Relationship between the dietary intake, serum, and breast milk concentrations of vitamin A and vitamin E in a cohort of women over the course of lactation

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Abstract

This study evaluated the nutritional status of lactating women with regard to vitamins A and E and the relationship between dietary intake and concentrations in serum and milk. A longitudinal study was conducted with 43 women at a hospital in northeastern Brazil. Blood and milk samples and food intake recalls were obtained at three moments during the breastfeeding period. Retinol and alpha-tocopherol were analysed by high-performance liquid chromatography. Dietary inadequacy was analysed according to the estimated average requirement, with intrapersonal variation adjusted by the multiple source method. Food intake was classified by quartiles of consumption. Serum retinol was 1.65 $\mu\text{mol/L}$, with 5% of low concentrations ($<0.7 \mu\text{mol/L}$) at the first collection. Serum alpha-tocopherol decreased from 30.18 to 25.49 $\mu\text{mol/L}$ at the third collection ($P = 0.008$), with an increase in the percentage frequency of deficiency ($<12 \mu\text{mol/L}$). Both vitamins maintained stable concentrations in milk at the different collection times, and the overall dietary inadequacy of vitamins A and E was 58% and 100%, respectively. There was a correlation only between vitamin A intake and serum retinol ($r = 0.403$, $P = 0.007$), and higher retinol concentrations were found in women classified in the highest consumption quartile ($P = 0.031$). Over the course of lactation, there was a high degree of inadequacy in vitamin intake and a reduction in serum alpha-tocopherol, whereas its concentrations in milk remained unchanged. Dietary intake of vitamin A has been shown to influence serum retinol, which underscores the importance of adequate nutrition and monitoring of vitamin deficiency during lactation.

KEYWORDS

alpha-tocopherol, breastfeeding, food consumption, lactation, retinol

1 | INTRODUCTION

During lactation, nutritional requirements are even higher than during pregnancy because of the need to replenish the substantial quantities of nutrients secreted in breast milk. Thus, an increase in the dietary

intake of most nutrients, including vitamins A and E, is recommended for breastfeeding women (Guiné & Gomes, 2015; Institute of Medicine, 2000, 2001).

Vitamin A plays a role in vision, intercellular communication, cell growth, and cell differentiation (Erdman, Macdonald, & Zeisel, 2012).

As for vitamin E, it acts as a free-radical scavenger, combatting lipid peroxidation in lipoproteins and cell membranes (Traber & Atkinson, 2007), and is important for optimal immune function, inflammation control, and cognitive performance (Dror & Allen, 2011; Traber, 2014).

Vitamin A deficiency (VAD) is one of the major nutritional problems in the world and is considered a public health issue in Brazil and other developing countries (Organização Mundial da Saúde, 2013). VAD has been observed in lactating women during various stages of lactation, affecting up to 33% of the women studied (Lira, Lima, Medeiros, Silva, & Dimenstein, 2013; Ribeiro et al., 2010; Samba, Tchibindat, Gourmel, Houzé, & Malvy, 2013; Souza et al., 2012).

Vitamin E deficiency (VED) during lactation is not frequently reported in the literature. Low concentrations of serum alpha-tocopherol were observed in women in the immediate post-partum period, which is directly related to the vitamin E status of the newborns (Lira, Ribeiro, Grilo, Lima, & Dimenstein, 2012; Ribeiro et al., 2016). Over the course of lactation, in South Africa, 70% of lactating women presented VED (Papathakis, Rollins, Chantry, Bennish, & Brown, 2007), which demonstrates the importance of studying these micronutrients during the lactation phase as well as their relationship with breast milk. Breast milk should contain adequate amounts of vitamin A and vitamin E because newborns have low reserves of these vitamins in their bodies at birth (Debie, 2007). This makes breastfeeding an important strategy for combatting nutritional deficiencies in children (Grilo et al., 2015; Lima, Dimenstein, & Ribeiro, 2014).

