Eur J Nutr (2011) 50:195–202
DOI 10.1007/s00394-010-0129-4

ORIGINAL CONTRIBUTION

Breast milk tocopherol content during the first six months in exclusively breastfeeding Greek women

Angeliki Antonakou · Antonia Chiou · Nikolaos K. Andrikopoulos · Chrysa Bakoula · Antonia-Leda Matalas

Received: 6 April 2010 / Accepted: 30 July 2010 / Published online: 19 August 2010

Abstract
Purpose To determine tocopherol and fat content of Greek mother's milk during the first 6 months of exclusive breastfeeding and correlate with maternal diet characteristics.
Methods Milk samples and dietary records were obtained by mothers at 1st ($n = 64$), 3rd ($n = 39$) and 6th ($n = 23$) month postpartum. Milk tocopherol content was determined by high-performance liquid chromatography method (HPLC) and fat content by the crematocrit method.
Results Milk's α-tocopherol content at 1st, 3rd and 6th month postpartum was 8.3 ± 3.4, 8.1 ± 4.2 and 8.5 ± 4.7 μmol/L, while total tocopherol values were 8.9 ± 3.6, 8.7 ± 4.6 and 9.5 ± 5.6 μmol/L, respectively, and were closely related to milk's fat content. No significant differences were observed for α- and total tocopherol content in breast milk among the three time points. Maternal vitamin E dietary intake was 7.2 ± 3.7, 6.8 ± 3.5 and 10.9 ± 5.2 mg/day at 1st, 3rd and 6th month postpartum, respectively. Though vitamin E dietary intake was less than the recommended one, vitamin E content in breast milk was considered sufficient for infant needs. Milk tocopherol content was found to be associated only with mothers' total fat and saturated fat dietary intake.
Conclusion This study is among few in literature to determine tocopherol content of breast milk in European women and detect dietary factors that may influence its values. The only maternal dietary characteristic to affect breast milk tocopherol content was mothers' total fat intake, while tocopherol intake seems to have no effect.

Keywords Vitamin E · Tocopherols · Breast milk · Mediterranean diet

A. Antonakou (✉) · A. Chiou · N. K. Andrikopoulos · A.-L. Matalas
Department of the Science of Dietetics–Nutrition, Harokopio University of Athens, El. Benizelou 70, 17671 Athens, Greece
e-mail: a.anton@hua.gr

C. Bakoula
1st Department of Paediatrics, Children's Hospital "Agia Sophia", Medical School-University of Athens, Athens, Greece

Introduction

The term vitamin E refers to a group of eight chemically related compounds, that is α-, β-, γ-, δ-tocopherol and α-, β-, γ-, δ-tocotrienol, which differ both in structure and bioavailability [1]. α-Tocopherol is the most active form of vitamin E, while the other forms of vitamin E (β-,γ-, δ-tocopherols and tocotrienols) do not contribute in meeting the vitamin E demands; though absorbed by the intestine, they are not converted to α-tocopherol in the human body and are poorly recognized by the binding α-tocopherol transfer protein (α-TTP) in the liver [2–4]. Vitamin E is believed to function primarily as a chain-breaking antioxidant that prevents propagation of lipid peroxidation [5]. Vitamin E is found in several foods and overt deficiency is very rare, seen only in individuals unable to absorb the vitamin or with inherited abnormalities, such as abetalipoproteinemia [6].

Vitamin E demonstrates a multiple role in the functions of human body. First of all, it is involved in modulating immune function and as shown by in vitro studies it affects cell signalling, regulates gene expression and contributes in many metabolic processes [7]. High concentrations of α-tocopherol in habitual diet are associated with a reduction of risk associated to free radicals in disorders such as atherosclerosis, cancer, cataract, and cell damage related to

ischaemia and reperfusion [8]. Especially in newborns, vitamin E prevents oxidative damage in neonates suddenly exposed to higher oxygen levels than those in intrauterine environment [9]. Preterm infants are more sensitive to vitamin E deficiency and can develop clinical symptoms such as haemolytic anaemia, retrolental fibroplasias, intraventricular haemorrhage and bronchopulmonary dysplasia [10]. Premature babies of very low birth weight (<1,500 grams) might also be vitamin E deficient and therefore are at risk for complications, such as retinopathy and infections [11].

