Effects of different levels of vitamin C intake on the vitamin C concentration in human milk and the vitamin C intakes of breast-fed infants[1-4]

Lauri O Byerley, MS, RD and Avanelle Kirksey, PhD

ABSTRACT The influence of maternal intake of vitamin C on the vitamin C concentration in human milk and on the vitamin C intakes of breast-fed infants has not been demonstrated conclusively. This study examined these influences of diet and supplementation in 25 lactating women administered 90 mg of ascorbic acid for 1 day followed by 250, 500 or 1000 mg/day for 2 days or unsupplemented for 1 day followed by either 0 or 90 mg ascorbic acid supplement for 2 days. Vitamin C content in milk and urine was determined by the 2,4-dinitrophenylhydrazine method. Vitamin C intakes of infants were calculated from milk volume, as determined by the test-weighing method and from vitamin C levels in milk samples obtained at each feeding. Total maternal intakes of vitamin C, which exceeded 1000 mg/day or 10-fold the RDA for lactation (100 mg/day), did not significantly influence the vitamin C content in milk or the vitamin C intakes of infants. However, maternal vitamin C intake was positively correlated (r = 0.7) with maternal urinary excretion. These differences in milk and urine response to vitamin C intake suggest a regulatory mechanism for vitamin C levels in milk. *Am J Clin Nutr* 1985; 41:665–671.

KEY WORDS Lactation, supplementation, nutritional status, ascorbic acid, vitamin C

Introduction

The Nutrition Committee of the Canadian Pediatric Society (1) and the Committee on Nutrition of the American Academy of Pediatrics (2) have recommended breast-feeding as the method of choice for infants until four to six months of age except in cases where this type of feeding is contraindicated or unsuccessful. Furthermore, the Committee on Nutrition of the American Academy of Pediatrics (2) has reported guidelines for infant formula preparation in which the nutrient content of human milk was used as the standard reference. Precise knowledge of factors that can affect the nutrient content of human milk is, therefore, essential. Currently information concerning factors which may alter the vitamin C content of human milk is incomplete.

The Recommended Dietary Allowance (RDA) (3) of vitamin C for infants through 6 months of age, 35 mg/day, is based on an assumed intake of 850 ml/day of milk which contains approximately 30 to 55 mg of vitamin C/L. This estimate of vitamin C concentration in milk was obtained from studies (4,

[1] From the Department of Goods and Nutrition, Purdue University, West Lafayette, IN 47907.

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[3] An abstract of a portion of these results was reported in Fed Proc 1981;40:877.

[4] Address reprint requests to: Dr Avanelle Kirksey, Department of Foods and Nutrition, Stone Hall, Purdue University, West Lafayette, IN 47907.

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5) conducted more than 40 years ago and which suggested that vitamin C intake influences the concentration of the vitamin in milk. In recent years, ascorbic acid supplements have been used extensively, but their effect on the vitamin C content of human milk has not been demonstrated conclusively. The widespread use of supplements suggested the need to re-evaluate the influence of maternal intake of vitamin C on the concentration of this vitamin in milk. Since urinary excretion of vitamin C is known to parallel its intake (6), if ascorbic acid is excreted in urine after vitamin C supplementation without significant increases in milk this may indicate a control mechanism for the regulation of vitamin C content in milk. In the present study, the effects of different levels of vitamin C intake during lactation on the vitamin C concentration in milk, and on infant intake and maternal urinary excretion of the vitamin were investigated.

TABLE 2
Composition of multivitamin and multimineral supplement used in this study*

Vitamins	Quantity/tablet†
A	8000 IU
D	400 IU
E	30 IU
Ascorbic Acid	90, 250, 500 or 1000 mg
Folic Acid	0.8 mg
Thiamin	1.7 mg
Riboflavin	2 mg
Niacin (Niacinamide)	20 mg
B_6	5 mg
B_{12}	8 μg
Minerals	
Calcium	200 mg
Iodine	200 μg
Iron	60 mg
Magnesium	100 mg
Zinc	25 mg

* Supplement (C107558-01) supplied by Hoffman-LaRoche Inc, Nutley, NJ.
† One tablet per day.