The composition of these vitamins in milk is known to vary according to the stage of lactation, the period of feeding (Ribeiro & Dimenstein, 2004), prematurity (Rodrigues, 2016), and different populations (Gurgel et al., 2014; Jiang et al., 2016; Lima et al., 2014). Nevertheless, there is no consensus yet as to whether maternal nutritional status and dietary intake can directly affect the concentrations of these vitamins in milk. Some studies have not found such a relationship (Deminice et al., 2018; Jiang et al., 2016; Lima et al., 2017; Lira et al., 2012; Ribeiro et al., 2016; Vries, Pundir, McKenzie, Keijer, & Kussmann, 2018), whereas others have found positive or negative associations between these variables (Azeredo & Trugo, 2008; Fares et al., 2016). Most of these analyses were performed during the first 72 hr after delivery, in which the secreted milk is termed colostrum and contains high concentrations of vitamins independently of the levels circulating in the mother's body. It is important to explore this relationship during the entire breastfeeding period.

As these vitamins are of great importance during the stage of infancy, and because there is some reported evidence of low concentrations in breast milk (Azeredo & Trugo, 2008; da Silva et al., 2017; Lima et al., 2017; Lira et al., 2013; Medeiros et al., 2016; Ribeiro et al., 2010; Souza et al., 2012), it is important to explore the relationship between maternal nutritional status and milk composition over the course of lactation, mainly in the mature milk phase. Thus, the objective of this study was to evaluate the vitamin A and vitamin E nutritional status of women over the course of lactation, as well as the relationship between their serum and breast milk concentrations and habitual dietary intake.

Key messages

- Concentrations of maternal serum alpha-tocopherol were reduced over the course of lactation, demonstrating that greater nutritional care at this period is necessary.
- The alpha-tocopherol and retinol levels in mature milk remain unchanged during lactation, even in situations of low vitamin intake and low nutritional status. This suggests that maternal reserves are used to maintain adequate concentrations in milk, thus guaranteeing the infant's supply.
- Dietary intake during lactation influences circulating retinol, and it is important to maintain adequate nutrition during this period.

2 | METHODS

2.1 | Ethical aspects and sample universe

All stages of the study were approved by the Research Ethics Committee of the Onofre Lopes University Hospital at the Federal University of Rio Grande do Norte (CAAE 61189516.9.0000.5292). The study was developed in compliance with the ethical norms for research involving human beings.

Study participants were lactating women recruited from the paediatric outpatient clinic at the Onofre Lopes University Hospital (*Hospital Universitário Onofre Lopes*—HUOL) in Natal, Rio Grande do Norte, Brazil, from December 2016 to December 2017. Breastfeeding mothers were eligible to be included irrespective of practicing exclusive or partial breastfeeding. To be eligible, they must not have been diagnosed with any infectious disease or fat malabsorption syndrome, and they had to be non-smokers who did not take vitamin A or vitamin E supplements during lactation, after giving birth to a single newborn without birth defects. Mothers were excluded if they did not have enough milk or serum for analysis of vitamin levels, if they took daily supplements containing vitamin A or vitamin E, or if they used illicit drugs.

The sample size was calculated using G*Power Software (version 3.1.9.2; Faul, Erdfelder, Lang, & Buchner, 2007), determining a minimum sample size of 28 participants per group, considering the comparison between three dependent moments. For this calculation, we adopted more conservative criteria for a two-tailed test with alpha of 0.05, power of 0.80, and effect size of 0.80, in order to adjust for losses during the process of data collection.

A case was regarded as a loss to follow-up if a lactating mother withdrew from the research study or if she participated in fewer than three collections of breast milk and blood. Thus, 116 lactating women were initially recruited, and 43 (Figure 1) remained after exclusion criteria. Some collections were performed with 42 women because of insufficient milk production or because it was not possible to determine the food intake.

