 5 min at room temperature. The supernatant was aspirated carefully, leaving a semi-dry layer of beads at the bottom of the tube. An aliquot (500 μL) of each serum sample was added to the beads and mixed constantly and vigorously for 2 h at room temperature. At the end of 2 h, the microfuge tubes were centrifuged at 2940 $\times$ g for 5 min at room temperature and the supernatants were carefully aspirated without disturbing the bead layer. Cobalamin concentrations were determined in the supernatants by using a radioassay (Simultrac Radioassay; ICN Pharmaceuticals, Orangeburg, NY). The difference in cobalamin concentration between an aliquot of serum not subjected to bead treatment and an aliquot of bead-treated serum represents the concentration of holotranscobalamin II. The optimal assay conditions were determined in pilot experiments with ^{57}Co-cyanocobalamin radiolabelled holotranscobalamin II. We confirmed that the antibody-coated beads consistently removed >98% of holotranscobalamin II under these incubation conditions (data not shown). The mean ± SD for holotranscobalamin II in 22 nondeficient, normal adult volunteers was 77.8 ± 49.2 pmol/L, with an inclusive range of 28–225 pmol/L. The CVs within and between assays in nondeficient samples were 15% and 17%, respectively.

Serum creatinine was analyzed by the clinical laboratory at the University of California, Davis Medical Center to detect impaired renal function, which is associated with elevated plasma homocysteine and MMA concentrations (22, 23). All assays were performed with ≥2 serum or plasma quality controls (one low and one high).

Reference ranges

In clinical practice, the most commonly used reference ranges for plasma cobalamin are < 148 pmol/L to indicate low or deficient

TABLE 1
Characteristics of the total sample of Guatemalan children screened for plasma cobalamin and folate

	Boys ($n = 270$)	Girls ($n = 283$)	Total ($n = 553$)
Age (y)	10.2 ± 1.2[1]	10.1 ± 1.4	10.1 ± 1.3
Plasma cobalamin			
Value (pmol/L)	272 ± 117	297 ± 134[2]	285 ± 126
% Low (<162 pmol/L)	14	8	11
% Marginal (162–221 pmol/L)	24	19	22
Plasma folate			
Value (nmol/L)	29.0 ± 8.2	29.9 ± 9.3	29.4 ± 8.8
% Low (<6.8 nmol/L)	0	0	0
% Marginal (6.8–13.4 nmol/L)	1	1	1
Hemoglobin			
Value (g/L)	133 ± 10	133 ± 10	133 ± 10
% Anemic[3]	6	7	7
Hematocrit			
Value (%)	40 ± 3	40 ± 3	40 ± 3
% Anemic[4]	4	6	5

[1] $\bar{x} \pm$ SD.

[2] Significantly different from boys, $P = 0.009$ (unpaired t test).

[3] Defined as hemoglobin <118 g/L for children aged 8–11 y or <123 g/L for children aged 12 y (25, 26).

[4] Defined as hematocrit <35% for children aged 8–11 y or <37% for children aged 12 y (25, 26).

status, 148–221 pmol/L to indicate marginal or indeterminate status, and >221 pmol/L to indicate adequate or replete status. We used <162 pmol/L for low, 162–221 pmol/L for marginal, and >221 pmol/L for adequate status. The decision to use a slightly higher cutoff for the low range was made on the basis of reports that neurologic consequences of cobalamin deficiency are associated with values below this cutoff (10). Children with cobalamin deficiency were treated with vitamin supplements upon completion of the study. The reference ranges for plasma folate were <6.8 nmol/L for low, 6.8–13.4 nmol/L for marginal, and >13.4 nmol/L for adequate status (24).

There are no established reference ranges for either serum MMA or plasma homocysteine concentrations in children. Therefore, we used the 95th percentile of values (after the removal of 3 outliers) in children in the adequate-cobalamin group as the upper limit of the reference range; this was 624 nmol/L for serum MMA and 12.0 μmol/L for plasma homocysteine.

