Breast Milk Vitamin B-12 Concentrations in Guatemalan Women Are Correlated with Maternal but Not Infant Vitamin B-12 Status at 12 Months Postpartum1,2

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Abstract
In our previous studies, one-third of lactating Guatemalan women, infants, and children had deficient or marginal serum vitamin B-12 concentrations. Relationships among maternal and infant status and breast milk vitamin B-12, however, have not, to our knowledge, been investigated in such populations. Our purpose was to measure breast milk vitamin B-12 in Guatemalan women with a range of serum vitamin B-12 concentrations and explore associations between milk vitamin B-12 concentrations and maternal and infant vitamin B-12 intake and status. Participants were 183 mother-infant pairs breastfeeding at 12 mo postpartum. Exclusion criteria included mother <17 y, infant <11.5 or >12.5 mo, multiple birth, reported health problems in mother or infant, and mother pregnant >3 mo. Data collected on mothers and infants included anthropometry, serum and breast milk vitamin B-12, and dietary vitamin B–12. Serum vitamin B-12 concentrations indicated deficiency (<150 pmol/L) in 35% of mothers and 27% of infants and marginal status (150–220 pmol/L) in 35% of mothers and 17% of infants. In a multiple regression analysis, breast milk vitamin B-12 concentration was associated (P < 0.05) with both maternal vitamin B-12 intake (r = 0.26) and maternal serum vitamin B-12 (r = 0.30). Controlling for the number of breastfeeds per day and vitamin B-12 intake from complementary foods, infant serum vitamin B-12 was associated with maternal serum vitamin B-12 (r = 0.31; P < 0.001) but not breast milk vitamin B-12, implicating a long-term effect of pregnancy status on infant vitamin B-12 status at 12 mo postpartum. J. Nutr. 142: 112–116, 2012.

Introduction
Vitamin B-12 deficiency is emerging as a public health concern in many developing countries. A review of studies in Latin America revealed that it occurs in ~40% of women and their children due to their low intake of animal source foods (1). In a nationally representative Mexican National Nutrition Survey, we found that 26% of children <5 y and the same proportion of women were deficient (serum vitamin B–12 <150 pmol/L) and 18 and 22%, respectively, were classified with marginal status (150–220 pmol/L) (M. Anaya and L. Allen, unpublished data). A WHO Consultation identified infants, preschool children, and pregnant and lactating women as the groups most vulnerable to vitamin B-12 deficiency (2). The symptoms of vitamin-B12 deficiency include developmental delays, developmental regression, and poor growth. A collection of case studies concerning clinically vitamin B-12-deficient infants who were exclusively breastfed by strict vegetarian or pernicious anemic mothers revealed that these symptoms appear at around 4–7 mo of age (3).

Vitamin B-12 may be secreted in inadequate amounts in breast milk when the mother’s intake or stores are low. In previous studies conducted in a community adjacent to that in our current investigation, 12% of infants had deficient concentrations of vitamin B-12 by age 3 mo (4) and 62% by age 7 mo (2). Moreover, the infants who consumed cow milk more frequently as a substitute for breast milk had higher serum vitamin B-12 concentrations than infants receiving only breast milk (2). Whether this was due to low vitamin B-12 concentrations in breast milk or to the fact that the vitamin B-12 content per unit energy is higher in cows milk has not been determined. It was evident, however, that the infants with low serum vitamin B-12 at 12 and 21 mo had slower motor development and were shorter and weighed less compared to those with normal status (5).

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Participants and Methods

The field research was conducted jointly between the UC Davis and INCAP in Guatemala City. The study protocol was reviewed and approved by 2 ethical review committees in Guatemala and by the Human Research Protection Office at UC Davis. Informed consent was obtained from mothers at the screening visit, which preceded implementation of the study protocol.

