CLINICAL NUTRITION
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Total fat and alpha-tocopherol in breast milk: is there a relationship after supplementation with vitamin E?

Lipídeos totais e alfa-tocoferol no leite maduro: existe relação após suplementação com vitamina E?

Abstract

Introduction: Fat intake during lactation may modify the concentration of vitamin E in human milk, probably because both nutrients are fat soluble and also share similar pathways of distribution, metabolism and molecular action. It is known that the high maternal vitamin E intake through supplementation alters alpha-tocopherol concentration in breast milk, but the relationship with human milk lipids has not yet been studied. ***Objective***: This study aims to evaluate the association between alpha-tocopherol and total fat in breast milk after maternal supplementation with megadoses of vitamin E. ***Method***: This study is part of a clinical trial composed of lactating women from a public outpatient clinic in the city of Natal, RN, Brasil. The group studied were 26 lactating women from a supplemented group whom were given a single dose of 800 IU of RRR-alpha-tocopherol (588 mg) between 30 and 90 days postpartum. Breast milk was collected before and 24h after supplementation. The food intake was assessed by a 24-hour Dietary Recall, the concentration of alpha-tocopherol was determined by high-performance liquid chromatography, and lipid concentration was determined by a creamatocrit analyzer. ***Results***: All participants had inadequate vitamin intake (<16mg). Alpha-tocopherol concentration in milk increased from 294.8 µg/dL to 631.6 µg/dL after supplementation ($p = 0.002$). No relationship was found between alpha-tocopherol and total fat in breast milk before and after RRR-alpha-tocopherol supplementation. **Conclusion**: Therefore, vitamin E supplementation did not show a relationship between alpha-tocopherol and total fat in breast milk.

Keywords: Lactation. Fatty acids. Food intake. Nutrient supplementation.

Resumo

Introdução: O consumo de lipídeos durante a lactação pode alterar a concentração de vitamina E do leite materno, provavelmente, por ambos os nutrientes possuírem caráter lipossolúvel e compartilharem vias similares de distribuição, metabolismo e ação molecular. Sabe-se que o alto consumo materno de vitamina E via

suplementação altera a concentração de alfa-tocoferol no leite, porém ainda não foi estudado se essa suplementação afetaria a quantidade de lipídeos do leite. ***Objetivo:*** Avaliar a associação entre as concentrações de alfa-tocoferol e de lipídeos totais no leite materno após suplementação materna com megadose de vitamina E. ***Método:*** O presente estudo é parte de um ensaio clínico randomizado, composto por lactantes atendidas em ambulatório público de Natal-RN. O recorte foi realizado com 26 lactantes do grupo suplementado, as quais receberam dose única de 800 UI de RRR-alfa-tocoferol (588 mg) entre 30 e 90 dias pós-parto, sendo coletado leite antes e 24h após a suplementação. O consumo alimentar foi obtido pelo recordatório de 24h, o alfa-tocoferol foi analisado por cromatografia líquida de alta eficiência e a concentração de lipídeo foi determinada por crematócrito. ***Resultados:*** Todas as mulheres apresentaram consumo inadequado de vitamina E (<16mg). A concentração de alfa-tocoferol no leite aumentou de 294,8 µg/dL para 631,6 µg/dL após a suplementação (p = 0,002), e não foi encontrada relação entre o alfa-tocoferol e lipídeos totais no leite antes e após a suplementação. ***Conclusão:*** A suplementação de vitamina E não evidenciou relação entre alfa-tocoferol e lipídeos totais no leite materno.

Palavras-chave: Lactação. Ácidos Graxos. Consumo Alimentar. Suplementação alimentar-

INTRODUCTION

Alpha-tocopherol is a fat-soluble compound, considered the bioactive form of vitamin E due to its greater biological activity in the natural form as RRR-alpha-tocopherol, and therefore the only isomer studied to contribute to the nutritional requirement of vitamin E.[1,2] Among its roles is that of biological antioxidant, due to the protection offered to polyunsaturated fatty acids (PUFAs) and lipoproteins against oxidation and lipid peroxidation reactions, being essential in a period of high oxidative stress, such as pregnancy and birth.[3,4]

The transfer of vitamin E from the mother to the fetus during pregnancy is limited and, as a consequence, the child has low body storage of this vitamin at birth. Thus, in view of the recommendation of exclusive consumption of breast milk during the first 6 months of life, breast milk must provide a sufficient amount of vitamin E, aiming to meet the infant's daily need and prevent diseases linked to vitamin E deficiency, such as hemolytic anemia, ventricular hemorrhage and lung diseases.[5-7]

The literature reports that vitamin E levels in human milk may change according to the stage of lactation (colostrum, transition and mature) and maternal diet, as found in a study by Mata et al.,[8] in which was observed a relationship between maternal intake of polyunsaturated fatty acids (PUFAs) and alpha-tocopherol levels in mature milk. However, the relationship between the lipid profile and the level of alpha-tocopherol in breast milk has not yet been fully elucidated.