The reference range used for plasma holotranscobalamin II is derived from data collected by Goh et al (19) with the indirect immunoadsorption method described above. A plasma holotranscobalamin II value <18 pmol/L was highly specific and sensitive for detecting patients with cobalamin deficiency (plasma cobalamin concentration <150 pmol/L). Additional indicators of cobalamin deficiency were elevated serum MMA, presence of intrinsic factor antibodies, an abnormal Schilling test, clinical signs of cobalamin deficiency, or any combination of these indicators.

Anemia was defined as a hemoglobin concentration <118 g/L for children aged 8–11 y and <123 g/L for children aged 12 y, or a hematocrit <35% for children aged 8–11 y and <37% for children aged 12 y. These values were selected on the basis of World Health Organization guidelines (25), plus a 0.3 g/L correction of hemoglobin and a 1.0% correction of hematocrit for the altitude of 1500 m (26).

Statistical methods

Data are expressed as means ± SDs. Logarithmic or square-root transformation was used to normalize data with skewed distributions (plasma cobalamin, folate, MMA, homocysteine, and holotranscobalamin II) before statistical analysis. To assess mean differences between the sexes for continuous variables, an unpaired t test was used. One-way analysis of variance (ANOVA) was used for multiple comparisons of means for the low-, marginal-, and adequate-cobalamin groups. Chi-square tests were conducted to test for differences in percentages among groups. If a significant difference was found with ANOVA or chi-square, we performed a post hoc multiple comparison with Bonferroni correction. Correlations were calculated to obtain Pearson's product-moment correlation coefficients. Statistical significance was set at $P < 0.05$, except for when the Bonferroni correction was used for multiple comparisons, in which case statistical significance was set at $P < 0.0167$. Analyses were performed with STATVIEW (version 5.0.1, SAS Institute Inc, Cary, NC).

RESULTS

Screening

The 553 children screened (49% boys and 51% girls) ranged in age from 8 to 12 y (mean: 10.1 ± 1.3 y) (**Table 1**). Low plasma cobalamin concentrations (<162 pmol/L) were found in 60 of the 553 subjects (11%). An additional 122 subjects (22%) had plasma cobalamin concentrations in the marginal range (162–221 pmol/L). None of the subjects had a low plasma folate concentration (<6.8 nmol/L), and only 6 (1%) had plasma folate in the marginal range (6.8–13.4 nmol/L). Of these children with marginal plasma folate, 1 had low and 4 had marginal plasma cobalamin. A two-factor ANOVA with post hoc Bonferroni correction was performed to discriminate between the effects of age, sex, and their interaction on plasma cobalamin and folate. The interaction between age and sex was not significant for either plasma cobalamin or plasma folate ($P = 0.584$ and $P = 0.596$, respectively). Plasma concentrations of both vitamins became progressively lower with increasing subject age ($P < 0.0001$ for cobalamin and $P = 0.006$ for folate). Mean plasma cobalamin concentration in 12-y-olds was significantly lower than concentrations in 8-, 9-, and 10-y-olds, and mean plasma folate concentration in 12-y-olds was significantly lower than concentrations in 8- and 9-y-olds (ANOVA with Bonferroni correction, $P < 0.005$). After age was controlled for, girls had a significantly higher mean plasma cobalamin concentrations than did boys (two-factor ANOVA, $P = 0.008$); however, there was no significant difference in the mean plasma folate concentration between girls and boys (two-factor ANOVA, $P = 0.527$).

Plasma cobalamin and folate concentrations were significantly correlated ($r = 0.278$, $P < 0.0001$). We found weaker, but still significant, correlations between plasma cobalamin and hemoglobin ($r = -0.160$, $P = 0.0002$), plasma folate and hemoglobin ($r = -0.101$, $P = 0.020$), and plasma folate and hematocrit ($r = -0.094$, $P = 0.040$). The prevalence of anemia was 4–7% on the basis of hemoglobin or hematocrit.

