Higher Absorption of Vitamin C from Food than from Supplements by Breastfeeding Mothers at Early Stages of Lactation

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Abstract: The aim of the present study was to determine the effect of vitamin C supply in the diet of lactating women on vitamin C concentrations in human milk ($n = 97$) sampled at different stages of lactation. Vitamin C levels were measured by liquid chromatography. Dietary intake of vitamin C was determined based on 3-day food dairies kept by breastfeeding mothers. Maternal dietary intakes of vitamin C from natural sources on lactation day 90 ($n = 18$) were significantly higher than on lactation days 15 ($n = 42$) and 30 ($n = 37$). The number of women taking vitamin C supplements decreased in successive stages of lactation. The average daily intake of vitamin C was estimated at 119 mg, but nearly 20% of mothers consumed less than 50 mg of vitamin C per day. No significant correlations were observed between lactation stage and vitamin C levels in breast milk ($r = 0.110$, $p = 0.064$). The average vitamin C concentrations in human breast milk were determined at 50.9 mg / L, and were not higher than 80.6 mg / L regardless of lactation stage and maternal intake of vitamin C. Vitamin C excretion into breast milk is regulated to prevent exceeding saturation level. The vitamin C concentration in milk was positive correlated with maternal intake of vitamin C from food, in the case of non-supplemented diet ($r = 0.402$, $p = 0.041$). Our results suggest that vitamin C occurring in food is much better absorbed and passes into breast milk than vitamin C from supplements.

Keywords: Breastfeeding, vitamin C, daily intake, supplements, lactation

Introduction

Breast milk is considered an ideal and most nutritionally complete food for both term and preterm infants up to 6 months of age [1]. It contains most nutrients needed by infants, including vitamin C. Vitamin C (ascorbic acid AsA and dehydroascorbic acid DHAsA) performs various functions and is involved in more than 300 processes in the human body. The biological roles of vitamin C are based on its reducing properties. Vitamin is specifically required by eight human enzymes that synthesize and metabolize collagen, hormones (such as adrenaline and serotonin) and carnitine, including enzymes that synthesize and modulate selected components of the nervous system, f acilitate the synthesis of corticosteroids and carnitine, the conversion of cholesterol to bile acids and the biosynthesis of connective tissue components, including elastin, fibronectin, proteoglycans and the bone matrix [2,3,4].

Vitamin C effectively neutralizes reactive oxygen species (ROS), reactive nitrogen species (RNS), singlet oxygen and hypochlorite, thus protecting tissues that are subjected to high oxidative stress [2,3]. Vitamin C also offers indirect antioxidant protection by regenerating other biological antioxidants, such as glutathione and α-tocopherol, to their active state. In foetuses, vitamin C deficiency can lead to abnormalities in brain and nervous system development, which can contribute to concentration problems and learning difficulties later in life [5]. Recent studies have shown association between very low levels of vitamin C in elderly people, especially the male gender and increased symptoms of depression [6].

The Dietary Reference Intake for vitamin C is 75 mg / day for adult females, 85 mg/day for pregnant women,

https://doi.org/10.1024/0300-9831/a000436

120 mg / day for breastfeeding women and 40–50 mg / day for infants up to 12 months of age [7]. According to the Institute of Medicine of the National Academies [7], the median dietary intake of vitamin C for adults is 102 mg / day in the United States and 72 mg / day in Canada.

In our previous studies [8,9], we found that concentrations of vitamins A and E decreased progressively as lactation advanced, whereas the total antioxidant capacity of human milk increased during lactation. Therefore, the aim of this study was to determine the concentrations of vitamin C in human breast milk sampled at different stages of lactation and to compare them with vitamin C levels in the diet of lactating women (intake from food and supplements).

Materials and Methods

Ethics

All procedures were approved by the Local Ethics Committee of the Medical University in Gdansk. The subjects had given their written informed consent before the beginning of experimental procedures.