FIGURE 1 Research design regarding data collection

2.2 | Data collection

The collections were performed at the HUOL's paediatric clinic or during a scheduled home visit, if there was prior consent. Data collection occurred at three different moments in time: the first collection occurred between 25 and 74 days after birth, because this is the stage when breastfeeding is established; the second collection occurred about 30 days after the first one; and the last collection was scheduled to 30 days after the second one, in order to ensure that the woman would still be breastfeeding, considering that in Brazil, the average duration of exclusive breastfeeding is 2 months, whereas partial breastfeeding is practised for 9 months (Brasil. Ministério da saúde. Centro Brasileiro de Análise e Planejamento, 2009).

Milk and blood samples and the anthropometric measures (weight and height) of the lactating women were obtained, and the participants were interviewed and completed a semistructured form to collect data on socioeconomic status, childbirth, clinical history, and diet. Maternal and infant dietary information, as well as maternal milk and blood samples, were acquired at all follow-up collections, when the mother's weight was also assessed (Figure 1).

Two millilitres of breast milk were collected under fasting by manual expression from a single breast that had not been recently nursed. The milk was collected before and at end of the feeding (represented by the final moment of one breastfeeding session). A 5-ml blood sample was drawn, under fasting, via the brachial vein, through venipuncture. All biological samples were collected in polypropylene tubes packed in aluminium foil, after which they were transported in refrigeration units. Breast milk was stored at -20°C until the time of analysis. Prior to storage, the blood samples were centrifuged for

10 min (at 1914 g), with subsequent separation of serum aliquots for vitamin analysis.

After the data collection, all participants were provided with guidance by the Extension Project linked to the research, addressing the management of lactation, milk donation, healthy food, and food sources of vitamin A and vitamin E.

2.3 | Chemical analysis

The extractions of alpha-tocopherol and retinol from serum, as well as the extraction of alpha-tocopherol from the milk, were performed according to the method adapted from Ortega, López-Sobaler, Quintas, Martínez, and Andrés (1998), in which 95% ethanol (Vetec, Rio de Janeiro, Brazil) was used for protein precipitation. Hexano PA (Vetec, Rio de Janeiro, Brazil) was used as an extractive reagent. The retinol extraction from the milk samples was performed according to the method adapted from Giuliano, Neilson, Kelly, and Canfield (1992), using 95% ethanol (Vetec, Rio de Janeiro, Brazil), as well as Hexano PA (Vetec, Rio de Janeiro, Brazil) and potassium hydroxide 50% (Vetec, Rio de Janeiro, Brazil) for retinyl ester hydrolysis.

After evaporation in an atmosphere of nitrogen, the residues were dissolved in 250 μl of absolute ethanol (Vetec, Rio de Janeiro, Brazil) for analysis of retinol and alpha-tocopherol in serum and retinol in the milk. Alternately, residues were dissolved in 250 μl of dichloromethane (Vetec, Rio de Janeiro, Brazil):methanol (Sigma-Aldrich, USA) 2:1 (v:v) for analysis of alpha-tocopherol in the milk, followed by high-performance liquid chromatography analysis.

The high-performance liquid chromatography used a Shimadzu chromatograph (Shimadzu, Kyoto, Japan) and a C18 LiChrospher® 100 RP-18 (5 µm) column (Merck, Darmstadt, Germany). The chromatogram was developed in isocratic elution with methanol as the mobile phase and flow rate of 1.0 ml/min, at a wavelength of 325 nm for retinol and 292 nm for alpha-tocopherol. The vitamins in the samples were identified and quantified by comparing their areas under the chromatographic curve with the standard area (Sigma-Aldrich, Brazil). The concentration of the standard sample was confirmed by the specific extinction coefficient of retinol (ϵ 1%, 1 cm = 1,780 to 325 nm) and alpha-tocopherol (1%, 1 cm = 75.8 to 292 nm) in absolute ethanol (Vetec, Rio de Janeiro, Brazil).

