The effects of vitamin C, vitamin B_6, and vitamin B_{12} supplementation on the breast milk and maternal status of well-nourished women[1-4]

M. Rita Thomas,[5] Ph.D., Joanne Kawamoto,[6] M.S., Sharon M. Sneed,[6] M.S., and Randi Eakin,[6] B.S.

ABSTRACT The effects of vitamin supplements and/or diet on the levels of vitamin C, vitamin B_6, and vitamin B_{12} in milk and blood of lactating women were determined. At the end of gestation, subjects were divided into two lactation groups: supplemented (10 subjects) and nonsupplemented (seven subjects). Milk samples were collected from 5 to 7 days and 43 to 45 days postpartum. Fasting blood samples were drawn at 8 and 46 days postpartum for vitamin C, B_6, and B_{12} status measurements. Dietary records of all foods consumed by the subject were kept for 4 days at 1 and 6 weeks postpartum. The vitamin B_6 level in breast milk of the unsupplemented group of mothers was significantly lower ($P < 0.05$) than the supplemented group of women at 5 to 7 days postpartum. Vitamin B_{12} concentration in milk of nonsupplemented mothers at 43 to 45 days postpartum was significantly lower ($P < 0.05$) than the supplemented group of women at 43 to 45 days postpartum. None of the milk values or the maternal blood levels measured in the women was less than published norms for vitamin C, vitamin B_6, and vitamin B_{12}. *Am. J. Clin. Nutr.* 32: 1679–1685, 1979.

Studies have shown that changes in maternal diet can influence the nutrient level of breast milk, especially with regard to the water-soluble vitamins. Diets supplemented with these vitamins have been associated with increases in concentration of the water-soluble vitamins in breast milk (1). Information on the influence of the maternal diet on the vitamin content of breast milk is still inadequate, especially involving the water-soluble vitamins (1).

Investigations involving undernourished lactating women of Indian origin have revealed that dietary supplementation with ascorbic acid and thiamin caused an increase in the concentration of these vitamins in breast milk (2, 3). The content of riboflavin and vitamin B_{12} in breast milk were also found to increase with supplemental ingestion of riboflavin and vitamin B_{12} (4). Metz (5) found that the supply of folic acid to the breast milk from the mother's intake takes precedence over other maternal tissues.

Although published data are available on the benefits of water-soluble vitamin supplements to undernourished women, well-nourished women ingest water-soluble vitamin supplements and for several of the vitamins their benefits to these women have not been assessed. The influence of vitamin B_6 intake on the content of that vitamin in breast milk was studied in a group of 19 lactating women (6). Results showed that intakes of less than the Recommended Dietary Allowances (RDA) (7) for vitamin B_6 significantly lowered the vitamin B_6 content in breast milk,

[1] From the Nelda Childers Stark Laboratory for Human Nutrition Research, Department of Nutrition and Food Sciences, Texas Woman's University, Denton, Texas 76204.

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[4] Address reprint requests to: M. Rita Thomas, Ph.D., Assistant Professor in Nutritional Science, College of Health, University of Utah, Salt Lake City, Utah 84112.

[5] Present address: College of Health, University of Utah, Salt Lake City, Utah 84100. [6] Graduate Research Assistant.

but intakes two to five times the RDA did not significantly raise the level of that vitamin in the milk. The present study was undertaken to investigate the effects of vitamin supplements and/or diet on the vitamin C, B_6, and B_{12} content of breast milk and maternal status at 1 and 6 weeks postpartum.

Materials and methods

Description of study

At the end of gestation 17 subjects volunteered for either of two lactation groups: supplemented (10 subjects) and nonsupplemented (7 subjects). The supplemented mothers were given Natalins, multivitamin and multimineral supplement (Table 1) from parturition. Milk samples were collected for 3-day periods at 1 and 6 weeks postpartum. Milk was expressed four times per day at 4-hr intervals. Fasting blood samples were drawn for vitamin B_6, B_{12}, and C analysis at 1 and 6 weeks postpartum. Dietary records of all foods consumed by the subjects were kept for 4 days at 1 and 6 weeks postpartum, 1 day before milk collection and the 3-day periods of milk collection.

Subjects

Prospective subjects (18 to 35 years of age) were recommended for participation in the study through pediatricians and obstetricians in the Denton/Dallas/Fort Worth area and through the LaLeche League directors of the area. Subjects were not allowed to participate in the study unless their physician agreed and biochemical tests revealed no need for vitamin supplements. Women could not participate who were on routine medication or had been on oral contraceptive agents for more than 2 years before the present conception. All subjects were briefed thoroughly on their participation in the study as outlined by the Human Subject Review Committee at the Texas Woman's University before they were allowed to sign consent forms.