Participants

This cross-sectional study was conducted between February and June in the cooperation with the Obstetrical Ward of the Regional Specialist Hospital in Gdansk. The surveyed group comprised healthy postpartum women older than 18 years who were planning to exclusively breastfeed their children for longer than one month. The inclusion criteria were as follows: full-term normal spontaneous vaginal delivery, uncomplicated gestation, good health status of the newborn (Apgar score ≥8 in the first minute of life) with normal birth weight (2.500–4.000g) and length. The participants‘ characteristics are presented in Table 1. The exclusion criteria included acute and chronic disorders (including gestational diabetes and atopic dermatitis), pharmacotherapy other than vitamin supplementation, dietary restrictions (elimination of selected products due to allergy in mother or child) and maternal smoking.

The study included 97 breast milk samples from 42 lactating women, residents of Gdansk (northern Poland). Experimental material was sampled from mothers on lactation day 15, 30, and on day 90. On lactation days 30 and 90, milk samples were not collected from all women because some mothers had finished breastfeeding, some participants met exclusion criteria on account of pharmacotherapy, acute infection or voluntary change of diet due to symptoms of allergy or food intolerance in their children.

Table 1. Participant characteristics. The parameters are presented as arithmetic means (±SD) and the corresponding value ranges are given in parentheses.

Parameter	Value*
No. of subjects	42
Maternal age (years)	26.2 ± 2.9 (18 – 41)
Maternal body weight at delivery (kg)	75.6 ± 9.5 (58 – 100)
Parity (n)	1.75 ± 0.98 (1 – 4)
Gestational age at birth (weeks)	39.1 ± 0.98 (38 – 42)
Birth weight (g)	3504 ± 483 (2720 – 3990)
Apgar score in the first minute (points)	9.74 ± 0.47 (8 – 10)
Vitamin supplementation (%)	
day 15	73.8 (frequencies 31 / 42)
day 30	56.8 (frequencies 21 / 37)
day 90	33.3 (frequencies 6 / 18)

*Values are presented as the mean ± standard deviation within the applicable range (in parentheses).

Breast milk sampling

Breast milk samples were collected on lactation days 15 (*n*=42), 30 (*n*=37) and 90 (*n*=18). The mammary gland was evacuated completely with the aid of an electric breast pump two hours after the first morning feeding (between 5 a.m. and 7 a.m.). The contents of the pump were gently stirred, 1 ml samples were collected and placed in a sterile container. The remaining milk was fed to the infants. At home, the samples were frozen at -18°C, delivered to the laboratory within six hours after collection and immediately frozen at -80°C until analysis, but not longer than for 2 months. Sample storage at freezing (-18°C) and ultra-low temperatures (-80°C) minimizes the loss of vitamin C in human milk [10].

Dietary intake of vitamin C

Dietary intake of vitamin C was determined directly before milk sampling based on 3-day nutritional diaries kept by the mothers. Diary entries were an abundant source of in-

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formation about the vitamin C intake of the women participating in the study. The results were processed using Dieta 4.0 software (National Institute of Food and Nutrition, Poland).

Determination of ascorbic acid content by HPLC

Vitamin C content was analysed by high performance liquid chromatography (HPLC) with the use of the Vitamin C HPLC Kit (Immundiagnostic AG, Bensheim, Germany). Milk samples of 0.2 ml were mixed with 0.2 ml precipitating reagent (PREC), left for 10 minutes at 4°C and centrifuged at 10.000 x g for 10 minutes. A supernatant was used in HPLC analysis.

A quantitative analysis of the ascorbic acid content of human milk samples was carried out in a Dionex liquid chromatograph equipped with a Rheodyne injector (model 7725i, Rheodyne®, Rohnert Park, CA, USA) and a SpectraSYSTEM UV3000HR detector (Thermo Electron Corporation, San Jose, CA, USA). A 25µl portion of the sample was injected onto a RP-18 column (Merck, Darmstadt, Germany) (125 x 4.0 mm I.D., 5 µm particle size), protected with a guard cartridge (C18, 5 µm), at the temperature of 30°C. Separation was performed by isocratic elution at 0.7 mL / min flow rate of the mobile phase and detection wavelength of 254 nm. Vitamin C retention time was 2.6 minutes. The results were expressed in mg / L of the sample.