Participants were inhabitants of a low-income, peri-urban neighborhood, San Jose La Comunidad, near Guatemala City. Our previous studies in this area revealed a high prevalence of vitamin B-12 deficiency in mothers and infants (4,6). Eligibility criteria included: mother aged ≥17 y and infant 11.5–12.5 mo, singleton birth, no reported health problems, no severe developmental delays (baseline Bayley II Mental Score <64), no severe stunting (HAZ <−3.0) or wasting (WHZ <−2.0), normal infant hemoglobin concentration (≥95 g/L, corrected 5.0 g/L for altitude, 1386 m), and mother not pregnant or <3 mo pregnant.

A total of 299 women were recruited for the intervention study, of whom 239 (80%) were still lactating and 210 (70%) produced a baseline milk sample. Vitamin B-12 was analyzed in breast milk of 183 of these women for whom there were complete data on all other variables measured in this substudy.

Anthropometry.

Two trained field workers measured the recumbent length of each infant to the nearest 0.1 cm using a wooden length-measuring board. Weight was taken to the nearest 0.01 kg with an electronic weighing scale (Tanita) and minimal clothing. Maternal height (to 1 cm) and weight (to 0.1 kg) were measured in the field office after removal of shoes and heavy clothing.

Socioeconomic data.

Socioeconomic status was determined based on information obtained from both parents when possible by using a questionnaire validated by INCAP (6). This included education, occupation, income, characteristics of the home, and living conditions.

Dietary intake.

A trained examiner administered a semiquantitative FFQ previously validated in this community to assess maternal vitamin B-12 intake (6). The women reported their consumption (frequency and usual amount) of items from a list of the foods, including fortified items, which provide vitamin B-12 in this community, and intake of supplements over the past month. The USDA food composition database (7) and food tables specific to Central America (8) were used to estimate vitamin B-12 intake from these sources. The vitamin B-12 in organ meats (beef and chicken liver, kidney, brain, and intestines) was calculated as 20% of the actual content, because absorption from vitamin B-12-rich foods is approximately one-fifth as efficient as from most food sources of the vitamin (9). The breastfeeding status of infants was proxied as the number of breastfeeding events over the previous 24 h reported by mothers. Each mother was also queried about any foods or liquids given to her infant during the same time period.

Sample collection.

Nonfasting maternal and infant blood samples (6 mL) were collected by venipuncture from the inner arm (mothers) or the back of the hand (infants) in trace mineral-free heparinized vacutainers (Becton-Dickinson). The samples were placed on ice in the dark and transported to INCAP, where serum was separated by centrifugation, aliquoted into 1-mL vials, and frozen at −55°C for subsequent analysis at UC Davis. Breast milk was collected from the mothers in the field office with no restriction regarding time since last feed. After cleaning the nipple, each woman hand-expressed her milk (~50 mL) into a presterilized and freezer-safe bag with a leak-proof seal.

Analysis of vitamin B-12 in serum and breast milk.

The serum vitamin B-12 concentration was measured at UC Davis by a competitive protein-binding radioassay with hog intrinsic factor as the vitamin B-12 binder (Folate/B-12 SimulTRAC Boil RIA kits, Biomedicals). Cutoffs of <150, 150–220, and ≥220 pmol/L were used to classify mothers and infants as having deficient, marginal, or adequate vitamin B-12 status, respectively (10). Breast milk samples were analyzed for vitamin B-12 using a recently published method (11). Briefly, milk was pretreated with cobinamide-coated, epoxy-activated, hydrophilic sepharose to remove the vast majority of apo-HC, because this would otherwise influence the accuracy of the analysis. The vitamin B-12 concentration was analyzed using an Advia Centaur Analyzer (Siemens Medical Solutions Diagnostics), which has a low-end detection limit of 50 pmol/L (imprecision <10%).

Statistical analysis.

SPSS (version 15.0 for Windows) was used to perform the statistical analysis. A nonparametric Kruskal-Wallis test was used to find overall differences among vitamin B-12 status groups and a Mann-Whitney U test detected significant differences between groups. Multiple linear regressions were used to test the predictors of breast milk vitamin B-12 concentration and serum vitamin B-12 (maternal vitamin B-12 intake and serum vitamin B-12). The infant outcomes were controlled for number of breastfeeds per day and vitamin B-12 intake from complementary foods. Infant anthropometric variables were not included as covariates, because they were not significantly associated with vitamin B-12 status. P ≤ 0.05 was considered significant for all analyses. All variables were assessed for conformance to the normal distribution and transformations were performed as necessary.