The calibration curve was made using standard micronutrient solutions (Sigma®). The linearity of the analytic method was confirmed by the determination of the statistical significance of the calibration curve coefficient at the concentration of 3.3–104.4 ng/20 µl ($r^2 = 0.9999$) for retinol and 50.1–1,201.7 ng/20 µl ($r^2 = 0.9971$) for alpha-tocopherol. The limit of quantification was reached at the concentrations of 0.04 and 0.05 µmol/L and the limit of detection at 0.04 and 0.05 µmol/L for retinol and alpha-tocopherol, respectively. The coefficient of variation was 4% for milk and serum samples. The recovery test was 93.3% in milk and 107% in serum for retinol and, for alpha-tocopherol, 97.6% in milk and 95.7% in serum.

Concentrations of maternal serum retinol below 0.7 and 1.05 µmol/L in milk were indicative of low vitamin A concentrations (West, Eilander, & Lieshout, 2002; World Health Organization (WHO), 1996). Vitamin E concentration was classified as low when serum alpha-tocopherol values <12 µmol/L were found (Institute of Medicine, 2000).

2.4 | Food intake assessment

The vitamin A and vitamin E intake of the lactating women was evaluated by three 24-hr dietary recalls obtained during data collection, which consisted of reporting all foods, supplements, and beverages consumed in the 24-hr period prior to the interview. The home measures described were converted to grams or millilitres based on standards adopted in other books and texts (Araújo & Guerra, 2007; Brasil, 2011; Tomita & Cardoso, 2002).

The food surveys were analysed using the Virtual Nutri Plus software (virtualnutriplus.com.br/). Intrapersonal variation was corrected through the multiple source method (Department of Epidemiology, German Institute of Human Nutrition, Potsdam-Rehbrücke, version 1.0.1, <https://msm.dife.de/>). The habitual consumption of vitamin A and vitamin E was determined from the three 24-hr dietary recalls, with intake adjusted for the energy content of the diet. This was obtained by the residual method, using SPSS software, version 21.0.

The prevalence of inadequacy of habitual nutrient intake was evaluated according to the estimated average requirement, calculated from the area of the curve that corresponds to the proportion of individuals with dietary inadequacy. It was evaluated in the normal distribution table (z-score), adopting 900 µg/day of vitamin A and 16 mg/day of vitamin E for lactating women (Institute of Medicine, 2000, 2001).

To evaluate the relationship between diet and biochemical indicators, the lactating women were divided by quartiles of consumption, into two groups: the lowest (up to the 25th percentile) and the highest (above the 25th percentile) quartile of vitamin intake. The 25th percentile was considered to refer to values of 721.6 µg/day and 6.6 mg/day for vitamin A and vitamin E, respectively.

2.5 | Statistical analysis

Statistical analysis was performed using the IBM SPSS statistical software version 21.0 for Windows (SPSS Inc., Chicago, IL, USA). The Kolmogorov–Smirnov normality test was applied to verify the distribution of the variables. The numerical results were expressed as mean (standard deviation) and the categorical results were expressed in absolute and percentage frequencies.

Analysis of variance with Tukey post hoc test was used to verify the mean differences in the values at the three collection times. To evaluate the relationship between serum and breast milk concentrations and habitual dietary intake of vitamins, the Pearson's correlation coefficient was calculated. In addition, the *t* test on independent samples was performed to compare the mean concentrations of retinol and alpha-tocopherol in serum and breast milk, according to the food intake groups of the respective vitamins. Differences were considered significant when $P < 0.05$.

3 | RESULTS

3.1 | General characteristics

The mean age was 27.8 (7.4) years old. The majority of the women had complete secondary education and family income level below the minimum wage; 63% of them were overweight or obese (World Health Organization (WHO), 2000) at the first collection, the mean gestational period at delivery was 36 (3.0) weeks, and 60% had a caesarean delivery; 86% of the women practised exclusive breastfeeding during the first data collection period (Table 1), and the percentages decreased to 74% and 73% at the second and third moments of data collection, respectively. The majority of the newborns (85%) had adequate weight for their gestational age, according to the birth weight/gestational age index (Villar et al., 2015; WHO, 2006).