Performance characteristics and linearity

The limit of detection (LOD) was 0.25 µg of vitamin C per mL and the limit of quantification (LOQ) was 0.75 µg / mL (three times the LOD value). The calibration curve was linear in the range of 0.0–250 µg / mL. The sensitivity was 0.4 µg of vitamin C in 1 mL of milk. The quantification process was performed in a commercial calibrator (vitamin C HPLC Kit). The amounts of native vitamin C were calculated based on sample peak areas, and peak areas were integrated by the external standard method.

Statistical analysis

Calculations were performed using Statistica 8 (StatSoft®, Poland) software. To the characterize of the samples the measures of central tendency (the mean, the median) and dispersion (the standard deviation) were used. P-values ≤ 0.05 were considered statistically significant. Normal distribution of continuous variables was evaluated with the use of the Kolmogorov–Smirnov test. Dietary intake of vitamin C and the vitamin C content of breast milk were compared by one-way ANOVA and Tukey's post-hoc test. Pearson's linear coefficient of correlation was calculated to describe the relationship between the dietary intake of vitamin C and the vitamin C content of breast milk.

Table 2. The participants' vitamin C intake from food and supplements [mg/day]

	Lactation day			
	15 N=42	**30** N=37	**90** N=18	**Mean** N=97
Intake from food	55.7^{a} ± 47.6	54.1^{b} ± 35.0	74.0^{a,b} ± 46.1	58.5 ± 43.4
	(8.53 – 244)	(9.67 – 186)	(20.8 – 199)	(8.5 – 244)
	42.4	45.0	60.1	46.6
Intake from supplements	73.3^{c} ± 53.1	59.7^{c} ± 59.3	31.1^{c} ± 51.9	60.3 ± 56.9
	(0 – 180)	(0 – 180)	(0 – 180)	(0 – 180)
	100	60.0	0	60.0
Dietary intake (food + supplements)	129 ± 67.0	114 ± 66.3	105 ± 54.4	119 ± 64.7
	(16.2 – 272)	(16.1 – 226)	(37.1 – 219)	(16.1 – 272)
	125	110	113	122

Values presented as mean ± standard deviation, range (in parentheses) and median;
$^{a, b, c}$ Statistically significant differences ($p < 0.05$; Tukey post-hoc test).

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Results

Dietary intake of vitamin C

The subjects‘ vitamin C intake from food and supplements is presented in Table 2. The mean daily intake of vitamin C was 118.8±64.7 mg during all stages of lactation. This maternal intake was near to the recommended dose for breastfeeding women, but nearly 20% of mothers consumed less than 50 mg of vitamin C per day. Vitamin C intake from food on lactation day 90 was about 74.0 mg / day and was significantly higher than on days 15 (55.7 mg / day) and 30 (54.1 mg / day) ($p=0.0197$, $p=0.0293$, Table 2). The main declared dietary sources of the analysed vitamin were fresh fruit, juice and vegetables. Approximately 74% of the subjects took vitamin C supplements at the first stage of lactation, 57% mothers used supplements after lactation day 30, and only 33% subjects – after lactation day 90 (Table 1). Lactating women used one tablet per day of six different types of supplements containing 60 to 180 mg vitamin C per tablet. One type contained 60 mg, three types - 100 mg, one - 110 mg and one 180 mg vitamin C per tablet. In the group of women who did not receive dietary supplements, the average daily vitamin C intake was 67.6±50.9 mg.

Concentrations of vitamin C in breast milk

The mean vitamin C content of human milk was 50.9±16.5 mg / L (Table 3). No significant differences in the vitamin C content of breast milk were observed between the samples collected on lactation days 15, 30 and 90. The mean vitamin C content of milk obtained from women who claimed to be taking vitamin C supplements at the time of sampling did not differ significantly from that determined in the milk of subjects without supplementation (49.9 mg / L vs. 52.3 mg / L, $p=0.241$, Table 3).