Results

Characteristics of households.

The study population was primarily Ladino, a distinct ethnic group of nonindigenous Spanish-speaking Guatemalans. The median yearly income was US $1800, of which the majority was spent on food (Table 1). All households had electricity and 44% had an indoor toilet. More than one-half of the households shared an outdoor kitchen with their neighbors. Most of the mothers (78%) did not work outside the home and their level of education was low. The infants were moderately stunted but had close to normal WHZ.

Maternal and infant vitamin B-12 status.

The median maternal serum vitamin B-12 concentration was 173 pmol/L (range 57–847 pmol/L) (Table 2). Values indicated deficiency in 35% of mothers and a marginal vitamin B-12 status in an additional 35%. Median dietary intake of vitamin B-12 was 2.6 μg/d, with 46% consuming less than their EAR of 2.4 μg/d.

Concentrations of vitamin B-12 in breast milk were very low, with values below the limit of detection of the assay (≤50 pmol/L) in 65%. Making the assumption that concentrations

8 Abbreviations used: apo-HC, unsaturated haptocorrin; EAR, Estimated Average Requirement; HAZ, height-for-age Z-score; INCAP, Institute of Nutrition of Central America and Panama; UC Davis, University of California, Davis; WHZ, weight-for-height Z-score.
TABLE 1 Characteristics of the household, mothers, and infants at 12 mo postpartum

<table>
<thead>
<tr>
<th>Characteristic</th>
<th>Persons in household, n</th>
<th>Children &lt;5 y old, n</th>
<th>Household income, $/y</th>
<th>Food expenditure, $/d</th>
<th>Mother’s BMI, kg/m²</th>
<th>Pregnancies, n</th>
<th>Mother’s education, y</th>
<th>Food expenditure, $/y</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>4 (2–10)</td>
<td>1.0 (1–3)</td>
<td>1800 (200–8000)</td>
<td>4.7 (1.1–6.2)</td>
<td>25 (17–43)</td>
<td>6 (0.9)</td>
<td>3 (1–10)</td>
<td>25 (16–42)</td>
</tr>
<tr>
<td>Infant’s age, mo</td>
<td>12.3 (11.7–13.6)</td>
<td>–1.45 (–3.30 to 0.87)</td>
<td>–0.46 (–2.09 to 2.81)</td>
<td>12 (0–30)</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

1 Values are median (range); n = 183. HAZ, height-for-age Z-score; WHZ, weight-for-height Z-score.

<50 pmol/L were measured accurately, median values were significantly lower in milk from mothers with serum vitamin B-12 in the deficient and marginal categories than in those with adequate status. In the mothers with serum vitamin B-12 indicating an adequate status, the median breast milk concentration was only 69 pmol/L (min <50 pmol/L, max 1300 pmol/L), with 44% of the values <50 pmol/L. Using the same cutoffs for serum vitamin B-12 in infants as in mothers, there was a substantially higher prevalence (~50%) of serum vitamin B-12 indicating deficiency in infants born to mothers with either a low or a marginally low serum vitamin B-12 level (Table 2).

There were no significant differences in maternal dietary intake of vitamin B-12 or frequency of breastfeeding across the 3 maternal vitamin B-12 status groups. The infants were being breastfed a median of 12 times/d with a median vitamin B-12 intake of 0.9 μg/d from complementary foods, of which 0.8 μg/d was from unfortified dry cow milk. This intake is 180% of the current adequate intake for infants aged 7–12 mo (0.5 μg/d) and 128% of the EAR for children 1–3 y (0.7 μg/d). Infant Z-scores did not differ by maternal vitamin B-12 status category.