Methods

Subjects and design

Twenty-five well-nourished women volunteered to participate in this study. Their ages ranged from 20 to 36 years with a mean of 28 years. Stage of lactation ranged from 7 to 13 weeks ($\bar{x}$ = 11 wk) except for one subject who was 20 weeks postpartum. Human milk was the sole source of nutrients for all of the infants who were breast-fed 5 to 10 times a day with a mean feeding of 7 times. Informed consent was obtained from each woman before her entry into the study. All procedures were approved by the University Committee on the Use of Human Subjects.

Subjects were randomly assigned to one of five experimental groups (**Table 1**). Eight subjects who did not take vitamin/mineral supplements prior to the study either received no supplement for the 3-day period (group 1), or received no supplement for one day followed by a supplement containing 90 mg ascorbic acid (**Table 2**) for two days (group 2). Two other subjects discontinued taking their vitamin/mineral supplements for one week prior to the start of the study in order to participate in one of these two groups. Subjects who had taken supplements previously were assigned to experimental groups 3, 4, or 5 to receive a 90 mg ascorbic acid supplement for one day followed by either 250, 500 or 1000 mg ascorbic acid supplement for two days.

The lowest level of ascorbic acid supplement, 90 mg, was used since this level was routinely used in earlier studies (4, 7, 8) and is also similar to levels found in prenatal supplements which are often continued during lactation. The 1000 mg level of ascorbic acid was chosen as a megadose level since it is 10 times the RDA of 100 mg/day for lactating women. Other levels of supplementation were selected as incremental doses between the extremes.

TABLE 1
Design of study

Experimental group	No of subjects	Ascorbic acid supplement: Day of study 1	2	3
			mg/day	
1	5	0	0	0
2	5	0	90	90
3	5	90	250	250
4	5	90	500	500
5	5	90	1000	1000

Dietary analysis

A record of food and supplement intake was kept by each woman starting with the evening meal before day 1 and concluding with the morning meal before the final milk collection on day 4. Nutrient content of the diets was determined by use of Agriculture Handbook No 456 (9).

Collection of milk and urine samples

Mothers expressed 5–10 ml of milk at each feeding following milk let down. Samples were obtained either manually or by use of a breast pump (Loyd-B-Pump, LOPUCO LTD, Woodbine, MD) and were collected into amber colored vials with airtight seals. Milk samples were kept refrigerated or frozen until collected from the mothers. In the laboratory, 4% metaphosphoric acid was added to stabilize the vitamin C. Urine was collected separately at each urination and stored in plastic containers to which 90 mg oxalic acid had been added to stabilize the vitamin C. Both milk and urine samples were frozen at −20°C until analysis.

Biochemical analysis

The vitamin C content of milk and urine samples was determined by use of a modified procedure of the 2,4-dinitrophenylhydrazine method developed by Roe and Kuether (10).

Estimation of milk volume and infant intake of vitamin C

Milk volume was estimated by the test-weighing procedure. Infants were weighed on a pediatric scale (Detecto Clinical Infant Scale, Brooklyn, NY) which was calibrated to 10 g accuracy in the home. The difference in weight of the infant before and after each feeding, without a change in clothing, was assumed to be the weight of milk consumed by the infant. The volume of milk consumed by the infant was estimated by dividing the weight of milk consumed by the specific gravity of human milk (1.031).

A daily estimate of vitamin C intake of the infant was obtained by multiplying the volume of milk intake at each feeding by the vitamin C concentration of milk obtained at each of the feedings. Vitamin C intakes for the feedings were then totaled for a 24 h period.

TABLE 3
Maternal intakes of vitamin C

Ascorbic acid supplement	Dietary intake	Total intake
mg/day	*mg/day*	*mg/day*
0	156 ± 115*	156 ± 115†
90	112 ± 77	202 ± 77†
250	166 ± 87	416 ± 87‡
500	80 ± 70	580 ± 70‡
1000	123 ± 96	1123 ± 96‡

* Mean ± SD; (n = 5 per group).
† Mean differ significantly ($p < 0.05$) from means of 250, 500 and 1000 mg supplemented groups.
‡ Means differ significantly ($p < 0.05$) from means of all other groups.