Lipid is present in breast milk as fat globules, which are synthesized by mammary alveolar cells by induction of prolactin, and compose an average of 3% of breast milk content and contribute to 45-55% of the total energy supplied to the infant.[9,10] Thus, breast milk is an important supplier of energy and nutrients to newborns, for it contributes to the development of their central nervous system, regulation the risk of inflammation and infection, reducing the risk of metabolic diseases, and cardiovascular disease in adulthood.[11]

Rebouças et al.[12] found that high consumption of vitamin E through supplementation increased the concentration of alpha-tocopherol in breast milk, although the effect of this supplementation on the composition and protection of milk lipids has not yet been addressed.

Knowing the existence of a relationship between vitamin E and lipids, in which both have a lipid-soluble character and share similar pathways for distribution, metabolism and molecular action,[4] as well as the protection provided by this vitamin to lipids,[3] this study's hypothesis is that there is an influence of maternal consumption of vitamin E on the association between alpha-tocopherol concentrations and total fat in breast milk in a situation of maternal supplementation with megadose of vitamin E.

Thus, this study aims to evaluate the association between alpha-tocopherol and total fat in breast milk after maternal supplementation with megadose of vitamin E.

METHODOLOGY

Study type

This study is part of the randomized clinical trial from the Master's dissertation of the UFRN Graduate Program in Biochemistry entitled "Effect of supplementation with 800 IU of alpha-tocopherol in the whey and milk of lactating women".[12] Data collection happened in the years of 2017 and 2018 and was approved by the Committee for Ethics in Research of the *Universidade Federal do Rio Grande do Norte* (Federal University of Rio Grande do Norte) under protocol number 2.327.614 (CAAE 76779217.1.0000.5537) and registered on the *Registro Brasileiro de Ensaios Clínicos* (Brazilian Registry of Clinical Trials) – ReBEC, under code RBR-38nfg2.

Selection of participants

The participants selected were lactating women who were 30 to 90 days postpartum, attended at the Pediatrics Outpatient Clinic of the *Hospital Universitário Onofre Lopes* (Onofre Lopes University Hospital - HUOL), in Natal, RN. To meet inclusion criteria for selection, the lactating women had to: 1) be breastfeeding their child, 2) be residing in Natal (RN) or metropolitan regions, 3) not have the diagnosis of diseases (hypertension, diabetes, neoplasms, diseases of the gastrointestinal and hepatic tract, syphilis, HIV positive); 4) be non-smokers; and 5) having undergone single births of newborns not presenting malformations. Those women who used daily supplements containing vitamin E during lactation, users of illicit drugs and those who did not have enough milk for analysis were excluded from the study. There were also losses of participants linked to follow-up when a second collection – which should be performed at the participant's home – was not possible to be done. Losses also happened due to not being able to perform analyzes in the centrifuge according to the creamatocrit method, owing to samples' non-homogeneity.

The study objectives were explained to the eligible participants, who signed an Informed Consent Form (ICF), upon agreeing to participate.

Intervention and data collection

The lactating women received two 400-IU capsules of RRR-alpha-tocopherol acetate (CVS Health, Woonsocket, USA) (Annex A), totaling an 800-IU dose, equivalent to 588 mg of alpha-tocopherol. The data collection stages took place in two moments: the first (collection 1), between 30 and 90 days postpartum, and the second (collection 2), on the day after the collection 1, at the participant's home, after prior scheduling. Vitamin E capsules were delivered at the time of collection 1 and the choice of the time of collection 2 occurred in order to generate a situation of high vitamin E intake to verify a possible association between alpha-tocopherol and the breast milk lipid. At both moments, breast milk and dietary information were collected, but only at collection 1 a semi-structured form surveying socioeconomic, anthropometric, health and delivery information (Annex B) was applied.

ANNEX A – Vitamin E supplement.