Metabolite substudy

By design, there were no significant differences in age, grade, or sex among the 3 groups of children stratified by plasma cobalamin

TABLE 2
Descriptive characteristics of children with low, marginal, and adequate plasma cobalamin concentrations matched on the basis of age, sex, and grade in school[1]

| | Plasma cobalamin | | |
	Low (<162 pmol/L)	Marginal (162–221 pmol/L)	Adequate (>221 pmol/L)
Age (y)	10.8 ± 1.2^2	10.8 ± 1.1	10.7 ± 1.2
Sex (% boys)	60	57	59
Grade in school	3.8 ± 1.2	3.8 ± 1.2	3.8 ± 1.3
Height (cm)	137 ± 8.3	138 ± 9.2	135 ± 9.0
Weight (kg)	34.9 ± 7.6	35.0 ± 8.1	32.7 ± 7.0

[1] $n = 60$ in each group. There were no significant differences among the cobalamin groups with respect to any of the variables, $P > 0.05$ (ANOVA and chi-square).
[2] $\overline{x} \pm$ SD.

concentrations (**Table 2**). Heights and weights of the children did not differ among the groups.

Prevalence of abnormal values

The mean serum MMA concentration for all children in the metabolite substudy was 479 ± 315 nmol/L, and 22% had a value >624 nmol/L. Serum MMA and plasma homocysteine concentrations of children in the low-cobalamin group were significantly higher on average than those of children with plasma cobalamin in the adequate range (**Table 3**). The percentage of children with elevated serum MMA was significantly higher in the low-cobalamin group compared with the adequate-cobalamin group (32% and 5%, respectively; chi-square with Bonferroni correction, $P = 0.0003$) and in the marginal-cobalamin group compared with the adequate-cobalamin group (28% and 5%, respectively; chi-square with Bonferroni correction, $P = 0.002$) (**Table 4**).

The mean ($\pm$ SD) plasma homocysteine concentration for all children in the metabolite substudy was 9.4 ± 2.5 μmol/L, and 9% of children had a value > 12.0 μmol/L. Homocysteine concentrations > 12.0 μmol/L were found in a significantly higher proportion of children in the low-cobalamin group than in the adequate-cobalamin group (19% and 2%, respectively; chi-square with Bonferroni correction, $P = 0.002$). Elevations in serum MMA, plasma homocysteine, or both were found in approximately one-third of children with low and marginal plasma cobalamin, but in only 7% of those in the adequate group (Table 4). In unadjusted

analyses, boys had a significantly higher mean plasma homocysteine concentration than did girls (9.7 and 8.9 μmol/L, respectively; unpaired *t* test, $P = 0.046$).

Elevated serum MMA and plasma homocysteine concentrations do not appear to be caused by impaired renal function, because only one child had an elevated serum creatinine concentration and his serum MMA and plasma homocysteine concentrations were in the normal ranges. Mean plasma holotranscobalamin II concentrations were significantly lower in children with low cobalamin than in children with marginal or adequate cobalamin concentrations (Table 3).

Mean plasma folate was significantly higher in the adequate-cobalamin group than in the low-cobalamin group (Table 3). There was significantly less anemia in the low-cobalamin group than in the adequate-cobalamin group on the basis of hemoglobin concentrations (chi-square with Bonferroni correction, $P = 0.015$), but not on the basis of hematocrit values.

Associations among biochemical values

Plasma cobalamin concentration correlated strongly with holo-transcobalamin II concentration ($r = 0.528$, $P < 0.0001$) across the cobalamin-status groups. However, when this correlation was tested within groups, it was significant only within the adequate-cobalamin group ($r = 0.366$, $P = 0.004$). Plasma cobalamin correlated negatively with both serum MMA ($r = -0.217$, $P = 0.004$) and plasma homocysteine concentration ($r = -0.201$, $P = 0.008$) (**Figures 1** and **2**). Plasma holotranscobalamin II correlated negatively with plasma homocysteine ($r = -0.187$, $P = 0.02$) and serum MMA ($r = -0.159$, $P = 0.04$). Serum creatinine correlated with plasma homocysteine ($r = 0.170$, $P = 0.03$), but not with serum MMA ($r = 0.014$, $P = 0.860$). Plasma homocysteine had a stronger negative correlation with plasma folate ($r = -0.371$, $P \leq 0.0001$) than with cobalamin ($r = -0.201$, $P = 0.0079$).

DISCUSSION

The present study was conducted to assess the prevalence of low plasma cobalamin concentrations in peri-urban Guatemalan schoolchildren and to determine whether low cobalamin values were associated with indicators of abnormal biochemical function. In this population, 11% had low plasma cobalamin and an additional 22% had marginal values. A decline in plasma cobalamin concentrations with increasing age was observed; this pattern is consistent with that reported in healthy Belgian schoolchildren (27).