Dietary intake and vitamin C content of breast milk

No significant correlation was observed between the dietary intake of vitamin C and vitamin C concentrations in breast milk ($r=0.11$, $p=0.064$, Figure 1a) or in the parts of participants which took vitamin C supplements ($r=0.09$, $p=0.073$, Figure 1b). However, in the group of women whose only source of vitamin C was a natural, unfortified diet a weak, but significant correlation between the content of vitamins in the diet and the level of its prevalence in milk was observed ($r=0.402$, $p=0.041$, Figure 1c).

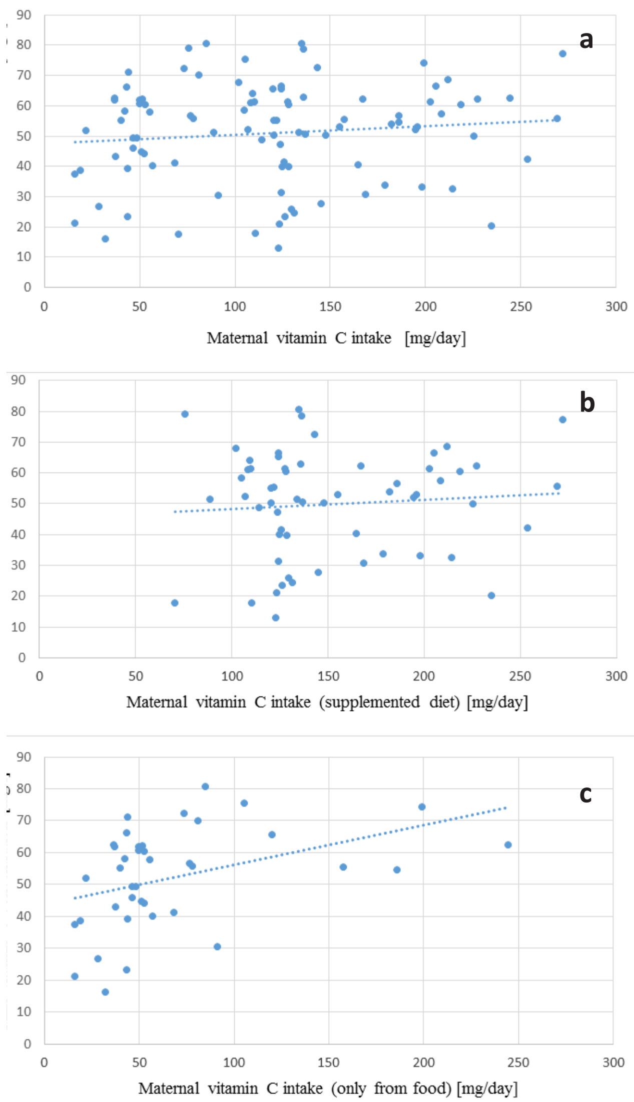

Figure 1. Relationship between breast milk vitamin C concentration and maternal vitamin C intake a) all samples, b) vitamin C supplemented diets, c) non- supplemented diets.

Discussion

The studied subjects‘ mean maternal intake of vitamin C on lactation day 90 was non-significantly lower than on days 15 and 30 ($p=0.077$ and $p=0.303$ respectively). However, the proportions between the amount of vitamin C from food products and from supplements in participants’ diet have changed. Vitamin C intake from food on lactation day 90 was significantly higher than on days 15 and 30 (Table 2). As stated in an mother’s dietary entries, due to dietary restrictions in early stages of lactation when the

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Table 3. The Vitamin C Content of Participants' Breast Milk [mg/L].