TABLE 1
Vitamin and mineral content of daily supplement[a]

Vitamin or mineral	Quantity	Percentage of RDA[b]
Vitamin A	8000 IU	133
Vitamin D	400 IU	100
Vitamin E	30 IU	200
Vitamin C	90.0 mg	113
Folic acid	0.8 mg	133
Thiamin	1.7 mg	121
Riboflavin	2.0 mg	133
Niacin	20.0 mg	111
Vitamin B_6	4.0 mg	160
Vitamin B_{12}	8.0 μg	200
Calcium	200.0 mg	17
Iodine	150.0 μg	100
Iron	45.0 mg	250
Magnesium	100.0 mg	22

[a] Natalins Multivitamin and Mineral Supplement, Mead Johnson, Evansville, Ind.; one tablet. [b] For lactating women.

Diet records and sample collection

The dietary intake records of each woman from 4 to 7 and 42 to 45 days postpartum were analyzed by the computer tape of Handbook Eight (8), the Ohio State Data Tape (9), and published nutrient analyses from food companies (10).

Milk samples were collected from 5 to 7 and 43 to 45 days postpartum. The milk was expressed immediately before taking the vitamin pill in the supplemented group in the morning at 0 time and 4, 8, and 12 hr thereafter. Those mothers not taking supplements expressed milk at corresponding times. Before the mothers nursed their infants, 25 ml of fore milk was expressed from both breasts into opaque containers, and frozen immediately at −10 C until analyzed. After an 8-hr fast, blood samples were drawn after the 3-day milk sampling was completed.

Biochemical analyses

When possible the methods from the Infant Formula Council (11) and the Association of Vitamin Chemists (12) were used for vitamin analyses.

The concentration of plasma and milk ascorbic acid was measured photometrically by the method of Zannoni et al. (13). The Bio-Rad Radioimmunoassay Kit for vitamin B_{12} was used for serum and milk measurement of the vitamin. It used a modification of the radioisotopes dilution procedure described by Lau et al. (14). Vitamin B_6 status was determined by the in vitro stimulation of EGPT the procedure used being adopted primarily from Woodring and Storvick (15), Dirige and Beaton (16), and Sauberlich et al. (17). Total vitamin B_6 content was measured by *Saccharomyces uvaris* in a microbiological assay. The procedure was based on the methods of Storvick et al. (18), Thiele and Brin (19), and Toepfer and Polansky (20). Growth of *S. uvaris* was measured as turbidity at 550 mμ in a spectrophotometer. Because of the sensitivity of vitamin B_6 to light, all procedures were carried out in a darkened room.

Statistical analysis

Milk samples were analyzed by a 3 factor ($2 \times 2 \times 4$) design with repeated measures on the last two factors (21). Blood data were analyzed by a 2×2 factorial design with trends analysis on the two factor level.

Results

Vitamin B_6

The mean dietary intake of vitamin B_6 was less than 60% of the RDA for the lactating woman which is 2.5 mg (7). At 42 to 45 days postpartum the nonsupplemented women consumed 34% of the RDA while the supplemented women ingested 44% of the RDA (Table 2). Although the nonsupplemented women consumed less dietary vitamin B_6

TABLE 2
Vitamin B_6 intake, maternal EGPT index, and milk concentration of lactating women

Vitamin indices	Nonsupplemented		Supplemented	
	5-7 Days	43-45 Days	5-7 Days	43-45 Days
Vitamin B_6 Intake (mg)	1.45	0.84	5.69[a]	5.11[b]
	(0.62)[c]	(0.22)	(0.65)	(0.35)
EGPT index	1.30	1.42	1.46	1.49
	(0.33)	(0.40)	(0.38)	(0.32)
Milk concentration (µg/liter)	128[d, e]	204	225	237
	(59)	(53)	(87)	(57)

[a] Dietary intake without supplementation was 1.69 (0.65). [b] Dietary intake without supplementation was 1.11 (0.35). [c] Mean and SE of seven subjects in the supplemented group and six subjects in the nonsupplemented group [d] $P < 0.01$, significantly lower than corresponding supplemented measurement. [e] $P < 0.05$, significantly lower than corresponding measurement in the same group.

both at 5 to 7 days postpartum and 43 to 45 days postpartum, this difference was not significant (Table 2).