Since vitamin E plays a crucial role in neonates' good health and development, provision of adequate vitamin E amounts through food is therefore of evident importance. The supreme food for infants up to sixth months of life is considered to be breast milk [12]. Breast milk from healthy, well-nourished women contains virtually all nutrients necessary for newborn infants as well as a variety of growth and immune factors. Colostrum and mature milk contain all vitamins required for optimal health, though their concentration varies markedly from one mother to another [13]. It is of great importance that infants are solely breastfed, as essential nutrients such as vitamin E do not consistently cross the placental barrier during pregnancy [14] and should be provided after birth through breast milk or milk formulas. This is the reason why it is proprietary by European law that milk formulas for newborns and infants should be enriched with α-tocopherol succinate esters [15]. Macronutrient composition of human milk is influenced by a number of factors, such as geographical area [16], duration of lactation [17], parity [18], and maternal diet [19, 20]. Likewise, the vitamin content of human milk has also been reported to be influenced by the stage of lactation and maternal diet [10].

The tocopherol content of human milk specifically has been the subject of studies conducted in some European and American regions [21–23]. Most of these studies have focused on the milk provided during the first days of lactation [24–27]. To the best of our knowledge, so far only two studies have been conducted concerning mature human milk in Europe during the last decade and only one in southern Europe [23, 24]. These studies showed a great variation of a-tocopherol content, i.e. being 2.27 ± 0.77 μmol/l for Spanish women [23] to 13.2 ± 5.1 μmol/l [24] for German women. The present study aimed to assess breast milk's tocopherol content at three different time periods during the first 6 months of lactation among Greek mothers who were exclusively breastfeeding and to evaluate its association to maternal diet characteristics.

Subjects and methods

In this prospective study, mothers who delivered healthy term newborns (>37 weeks of gestation) weighing >2.5 kg at antenatal clinics in the area of Athens and were planning to exclusively breastfeed their infants were recruited. Mothers were approached by a member of the research group during the last trimester of their pregnancy and were thoroughly informed about the study protocols and aims. They were asked to sign an informed consent form. Ethical approval was obtained by the Harokopio University Ethics Committee and permission to approach the mothers was secured from the clinic's executive board.

Mothers participating in the study had no medical history and were not receiving any vitamin E supplements during their pregnancy or postpartum. Samples of milk were obtained at three different time points. First sampling was performed at 20–30 days postpartum (1st month, $n = 64$). The second milk sampling was done on 3rd month postpartum and concerned $n = 39$ women who continued to breastfeed. Third sampling was done on 6th month postpartum and included $n = 23$ women who still continued to breastfeed.

Data collection

Anthropometric data

Data were collected during early morning hours. Weight and height were measured with the subjects wearing only underwear and with the use of a digital electronic balance (range 0.1–150 kg) and a tape measure (range 0–200 cm). Body mass index (BMI) (kg/m^2) was thus calculated. Pre-pregnancy weight was derived from the subjects' medical record kept at the antenatal clinic.

Dietary records analysis

Dietary intake was assessed using a 3-day dietary record at 1st, 3rd and 6th month postpartum. Participants were thoroughly explained how to fill in the 3-day dietary record, how to measure the proportions of food and how important was not to miss out any food or snack. They were also advised not to change their habitual diet during the 3 days of recording. Consequently, mothers recorded the type and amount of food and beverages consumed for two consecutive weekdays and one weekend day, using standard household measures (cups, tablespoons, etc.). On site, a member of the research team reviewed the records with the respondent to clarify entries, number and size of servings, and forgotten foods. Clarification of foods involved the use of food models, pictures, and measuring devices. Traditional Greek foods were also included in the food database. The 3-day dietary records were analysed using the Nutritionist V diet analysis software (FirstData-Bank Inc, San Bruno, California, US) to estimate intakes of energy, carbohydrate, fat, protein and vitamin E.

Milk sampling

After completion of dietary records, a member of the research team visited mothers at home and a total of 30 mL of foremilk was collected from one breast by an electric breast pump (mini electric breast pump, MEDELA Inc, USA). Milk was placed in three dark sterile plastic tubes without preservatives and was immediately transferred in a cool-box to Harokopio University, where it was stored at −80 °C until analysed. Literature reports that serum vitamin E remains stable at −80 °C [28]. Home visits were made during morning hours and mothers were instructed not to have breastfed their infants for at least 2 h prior to using the breast pump, in order to obtain a significant amount of milk fat [29, 30].

Other data

The date of each subject's last menstruation plus data from their first ultrasound were used to establish gestational age at recruitment. Any pathology during pregnancy was also recorded. Mothers' age, educational level, number of children previously born, and use of tobacco were recorded during the first interview.

Laboratory methods

Materials

Methanol and hexane of analytical grade, ethanol, propanol-2, acetonitrile, and methanol of high-performance liquid chromatography (HPLC) were purchased from Merck (Darmstadt, Germany). α-tocopherol, δ-tocopherol, and α-tocopherol acetate were obtained from Aldrich (Steinheim, Germany), while γ-tocopherol was purchased from Fluka (Steinheim, Germany).

