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VITAMIN B_{12} STATUS OF EAST INDIAN VEGETARIAN LACTATING WOMEN LIVING IN THE UNITED STATES

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ABSTRACT

The purpose of this study was to determine the vitamin B_{12} status of vegetarian, lactating women who had immigrated to the US from India. Women exclusively breast-feeding their 4-20 week old infants were studied [8 lactovegetarians (VEG group), 11 omnivorous women (CONTROL group)]. Fasting maternal blood and breast milk samples were collected and analyzed for B_{12} concentrations; a random urine sample was collected from infants to determine methylmalonic acid (MMA) concentration. All subjects, except three VEG, reported taking vitamin supplements containing 2 to 25 μg of vitamin B_{12} regularly during pregnancy and lactation. Vitamin B_{12} intake from dietary sources and total intake (dietary plus supplement) was significantly lower in the VEG group compared to CONTROL subjects (1.35 ± 0.27 vs 2.61 ± 0.23, $p<0.01$ and 4.60 ± 1.43 vs 11.70 ± 2.47 μg/d, $p = 0.04$; respectively). Serum and milk vitamin B_{12} concentrations in VEG women were significantly lower than the CONTROL group (264 ± 52 vs 531 ± 78 pmol/L, $p = 0.02$ and 277 ± 39 vs 544 ± 104 pmol/L, $p = 0.05$; respectively). After controlling for diet, the mean milk vitamin B_{12} concentration of mothers at 16-20 wks postpartum was significantly lower than mothers 4-8 wks postpartum ($p = 0.04$). Assuming that infants consumed 750 ml of milk/d, 75% of infants of VEG mothers and 27% of infants of CONTROL mothers were not consuming the RDA for vitamin B_{12}. All MMA concentrations were within normal limits except for one infant whose mother consumed a VEG diet. These results suggest the importance of supplementation with vitamin B_{12} during lactation among lactovegetarian women.

KEY WORDS: Vitamin B_{12} Lactation Vegetarians Infant Nutrition Human Milk

INTRODUCTION

Vitamin B_{12} deficiency during lactation may be more prevalent than previously thought, especially among women consuming low amounts of animal protein and/or living in developing countries (1). Several cases of vitamin B_{12} deficiency in exclusively breast-fed infants have been reported in India as well as in other parts of the world. Most of these cases have been traced to maternal B_{12} deficiency, usually from consuming vegetarian diets (2-8). Theoretically, newborn infants of B_{12} depleted mothers will have insufficient neonatal stores of the vitamin. After birth, their status may be further compromised since a positive relationship between maternal serum and milk B_{12} concentrations has also been reported (6, 8-12). In addition, elevated concentrations of urinary methylmalonic acid (MMA), a biochemical indicator of vitamin B_{12} deficiency, have been reported in exclusively breast-fed infants of vegetarian mothers with decreased vitamin B_{12} status (2, 8, 11, 13, 14).

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Presently, the Institute of Medicine recommends a supplement of 2.6 μg of vitamin B_{12} daily for complete vegans, but not lactovegetarians (15). However, studies in India have shown that lactovegetarians have lower serum B_{12} concentrations than omnivores, and breast milk B_{12} concentrations are lower in lactovegetarian mothers than in omnivorous mothers (16-19). The milk B_{12} concentrations of East Indian lactovegetarian mothers living in the United States (US) may not be as low as concentrations in India, because of the availability of prenatal vitamin supplements and dairy products. However, it is not known whether East Indians increase their consumption of dairy products and regularly take their prenatal vitamins during pregnancy and lactation when they immigrate to the US. Therefore, the purpose of this study was to determine the vitamin B_{12} status of East Indian lactovegetarian mothers living in the US.

METHODS AND MATERIALS

Subjects

Lactating women, who were free from any chronic illness, did not smoke, and gave birth to healthy, full term (37-42 weeks) infants were recruited for the study. Infants were at least 4 weeks of age and were exclusively breast-fed at the time of sample collection. The vegetarian group (VEG) consisted of eight East Indian women consuming either a lactovegetarian diet (5 women) or a lacto-ovovegetarian diet (3 women consumed one egg/week). Five East Indian women and 6 Caucasian women were in the omnivorous group (CONTROL). VEG mothers were predominantly Hindu and lifelong vegetarians. This study was approved by the Human Subjects Review Board at the University of North Carolina at Greensboro.