	Lactation day			
	15	**30**	**90**	**Mean**
All samples	N = 42	N = 37	N = 18	N = 97
	50.3 ± 18.3	50.4 ± 15.3	53.1 ± 16.4	50.9 ± 16.5
	(13.0 – 80.5)	(20.1 – 80.4)	(23.2 – 75.3)	(13.0 – 80.5)
	51.2	51.9	56.7	53.8
Diet without supplements	N = 11	N = 16	N = 12	N = 39
	43.4^{a,b} ± 17.1	56.3^{a} ± 12.9	55.2^{b} ± 15.7	52.3 ± 15.7
	(16.1 – 69.9)	(37.5 – 80.4)	(23.2 – 75.3)	(16.1 – 80.4)
	44.0	57.2	56.7	55.5
Diet with supplements	N = 31	N = 21	N = 6	N = 58
	52.7^{c} ± 17.7	46.0^{c} ± 15.7	48.8 ± 18.5	49.9 ± 17.1
	(13.0 – 79.0)	(20.1 – 68.5)	(23.4 – 66.5)	(13.0 – 80.5)
	53.8	51.2	57.6	52.5

Values presented as mean ± standard deviation, range (in parentheses) and median;
^{a,b,c} Statistically significant differences ($p < 0.05$; Tukey post-hoc test).

mothers limited their consumption of fresh fruit and vegetables in fear of inducing an allergic or gastric reaction in their children. Those choices could reduce the content of essential nutrients, such as vitamins, in the diets of lactating mothers.

The number of samples was quite low on lactation day 90 because Polish women generally do not breastfeed for long periods of time. In a study by Wilińska et al. [11], 87% of mothers breastfed on the third day postpartum, 59% breastfed for 4 months postpartum, but in the latter group, only 31% of babies were exclusively breastfed. Only 14% of children were exclusively breastfed for 6 months postpartum. Similar observations were made in Spain. According to the Spanish National Health Survey, the estimated prevalence of breastfeeding was 68.4% six weeks after birth and 52.48% and 24.72% three and six months postpartum, respectively [12]. By contrast, in Norway, almost 100% of babies are breastfed at birth, 80% are breastfed up to 6 months and 46% up 12 months postpartum [13].

More women used C supplements in early stages of lactation. Approximately 74% of the analysed mothers took vitamin C supplements in the first month postpartum, but only 33% of them continued their supplementation regime on lactation day 90. The use of dietary supplements during lactation is very poorly documented. Limited data suggest that usage is dependent on demographic, economic and social factors [14]. Stultz et al. [15] studied the intakes of medication (including vitamin supplements) by lactating women in the United States to reveal that the majority of breastfeeding women (nearly 73%) took multivitamin supplements. The above authors also observed that significantly more women used dietary supplements during pregnancy than during lactation.

The average daily intake of vitamin C in the analysed group met the daily recommendations for breastfeeding women. However, in the group of women who did not take vitamin preparations, the average vitamin C intake was equal to only 56% DRI for lactating women. Our results clearly show that a standard diet consumed by lactating women without additional supplementation does not provide optimal amounts of vitamin C. Vitamin C is one the most commonly used food supplements [16,17].

Szponar et al. [18] determined the average daily vitamin C intake of Polish women at 82.1 mg on an annual basis, which corresponds to nearly 133% DRI for females with low levels of physical activity (60 mg/day). In a nationwide study, the average vitamin C intake exceeded the values recommended for males and females from all age groups. Despite the above, an analysis of vitamin intake distribution revealed a vitamin C deficiency in 40% of the population [18]. Our results indicate that breastfeeding women should increase their vitamin C intake, preferably

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from natural sources, such as fruit and vegetables. Mothers of children suffering from allergies or digestive problems are advised to take vitamin C supplements.

Salmenpera [19] reported a significant decrease in the vitamin C content of human milk between colostrum (61.8±9.9 mg/L) and the fourth month of lactation (49.7±10.6 mg/L). Ahmed et al. [20] observed a gradual but statistically insignificant decline in the vitamin C content of breast milk sampled from Bangladeshi mothers during the first four weeks of lactation: from 35.2±5.6 in colostrum to 30.3±6.7 mg/L in mature milk. In our study, we found no significant differences in the vitamin C content of breast milk between lactation stages (weeks 2 and 11). Vitamin C concentrations in human milk increased from about 50.3 mg/L on lactation day 15 to 53.0 mg/L on day 90, but the noted difference was not statistically significant (p=0.291). The mean vitamin C content of human milk was 50.9 mg/L across all lactation stages.