EGPT indexes were not significantly different between the two groups of women or from measurements in the same groups at 1 week to 6 weeks postpartum (Table 2). Although the EGPT index was lower in the nonsupplemented group as compared to the supplemented group, this difference was not significant (Table 2).

Milk concentration differed significantly ($P < 0.05$) between the two groups at 5 to 7 days postpartum. The milk from the nonsupplemented group of women contained 225 µg of vitamin B_6 per liter while the milk from the supplemented mothers contained 128 µg of pyridoxine per liter (Table 2). When the milk was measured for pyridoxine at 43 to 45 days, the concentration of the vitamin significantly increased ($P < 0.05$) in the group consuming only dietary pyridoxine. The concentration of pyridoxine in milk of women in the supplemented group remained constant at 225 µg/liter at 5 to 7 days to 237 µg/liter at 43 to 45 days. The difference in pyridoxine concentration between the groups of mothers was not significant at 43 to 45 days postpartum.

When women consumed vitamin B_6 from the diet (nonsupplemented group), the milk levels of pyridoxine remained relatively constant throughout the day (Fig. 1). When the vitamin pill was ingested in addition to the diet there was a significantly higher ($P <$ 0.05) concentration of the vitamin in the milk measured at 4 and 8 hr after the pill was taken. At 43 to 45 days postpartum the milk concentration of vitamin B_6 peaked at 8 hr after the vitamin supplement was taken (Fig. 1).

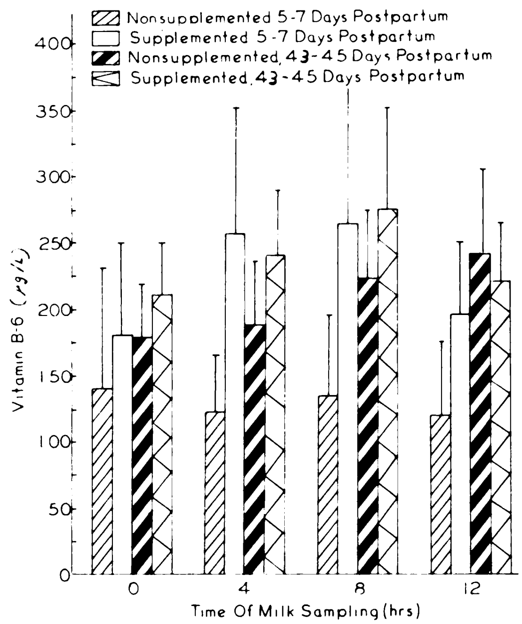

FIG. 1. Vitamin B_6 concentration of breast milk of seven supplemented and six nonsupplemented women, SEM.

Vitamin C

All women in both groups consumed more than the RDA for vitamin C. The unsupplemented group of women consumed two times the RDA of 80 mg (7) and the supplemented group of women ingested three times the RDA (Table 3).

The plasma vitamin C level of the women consuming only dietary vitamin C was significantly ($P < 0.01$) lower at 7 days postpar-

TABLE 3
Vitamin C intake, maternal plasma levels, and milk concentration of lactating women

Vitamin indices	Nonsupplemented		Supplemented	
	5–7 Days	43–45 Days	5–7 Days	43–45 Days
Vitamin C Intake (mg)	203	174	264[a]	215[b]
	(71)[c]	(67)	(66)	(97)
Plasma levels (mg/100 ml)	1.55[d,e]	3.86	3.09	2.14
	(0.86)	(1.12)	(1.09)	(68)
Milk concentration (mg/liter)	74.3	61.1	58.4	87.2
	(36)	(36)	(34)	(50)

[a] Dietary intake without supplementation was 174 mg (66, SD). [b] Dietary intake without supplementation was 122 mg (98, SD). [c] Mean and SE of seven subjects in the supplemented group and six subjects in the nonsupplemented group. [d] $P < 0.01$, significantly lower than corresponding supplemented measurement. [e] $P < 0.01$, significantly lower than corresponding measurements at days 43 to 45 or 5 to 7 in the same group.

tum than the supplemented group, 1.55 versus 3.09 mg/100 ml, respectively (Table 3). At 6 weeks postpartum the nutritional status of both groups of women was not significantly different as reflected by plasma vitamin C levels, but the vitamin C concentration in the serum, of the supplemented group of women was lower than the nonsupplemented group of women. The women's vitamin C status of the nonsupplemented group increased significantly ($P < 0.01$) from 1 to 6 weeks postpartum.

Milk levels of vitamin C were not significant between groups or between 5 to 7 and 43 to 45 days postpartum (Table 3). Milk levels of vitamin C remained constant throughout the day as there was no reflection of the vitamin C from the vitamin pill at 4 or 8 hr postpartum (Fig. 2).