Ingredients

Vitamin E 400 IU (As D-Alpha Tocopheryl Acetate). Other Ingredients: Gelatin (Bovine), Glycerin, Soybean Oil.

Nutritional Facts

Serving Size: 1softgels
Servings Per Container: 100

Nutrient	Amount per Serving	% Daily Value
Vitamin E (as D-Alpha Tocopheryl Acetate)	400 IU	1333%

Collection of biological material

Three milliliters (3 mL) of mature milk were obtained through the manual expression of a single breast which had not been recently offered during breastfeeding. The first milk ejections were discarded in order to avoid changes in the fat content. The material was collected in a polypropylene tube wrapped in aluminum foil and transported under refrigeration to the Food and Nutrition Biochemistry Laboratory of the Biochemistry Department – Biosciences Center (UFRN), where it was stored at -20ºC until the time of analyses.

Information obtained through the form

A semi-structured questionnaire (Annex B) was used to obtain socioeconomic (income, marital status, and education) and health (age, birth information and clinical history) data. Anthropometric information was also collected (weight, height, and body mass index), about childbirth (type and parity), as well as family income, which was used to obtain *per capita income* by dividing family income by the number of residents of the household. Families with a gross monthly income between 85.01 BRL (Brazilian Real) and 170.00 BRL per person were considered to be in poverty.[13] Family income was expressed in minimum wage, considering the value of the monthly minimum wage in Brazil in 2018 (954.00 BRL), and families that received up to 3 minimum wages were classified as > 1 minimum wage.

ANNEX B – Semi-structured survey questionnaire.

CENTRO DE BIOCIÊNCIAS
DEPARTAMENTO DE BIOQUÍMICA
PÓS-GRADUAÇÃO EM BIOQUÍMICA

QUESTIONÁRIO DO PROJETO "EFEITO DA SUPLEMENTAÇÃO COM 800 UI DE VITAMINA E SOBRE O ALFA-TOCOFEROL DO SORO E LEITE DE MULHERES LACTANTES"

Nº: _____

001	Número do questionário:	__ __ __	[ ]
002	Nome e código do entrevistador	_____	[ ]
003	Data de aplicação do questionário	__/__/____ (dd/mm/aaaa)	__/__/__ (Dia/Mês/Ano)
	CARACTERIZAÇÃO DA ENTREVISTADA		
004	Nome: _____ Nome da criança: _____		
005	Endereço: _____ _____ Telefone: _____ Email: _____		
006	Qual sua idade em anos completos?	__ __	[ ]
007	Atualmente qual seu estado civil?	01. Solteira 02. Casada/ União estável 03. Divorciada 04. Viúva	[ ]
008	Qual seu nível de escolaridade?	01. Analfabeta 02. Ensino Fundamental Incompleto 03. Ensino Fundamental Completo 04. Ensino Médio Incompleto 05. Ensino Médio Completo 06. Graduação Incompleta 07. Graduada 08. Pós-graduada 00. Não se aplica	[ ]
009	Quantos anos de estudo?	__ __	[ ]
010	Qual sua ocupação?	01. Trabalha _____ 02. Dona de casa 03. Não trabalha	[ ]
011	Número de moradores na casa? (incluindo o bebê)	__ __	[ ]
012	Qual o rendimento mensal da sua família? (Devem ser somados todos os rendimentos	01.Sem renda 02. Até 1 salário mínimo (até R$ 880,00) 03. De 1 a 3 salários mínimos (de R$ 880,00 a R$ 2.640,00)	