Correlations among vitamin B-12 status indicators. Regression analyses were conducted to test a theoretical model predicting that maternal vitamin B-12 intake and maternal serum vitamin B-12 concentration would affect the amount of the vitamin in breast milk and subsequently in infant serum (Fig. 1). Although infant serum vitamin B-12 was positively correlated with breast milk concentrations of the vitamin in a bivariate analysis (r = 0.29; P < 0.0001), it was also correlated with intake from complementary foods. When vitamin B-12 consumption from complementary foods and number of breastfeeding episodes per day was controlled for in the multiple regression analysis, both maternal intake and maternal serum vitamin B-12 correlated with milk vitamin B-12 concentration, but infant serum vitamin B-12 concentration was not correlated with milk vitamin B-12. Rather, infant serum vitamin B-12 was significantly predicted by maternal serum vitamin B-12 concentration and intake of the vitamin from complementary foods.

Discussion

To the best of our knowledge, this study is the first to analyze and compare breast milk vitamin B-12 concentrations to maternal intake and serum concentrations, and infant status in mother–infant dyads from a community with chronically poor vitamin B-12 status. Additionally, because accurate assessment of vitamin B-12 in human milk is difficult due to its high concentration of haptocorrin, a strong vitamin B-12 binder, we used a new, validated method for analyzing the concentration of vitamin B-12 in human milk.

The data revealed serum vitamin B-12 concentrations that suggest a high prevalence of vitamin B-12 deficiency in both the mothers and infants, thereby supporting our previous studies in this community (2, 4). Approximately two-thirds of the women had low or marginal serum vitamin B-12 concentrations. Although maternal depletion may have been exacerbated by the long duration of breastfeeding, our previous studies found a

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TABLE 2 Vitamin B-12 status, intake, and infant Z-scores of Guatemalan mother-infant pairs at 12 mo postpartum grouped by maternal serum vitamin B-12 concentration

<table>
<thead>
<tr>
<th>Vitamin B-12 Intake</th>
<th>Deficient</th>
<th>Marginal</th>
<th>Adequate</th>
</tr>
</thead>
<tbody>
<tr>
<td>Maternal serum, pmol/L</td>
<td>121a (57–147)</td>
<td>176a (149–217)</td>
<td>291a (221–847)</td>
</tr>
<tr>
<td>Breast milk, pmol/L</td>
<td>22a (0–738)</td>
<td>25a (0–644)</td>
<td>63a (50–1300)</td>
</tr>
<tr>
<td>Infant serum, pmol/L</td>
<td>143a (45–471)</td>
<td>161a (48–545)</td>
<td>235a (98–866)</td>
</tr>
<tr>
<td>% Deficient</td>
<td>53a</td>
<td>44a</td>
<td>19a</td>
</tr>
<tr>
<td>% Marginal</td>
<td>23</td>
<td>28</td>
<td>22</td>
</tr>
</tbody>
</table>

Vitamin B-12 intake

- Maternal intake, μg/d: 2.4 (0.2–23)
- Breastfeeding, times/d: 14 (0–30)
- Infant intake, μg/d: 1.0 (0.1–4)

Infant Z-scores

- HAZ: –1.45 (–2.79 to 0.62)
- WAZ: –1.34 (–2.06 to 2.62)
- WHZ: –0.46 (–1.83 to 2.81)

1 Values are medians (range) or percentage. Values in a row with superscripts without a common letter differ, P < 0.05. HAZ, height-for-age Z-score; WAZ, weight-for-age Z-score; WHZ, weight-for-height Z-score.
2 Maternal serum vitamin B-12: deficient, <150 pmol/L; marginal, 150–220 pmol/L; adequate, >220 pmol/L.
3 From complementary foods.
similar prevalence of low serum vitamin B-12 after 3 mo of lactation (4). Although there is some uncertainty concerning the appropriate cutoffs for diagnosing vitamin B-12 deficiency in infants, data from Norway that included measures of serum methylmalonic acid indicated that by age 12 mo, it is appropriate to use the same cutoffs as in adults (11). Based on this approach, serum levels of vitamin B-12 in the infants indicated that 39% were deficient, 24% were marginal, and 36% had normal status. Interestingly, maternal serum vitamin B-12 was still strongly correlated with infant serum vitamin B-12 at 12 mo postpartum, whereas milk B-12 concentration was not, suggesting a continuing influence of maternal status originating in utero or early postnatal life. The observation that infant serum vitamin B-12 was correlated with intake of vitamin B-12 from complementary foods suggests that these foods (primarily unfortified, dry cow milk) provided substantially more of the vitamin than breast milk even though breastfeeding was still occurring about 12 times/d. Milk vitamin concentrations may also have varied throughout the day in response to maternal intake in meals, which would not have been captured in our milk collection protocol and could have reduced the strength of any associations.