Statistical analysis

Data were analyzed using subprograms of the Statistical Package for Social Sciences (11). One-way analysis of variance was used to determine differences in dietary intake and total vitamin C intake among the five exper-

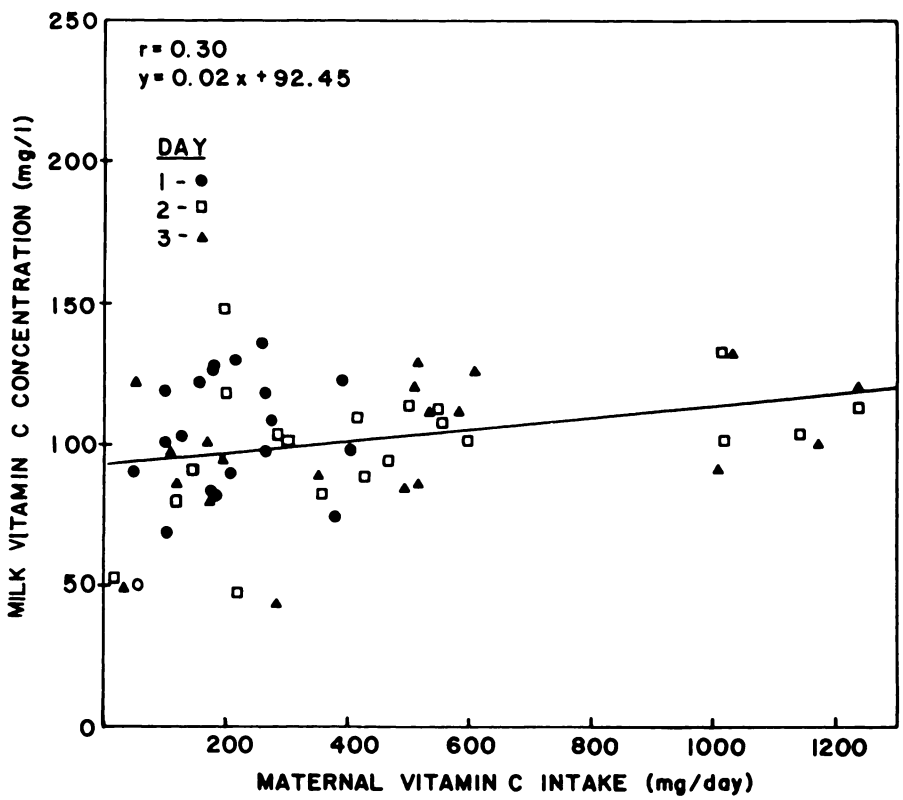

FIG 1. Relationship between milk vitamin C concentration and maternal vitamin C intake.

imental groups. Multivariate analysis of variance was used to determine differences in milk volume, vitamin C concentration in milk, infant intake of vitamin C and maternal urinary excretion of vitamin C among the five experimental groups. If the F value was significant ($p = 0.05$), then the Newman-Keuls test was used to distinguish differences among means.

Results

Maternal intake

Dietary intakes of vitamin C were not significantly different among the five treatment groups (**Table 3**). The overall mean intake was 127 ± 84 mg/d which is somewhat higher than the RDA (3) of 100 mg/d for lactating women. Individual intakes ranged from 4 to 285 mg/d. Total vitamin C intake from diet plus supplement did not differ significantly between non-supplemented women and those receiving a 90 mg ascorbic acid supplement. As expected, total vitamin C intakes of women who consumed 250, 500 or 1,000 mg ascorbic acid supplements differ significantly ($p = 0.05$) from each other and also differed from those who consumed 90 mg of vitamin C.

Since vitamin C is present in high concentration in several common foods which can alter intake appreciably, total maternal vitamin C intake was determined by adding the amounts provided by diet to that provided by supplements. Women were then divided for subsequent evaluation into five groups according to their total vitamin C intakes: 100, 100–199, 200–399, 400–999, ≥1000 mg/d.

Vitamin C concentration in milk

Vitamin C concentrations in milk ranged from 44 to 158 mg/L but were not correlated significantly to maternal intakes of vitamin C (**Fig 1**). Mean vitamin C concentrations of milk did not differ significantly among the five treatment groups who consumed different levels of vitamin C (**Fig 2**).