Tocopherols' analysis and determination

The experimental procedure adopted was based on previous published methods [10, 26, 30] and modified as follows: Samples were allowed to thaw and come to room temperature on a shaker to prevent separation of the fat and aqueous phases [25]. Pooled milk (1.5 mL) was mixed with 12% pyrogallol in ethanol (1 mL) and then hexane (2 mL) and α-tocopherol acetate in methanol (100 μL, 0.5 mg/mL were added). The mixture was sonicated at room temperature for 6 min and then it was vortexed for 10 min, centrifuged for 15 min at 2,000 rpm, and the upper organic layer was collected. This step was repeated twice, adjusting the centrifuge at 3,000 rpm. The combined organic extracts were evaporated to dryness under a stream of nitrogen, the residue was re-suspended in 200 μL propanol-2, and vortexed for 30 s. Samples were transferred into sealable autoinjection vials (250 μL). All analyses were conducted under subdued lighting to avoid degradation of vitamin E. For tocopherol determination, an HPLC system (Agilent Technologies, model 1050, Waldbronn, Germany) combined with quaternary pump, auto-sampler, diode array detector (HP-1050), fluorescence detector (HP-1046A), and data analysis software was used. A quaternary solvent system consisting of o-phosphoric acid, methanol, acetonitrile and propanol-2, with gradient elution on a Nucleosil C18 100 (125 × 4.6 mm) column (Macherey–Nagel, Düren, Germany) was used as previously described [31]. Aliquots of 50 μL were subjected to reversed phase HPLC analysis with UV (280, 295 nm) and fluorescence detection (λex = 295 nm, λem = 330 nm). External standard quantification was performed based on a series of five different standard concentrations of α-, γ-, and δ-tocopherol. β- and γ-tocopherols eluted as overlapped peaks and were quantified based on γ-tocopherol standard curve. All analyses were duplicated.

Milk fat determination

Fat in milk samples was analysed according to creamatocrit method [32]. The same researcher read all the creamatocrits, and the numerical formula defined by Lucas et al. [32] was used to estimate the fat content of whole milk.

Statistical analysis

The primary-dependent variable was concentration of vitamin E (sum of α, $\beta + \gamma$ and δ-tocopherols) in mature milk. Equality of means within the three measurements (first, third and sixth months) for vitamin E and three tocopherols was tested with *repeated measures analysis of variance* (*ANOVA*). Concentration of α-tocopherol was compared with that of other published studies with use of *independent samples t-test*. The necessary prerequisite for this comparison was that studies reported the mean values, standard deviation and their sample size. Finally, the effect of food and energy intake on concentration of vitamin E in mature milk was tested with the *general linear model* (*univariate analysis of variance*) using the intake values as covariates. Data are reported as means ± SD. The level at which differences were considered significant was $p = 0.05$. All analyses were performed with the use of SPSS version 17.0 software (SPSS Inc, Chicago, Il, USA).

Results

Maternal characteristics

Mothers' mean age was 32.5 ± 3.1 years. All participants were married, the great majority (94.3%) was employed,

two-thirds (64%) of them had university degrees and 78% were first time mothers. Anthropometric characteristics of the initial sample ($n = 64$) are presented in Table 1. Some mothers (4%) had BMI less than 18.5, while 6% were overweight (BMI > 25) before pregnancy.

Breast milk tocopherol and fat content

Variability of α-, $\beta + \gamma$-, δ-tocopherol and vitamin E content (defined as the sum of tocopherols' content) in mature milk is presented in Table 2. Repeated ANOVA measures revealed that tocopherol content did not differ significantly among the three time points, thus indicating that all four tocopherols remained constant throughout the 6-month period. In addition, milk's vitamin E and fat content as well as the ratio of vitamin E to milk fat content were found not to differ among the three time points. The use of Pearson's correlation coefficient (r) revealed a moderate to strong positive correlation between milk's vitamin E and fat content at the 1st, 3rd and 6th month postpartum ($r = 0.546$, 0.701, and 0.559, respectively).

Dietary parameters

Results from analysis of the 3-day food diaries are presented in Table 3. Repeated ANOVA measures revealed that dietary parameters did not differ significantly among the three time points. Dietary parameters used were the number of meals consumed, variety of foods, energy intake, caloric contribution of carbohydrate, protein, fat (saturated–monounsaturated [MUFA]–polyunsaturated [PUFA]), and vitamin E dietary intake. Dietary intake of vitamin E for the 1st-, 3rd- and 6th-month postpartum was 7.2 ± 3.7, 6.8 ± 3.5 and 10.9 ± 5.2 mg/day, respectively.