	das pessoas da família que moram na mesma casa)	04. De 3 a 5 salários mínimos (de R$ 2.640,00 a R$ 4.400,00) 05. De 5 a 7 salários mínimos (de R$ 4.400,00 a R$ 6.160,00) 06. De 7 a 10 salários mínimos (de R$ 6.160,00 a R$ 8.800,00) 07.De 10 a 20 salários mínimos (de R$ 8.800,00 a R$17.600,00) 08. Acima de 20 salários mínimos (acima de R$ 17.600,00) 09. Não sabe 00.Não respondeu	[ ]
013	Número de filhos?	__ __	[ ]
014	Número de abortos?	__ __	[ ]
015	Utilizou algum medicamento, vitamina ou suplemento durante a gestação? Se sim, qual?	01. Sim _____ 02. Não	[ ]
016	Realizou acompanhamento nutricional após o parto? Se sim, quantas vezes?	01. Sim _____ 02. Não	[ ]
017	Utilizou algum medicamento, vitamina ou suplemento após o parto? Se sim, qual?	01. Sim _____ 02. Não	[ ]
DADOS DO PARTO			
018	Qual foi seu peso na última consulta da gestação?	_____kg 00. Não se aplica	[ ]
019	Data do parto/ Horário	__/__/__ (dd/mm/aaaa) ___ h	__/___/____
020	Tipo de parto	01. Normal 02. Cesário 00. Não se aplica	[ ]
021	Sexo do Recém-nascido (RN)	01. Masculino 02. Feminino	[ ]
022	Peso ao nascer do RN	kg	[ ]
023	Comprimento nascer do RN	_____cm	[ ]
024	Estado nutricional do RN	01. <1500g muito baixo peso 02. 1500-2500g baixo peso 03. 2500-4000g adequado 04. >4000g Macrossomia	[ ]
025	Idade gestacional (capurro do bebê ou USG) – prontuário do Bebê	_____ sem	[ ]
026	Intercorrências da criança Ao nascer	_____ _____ _____	
DADOS ANTROPOMÉTRICOS			

027	Altura atual	_____ m 00. Não se aplica	[ ]
028	Peso atual (30-60 dias)	_____kg 00. Não se aplica	[ ]
029	Estado nutricional atual (30-60 dias): IMC = _____	01. Baixo peso (IMC<18,5 kg/m2) 02. Normal (IMC 18,5 - 24,9 kg/m2) 03. Sobrepeso (IMC 25 - 29,9 kg/m2) 04. Obesidade (IMC ≥ 30,0kg/m2) 00. Não se aplica	[ ]
030	Perda peso:	01. Sim _____ 02. Não	[ ]
031	Intercorrências:	_____ _____	
ALIMENTAÇÃO DA CRIANÇA			
032	Alimentação infantil dia 30-60 _____ _____ _____ _____ _____	01.Aleitamento materno exclusivo enteral 02.Aleitamento materno exclusivo oral 03.Aleitamento misto (oral e enteral) 04.Aleitamento materno (LM e outros leite 05.Leite materno + suplementos 06.Fórmula infantil ou outro leite 07.Alimentação complementar 08.Dieta zero 00. Não se aplica	[ ]
DADOS DA COLETA			
033	Sangue 30-60 dias	Data da Coleta: __ / ___ / _____ Hora: ___: ____	TOH [ ]
034	Leite 30-60 dias	Data da Coleta: __ / ___ / _____ Hora: ___: ____	TOH [ ]
035	Sangue 24h	Data da Coleta: __ / ___ / _____ Hora: ___: ____	TOH [ ]
036	Leite 24h	Data da Coleta: __ / ___ / _____ Hora: ___: ____	TOH [ ]

Data: _____ Assinatura do entrevistador:

Data: _____ Assinatura do entrevistador:

Assessment of anthropometric nutritional status

The weight and height of the lactating women were collected following the recommendations of the Ministry of Health / *Sistema de Vigilância Alimentar e Nutricional* (Food and Nutrition Surveillance System) – SISVAN.[14] To assess the anthropometric nutritional status, the body mass index (BMI) was considered, calculated from the current weight and maternal height using the formula below and classified as underweight (< 18.5 kg/m^2), normal weight (≥ 18.5 and < 25 kg/m^2), overweight (≥ 25 and < 30 kg/m^2) and obesity (≥ 30 kg/m^2).[14]

$$\text{Body Mass Index (BMI)} = \frac{\text{Weight (Kg)}}{\text{Height}^2\ \text{(m)}}$$

Assessment of food consumption

For the food consumption assessment, a 24-hour Dietary Recall (24HR) was used – a toll to that includes the investigation of all foods, beverages and supplements consumed by the interviewee in the 24 hours prior to the interview (Annex C). The analysis of the 24RH was performed using the "Virtual Nutri Plus" software, in which a food database was built based on a table developed from the chemical information of the food, obtained by the TACO food composition table[15] and food composition table of the U.S. Department of Agriculture (USDA).[16] In the database there was information on energy, lipids, types of fatty acids (saturated, monounsaturated and polyunsaturated) and vitamin E. The intake of such nutrients was obtained by averaging the two 24-hour recalls applied.