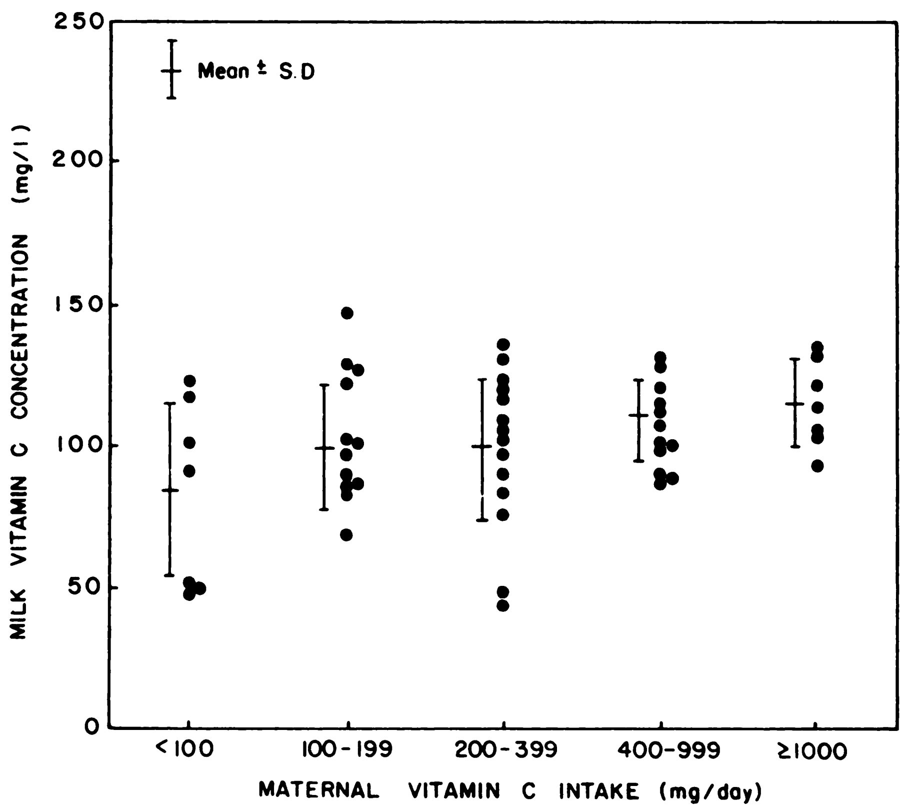

FIG 2. Vitamin C concentration of milk in relation to different levels of maternal intake of vitamin C.

Volume of milk intake

Mean volume of milk intake by infants in this study was below 850 ml/d, the volume estimate used in determining the RDA for infants. Individual intakes of milk by infants varied from 330 to 1018 ml/d. Since there were no significant differences in milk volume intake among the groups, the level of maternal intake of vitamin C appeared to have no effect on this parameter.

Vitamin C intakes of infants

Mean vitamin C intakes of infants ranged between 49 ± 9 and 86 ± 11 mg/d and were not statistically different among the five levels of maternal vitamin C intake (**Table 4**). However, intakes tended to be higher for infants whose mothers consumed ≥1000 mg vitamin C/d compared to infants whose mothers consumed less than this amount.

TABLE 4
Intakes of milk and vitamin C by infants

Maternal intake	No of observations	Volume of milk intake	Vitamin C intake
mg/day		*ml/day*	*mg/day*
<100	7	588 ± 46*	49 ± 9
100–199	15	601 ± 49	65 ± 8
200–399	15	619 ± 45	60 ± 7
400–999	15	662 ± 33	71 ± 5
≥1000	8	750 ± 68	86 ± 11

* Mean ± SEM.

Urinary excretion of vitamin C

Maternal excretion of vitamin C in urine was correlated significantly (r = 0.70) with maternal intake of vitamin C (**Fig 3**). Women who consumed ≥1000 mg vitamin C per day excreted significantly more (p = 0.05) vitamin C in urine than women who consumed 100 mg vitamin C per day (**Fig 4**).