TABLE 3
Biochemical and hematologic values in children with low, marginal, and adequate plasma cobalamin concentrations matched on the basis of age, sex, and grade in school[1]

| | Plasma cobalamin | | |
	Low (<162 pmol/L)	Marginal (162–221 pmol/L)	Adequate (>221 pmol/L)
Serum MMA (nmol/L)	555 ± 396^a	534 ± 317^a	350 ± 147^b
Plasma homocysteine (μmol/L)	10.2 ± 3.5^a	$9.2 \pm 1.8^{a,b}$	8.8 ± 1.9^b
Plasma holotranscobalamin II (pmol/L)	26.5 ± 14.3^a	40.0 ± 21.9^b	53.8 ± 24.1^c
Plasma folate (nmol/L)	25.8 ± 9.2^a	$27.9 \pm 8.0^{a,b}$	31.8 ± 10.2^b
Hemoglobin (g/L)	135 ± 8	135 ± 11	132 ± 14
Hematocrit (%)	40 ± 2	40 ± 2	40 ± 3

[1] $\overline{x} \pm$ SD; $n = 60$ in each group. MMA, methylmalonic acid. Values in the same row with different superscript letters are significantly different, $P < 0.05$ (ANOVA with Bonferroni correction).

TABLE 4
Prevalence of coexisting abnormal concentrations of metabolites, plasma holotranscobalamin II, and folate in Guatemalan children with low, marginal, and adequate plasma cobalamin concentrations matched on the basis of age, sex, and grade in school[1]

	Plasma cobalamin		
	Low (<162 pmol/L)	Marginal (162–221 pmol/L)	Adequate (>221 pmol/L)
	n (%)[2]		
Elevated concentrations			
Serum MMA (>624 nmol/L)	17/53 (32)[a]	16/58 (28)[a]	3/56 (5)[b]
Plasma homocysteine (>12.0 μmol/L)	10/53 (19)[a]	4/58 (7)[a,b]	1/60 (2)[b]
Both MMA and homocysteine	5/53 (9)	2/58 (5)	0/56 (0)
MMA, homocysteine, or both	22/53 (42)[a]	18/58 (31)[a]	4/58 (7)[b]
Low concentrations			
Holotranscobalamin II (<18 pmol/L)	15/49 (31)[a]	6/58 (10)[b]	3/60 (5)[b]
Plasma folate (<6.8 nmol/L)	0/60 (0)	0/60 (0)	0/59 (0)
Hemoglobin[3]	0/57 (0)[a]	5/58 (9)[a,b]	6/60 (10)[b]
Hematocrit[4]	0/50 (0)	1/48 (2)	2/46 (4)

[1] MMA, methylmalonic acid. Values in the same row with different superscript letters have significantly different percentage abnormal, $P < 0.05$ (chi-square with Bonferroni correction).
[2] No. of subjects with abnormal values divided by total no. of subjects, with % abnormal in parentheses.
[3] Defined as hemoglobin <118 g/L for children aged 8–11 y or <123 g/L for children aged 12 y (25, 26).
[4] Defined as hematocrit <35% for children aged 8–11 y or <37% for children aged 12 y (25, 26).

Low plasma cobalamin was associated with elevated serum MMA and plasma homocysteine, and with lower plasma holo-transcobalamin II. There are no universally accepted reference values for these analytes, especially in children. van Dussel-dorp et al (4) reported mean serum MMA concentrations of 160 nmol/L in 94 Dutch adolescents consuming an omnivorous diet since birth and 270 nmol/L in 73 adolescents who had consumed a macrobiotic diet in early childhood. The authors used an upper-limit reference value of 410 nmol/L, selected on the basis of the 95th percentile of values in the omnivorous children. In the present Guatemalan study, the mean serum MMA concentration across the 3 cobalamin-status groups (479 ± 315 nmol/L) was much higher than in the Dutch