For the calculation of the estimated daily intake of lipids by infants, the volume of milk ingested (expressed in volume of milk compatible with the daily intake by an infant) was considered. It was then compared to the Adequate Intake (AI) cutoff point for 0-6-month-old children, for whom the recommendation is 31g/day.[17] For this calculation, it was considered that an infant in the first 6 months of life ingests a daily volume of milk of 780 mL.[17]

ANNEX C – 24-hour dietary recall to assess lactating women's food consumption

Nº: _____

( ) 30-60 dias

RECORDATÓRIO 24 HORAS PARA MÃE ( ) Dia seguinte - 24h

Nome: _____

Peso: _____ Altura: _____

REFEIÇÃO /HORÁRIO/ LOCAL	ALIMENTO	MEDIDA CASEIRA	QTDE	OBS.

DATA: _____/ _____/ _____ Responsável:

. _____

Extraction and analysis of breast milk alpha-tocopherol

Alpha-tocopherol extraction

Alpha-tocopherol was extracted from breast milk according to the method adapted from Ortega et al.[18] To each 1.0 mL of breast milk, 1.0 mL of 95% ethanol (Vetec, Rio de Janeiro, Brazil) was added for protein precipitation and 2 mL of hexane p.a. (Vetec, Rio de Janeiro, Brazil) to extract the lipid fraction, in which alpha-tocopherol is found. The samples were homogenized for 1 minute by vortexing, and centrifuged for 10 minutes at 4,000 rpm to separate the supernatant layer of approximately 2mL, which was later removed to a second tube. This step was performed three times, and at the end a total of 6 mL of supernatant was collected, of which only 4 mL were placed in a third tube and evaporated in a water bath at 37ºC. The dry extract was dissolved in 250 µL of dichloromethane (Vetec, Rio de Janeiro, Brazil): methanol (Sigma-Aldrich, United States) 2:1 (v:v) for analysis of breast milk alpha-tocopherol by High Performance Liquid Chromatography (HPLC).

High performance liquid chromatography analysis

To determine the breast milk alpha-tocopherol concentration, a Shimadzu chromatograph (Shimadzu, Kyoto, Japan) was used, consisting of an LC-20 AT Shimadzu pump (Kyoto, Japan), coupled to an SPD-10A UV-VIS detector (Shimadzu, Kyoto, Japan) and a CBM 20A communicator. For chromatographic separation, reversed-phase C^{19} column (LiChroCART 250-4; Merck, Darmstadt, Germany) was used.

During the analysis, the data obtained were processed by the LC Solution software (Shimadzu Corporation). The mobile phase used for the vitamin E analysis in the breast milk samples consisted of 100% methanol in an isocratic system with a flow rate of 1.0 mL/min. Detection occurred at a wavelength of 292 nm for alpha-tocopherol, being the vitamin E identified and quantified in the samples by comparing the peak area obtained on the chromatogram with the area of the alpha-tocopherol standard (Sigma- Aldrich, Brazil). The retention time followed was 10.3 minutes for alpha-tocopherol, and the vitamin E values in the samples were expressed in µg/dL.

Determination of fat content

To determine the fat content of mature breast milk, we used the creamatocrit method described by Lucas et al,[20] in which the milk samples were vortexed for 30 seconds and placed in microcapillary glass tubes (triplicate) and underwent centrifugation at 12,000 rpm for 15 minutes to separate the serum and cream. After that, the length of the cream column (smallest column) and the length total column of the product (cream column + serum column, expressed in mm) were measured using a ruler (mm). The fat content was obtained using the following formulas:

$$\text{Cream content (\%)} = \frac{\text{Cream column (mm) x 100}}{\text{Total product column (mm)}}$$

$$\text{Fat content (g/dL)} = \frac{\text{Cream content (\%)} - 0.59}{1.46}$$

Statistical analysis

Statistical analysis was performed in the Statistical Package for the Social Sciences (SPSS) software. The Kolmogorov -Smirnov test was applied to assess the distribution of samples regarding the degree of normality, and continuous variables were expressed as mean and standard deviation. To assess the association between fat and alpha-tocopherol in breast milk before and after supplementation, the Pearson correlation test was performed. The alpha-tocopherol/total fat ratios were calculated from the values of total alpha-tocopherol and total fat, and the means and standard deviation were expressed in µg of alpha-tocopherol/g of fat. To verify differences between the proportions of nutrients at the two times, a paired Student's T-Test was performed. All differences were considered significant when $p \leq 0.05$.