The effect of dietary parameters on the tocopherol content of breast milk was tested by applying the *general linear model*. Results during the 1st month are shown in Table 4. Results demonstrate a significant effect of saturated and total fat dietary intake on vitamin E concentration in human milk at 1st month of lactation. In addition, there was a strong trend detected between monounsaturated fat intake and the vitamin E content of human milk at 1st month of lactation. Nevertheless, these effects were not maintained over the 3rd- and 6th-month. No other significant correlations between dietary parameters and milk's vitamin E concentration were detected over the entire 6-month period of exclusive breastfeeding.

Table 1 Age and anthropometric characteristics of subjects

	Mean ± SD	Range
Age (years)	32.5 ± 3.1	25–39
Height (m)	1.67 ± 0.06	1.52–1.80
Body weight (kg)	62.3 ± 11.5	45–106
BMI (kg/m^2)	22.2 ± 4.1	17.4–36.6
Underweight	6.3% ($n = 4$)	
Normal	78.1% ($n = 50$)	
Overweight	10.9% ($n = 7$)	
Obese	4.7% ($n = 3$)	
Birth weight (g)	3,266 ± 368	2,500–4,380

Discussion

This is one of a few studies to report on the milk tocopherol content among European women and is the first longitudinal study to report on the milk tocopherol content of solely breastfeeding Mediterranean women for up to 6 months postpartum.

Milk content of vitamin E and α-tocopherol in Greek women is similar to that reported in literature. Milk vitamin E content (8.9 ± 3.6 μmol/L, or 3.8–4.1 mg/L in the 1st month postpartum) found in the present study is similar to that reported by Bates and Prentice [33] in their meta-analysis. Additionally, although vitamin E intake of our subjects was lower than the RDA (Recommended Dietary Allowances) value for lactating women [34], vitamin E content of their milk does show to meet the recommendations of both National Research Council (3 mg/day) [35] and Food and Nutrition Board (4 mg/day) [36] for infants up to 6 months of age. At the same time, it is almost tenfold higher than the lower normal limit recorded, i.e. 0.9 μmol/L [37]. These calculations were based on the generally acknowledged fact that the volume of daily produced milk is between 750 and 950 mL/day [16]. A comparison of α-tocopherol concentration in the mature milk of our sample at 1st month (8.3 ± 3.4 μmol/L) postpartum to that of mothers in other nine countries is given in Table 5. Overall, a-tocopherol levels recorded in our study are on the average of values recorded in other published studies.

Dietary intake of vitamin E in the 1st-, 3rd- and 6th-month of lactation was 7.2 ± 3.7, 6.8 ± 3.5 and 10.9 ± 5.2 mg/day, respectively. These values are similar to those found by Murphy and Abrams [38] (8.5 ± 1.2 mg/day) as well as by Schiff et al. [39] (11.3 ± 7.5 mg/day). Only 2/64 (3.1%) of our subjects had a vitamin E dietary intake above recommended levels [34]. Other authors have also reported dietary intakes of vitamin E in pregnant women [40, 41] and lactating mothers [42] lower than the recommended [34]. That is the reason why Black et al. [41] commended that established Recommended Intakes (RIs) are probably higher than necessary and therefore deficiency is unlikely to occur.

Table 2 Human milk content (mean ± SD (range)) of a-, ($\beta + \gamma$-), δ- tocopherol, vitamin E, and fat at 1st, 3rd, and 6th month postpartum; Vitamin E/milk fat ratio and results obtained from repeated measure ANOVA

Concentrations (μmol/l)	1st month ($n = 64$)	3rd month ($n = 39$)	6th month ($n = 23$)	p value
α-tocopherol	8.3 ± 3.4 (3.0–22.1)	8.1 ± 4.2 (2.6–19.0)	8.5 ± 4.7 (2.4–21.4)	0.989
($\beta + \gamma$)-tocopherol	0.59 ± 0.33 (0.13–1.56)	0.76 ± 0.48 (0.15–1.8)	1.02 ± 1.25 (0.18–5.95)	0.116
δ-tocopherol	0.018 ± 0.047 (0.001–0.26)	0.007 ± 0.02 (0.001–0.1)	0.009 ± 0.02 (0.001–0.1)	0.374
Vitamin E[a]	8.9 ± 3.6 (3.1–23.5)	8.7 ± 4.6 (2.9–20.5)	9.5 ± 5.6 (2.6–27.2)	0.894
MFAT (g/l)	31.5 ± 16.6 (5.7–101.1)	27.3 ± 18.0 (5.8–70.4)	23.5 ± 12.4 (5.8–46.8)	0.422
VitE/MFAT (mg/g)	0.14 ± 0.06 (0.04–0.39)	0.18 ± 0.09 (0.07–0.45)	0.20 ± 0.12 (0.06–0.62)	0.145