3.2 | Status of vitamin A and vitamin E

The mean serum retinol and maternal breast milk retinol values were 1.66 and 1.57 µmol/L in all collections ($P > 0.05$), respectively. The mean values showed no difference over the course of lactation (Figure 2a). Low serum concentrations of retinol were found only in the first collection, equivalent to 5%. Concentrations of retinol below 1.05 µmol/L were identified in 12%, 14%, and 12% of the milk at the three collection times, respectively. The habitual vitamin A intake during lactation was 852.7 (274.6) µg/day, and dietary inadequacy was observed in 58% of the lactating mothers.

Serum alpha-tocopherol values were 30.18 (8.8) µmol/L at the first collection time. The values decreased to 25.49 (9.3) µmol/L at

TABLE 1 Socioeconomic, demographic and health-related characteristics, and prevalence of inadequate intake of vitamins of lactating women. Natal, Rio Grande do Norte, Brazil, 2016–2017

Characteristics	n	%	[95% CI]
Level of education of the mother			
Incomplete primary education	7	16.3	[7.0, 27.9]
Complete primary education	5	11.6	[2.3, 20.9]
Incomplete secondary education	7	16.3	[7.0, 27.9]
Complete secondary education	18	41.9	[25.6, 55.8]
Complete higher education	6	14.0	[4.7, 25.6]
Family income level ^a			
<1 minimum wage	23	53.5	[37.2, 69.8]
>1 minimum wage	20	46.5	[30.2, 62.8]
Number of children birthed by the mother			
First child (1 child)	20	46.5	[32.6, 62.8]
Multiple children (>1 child)	23	53.5	[37.2, 67.4]
Type of birth			
Natural	17	39.5	[25.6, 55.8]
Caesarean	26	60.5	[44.2, 74.4]
Classification of maternal BMI at the first collection ^b			
Low weight	2	4.7	[0.0, 11.6]
Normal	14	32.6	[18.6, 48.8]
Overweight	10	23.3	[11.6, 37.2]
Obese	17	39.5	[25.6, 53.5]
Type of maternal breastfeeding at the first collection			
Exclusive maternal breastfeeding	37	86.0	[74.4, 95.3]
Maternal breast milk and other milks ^c	5	14.0	[4.7, 25.6]
Vitamin A intake µg/day (mean [SD])	852.7 (274.6)		[768.1, 937.2]
Prevalence of inadequate habitual consumption of vitamin A	—	58	—
Vitamin E intake mg/day (mean [SD])	7.0 (1.0)		[6.7, 7.4]
Prevalence of inadequate habitual consumption of vitamin E	—	100	—

Note. n: number; CI: confidence interval; BMI: body mass index; SD: standard deviation.

^aBrazilian minimum wage per month = US\$ 291.5.

^bWHO Classification, 2000.

^cIncluding cow's milk, goat's milk, and formula.

FIGURE 2 Concentrations of retinol and alpha-tocopherol in serum and breast milk of lactating women over the course of lactation. Natal, Rio Grande do Norte, 2016–2017. (a) Retinol concentrations in serum and maternal milk at Collection 1 (25 to 74-day post-partum), Collection 2 (30 days after Collection 1), and Collection 3 (30 days after Collection 2). Analysis of variance test with Tukey post hoc test. (b) Concentrations of alpha-tocopherol in serum and maternal milk at Collection 1 (25 to 74-day post-partum), Collection 2 (30 days after Collection 1), and Collection 3 (30 days after Collection 2). Analysis of variance test with Tukey post hoc test. *Significant difference between the mean values, considering $P < 0.05$

the end of the study period ($P = 0.008$; Figure 2b), with a consequent increase in the percentage of low concentrations of vitamin E over the course of lactation, going from no cases at the first collection time to 7% of the lactating mothers at the second and third

collection times. The concentration of alpha-tocopherol in breast milk was around 8 µmol/L in all collection samples, and there was no difference in the mean values over the course of lactation ($P = 0.737$; Figure 2b).