Published values for the concentration of vitamin B-12 in human milk are sparse and highly variable as a result of different analytical methods, different diets, and differences in maternal vitamin B-12 supplementation during pregnancy and/or lactation (12–14). Human milk has a much higher vitamin B-12 binding capacity than serum because of its higher content of apo-HC. Our previous analysis of vitamin B-12 in human milk using different commercially available platforms for analysis showed that >10 nmol/L apo-HC affected the analytical results. Removal of apo-HC by absorption to cobinamide-coated Sepharose resolved this problem and was therefore used prior to analysis in the present study (10).

A limitation of our milk vitamin B-12 analyses is that instruments in clinical laboratories are designed to measure the vitamin in serum, and a high percentage (65%) of our samples fell at or below the 50 pmol/L lower limit of detection. Whereas values below this limit are of questionable accuracy, for the purposes of the current research, this is of limited importance; there is little doubt that vitamin B-12 is almost nonexistent in breast milk from deficient and marginal status mothers. Although our milk collection method did not control time of day or time of feeding, in the one study considering this question, vitamin B-12 concentrations did not differ between fore- and hind-milk or with time of day (15). A high, intra-individual variability in concentrations suggests that more samples need to be collected from each woman in future studies.

Accepting the validity of the dietary assessment, the proportion of women with marginal or deficient vitamin B-12 status based on their serum vitamin B-12 appears to be somewhat discrepant with their intake of the vitamin. However, about one-half of the group did not meet their EAR. Also, maternal serum concentrations of vitamin B-12 reflect long-term intake and it is possible that these women did attempt to improve their nutrient intake during pregnancy and lactation, e.g. by increasing their intake of vitamin-fortified cereals such as Incaparina.

To date, the only group whose breast milk vitamin B-12 was analyzed using the same method as that of the Guatemalan women was a group of 24 healthy women 1–3 mo postpartum and residing in the San Francisco region of California. The California participants were of low socioeconomic status and mixed ethnicity. Although the samples were collected at an earlier stage of lactation, vitamin B-12 concentrations in milk do not change greatly after falling from their highest values in colostrum (15). It is also true that most of the Californian women had taken multiple micronutrient supplements during pregnancy and lactation, which provided 6 µg/d of vitamin B-12. These supplements may have increased maternal serum and liver stores of the vitamin and, subsequently, concentrations in breast milk. Forty percent of the milk samples contained >800 pmol/L vitamin B-12, with a median of 565 pmol/L and none had a value ≤50 pmol/L.

DRI for vitamin B-12 during lactation and infancy are based on the concentration of vitamin B-12 in breast milk. The value used in the current DRI is 0.42 pg/mL (315 pmol/L) based on sparse data, i.e., samples from 9 unsupplemented Brazilian women, and presumably inadequate removal of apo-HC prior to analysis of vitamin B-12. Serum vitamin B-12 concentrations in breast-fed infants have been reported as lower than in those who are formula fed (16). These data do not call into question the well-established superiority of human milk for the nutrition of human infants (17). Our results do, however, suggest a need to improve the vitamin B-12 status of lactating women. In the Guatemalan situation, options still to be evaluated include maternal supplementation during pregnancy and/or lactation, infant supplementation starting soon after birth, and fortification of the food supply.

Acknowledgments
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