Dietary intake and anthropometric measurements

Vitamin B_{12} intake was estimated at the same time breast milk and blood samples were collected. Using a food-frequency questionnaire, researchers interviewed subjects to determine the number of daily servings of dairy products and weekly servings of meat (including fish and poultry) and eggs consumed during lactation. Intake of vitamin B_{12} supplements by mothers during pregnancy and lactation was also recorded. The estimated daily dietary intake of vitamin B_{12} was calculated by multiplying the number of servings of dairy products, meat (including fish and poultry) and eggs consumed by the average vitamin B_{12} content in one serving of each of these food groups, determined using food composition tables (20). The amount of vitamin B_{12} assigned to the meat, fish, and poultry group was 1.3 μg/serving, the dairy group was 0.72 μg/serving and 0.5 μg was the estimate for each egg consumed. While food frequency questionnaires provide a better qualitative rather than quantitative indicator of food intake, this method is appropriate to distinguish between gross estimates of intake, which one sees with vitamin B_{12} and omnivores and lactovegetarians. Infants' weights were measured to the nearest 10 g on a portable, digital balance (Seca, model 735) and lengths were measured to the nearest centimeter on an infant measuring board.

Serum and milk B_{12} concentrations

A fasting blood sample was collected from each subject in the morning. Serum was separated from the blood and frozen at -20°C. Mothers expressed all the milk from one breast at the first feeding of the day and samples were frozen at -20°C. Vitamin B_{12} concentrations in the serum and milk were determined using a radioassay technique that employs purified hog intrinsic factor as binder (Quantaphase B_{12}, Bio-Rad, Greensboro, NC). Samples were analyzed in duplicate and the coefficient of variation for the serum and milk assay was 2.6% and 2.3%, respectively. Although this method is intended for determination of cobalamin in blood serum, it is also adequate for assessing this vitamin in milk (21).

Methylmalonic acid concentrations

To determine MMA concentrations, a urine sample from all infants was collected using pediatric urine bags. Samples were frozen at -20°C until analyzed. Semiquantitative urinary MMA

concentrations and creatinine concentrations were determined by the methods described by Ibbott (22). MMA was extracted from acidified urine and separated from other urine components by thin layer chromatography. The MMA was visualized on the chromatogram using Fast Blue B and the amount present approximated by comparison with standards (American Medical Laboratories, Inc., Chantilly, Virginia). Results were expressed as urinary MMA concentrations/mmol creatinine.

Statistical analysis

Student's T test, Chi-square test and ANOVA were used to compare variables between the groups. Regression analysis techniques were used to determine significant relationships between variables. Results are reported as means ± SEM.

RESULTS

Nineteen women completed all measurements; however, a blood sample was not obtained from one CONTROL subject. Five East Indian women and 6 Caucasian women were in the omnivorous group; therefore this group was subdivided to determine if any ethnic differences existed in the vitamin B_{12} status of the women. There were no significant differences in subjects' characteristics between groups (see Table 1).

The mean dietary vitamin B_{12} intake of the VEG group was significantly lower than the CONTROL group (see Table 1). The intake of the East Indian and Caucasian omnivorous mothers was similar. VEG and CONTROL groups consumed an average of 1-2 servings of dairy products per day. In addition, the omnivorous mothers consumed 3-6 servings of meat (including fish and poultry) per week, and 3-6 servings of eggs per week. There was no significant correlation between the consumption of number of servings of dairy products per day and milk B_{12} concentrations among all the subjects. In the omnivorous mothers, there was no significant correlation between number of daily servings of meat and eggs and milk B_{12} concentrations.

Intake of vitamin B_{12} from supplements ranged from 2.2 to 25 μg/d. All mothers except three vegetarians reported taking prenatal vitamins regularly (one per day) during pregnancy and lactation. Total vitamin B_{12} intake was estimated for each subject from reported dietary intake and supplement use. VEG women's mean total intake of B_{12} was significantly less than the CONTROL subjects' intake (see Table 1). However, there was no significant difference between East Indian and Caucasian omnivores' total B_{12} intake. There was no correlation between total B_{12} intake and serum B_{12} or milk B_{12} concentrations.