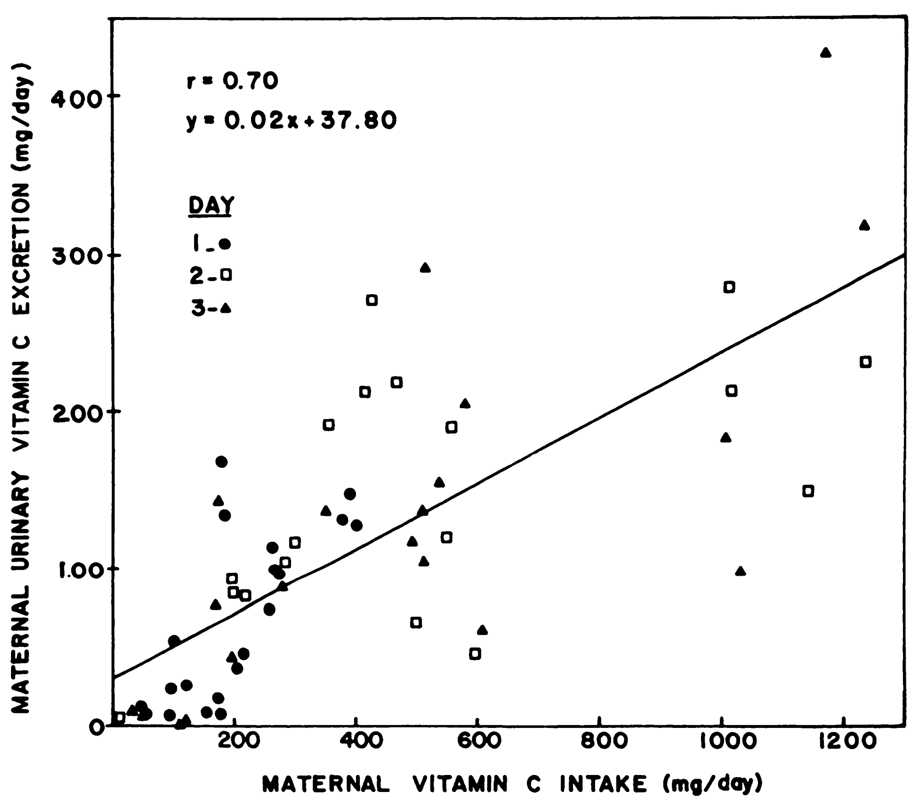

FIG 3. Relationship between maternal urinary vitamin C excretion and maternal vitamin C intake.

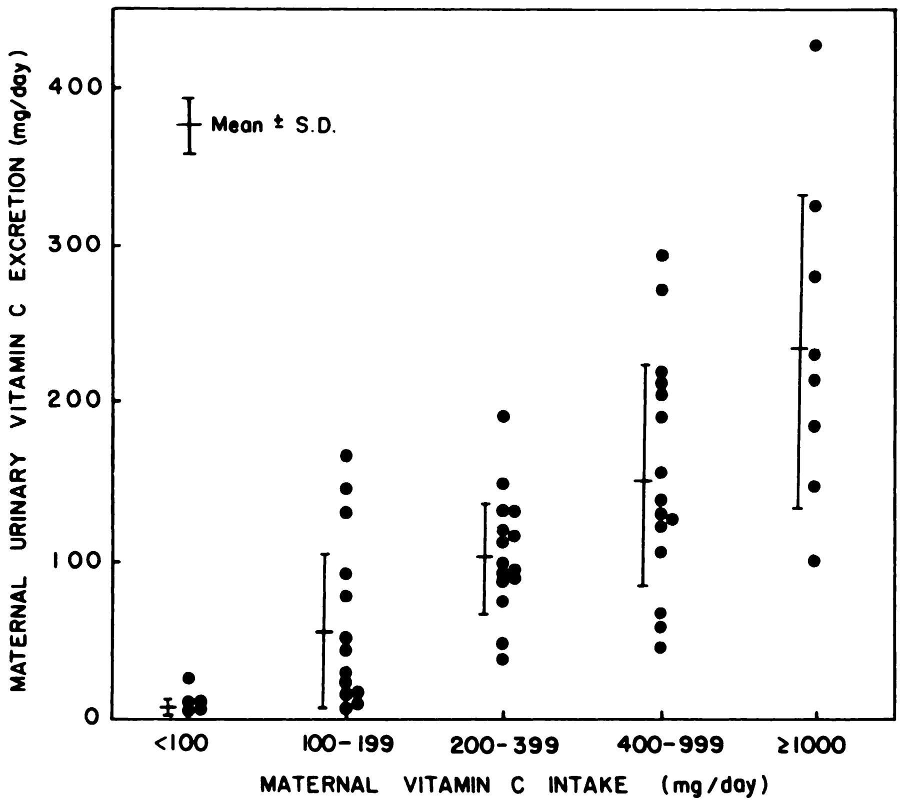

FIG 4. Maternal urinary vitamin C excretion in relation to maternal vitamin C intake.