Hoppu et al. [21] reported a positive correlation between vitamin C intake from the maternal diet and vitamin C concentrations in breast milk. The above authors observed that vitamin C levels in milk were affected only by vitamin C intake from the mother‘s diet, whereas the intake from supplements had no effect on the above. In our study were observed the similar occurrence. No significant correlations between vitamin C intakes and vitamin C concentrations in breast milk were observed when intakes from the diet and nutritional supplements were analysed. But vitamin C concentration in milk correlated significantly to maternal intake of vitamin C from non-supplemented diet (Figure 1). However, it should be noted that the vitamin C content of human milk ranged from 13.0 mg/L to max. 80.5 mg/L (average 50.9 mg/L) throughout lactation despite significant differences in the mother‘s dietary intake of vitamin C (from 16.1 to 272.3 mg/day). The vitamin C average content of human milk remains statistically constant even when vitamin C is ingested in high doses. Our results indicate that the content of vitamin C in human milk is regulated. The results of this study collaborate the findings of other authors. Byerley and Kirksey [22] observed that vitamin C levels in milk did not increase in response to an increase in the maternal intake of vitamin C (supplements of 250, 500 or 1000 mg vitamin C/day), whereas urinary excretion of vitamin C increased significantly. These studies indicate that vitamin C excretion into breast milk is regulated to prevent exceeding a saturation level. Daneel-Otterbech et al. [23] detected that the average AsA content of milk sampled from African women (Abidjan, Ivory Coast) was approximately 50% lower than that of European mothers (Zurich, Switzeland), 31 mg/kg and 63 g/kg of milk, respectively. The consumption of relatively high doses of vitamin C produced a modest response in European women (up to approximately 70 mg/kg), whereas a 3-fold increase was noted in the vitamin C content of milk samples from African women (max. 46 mg/kg). Tawfeek et al. [24] observed seasonal variations in the vitamin C content of the milk of nursing mothers in Baghdad, which was much higher in summer (max. 39 mg/L). The above findings suggest that the vitamin C content of human milk is influenced by the long-term diet which, in turn, is affected by the women‘s place of residence.

Conclusions

Depending on the stage of lactation, human breast milk contained mean from 50.3 to 53.0 mg/L of vitamin C, but the observed changes in vitamin C levels were not statistically significant. The mean vitamin C contents of milk obtained from mothers with and without supplementation were the same level. Despite significant differences in vitamin C daily intake among breastfeeding mothers and vitamin C levels in breast milk, no significant correlations were observed between vitamin C consumption and the vitamin C content of milk. But vitamin C concentration in milk was positive correlated to maternal intake of vitamin C from foodstuff, only in the case of non-supplemented diet. This result might indicate that the content of vitamin C in milk can increase to saturated level, but only in response to increasing the natural sources of vitamin C. Our results suggest that vitamin C occurring in food is much better absorbed by mother’s body and passes into breast milk than vitamin C from supplements.

The diets of breastfeeding women from northern Poland are not balanced and are deficient in vitamin C. Lactating women‘s dietary intakes of vitamin C fulfil the nutritional requirements of the child, but they do not meet the nutritional needs of the mother. The results of our study clearly indicate that lactating women need nutritional guidance. A well-balanced diet rich in fruit and vegetables should be an adequate source of vitamin C. Natural sources of vitamin C, including fruit, berries and vegetables, also contain other health-promoting compounds, such as carotenoids, flavonoids and folic acid. Lactating women should choose diets that are varied, balanced and natural.

Acknowledgements

The authors are thankful to participating mothers for their time and effort in providing milk samples and dietary records.

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Conflict of Interest

The authors declare that there are no conflicts of interest.

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