Maternal serum and milk vitamin B_{12} concentrations were significantly lower in the VEG group than the CONTROL subjects (see Table 1). Among the CONTROL subjects, the mean serum B_{12} concentrations of the East Indian mothers were significantly lower than the Caucasian mothers; however, there was no significant difference in mean milk B_{12} concentrations between the two subgroups. There was a trend toward a positive relationship between breast milk and maternal serum vitamin B_{12} concentrations (see Figure 1).

To see if there were differences in milk B_{12} concentrations with the duration of lactation, all subjects were divided into two groups: 1) 4 to 8 weeks postpartum (PP) (n=8) and 2) 16 to 20 weeks PP (n=8). After controlling for the type of diet the mothers consumed (vegetarian vs omnivorous), the mean milk B_{12} concentrations were significantly higher in mothers at 4 to 8 weeks PP than in mothers at 16 to 20 weeks PP (613 ± 96 vs 328 ± 95 pmol/L, respectively; $p=0.04$). When controlling for the duration of lactation, milk B_{12} concentrations of lactovegetarian mothers were still significantly lower than the omnivorous mothers (211 ± 86 vs 592 ± 73 pmol/L, respectively; $p=0.006$). Parity did not have a significant effect on either milk or serum B_{12} concentrations.

We were unable to collect adequate amounts of urine from two infants. Urinary MMA was detected in only 5 urine samples from the remaining 17 infants. Except for one sample, all the

values were within normal range (0.4-23 μmol/mmol creatinine, determined from 62 infants, 6 mos of age, ref. 14). This range is wider than that reported for adults (0.7-3.2 μmol/mmol creatinine) or children (2.0-5.1 μmol/mmol creatinine); however, it is hypothesized that the higher urinary MMA concentrations among breast-fed infants may be due to the higher concentration of odd-chain fatty acids present in human milk (14). Odd-chain fatty acids are oxidized to propionate, the main precursor to MMA. This may cause an elevation in urinary MMA.

TABLE 1
Subjects' Characteristics and Vitamin B_{12} Status*

	Groups			
	Lactovegetarian (n=8)	Omnivores (n=11)		
		All (n=11)	East Indian (n=5)	Caucasian (n=6)
Maternal characteristics				
Age (y)	27.3 ± 0.9	29.1 ± 1.1	31.6 ± 1.5	27.0 ± 0.9
Parity				
1	62%	91%	80%	100%
2	38%	none	none	none
3	none	9%	20%	none
Anemia during pregnancy	50%	36%	40%	33%
Infant characteristics				
Birth weight (kg)	3.08 ± 0.20	3.19 ± 0.16	3.03 ± 0.22	3.33 ±0.23
Age (mo)	2.4 ± 0.4	3.3 ± 0.4	2.8 ± 0.6	3.7 ± 0.4
Vitamin B_{12} status				
Intake of vitamin B_{12} (μg/d)				
Dietary	1.4 ± 0.3#	2.6 ± 0.2	2.8 ± 0.4	2.5 ± 0.3
Total**	4.6 ± 1.4#	11.7 ± 2.5	8.1 ± 0.5	14.7 ± 4.3
Serum vitamin B_{12} pmol/L	264 ± 52#	531 ± 78	368 ± 31##	640 ± 109
Milk vitamin B_{12} pmol/L	277 ± 39#	544 ±104	611 ± 210	489 ± 93

* Values are means and standard errors of the mean.
# Significantly different from omnivore controls, $p < 0.05$, from *t*-test.
**Total intake of vitamin B_{12} is the sum of the dietary intake and the amount in the supplement.
##Significantly different from control Caucasians ($p = 0.04$) but not from East Indian vegetarians.

The urine sample with elevated urinary MMA concentration (24 μmol/mmol creatinine) was from an East Indian infant whose mother consumed a lactovegetarian diet. Her milk B_{12} concentration was 274 pmol/L and her serum B_{12} concentration was 122 pmol/L. Her vitamin B_{12} intake from dairy foods was estimated to be 1.08 μg/d; however, she reported that she took a supplement containing 10 μg of B_{12} daily. On the other hand, there was one East Indian lactovegetarian mother who reported that she did not take a vitamin supplement or consume dairy products daily. Her serum B_{12} concentration was 130 pmol/L, and her milk B_{12} concentration was 326 pmol/L. However, urinary MMA concentration in her infant's urine sample was nondetectable. Weight and height of all infants were adequate at the time of sample collection (between the 10th and the 95th percentile of the growth charts from the National Center for Health Statistics).