MFAT milk fat content, *VitE/MFAT* ratio of vitamin E to milk fat content

[a] Vitamin E was calculated as the sum of α-, ($\beta + \gamma$)- and δ-tocopherol

Table 3 Results from analysis of the 3-day food diaries results obtained from repeated measure ANOVA

	1st month ($n = 64$)	3rd month ($n = 39$)	6th month ($n = 23$)	p value
Number of meals/day	5.85 ± 1.5	5.58 ± 1.4	5.83 ± 1.9	0.125
Number of different foods/3 days	18.4 ± 4.7	17.9 ± 4.4	18.6 ± 3.8	0.895
Energy intake (EI) (kcal)	1961.4 ± 490.8	2044.9 ± 463.6	1999.7 ± 660.6	0.48
EI/body weight (kcal/kg)	29.2 ± 8.7	31.1 ± 8.1	30.3 ± 7.4	0.335
Carbohydrates (% EI)	44.7 ± 6.4	45.2 ± 7.3	46.78 ± 7.3	0.744
Proteins (% EI)	16.2 ± 3.4	15.5 ± 3.2	14.4 ± 2.8	0.203
Total fat (% EI)	38.5 ± 6.4	38.7 ± 7.1	38 ± 7	0.981
SAT (% total fat)	13.4 ± 2.8	13.1 ± 3	12.1 ± 3.1	0.353
MUFA (% total fat)	16 ± 2.8	16.6 ± 5.8	16.3 ± 4.9	0.917
PUFA (% total fat)	5.6 ± 1.6	5.4 ± 1.5	6 ± 4.1	0.627
Vitamin E (mg/day)	7.2 ± 3.7	6.8 ± 3.5	10.9 ± 5.2	0.118

SAT Saturated fatty acids, *MUFA* Mono-unsaturated fatty acids, *PUFA* Poly-unsaturated fatty acids

Table 4 Correlation of dietary intake parameters with the concentration of vitamin E in mature milk at 1st month of lactation

	p-value	Correlation coefficient (r)
Number of meals/day	0.474	−0.212
Number of different foods/3 days	0.635	0.098
Energy intake (EI)	0.559	−0.027
EI/body weight	0.618	−0.021
Carbohydrates (% EI)	0.081	−0.301
Proteins (% EI)	0.088	0.085
Total fat (% EI)	0.047*	0.244
SAT (% total fat)	0.034*	0.300
MUFA (% total fat)	0.062	0.195
PUFA (% total fat)	0.387	0.092
Vitamin E	0.745	0.002

SAT Saturated fatty acids, *MUFA* Mono-unsaturated fatty acids, *PUFA* Poly-unsaturated fatty acids

* Significant at the level of $p < 0.05$

Although potentiality of several parameters in affecting human milk vitamin E content has been assessed in a number of studies, so far the only strong parameter proven is the stage of lactation. The most remarkable differences occur between the concentration of vitamin E found in colostrum, transitional milk, and mature milk [22, 23, 25, 26]. Only few studies have been designed for large periods of lactation. In fact, in only one study vitamin E status during the 2nd and 4th month of lactation was investigated [43]. Results of this latter study are in accordance with our findings; vitamin E seems to remain practically constant during the first 6 months of lactation, with the exclusion of the first 20 days postpartum. Likewise, literature review shows that the majority of macronutrient content of human milk is not significantly affected by the stage of lactation after the first and up to the sixth month postpartum [16, 17].

In the present study, based on results of the general linear model adopted for statistical interpretation of our findings, no parameter was found to significantly affect

Table 5 Human milk α-tocopherol content: comparison of α-tocopherol values between the present study and other published studies (mean ± SD, sample size *n*)

Country	Reference	α-tocopherol (μmol/L)	*p*-value	*t*-value
Greece ($n = 64$)	Present study	8.3 ± 3.4	–	
USA ($n = 19$)	Gossage et al. [25]	↑9.4 ± 1.2	0.037	2.12
Germany ($n = 21$)	Schweigert et al. [24]	↑13.2 ± 5.1	0.001	4.09
Curacao ($n = 47$)	Muskiet et al. [47]	↑14.4 ± 12.5	0.002	3.25
Dominica ($n = 10$)	Muskiet et al. [47]	10.6 ± 4.2	NS	1.64
Belize ($n = 10$)	Muskiet et al. [48]	12.2 ± 10.9	NS	1.12
Iceland ($n = 77$)	Olafsdottir et al. [43]	↑9.6 ± 4.1	0.045	2.02
Spain ($n = 18$)	Ortega et al. [23]	↓2.2 ± 0.7	<0.001	12.6
Cuba ($n = 21$)	Macias and Schweigert [26]	↓2.7 ± 1.1	<0.001	5.6
Canada ($n = 60$)	Tijerina-Saenz et al. [49]	↓5.39 ± 0.25	<0.001	6.85