RESULTS

For this study, 26 lactating women were selected after exclusion criteria and losses (inadequate samples for performing the analyses), which is 35% less than the sample of the original study. The socioeconomic characterization is described in Table 1. The average age was observed to be 27 years, most had completed secondary education (42.3%) and 96.2% had a per capita family income of less than 1 minimum wage. Regarding the anthropometric nutritional status assessed by BMI, 26.9% of them were within the overweight range and 23% were within the obesity range.

Table 1. General characterization of the 26 lactating women supplemented with 800 IU of RRR-alpha-tocopherol. Natal, Rio Grande do Norte, Brazil, 2017-2018.

Characteristic	Lactating women supplemented N = 26	
	N	%
Maternal age, years (mean-SD*)	27 (6.8*)	-
Education level		
Incomplete primary education	5	19.2%
Complete primary education	1	3.8%
Incomplete secondary education	3	11.5%
Complete secondary education	11	42.3%
Incomplete higher education	4	15.4%
Complete higher education	2	7.7%
Marital status		
Single	9	34.6%
Married	17	65.4%
Family income[a]		
≤ 1 minimum wage	1	3.8%
>1 minimum wage	25	96.2%
Delivery type		
Vaginal	10	38.5%
Cesarean	16	61.5%
Maternal parity		
Primiparous	11	42.3%
Multiparous	15	57.7%
Maternal BMI classification (kg/m^2)		
Low weight	2	7.7%
Normal weight	10	38.5%
Overweight	7	26.9%
Obesity	6	23%

N: Number; *Standard deviation; BMI: Body Mass Index. [a]Brazilian minimum wage per month = 954 reais. > 1 minimum wage (up to 3 minimum wages).

Dietary indicators

The values referring to the consumption of each nutrient analyzed are shown in table 2. As for the consumption of vitamin E from food, it was observed that 100% of the lactating women had low consumption of the vitamin E (<16 mg/day). The mean intake of energy and other nutrients did not differ between collections.

Table 2. Dietary consumption values of lactating women (n=26) supplemented with 800 IU of RRR-alpha-tocopherol.

Characteristic	Lactating women supplemented N = 26	
	Mean (SD)	Mean (SD)
	0h	24h
Energy intake (Kcal/day)	2244.5 (705.5)	2178.7 (781.28)
Vitamin E intake (mg/day)	5.5 (2.9)	6.5 (3.64)
Total lipid intake (g/day)	69.5 (29.3)	71.5 (36.9)
Saturated fatty acid (g/day)	22.74 (14.26)	22.13 (13.9)
Monounsaturated fatty acid (g/day)	21.85 (10.67)	22.24 (11.7)
Polyunsaturated fatty acid (g/day)	12.73 (6.34)	16.21 (10.6)

N: number; SD: standard deviation.

Determination of the total fat and alpha-tocopherol contents in breast milk before and after vitamin E supplementation

When evaluating the concentration of alpha-tocopherol in breast milk at 0h (collection 1), the mean obtained was 294.81 (87.9) µg/dL; after supplementation with 800 IU of RRR-alpha-tocopherol, at the time 24h (collection 2), the mean was 631.57 (182.3) µg/dL ($p = 0.002$). Regarding the total fat content in the samples, the mean obtained before supplementation was 2.03 (1.3) g/dL and after it was 2.11 (1.6) g/dL ($p = 0.688$).

When calculating total fat consumption by infants through the mean concentration of this nutrient obtained and the estimated daily intake of about 780 mL of mature milk, a mean supply of 15.8g/780mL was observed in collection 1 and 16.5g/780mL in collection 2.

Regarding the mean concentration of alpha-tocopherol per total fat in breast milk, the same increase pattern was observed after supplementation ($p = 0.002$) (Figure 1); however, no significant correlation was found between alpha-tocopherol and fat content in breast milk before (Figure 2a) and after supplementation (Figure 2b).

Figure 1. Relationship alpha-tocopherol/total fat in breast milk at collection 1 and 2.