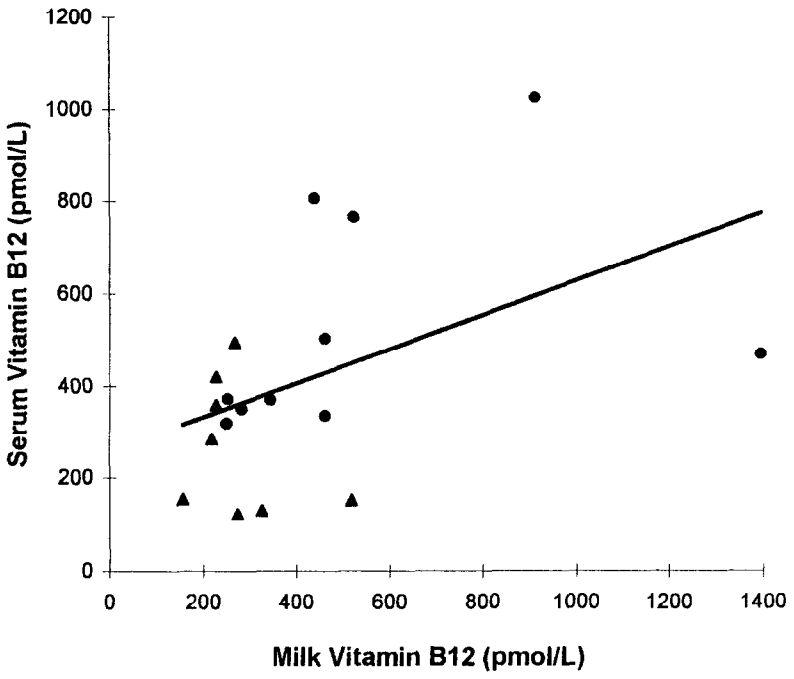

FIG. 1. Relationship between milk and serum vitamin B_{12} concentrations ($r = 0.46$, $p = 0.06$). • = omnivorous women, Δ = lactovegetarian women.

DISCUSSION

The results of this study indicate that lactovegetarian mothers have lower milk B_{12} concentrations than omnivorous mothers. This is in agreement with Jathar et al. (16) who observed lower milk B_{12} concentrations in East Indian lactovegetarian mothers compared to omnivorous mothers (66 vs 75 pmol/L, respectively). However, the mean milk B_{12} concentrations of our East Indian lactovegetarian mothers in the US were much higher than what Jathar et al. reported in India (277 vs 66 pmol/L, respectively). This may be explained by the supplementation of vitamins by the lactovegetarian mothers in our study. In India, the consumption of vitamins may be restricted due to economic factors.

Milk vitamin B_{12} concentrations were similar to those of other vegetarians and omnivores reported by Specker et al. (231 ± 94 pmol/L in milk of "strict" vegetarians and 378 ± 75 pmol/L in omnivores, ref. 11) and by Dagnelie et al. [266 ± 256 pmol/L in macrobiotic women (some who consumed dairy products, fish and eggs weekly) and 325 ± 173 pmol/L in omnivorous women, ref. 23]. The milk vitamin B_{12} concentrations of our omnivores (544 ±104 pmol/L) were similar to those reported by other researchers in the US. Sneed et al. (10) reported average concentrations of 406 ± 118 pmol/L in unsupplemented and 583 ± 177 pmol/L in supplemented low income women. Thomas et al. (12) reported average concentrations of 450 ± 125 pmol/L in unsupplemented and 812 ± 412 pmol/L in supplemented well-nourished women.

Our results showing the lack of correlation between the consumption of number of servings of dairy products and milk B_{12} concentrations confirmed similar findings by Dagnelie et al. (23). They reported vitamin B_{12} concentrations in breast-milk showed no relationship with frequency of consumption of dairy products. However, they reported a significant correlation between meat and fish consumption and milk B_{12} concentrations, which we did not observe.