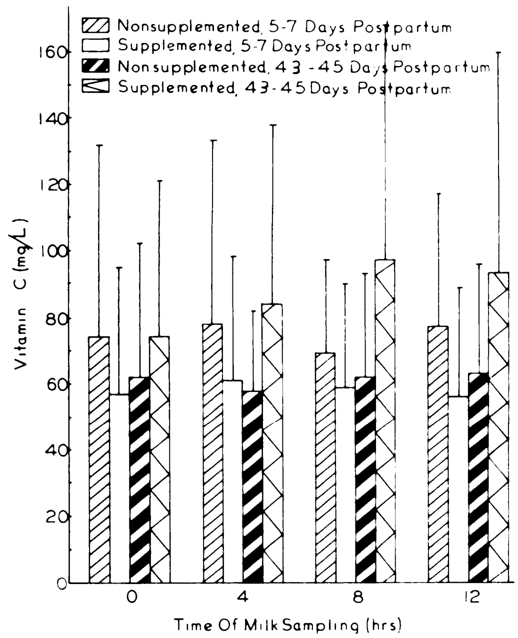

FIG. 2. Vitamin C profile of breast milk of seven supplemented and six nonsupplemented women, SEM.

Vitamin B_{12}

Dietary intake of vitamin B_{12} in the nonsupplemented group was 2.82 and 2.13 μg at 5 to 7 and 43 to 45 days, respectively (Table 4). These figures correspond to 71 and 53% of the RDA for the lactating woman. Since the SD's of the dietary intakes of vitamin B_{12} of both groups were so large, the difference between the groups both at 5 to 7 and 43 to 45 days postpartum was not significant (Table 4).

All serum values of vitamin B_{12} were normal in all subjects in this study (17). Their serum values ranged from 496 to 609 pg/ml of blood (Table 4).

The milk levels of vitamin B_{12} were not significantly different between the nonsupplemented and supplemented groups of women at 5 to 7 days postpartum (Table 4). At 43 to 45 days postpartum the concentration of vitamin B_{12} had declined in both groups from that value measured at 5 to 7 days postpartum. The nonsupplemented group of women not only reflected a significantly lower ($P < 0.05$) concentration of vitamin B_{12} in the milk at 43 to 45 days in comparison to the measurement of the same group of women at 5 to 7 days postpartum, but also to the value of the supplemented group of women at 43 to 45 days postpartum (Table 4).

The level of vitamin B_{12} in the supple-

TABLE 4
Vitamin B_{12} intake, maternal serum level, and milk concentration of lactating women

Vitamin indices	Nonsupplemented		Supplemented	
	5–7 Days	43–45 Days	5–7 Days	43–45 Days
Vitamin B_{12} intake (μg)	2.82 (0.99)[c]	2.13 (1.04)	11.67[a] (1.47)	11.86[b] (1.75)
Serum levels (pg/ml)	496 (88)	538 (102)	609 (181)	565 (91)
Milk concentration (μg/liter)	1.22 (0.41)	0.61[d,e] (0.17)	1.65 (0.63)	1.10 (0.57)

[a] Dietary intake without supplementation was 3.68 (1.47). [b] Dietary intake without supplementation was 3.86 (1.75). [c] Mean and SE of seven subjects in the supplemented group and six subjects in the nonsupplemented group. [d] $P < 0.05$, significantly lower than corresponding supplemented measurement. [e] $P < 0.05$, significantly lower than corresponding measurements at 5 to 7 days postpartum.

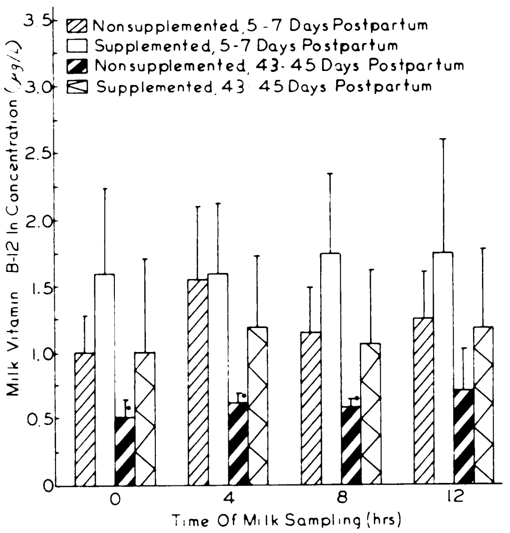

FIG. 3. Vitamin B_{12} levels of breast milk of seven supplemented and six nonsupplemented women, SEM. Concentration significantly lower ($P < 0.05$) than corresponding measurements at 5 to 7 days postpartum in both groups of women.