adolescents. The 95th percentile of serum MMA was 624 nmol/L for children with plasma cobalamin >221 pmol/L. MMA concentrations may be elevated by abnormal gut flora (28–30); propionic acid is a major metabolic product of colonic anaerobes, and MMA is produced from the breakdown of propionic acid. Nevertheless, the majority of serum MMA concentrations >624 nmol/L did occur in those children with a plasma cobalamin concentration <225 pmol/L. This 225 pmol/L value is very similar to that below which serum MMA increased in Dutch adolescents (4) and in elderly persons in the United States (31). An intervention study with supplemental cobalamin, limited-spectrum antibiotics, or both is needed to investigate the extent to which the generally higher average serum
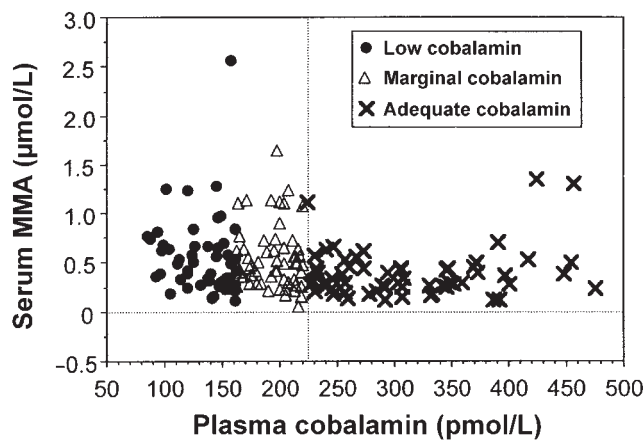
FIGURE 1. Correlation between plasma cobalamin and serum methylmalonic acid (MMA) concentrations in Guatemalan schoolchildren (*n* = 170); *r* = −0.217, *P* = 0.004. The majority of elevated serum MMA concentrations occurred when plasma cobalamin concentrations were <225 pmol/L.
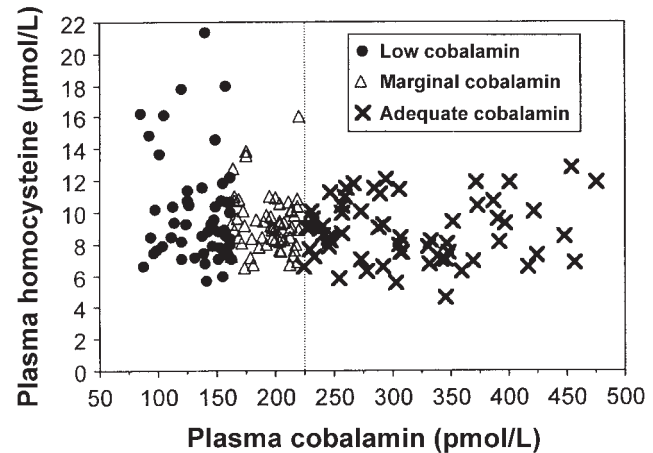
FIGURE 2. Correlation between plasma cobalamin and plasma homocysteine concentrations in Guatemalan schoolchildren (*n* = 172); *r* = −0.201, *P* = 0.008. The majority of elevated plasma homocysteine concentrations occurred when plasma cobalamin concentrations were <225 pmol/L.

MMA concentrations in Guatemalan children result from relatively poor cobalamin status or from differences in gut flora.

Reported values for plasma homocysteine in US and European children range from 4.8 to 8.3 μmol/L (4, 27, 32–36). Variations in these values may result from differences in analytic methods and statistical analyses, and from differences among the populations studied. Ethnic differences in plasma homocysteine have been documented in South African children aged 7–15 y (33), where black children had significantly higher homocysteine than did white children (5.8 and 5.1 μmol/L, respectively). In the present study, we found that the mean plasma homocysteine concentration (9.4 ± 2.5 μmol/L) in Guatemalan children was higher than that reported for children in the literature. In addition, the association between cobalamin and homocysteine was less strong than that between homocysteine and folate, even though none of the subjects had low plasma folate and only 1% had marginal values. The mean (±SD) serum folate for children in all cobalamin-status groups was 28.6 ± 9.4 nmol/L, which is similar to that reported in 600 healthy Turkish children aged 7–11 y (24 ± 12 nmol/L) (37) but is higher than the median value (14.6 nmol/L) reported in 94 omnivorous Dutch adolescents (4). Fortification of wheat flour with folic acid and the occasional consumption of chicken and beef liver could explain the low prevalence of low plasma folate concentrations in these Guatemalan schoolchildren.