Discussion

Regardless of the level of maternal intake of vitamin C mean vitamin C concentration in milk was approximately twice the minimum level of vitamin C recommended for infant formulas (2), and also twice the vitamin C content in certain infant formulas: Similac (Ross Labs, Columbus, OH) 55 mg/L; Enfamil (Mead Johnson, Evansville, IN) 55 mg/L; and SMA (Wyeth, Philadelphia, PA) 58 mg/L. Mean values from the present study for vitamin C concentration in human milk were approximately twice that (30 to 55 mg/L) used to estimate the RDA (3) for infants from birth to 6 months of age. The current allowances are based on earlier studies conducted by Selleg and King (4), Ingalls et al (5) and Tarjan et al (12). In recent years, the widespread availability of citrus fruits, greater awareness of vitamin C, and increased use of prenatal supplements may have led to improved ascorbic acid nutriture among many lactating women. This in turn could be reflected in higher levels of vitamin C in milk.

Short-term supplementation with ascorbic acid in this study did not significantly alter the vitamin C content of milk. Studies (4, 5, 13) conducted during the 1930's indicated that vitamin C content in milk increased when vitamin C intake was increased and that the level of vitamin C in human milk possibly reached a maximum concentration at around 80 mg/L milk (13, 4). The level of dietary vitamin C intake of the women who participated in those studies was not more than 20 mg/d prior to the studies. A study by Deodhar et al (14) also indicated that ascorbic acid supplementation increased the level of vitamin C in the milk of lactating Indian women who had been consuming approximately 23 mg vitamin C daily. Contrary to these findings, Thomas et al (7) reported no significant changes in the vitamin C content in milk of well-nourished lactating women who were given a 90 mg ascorbic acid supplement; however, total intake of vitamin C from diet plus supplement did not differ significantly between the non-supplemented and the supplemented group. Sneed et al (8) found that concentrations of vitamin C did not significantly increase when a supplement of 90 mg ascorbic acid/d was given to lactating women from low socioeconomic groups who consumed approximately 100% of the RDA for vitamin C. In agreement with findings from the latter studies, the present study indicated that ascorbic acid supplements of 250, 500 or 1000 mg/d did not significantly increase the concentration of vitamin C in milk of lactating women.

Vitamin C intakes of breast-fed infants were not significantly affected by different levels of maternal vitamin C intake. Intakes of infants were approximately twice the RDA of 35 mg vitamin C/d. Ingalls et al (5) estimated the vitamin C intake of infants to be 46 mg/d based on an assumed volume of milk intake of approximately 100 ml every 4 h. Selleg and King (4) reported that the vitamin C intake of infants was approximately 40 to 50 mg/d, calculated from an assumed milk intake of 621 ml/d.

Vitamin C intakes of only 3 of the 25 infants, who participated in the present study, were below the RDA for at least one day of the 3-day study period. The mothers of these infants did not take vitamin-mineral supplements before the study; therefore, diet was the mothers only source of vitamin C. Low vitamin C intakes for 2 of the infants was attributed to low maternal intake of vitamin C. Low vitamin C intake by the third infant, who was 20 weeks of age, may be associated with maturational changes in milk composition with stage of lactation since the mother's intake of vitamin C appeared to be adequate.

Vitamin C levels in milk did not increase in response to increasing maternal intake of vitamin C whereas urinary excretion of vitamin C increased significantly with increased levels of vitamin C intake. These differences in response of milk and urine to vitamin C intake suggested that mammary tissue became saturated with the vitamin. Vitamin C content in human milk failed to respond significantly to ten-fold increases in maternal intake of the vitamin. Thus a regulatory mechanism may be present in mammary cells to prevent an elevation in the concentration of vitamin C in milk beyond a certain saturation level.

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