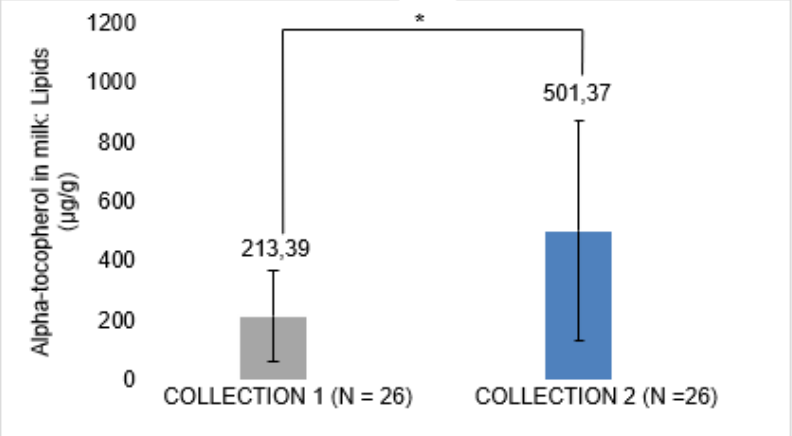
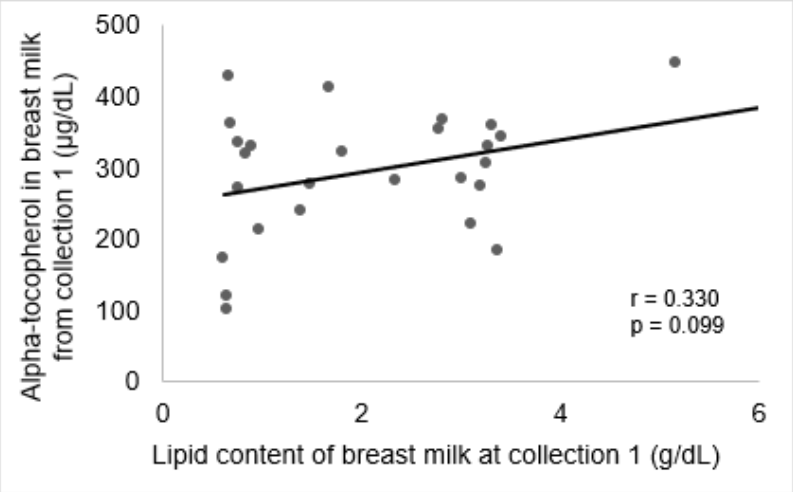
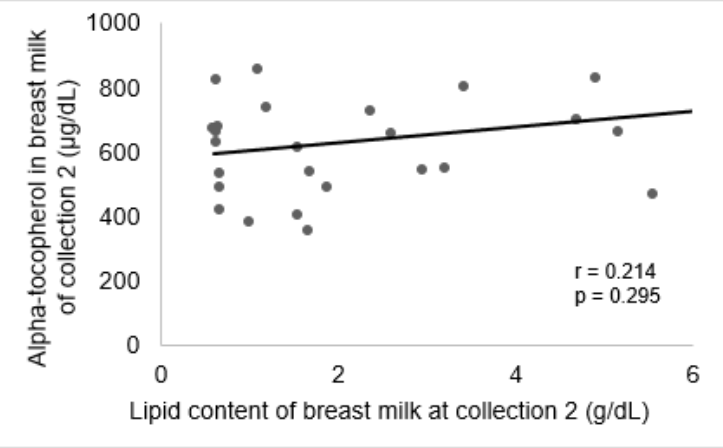


Figure 2. Relationship between alpha-tocopherol concentrations and total fat in breast milk.



(A) Representation of the alpha-tocopherol ratio and total fat in breast milk at time 0h, expressed in µg/dL.



(B) Representation of the alpha-tocopherol ratio and total fat in breast milk at 24h, expressed in µg/dL.

DISCUSSION

In this study, all lactating women had vitamin E consumption below the recommended level for the lactation phase (EAR = 16 mg/day), both before (5.5 mg/day) and after supplementation (6.5 mg/day), demonstrating a consumption below the recommendation, as reported by most studies conducted with this population.[8,21-23] It is noteworthy that the main dietary sources of vitamin E are vegetable oils, such as sunflower, peanut and corn, in addition to walnuts, almonds and sunflower seeds.[3]

Furthermore, after supplementation with 800 IU of RRR-alpha-tocopherol, it was possible to observe a significant increase of 114% in breast milk alpha-tocopherol. This result is extremely positive, since, although maternal vitamin E intake is deficient, with a megadose it is possible to increase the supply of this vitamin to infants. It should be highlighted that the protocol used in the supplementation of lactating women with 800 IU of RRR-alpha-tocopherol (equivalent to 588 mg of alpha-tocopherol) can be considered safe, as this dose does not exceed this nutrient's tolerable upper intake level (UL = 1000 mg/day) of vitamin E for this group. In addition, the increase of vitamin E in breast milk also confirms the effectiveness of supplementation, considering that in situations of non-supplementation, the concentration of alpha-tocopherol in breast milk tends to be lower, being higher in colostrum milk and lower in mature milk. This fact was observed in a study by Silva et al.,[24] in which the alpha-tocopherol concentrations in the different stages of lactation were quantified and the results showed decline in the vitamin E concentration as breast milk matures.