Specker et al. reported that an inverse relationship between infant urinary MMA concentrations and milk vitamin B_{12} concentrations existed when milk B_{12} concentrations were less than 362 pmol/L (11). They calculated that an infant consuming an average of 750 ml/d of breast milk containing 362 pmol/L would have an intake of approximately 271 pmol of vitamin B_{12}. In our study, seven of the eight lactovegetarians and four of the eleven omnivorous mothers had milk B_{12} concentrations <362 pmol/L. Assuming the average consumption of breast milk was 750 ml/day, six lactovegetarians' and three control subjects' infants did not consume the RDA for vitamin B_{12}. Black et al. (24) reported that 31 of 50 lactating women participating in the Mexico Collaborative Research Support Program had milk B_{12} concentrations <362 pmol/L, which they classified as deficient.

Despite this large percent of lactovegetarian women with low concentrations of vitamin B_{12} in their breast milk, only one infant had elevated urinary MMA concentration. Since vitamin B_{12} is necessary for the conversion of methylmalonyl-CoA to succinyl-CoA, elevated levels of urinary MMA indicate vitamin B_{12} deficiency. Specker et al reported that infants of strict vegetarian mothers with milk concentrations <221 pmol/L had higher urinary MMA concentrations (14). The reason we did not observe more infants with elevated urinary MMA may be due to the less sensitive method we used to determine methylmalonic acid. In addition, the infants in this study were younger (approximately 2 mos of age) than those studied by Specker et al. (approximately 7 mos of age). Younger infants may utilize vitamin B_{12} accumulated during gestation.

The risk of developing vitamin B_{12} deficiency in exclusively breast-fed infants of lactovegetarian mothers is greater in the later months of lactation for three reasons: 1) depletion of neonatal stores which may be low at birth in vegetarian infants; 2) increase in the vitamin B_{12} requirement as the infant grows older; and 3) decrease in milk B_{12} concentrations with increase in duration of lactation. While our results are cross-sectional and based on a small sample size and must be interpreted with caution, other longitudinal studies have reported a decline in vitamin B_{12} concentrations during lactation. Trugo and Sardinha (25), reported the mean milk B_{12} concentrations of nine women to be significantly higher in colostrum than in mature milk (350 pmol/L vs. 250 pmol/L, respectively; $P < 0.01$). Sneed et al. (10) measured samples at 5 to 7 days and 42 to 45 days postpartum in low income women. They reported a decline from 515 ± 140 to 406 ± 118 pmol/L in seven unsupplemented women and from 671 ± 184 to 583 ± 177 pmol/L in nine supplemented women. Thomas et al. (12) examined breast milk from well-nourished women at the same time points and reported a similar decline in milk vitamin B_{12} concentrations (from 900 ± 302 to 450 ± 125 in seven unsupplemented women and from 1217 ± 465 to 812 ± 421 in ten supplemented women). Thus, the problem of decreased vitamin B_{12} status of a newborn infant can be further compromised by decreased milk B_{12} concentrations and increased requirements during the later months of infancy.

The only difference observed between East Indian and Caucasian omnivorous mothers were in the serum B_{12} concentrations, despite no significant difference in total vitamin B_{12} intake. In addition, the serum levels were similar between the East Indian lactovegetarians and omnivores. This suggests that there may be ethnic differences, possibly in the binding proteins, leading to lower serum vitamin B_{12} levels in East Indian women. A recent study by Frery et al (26) observed an ethnic difference in plasma vitamin B_{12} levels of pregnant women: averages among blacks (n=23) were statistically greater than among non-blacks (n=165). Another ethnic difference may exist in the consumption of vitamin supplements. While all CONTROL subjects reported regular consumption of supplements, there may have been a difference in actual daily use between the groups. The milk concentration of vitamin B_{12} was not different between the East Indian and Caucasian omnivores, but was significantly lower in the East Indian vegetarians. This difference suggests an effect of total B_{12} intake. The small sample size and the imprecise estimate of intake

limits further analysis; however, the results are suggestive of an interaction between ethnicity and vitamin B_{12} intake.

Presently, the Institute of Medicine recommends a supplement of 2.6 μg of vitamin B_{12} daily for complete vegans, but not lactovegetarians. Results of this study suggest a need for supplementation in lactating, lactovegetarian women. Biochemical deficiency was observed in only one infant. However, five of the eight lactovegetarians in this study reported taking supplements during pregnancy and lactation. More cases of biochemical deficiency of vitamin B_{12} may have been detected if the mothers were not taking supplements and/or a more sensitive indicator of infant B_{12} status was measured. Future research is needed on the effect of supplementation on the vitamin B_{12} status of lactovegetarian mothers and their infants, given the importance of vitamin B_{12} in neural development.

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