The habitual vitamin E intake was 7.0 (1.0) mg/day, and the dietary inadequacy in the population studied reached 100%, demonstrating that all lactating women had vitamin E intake below nutritional recommendations (16 mg/day).

3.3 | The relationships between biochemical indicators and habitual intake with regard to retinol and alpha-tocopherol

When we correlated the serum vitamin concentrations with the respective breast milk and dietary intake at the final collection time of the study, we found a positive association between serum retinol and habitual vitamin A intake ($r = 0.403$, $P = 0.007$; Figure 3b).

When the women in the study were divided according to the vitamin A quartile classification, a significant difference was observed in the mean concentrations of serum retinol at the third collection time. Thus, lactating mothers who had higher concentrations of serum

retinol at the third collection time were in the higher vitamin A intake group. When dividing women according to the vitamin E intake rating (up to the 25th percentile vs. above the percentile), no significant differences were found in the concentrations of alpha-tocopherol at the third collection time (Table 2).

4 | DISCUSSION

Lactating women are considered to be a biologically vulnerable group at risk of presenting marginal or deficient nutritional status with regard to several micronutrients and related compounds (Allen, 2005). Therefore, monitoring of nutritional deficiencies and excesses is necessary to reduce the risks of complications for mothers and, consequently, for babies who are exclusively breastfed.

The concentrations of retinol in serum and breast milk observed in the study were adequate, with a mean of 1.66 $\mu\text{mol/L}$ in serum and 1.57 $\mu\text{mol/L}$ in milk; these are similar values to those found in

FIGURE 3 Correlations between biochemical indicators and the habitual intake of vitamin A and vitamin E of lactating women at the third moment of data collection. Natal, Rio Grande do Norte, Brazil, 2016–2017. (a) Correlation between retinol in maternal milk and the retinol in maternal serum. (b) Correlation between habitual vitamin A intake and the retinol in maternal serum. (c) Correlation between habitual vitamin A intake and the retinol in maternal milk. (d) Correlation between α -tocopherol in maternal milk and the α -tocopherol in maternal serum. (e) Correlation between habitual vitamin E intake and the α -tocopherol in maternal serum. (f) Correlation between habitual vitamin E intake and the α -tocopherol in maternal milk. r : Pearson's correlation coefficient; P value: level of significance

TABLE 2 Mean values (standard deviation) of serum retinol and serum alpha-tocopherol and maternal breast milk according to categories of habitual intake of these micronutrients in the maternal diet. Natal, Rio Grande do Norte, Brazil, 2016–2017

Biochemical indicators of retinol	Lowest intake of vitamin A ($\leq p25$)	Highest intake of vitamin A ($> p25$)	P value
Serum retinol ($\mu\text{mol/L}$)	1.48 (0.31), $n = 10$	1.75 (0.31), $n = 33$	0.031*
Retinol in breast milk ($\mu\text{mol/L}$)	1.40 (0.48), $n = 10$	1.67 (0.37), $n = 32$	0.126
Biochemical indicators of alpha-tocopherol	Lowest intake of vitamin E ($\leq p25$)	Highest intake of vitamin E ($> p25$)	P value
Serum alpha-tocopherol ($\mu\text{mol/L}$)	21.18 (8.69), $n = 11$	26.98 (9.11), $n = 32$	0.076
Alpha-tocopherol in breast milk ($\mu\text{mol/L}$)	8.57 (5.19), $n = 11$	9.05 (4.83), $n = 31$	0.794

Note. Independent samples *t* test.

*Significant difference between the mean values, considering $P < 0.05$.

the literature (Azeredo & Trugo, 2008; Kim et al., 2018; Matamoros, Visentin, Ferrari, Falivene, & Fasano, 2018; Meneses & Trugo, 2005; Souza et al., 2012). The percentage of low concentrations of retinol was around 5%, according to the serum indicator, which corroborated data from other studies of lactating women in Brazil (Azeredo & Trugo, 2008; Meneses & Trugo, 2005). In Brazil, VAD is considered a public health problem that has been monitored by the WHO, affecting a percentage of 10 to 20% of the population (Brasil. Ministério da Saúde. Secretaria de Atenção à Saúde. Departamento de Atenção Básica, 2013).