Knowing that lipids comprise the main energy source of breast milk, being associated with several benefits for the health of the infant, the consumption of this nutrient by infants ought to be adequate.[11,25] Therefore, the estimated average intake of 15.8 g/day in collection 1 and 16.5 g/day in collection 2 demonstrate low levels compared to the total fat requirement of 0-6-months-old children (31 g/day), possibly insufficient to meet the needs of this group during the period of exclusive breastfeeding.[26] Nonetheless, as these values are based on an estimate of daily consumption of 780 mL of breast milk by infants in the first 6 months,[17] increasing such volume may ensure a greater supply of vitamin E, reaching recommended levels.

Regarding the breast milk total fat content, this study observed a mean of 2.03 g/dL in collection 1 and 2.11 g/dL in collection 2, similar to the mean of 2.13 g/dL found by Rydlewski et al.[27] in a study conducted with lactating women in Paraná, Brazil. On the other hand, these values were lower than those found in a study carried out with South Korean lactating women, in which the mean obtained was 3.30 g/dL,[28] as well as by Bortolozo et al.,[29] whose mean found was 2.56 g/dL, but higher than that reported by Silva et al.,[30] (1.38 g/dL).

The results found in the aforementioned studies may be justified because vitamin E is a micronutrient with great inter and intraindividual variation, which may be influenced by maternal nutritional status, body composition, presence of pathologies, diet, collection period, adhesion of fat molecules on the walls of flasks, among others.[31,32]

According to our results, unlike what was exposed about the alpha-tocopherol concentration before and after supplementation, the total fat content did not change after supplementation ($p > 0.05$). The literature reports the existence of a relationship between the nutrients studied, as shown by Mata et al.,[8] who assessed the relationship between habitual fat consumption and vitamin E and the concentration of breast milk alpha-tocopherol. The authors observed that lactating women with higher intakes of polyunsaturated fatty acids had higher levels of alpha-tocopherol in mature milk, demonstrating an association between nutrients.

Besides that, it is also possible to identify a link between the nutrients studied in relation to the functions performed by both, since the lipid fractions play an essential role in the metabolism of vitamin E, participating in the absorption, transport and distribution of this vitamin.[4] On the other hand, vitamin E is also essential for

lipids, due to its action as an important biological antioxidant, capable of protecting polyunsaturated fatty acids (PUFAs) in cell membranes and lipoproteins against peroxidation caused in periods of high oxidative stress, such as during pregnancy and birth.[3,4]

Although the relationship between the two variables has already been reported in the literature, such as the influence of maternal consumption of lipid-rich foods on the breast milk alpha-tocopherol concentration and the protective action of this vitamin on lipids, our findings demonstrate that the opposite does not occur. That is, even in situations of vitamin E megadose supplementation, a significant increase in breast milk total fat was not observed. This probably occurs because the mammary gland synthesizes lipids to compose breast milk, in addition to those from the diet and maternal circulation. Another point observed is that the creamatocrit method used quantifies only the total content of milk lipids, making it necessary to carry out studies to analyze the behavior of lipid fractions when vitamin E supplementation occurs, since this composition may vary with the progression of lactation, with an increase in triglycerides and medium-chain fatty acids, and a decrease in long-chain fatty acids.[9]

Thus, the relationship between the lipid profile and the level of breast milk alpha-tocopherol has not yet been fully elucidated, yet studying that relationship is important for raising evidence about the factors that can affect breast milk composition, since with the control of these variables, deficiency in breastfeeding women and infants may be prevented.

CONCLUSION

Supplementation with 800 IU of RRR-alpha-tocopherol increased the concentration of breast milk vitamin E, but it did not affect breast milk total fat content. No significant association was observed between alpha-tocopherol and fat content in breast milk before and after supplementation with RRR-alpha-tocopherol..

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Contributors

Medeiros JO and Rebouças AS participated in data collection, analysis and interpretation, manuscript writing, review and approval of the final version. Dimenstein R and Rodrigues KDSR participated in the study's conception and design, data analysis and interpretation, review and final approval.

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