NS non-significant $t = \frac{m1-m2}{\sqrt{sd1^2/n1+sd2^2/n2}}$ where m1, sd1 and n1 are the mean value, standard deviation and size of our sample; m2, sd2 and n2 are the mean value, standard deviation and size of the second sample

Significant at the level of $p < 0.05$

vitamin E content of mature human milk throughout the first 6 months of lactation. This is in accordance with the literature showing that mature milk's lipid and fatty acid content is affected by dietary and non-dietary parameters, but not its vitamin E content [16, 26]. It is noteworthy that even the use of vitamin E supplements during pregnancy does not seem to affect tocopherol content of mature human milk, in contrast to its effect on transitional milk [23]. Accordingly, supplementation with β-carotene during lactation did not seem to affect α-tocopherol levels in human milk, either [25]. However, a transient raise of vitamin E content in human milk after a 3-day supplementation with α-tocopherol has been reported [44]. In the present study, no woman was taking any supplements of vitamin E throughout pregnancy and lactation.

In the present study, vitamin E content of breast milk in the first month of lactation was significantly correlated to total fat and saturated fat intake. These correlations were significant in the first month but not in the third and sixth month of lactation. The vitamin E content of human milk, however, did not show any correlation to the intake of PUFAs, which accompany vitamin E in many of its food sources. This may be explained by the fact that these women had a low PUFA/total fat intake ratio, typical of the Greek-Mediterranean diet [45], and that the predominant vitamin E sources in their diet are foods rich in monounsaturated and saturated fat; foods rich in vitamin E consumed by the mothers of the sample include olive oil, various green vegetables cooked with meat and fat, and typical Mediterranean desserts made with nuts and butter [46]. Mothers' wide use of olive oil in particular may explain the trend detected between the dietary intake of monounsaturated fat and vitamin E content of human milk during the first month postpartum.

The fact that these correlations weaken at the third month and disappear at the sixth month postpartum can be explained by the fact that the mothers who continued to breastfeed until the sixth month ($n = 23$) did not show any significant correlation of dietary intake with milk tocopherol content at any point of the study period. This means that if all women ($n = 64$) of the sample remained in the study until the sixth month, then the correlation that was revealed in the first month might have probably remained significant throughout the entire study period.

In conclusion, the dietary effects on tocopherol concentration of breast milk during nursing are still unknown. There is a great need for further longitudinal studies using larger samples to investigate the exact mechanism of vitamin E transfer from maternal plasma to breast milk. This is the first study conducted among Greek mothers and has shown that although dietary intake of vitamin E during lactation is lower than the RDA, vitamin E content of breast milk covers the infants' dietary requirements. This is an area that needs to be further explored, by designing studies that include data from pregnancy, lactation, and infancy. The establishment of any dietary parameters positively affecting the vitamin E content of human milk may help design health-care strategies in western countries in order to improve the quality of human milk and therefore the infants' health status.

Conflict of interest The authors declare that there are no conflicts of interest.