Deficient vitamin A values were found in the breast milk of a maximum of 14% of the lactating mothers, which is a result much lower than the results reported in the other states of Brazil, where proportions vary between 20% and 87% of the lactating mothers who were studied (Azeredo & Trugo, 2008; Deminice et al., 2018; Meneses & Trugo, 2005; Souza et al., 2012).

Of the lactating women who were studied, the percentage who had dietary inadequacy was 58%. These women may have a higher risk for low concentrations of vitamin A in their serum and breast milk, because low dietary intake means that hepatic retinol tends to be used, which impairs vitamin A status (Tanumihardjo et al., 2016). Therefore, it is imperative that lactating women maintain adequate vitamin A intake in order to avoid a loss of vitamin A in their breast milk in situations of prolonged dietary inadequacy. Adequate vitamin A intake can prevent VAD in the maternal–infant population.

Additionally, the present study found a relationship between habitual vitamin A dietary intake and the concentration of retinol in maternal serum (Figure 3b and Table 2), demonstrating that lactating mothers with higher intake of vitamin A had a higher concentration of vitamin A in their serum at the third collection time (the final period evaluated). This was the first study that showed this relationship by analysing women after 60 days of lactation. For lactating mothers in China, for the same time period of study and categorizing dietary intake by food groups from three 24-hr recalls, this association was not found (Jiang et al., 2016).

The positive relationship between dietary intake and serum retinol demonstrates the importance of a healthy and balanced diet, with adequate intake of food sources of vitamin A. Therefore, to reduce the incidence of VAD in lactating women, actions should be promoted that increase the availability and consumption of food sources of vitamin A, with consideration of economic, social, and cultural factors.

During lactation, a reduction in concentrations of alpha-tocopherol in maternal serum was observed, with a consequent increase in cases of

low vitamin concentrations (Figure 2b). This finding is likely related to a decrease in circulating lipids at this stage, or to a greater use of maternal vitamin E for milk secretion (Institute of Medicine, 2000; Schweigert, Bathe, Chen, Büscher, & Dudenhausen, 2004). This leads to a decrease in nutritional reserves if there is not adequate consumption of vitamin E. This inadequacy was found among the study participants who had vitamin E consumption of 7.0 mg/day, which was less than 50% of the daily recommendation (16 mg/day).

Despite this reduction in serum alpha-tocopherol concentrations during lactation, vitamin E in breast milk remained stable. There was no correlation between these variables and diet, even in situations of dietary inadequacy of vitamin E (< 16 mg/day). This finding reinforces the proposition that maternal vitamin E reserves are used to maintain adequate concentrations of the vitamin in milk, thus guaranteeing the supply of vitamin E to the baby (Traber, 2014).

The largest reserve of alpha-tocopherol in the body is in adipose tissue (about 90%), but the mechanisms involved in the transport of alpha-tocopherol to the mammary gland and in the secretion in milk are not fully understood. It is believed that part of the alpha-tocopherol reaches milk via low-density lipoprotein receptors, and another part may be transported through cell surface receptors (SR-B1) that bind to low-density lipoprotein and to high-density lipoprotein. There is also a suggestion that this occurs through lipoprotein lipase and that it follows the Michaelis–Menten kinetics by active transport (Debie, 2007; Dimenstein et al., 2010; Jensen, Johannsen, & Hermansen, 1999; Mardones & Rigotti, 2004). Although most of the women in the study are overweight or obese, increased fatty tissue deposition did not influence serum alpha-tocopherol concentrations, which proved similar ($P = 0.106$) in women with and without excess weight (data not shown). Additionally, the literature reveals that the vitamin status is not related to alpha-tocopherol adipose tissue (El-Sohemy et al., 2001).