Holotranscobalamin II is the transport protein for newly absorbed cobalamin and has been proposed as an early indicator of cobalamin deficiency and possibly of malabsorption of the vitamin (13, 14, 38). Fourteen percent of children in the metabolite substudy had a low holotranscobalamin II concentration; most (64%) of these children had low plasma cobalamin. However, our results do not support the idea that holotranscobalamin II is a more sensitive indicator of cobalamin depletion than is MMA (eg, in the marginal cobalamin group, about 3 times as many children had elevated MMA than had low holotranscobalamin II). Moreover, plasma holotranscobalamin II concentrations were less strongly correlated with serum MMA and plasma homocysteine than with plasma cobalamin. Further research is needed to determine the extent to which low holotranscobalamin II results from *1*) overall poor cobalamin status, *2*) recent or current malabsorption, or *3*) recent low intake of the vitamin.

The prevalence of anemia, determined on the basis of hemoglobin values, was lower in children with low plasma cobalamin than in those with marginal or adequate plasma cobalamin, and there was a weak negative correlation between plasma cobalamin and hemoglobin in the total population screened. This association was not significant in the metabolite substudy. Thus, there was no evidence that cobalamin deficiency is a cause of anemia in these children.

There is evidence that the severity of cobalamin-related neurologic symptoms is greater when subjects do not present with anemia (39, 40). Thus, the low prevalence of anemia and high prevalence of biochemical cobalamin deficiency in these children may make the early identification of cobalamin deficiency especially important in this and other populations in developing countries. Persons with cobalamin concentrations as high as 258 pmol/L have been found at increased risk of neurologic signs and symptoms of cobalamin deficiency, and of hyperhomocysteinemia (10, 31, 39, 41, 42).

Cognitive impairment can result from cobalamin deficiency (9, 10, 43, 44). Our evaluation of the psychoeducational performance of these Guatemalan children indicates that reasoning, short-term

memory, and perception were poorer in the low-cobalamin group than in the adequate-cobalamin group (45). Serum MMA was also negatively related to academic performance and adaptive function and positively related to attention problems (46). Also, humoral and cellular immune dysfunctions have been reported in disorders related to cobalamin deficiency (47–50). Cobalamin intervention studies are clearly needed to ascertain the extent of the adverse effects of cobalamin deficiency on the metabolic, neurologic, cognitive, and immune function of these children, and the reversibility of these effects with supplementation.

Factors that may contribute to poor cobalamin status include inadequate dietary intake and malabsorption of the vitamin. Most (80–90%) of the children in the present study consumed beef or chicken at least weekly, and 35% consumed eggs daily (unpublished observations). The average daily consumption of vitamin B-12 sources was ≈20 g meat (beef and chicken), ≈1 egg, and 58 mL milk. Vitamin B-12 intakes of omnivores are expected to be adequate even when animal product intake is relatively low, because of the efficient absorption and enterohepatic recirculation of the vitamin. However, this is uncertain because several studies reported a higher prevalence of low serum cobalamin in lactoovovegetarians than in omnivores (15, 51, 52). Another possibility is that cobalamin deficiency is caused by a malabsorptive condition that interferes with uptake of the vitamin from both the diet and the enterohepatic circulation. *Helicobacter pylori* infection, subsequent bacterial overgrowth, or both are conditions that have been associated with poor cobalamin status. We are currently evaluating these conditions and dietary intake of vitamin B-12 in this population.

In summary, the high prevalence of low and marginal plasma cobalamin concentrations was accompanied by signs of abnormal biochemical function. Serum MMA concentrations increased when plasma cobalamin concentrations were below ≈225 pmol/L, a value similar to those found in other population groups. These data suggest that attention needs to be paid to screening, treating, and preventing cobalamin deficiency in Guatemala and other developing countries. Further research is also needed to investigate the causes and consequences of cobalamin deficiency in children in these populations. ☘

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