References

1. Jansson L, Akesson B, Holmberg L (1981) Vitamin E and fatty acid composition of human milk. Am J Clin Nutr 34:8–13

2. Blatt DH, Leonard SW, Traber MG (2001) Vitamin E kinetics and the function of tocopherol regulatory proteins. Nutrition 17:799–805
3. Brigelius-Flohe R, Traber MG (1999) Vitamin E: function and metabolism. FASEB J 13:1145–1155
4. Traber MG, Kayden HJ (1989) Preferential incorporation of alpha-tocopherol vs. gamma-tocopherol in human lipoproteins. Am J Clin Nutr 49:517–526
5. Desai ID, Machlin LJ (1985) Vitamin E. In: Augustin J, Klein BP, Becker S, Venugopal PB (eds) Methods of vitamin assay, 4th edn. New York, Wiley, pp 255–281
6. Traber MG, Rader D, Acuff RV, Brewer HB Jr, Kayden HJ (1994) Discrimination between RRR and all-racemic-a-tocopherols labeled with deuterium by patients with abetalipoproteinemia. Atherosclerosis 108:27–37
7. Traber MG (2006) Vitamin E. In: Shils ME, Shike M, Ross AC, Caballero B, Cousins R (eds) Modern nutrition in health and disease, 10th edn. Lippincott Williams & Wilkins, Baltimore, pp 396–411
8. Escrivá A, Esteve MJ, Farré R, Frígola A (2002) Determination of liposoluble vitamins in cooked meals, milk and milk products by liquid chromatography. J Chromatogr A 947(2): 313–318
9. Crawford MA, Golfetto I, Ghebremeskel K, Min Y, Moodley T, Poston L (2003) The potential role for arachidonic and docosahexaenoic acids in protection against some central nervous system injuries in preterm infants. Lipids 38:303–315
10. Romeu-Nadal M, Morera-Pons S, Castellote AI, López-Sabater MC (2006) Determination of gamma- and alpha-tocopherols in human milk by a direct high-performance liquid chromatographic method with UV-vis detection and comparison with evaporative light scattering detection. J Chromatogr A 1114(1):132–137
11. Brion LP, Bell EF, Raghuveer TS (2003) Vitamin E supplementation for prevention of morbidity and mortality in preterm infants. Cochrane Database Syst Rev;4:CD003665
12. WHO (2003) Evidence on the long-term effects of breastfeeding. Systematic reviews and meta-analysis. Department of child and adolescent health and development, Switzerland
13. Worthington-Roberts B (1997) The role of maternal nutrition in the prevention of birth defects. J Am Diet Assoc 97(10 Suppl 2): S184–S185
14. Kolb E (1995) The significance of vitamin A for the immune system. Berl Munch Tierarztl Wochenschr 108(10):385–390
15. Chávez-Servína JL, Castellotea AI, Riverob M, López-Sabater MC (2008) Analysis of vitamins A, E and C, iron and selenium contents in infant milk-based powdered formula during full shelf-life. Food Chem 3:1187–1197
16. Fidler N, Salobir K, Stibilj V (2000) Fatty acid composition of human milk in different regions of Slovenia. Ann Nutr Metab 44(5–6):187–193
17. Allen JC, Keller RP, Archer P, Neville MC (1991) Studies in human lactation: milk composition and daily secretion rates of macronutrients in the first year of lactation. Am J Clin Nutr 54(1):69–80
18. Nommsen LA, Lovelady CA, Heinig MJ, Lönnerdal B, Dewey KG (1991) Determinants of energy, protein, lipid, and lactose concentrations in human milk during the first 12 mo of lactation: the DARLING study. Am J Clin Nutr 53(2):457–465
19. Meneses F, Trugo NMF (2005) Retinol, β-carotene and lutein + zeaxanthin in the milk of Brazilian nursing women: associations with plasma concentrations and influences of maternal characteristics. Nutr Res 25:443–451
20. Ortega RM, Andres P, Martinez RM, Lopez-Sobaler AM (1997) Vitamin A status during the third trimester of pregnancy in Spanish women: influence on concentrations of vitamin A in breast milk. Am J Clin Nutr 66:564–568
21. de Azeredo VB, Trugo NM (2008) Retinol, carotenoids, and tocopherols in the milk of lactating adolescents and relationships with plasma concentrations. Nutrition 24(2):133–139
22. Campos JM, Paixão JA, Ferraz C (2007) Fat-soluble vitamins in human lactation. Int J Vitam Nutr Res 77(5):303–310
23. Ortega RM, Lopez-Sobaler AM, Andres P, Martinez RM, Quintas ME, Requejo AM (1999) Maternal vitamin E status during the third trimester of pregnancy in Spanish women: influence on breast milk vitamin E concentration. Nutr Res 19(1):25–36
24. Schweigert FJ, Bathe K, Chen F, Büscher U, Dudenhausen JW (2004) Effect of the stage of lactation in humans on carotenoid levels in milk, blood plasma and plasma lipoprotein fractions. Eur J Nutr 43:39–44
25. Gossage CP, Deyhim M, Yamini S, Douglass LW, Moser-Veillon PhB (2002) Carotenoid composition of human milk during the first month postpartum and the response to b-carotene supplementation. Am J Clin Nutr 76:193–197
26. Macias C, Schweigert FJ (2001) Changes in the concentration of carotenoids, vitamin A, alpha-tocopherol and total lipids in human milk throughout early lactation. Ann Nutr Metab 45(2):82–85
27. Boersma ER, Offringa PJ, Muskiet FA, Chase WM, Simmons IJ (1991) Vitamin E, lipid fractions, and fatty acid composition of colostrum, transitional milk, and mature milk: an international comparative study. Am J Clin Nutr 53:1197–1204
28. Comstock GW, Alberg AJ, Helzlsouer KJ (1993) Reported effects of long-term freezer storage on concentrations of retinol, beta-carotene, and alpha-tocopherol in serum or plasma summarized. Clin Chem 39(6):1075–1078
29. Saarela T, Kokkonen J, Koivisto M (2005) Macronutrient and energy contents of human milk fractions during the first six months of lactation. Acta Paediatr 94:1176–1181
30. Schweigert FJ, Hurtienne A, Bathe K (2000) Improved extraction procedure for carotenoids from human milk. Int J Vitam Nutr Res 70(3):79–83
31. Andrikopoulos NK, Bruschweiler H, Felber H, Taeschler Ch (1991) HPLC analysis of phenolic antioxidants, tocopherols and triglycerides. J Am Oil Chem Soc 68:359–364
32. Lucas A, Gibbs JA, Lyster RL, Baum JD (1978) Creamatocrit: simple clinical technique for estimating fat concentration and energy value of human milk. Br Med J 1:1018–1020
33. Bates CJ, Prentice A (1994) Breast milk as a source of vitamins, essential minerals and trace elements. Pharmacol Ther 62(1–2): 193–220
34. National Institute of Health (2002) Dietary reference intakes for vitamin a, vitamin k, arsenic, boron, chromium, copper, iodine, iron, manganese, molybdenum, nickel, silicon, vanadium, and zinc. A report of the panel on micronutrients, subcommittees on upper reference levels of nutrients and of interpretation and uses of dietary reference intakes, and the standing committee on the scientific evaluation of dietary reference intakes. Food and Nutrition Board, Institute of Medicine, National Academy Press, Washington, DC
35. National Research Council (1989) Recommended dietary allowances. Subcommittee on the tenth edition of the RDAs. Food and Nutrition Board Commission on Life Sciences, 10th edn. National Academy Press, Washington, DC
36. Institute of Medicine (2000) Dietary reference intakes for vitamin C, vitamin E, selenium, and carotenoids. Food and Nutrition Board. National Academy Press, Washington, DC, pp 186–283
37. Anderson DM, Pittard WB (1985) Vitamin E and C concentrations in human milk with maternal megadosing: a case report. J Am Diet Assoc 85:715–717
38. Murphy SP, Abrams BF (1993) Changes in energy intakes during pregnancy and lactation in a national sample of US women. Am J Public Health 83(8):1161–1163