In this study, no association was found between serum alpha-tocopherol, milk, and dietary intake of vitamin E (Figure 3d–f). This result may indicate that, even in situations of low intake, alpha-tocopherol can be efficiently controlled, particularly during lactation, in order to participate in oxidative processes and to protect circulating lipids. This likely occurs through the use of body reserves and/or uptake from other tissues, such as uptake from tissues of the mammary gland (Traber, 2014).

Another factor that may affect the absence of an association between diet and vitamin E concentrations is that all the women in the study had a low intake of vitamin E. It is interesting to evaluate

these relationships between biochemical indicators and diet in situations of different levels of dietary intake. In addition, a limitation of the study methods with regard to evaluating food consumption was that the data could be affected by the interviewee's memory accuracy and under-reporting of nutrients in the information tables on the chemical composition of food. Such limitations are minimized for correction intrapersonal variability in nutrient intake (Fisberg, Slater, Marchioni, & Martini, 2005). It is worth noting that the 24-hr diet recall is an effective instrument for assessment of food intake when used in a series and when consumption is corrected for intrapersonal variability, as performed in this study (Ma et al., 2009; Shamah-Levy et al., 2016).

During lactation, the mammary glands have a degree of metabolic autonomy that ensures adequate composition of milk (Segura, Ansótegui, & Díaz-Gómez, 2016). However, if there is dietary inadequacy, it is important to verify whether a woman's milk can deliver adequate amounts of vitamin E up until the end of lactation.

It is interesting to note that there are few studies evaluating maternal vitamin E levels during lactation and their relation to diet (Antonakou, Chiou, Andrikopoulos, Bakoula, & Matalas, 2011; Aubuchon-Endsley, Kennedy, Gilchrist, Thomas, & Grant, 2015; Jiang et al., 2016; Martysiak-Żurowska, Szlagatys-Sidorkiewicz, & Zagierski, 2013). This dearth of studies exists despite the extreme importance of wide-ranging nutritional adequacy during the period of lactation to ensure the mother's health as well as the transfer of nutrients to breast milk for the infant's benefit.

This study found a relationship between dietary intake of vitamin A and serum retinol concentrations. Moreover, this was the first study to observe a reduction in concentrations of serum alpha-tocopherol over the course of lactation, whereas the vitamin composition of the mature milk remained unchanged. These results demonstrate the need to better understand the mobilization and use of retinol and serum alpha-tocopherol in breast milk, as well as to evaluate the status of these vitamins over the course of lactation in different populations, so that intervention measures can be implemented to offer nutritional assistance to lactating women and to place them in a nutritional risk group.

The results showed that lactating women are at nutritional risk for VAD and VED, and this was the first study to observe a reduction in serum alpha-tocopherol concentrations over the course of lactation, with a consequent increase in the percentage of low concentrations, whereas the composition of alpha-tocopherol in mature milk remained unchanged.

These results demonstrate the need to better understand the mobilization and use of retinol and serum alpha-tocopherol in breast milk, as well as to evaluate the status of these vitamins over the course of lactation in different populations, so that intervention measures can be implemented to offer nutritional assistance to lactating women and to place them in a nutritional risk group.

Measures that may be effective include food and nutritional education strategies as well as the promotion of better accessibility of foods that are sources of vitamins. Other measures include strategies to fortify food, the use of supplements when necessary, and a healthy and balanced diet. Changes in dietary intake may improve concentrations of vitamin A and vitamin E and may provide adequate levels of vitamins in human milk to protect the infant from deficiency in these vitamins.

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CONFLICTS OF INTEREST

The authors declare that they have no conflicts of interest.

CONTRIBUTIONS

AGS, RD, and KDR designed the research; AGS, AR, BM, and DS conducted the sampling procedures and data collection; AGS and KDR analysed the data, and AGS, KDR, and RD wrote the paper. All authors read and approved the final manuscript.

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