mented group did not increase significantly after the vitamin supplement was taken at 4, 8, or 12 hr (Fig. 3). The concentration of vitamin B_{12} measured in the milk at 0, 4, 8, and 12 hr in all groups reflected the total decline of vitamin B_{12} concentration from 5 to 7 to 43 to 45 days postpartum. The significantly lower concentration of vitamin B_{12} found in women at 43 to 45 days postpartum was reflected in those values measured at 0, 4, and 8 hr (Fig. 3).

Discussion

Both groups of women were consuming at least 100% of the RDA for the lactating woman for protein and energy (22). All nutrients were consumed by the women in adequate amounts except for vitamin B_6, vitamin B_{12}, folate, vitamin D, and zinc. Vitamin B_6 and B_{12} intakes of the women were below 70% of the RDA (7). These low levels may not be actual low dietary intakes but may reflect the incompleteness of the existing food tables (8, 9). Other authors have reported low dietary intakes of vitamin B_6 (6, 23).

West and Kirksey (6) found that intakes of less than the RDA (7) for vitamin B_6 significantly lowered the vitamin B_6 concentration in breast milk. For those women ingesting an average of 1.8 mg of vitamin B_6 the average concentration of the vitamin in the milk was 129 μg/liter. In this study the unsupplemented women consuming 1.45 mg of vitamin B_6 at 5 to 7 days postpartum reflected a level of 128 μg of vitamin B_6 per liter. When dietary vitamin B_6 intake decreased at 6 weeks postpartum in the nonsupplemented group of women the milk concentration of vitamin B_6 increased to 204 μg/liter which approximated that value found by West and Kirksey (6) in their subjects' milk during the first 3 months of lactation.

Both Karlin (24) and West and Kirksey (6) found an increase of vitamin B_6 in the milk of women after consuming a vitamin supplement. Karlin (24) found that a supplemental dose of 1000 times the RDA significantly increased the concentration of vitamin B_6 in the milk in a few hours. West and Kirksey (6) found peak levels of vitamin B_6 occurring 3 to 5 hr after a supplement was taken. In this study vitamin B_6 concentration in the milk was significantly increased at 4 and 8 hr after the supplement.

Supplementation studies with vitamin C and vitamin B_{12} in well-nourished lactating women are scarce, but Gopalan and Belavady (2) have supplemented malnourished women. Indian lactating women when supplemented with vitamin C reflected 30 to 40 mg/liter of vitamin C in their milk. In this study the amount of vitamin C in the dietary intake and vitamin supplements groups was two to three times the RDA for the lactating women (7), but the vitamin C concentration in the milk ranged from 58 to 87 mg of vitamin C per liter. The lowest concentration of vitamin C in the milk was reflected in the supplemented group of women.

Investigations (4, 25) have found that supplemental vitamin B_{12} does increase the concentration of vitamin B_{12} in the milk. In both cases the women were malnourished. Megaloblastic anemia due to a vitamin B_{12} deficiency has been reported in a breast-fed infant of a vegetarian mother (25). Although the women in the present study were well-nourished, the vitamin B_{12} concentration in the breast milk of the nonsupplemented women at 6 weeks postpartum was significantly lower than the supplemented group of women. Ford (26) has postulated that the vitamin B_{12} binding proteins in the mammary gland may act as trappers for plasma vitamin B_{12}. Since there is assumed to be an abundance of binding proteins, vitamin B_{12} could accumulate in the mammary gland (26).

The vitamin concentration of human milk has been reported and revised by Hartman and Dryden (27). All vitamin measurements on the milk of both groups of women were higher than published values (27) and those values used to set the admissible intakes for infants (28). Even though dietary intakes of vitamin B_6 and B_{12} were low, milk concentration of these vitamins were above published norms. The maternal status of the women for water-soluble vitamins was well within normal limits, thus indicating adequate vitamin C, B_{12}, and B_6 status (29). Although the values of some measurements, milk for vitamin B_6 and plasma for vitamin C, were lower than in the nonsupplemented group of women, those measurements did not differ from the supplemented group of women at 6 weeks postpartum. Diet alone increased those values.

For those healthy, well-nourished women with a history of short-term oral contraceptive use, vitamin C, B_6, and B_{12} concentrations in milk and the status of the mothers can be maintained on diet alone.

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