39. Schiff E, Friedman SA, Stampfer M, Kao L, Barrett PH, Sibai BM (1996) Dietary consumption and plasma concentrations of vitamin E in pregnancies complicated by preeclampsia. Am J Obstet Gynecol 175((4 Pt1)):1024–1028
40. Antal M, Regoly-Merei A, Varsanui H, Biro L, Sagi K, Molnar DV et al (1997) Nutritional survey of pregnant women in Hungary. Int J Vitam Nutr Res 67:115–122
41. Black AE, Wiles SJ, Paul AA (1986) The nutrient intakes of pregnant and lactating mothers of good socio-economic status in Cambridge, UK: some implications for recommended daily allowances of minor nutrients. Br J Nutr 56(1):59–72
42. de Pee S, West CE, Muhilal D, Karyadi D, Hautvast JGAJ (1995) Lack of improvement in vitamin A status with increased consumption of dark-green leafy vegetables. Lancet 346:75–81
43. Olafsdottir AS, Wagner KH, Thorsdottir I, Elmadfa I (2001) Fat-soluble vitamins in the maternal diet, influence of cod liver oil supplementation and impact of the maternal diet on human milk composition. Ann Nutr Metab 45(6):265–272
44. Kanno C, Kobayashi H, Yamauchi K (1989) Transfer of orally administered alpha-tocopherol into human milk. J Nutr Sci Vitaminol 35(6):649–653
45. Bondia-Pons I, Serra-Majem L, Castellote AI, López-Sabater MC (2007) Compliance with the European and national nutritional objectives in a Mediterranean population. Eur J Clin Nutr 61(12):1345–1351
46. Trichopoulou A, Psaltopoulou T, Orfanos P, Hsieh CC, Trichopoulos D (2007) Low-carbohydrate-high-protein diet and long-term survival in a general population cohort. Eur J Clin Nutr 61(5):575–581
47. Muskiet FA, Hutter NH, Martini IA, Jonxis JH, Offringa PJ, Boersma ER (1987) Comparison of the fatty acid composition of human milk from mothers in Tanzania, Curacao and Surinam. Hum Nutr Clin Nutr 41(2):149–159
48. Muskiet FA, Offringa PJ, Boersma ER (1988) Lipid content and fatty acid composition of human milk in relation to developing countries. In: Boersma ER, Huisjes HJ, Poortman HMC (eds) A holistic approach to perinatal care and the prevention of handicap. Erven B van der Kamp Publishers, Groningen, The Netherlands, pp 294–305
49. Tijerina-Sáenz A, Innis SM, Kitts DD (2009) Antioxidant capacity of human milk and its association with vitamins A and E and fatty acid composition. Acta Paediatr 98(11):1793–1798