Concentrations of alpha- and gamma-tocopherols in human breast milk during the first months of lactation and in infant formulas

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Abstract

The aim of this study was to determine the concentrations of alpha- and gamma-tocopherols in human breast milk samples from different periods of lactation and to compare them with tocopherol content in commercially available formulas for infants at corresponding ages. The study included 93 breast milk samples obtained on the 2nd (colostrum, $n = 17$), 14th ($n = 30$), 30th ($n = 27$) and 90th day of lactation ($n = 19$), along with 90 samples of commercially available initial and follow-on infant formulas. Concentrations of tocopherols were determined using normal-phase high-performance liquid chromatography. Depending on the stage of lactation, human breast milk contained 2.07–9.99 mg L^{-1} of alpha-tocopherol and 0.22–0.60 mg L^{-1} of gamma-tocopherol. Breast milk concentrations of alpha-tocopherol decreased with the time of lactation, while significant differences in gamma-tocopherol concentration were observed only between the 14th and 30th day of lactation. There was no significant correlation between the dietary intake of vitamin E and its estimated breast milk concentration, also in women who declared vitamin supplementation. Compared with colostrum, infant formulas were characterised by significantly lower concentrations of alpha-tocopherol and vitamin E. This finding indicates the need of additional vitamin E supplementation of bottle-fed infants during the initial 2–3 days of life.

Keywords: tocopherols, breast milk, lactation, dietary intake of vitamin E, infant formulas.

Introduction

Breast milk constitutes ideal first food for the infant, providing various nutritional, immunologic and emotional benefits. Volume and composition of human breast milk depend on the time of a day and vary throughout lactation and even during a single feeding (Emmett & Rogers 1997).

Breast milk contains most nutrients needed by infants, among them is vitamin E. This vitamin is particularly essential for pre-term neonates who have very low plasma levels of this compound due to immature lipoprotein metabolism and low transport capacity (Romeu-Nadal *et al*. 2006). Vitamin E is involved in antioxidant activity (e.g. inhibition of polyunsaturated fatty acid peroxidation in cell membranes) and immunomodulation (Meydani *et al*. 2005; Debier 2007; Antonakou *et al*. 2010). Additionally, vitamin E, the most potent lipid peroxyl radical scavenger, protects tissues against chromosome damage and DNA oxidation (Claycombe & Meydani 2001). Naturally occurring structures classified as having vitamin E antioxidant activity include eight chemically related compounds: alpha-, beta-, gamma-,

delta-tocopherol and alpha-, beta-, gamma- and delta-tocotrienol. These substances differ both in structure and in bioavailability, but all tocopherols that occur naturally have the RRR stereochemistry in the side chain (Institute of Medicine 2000). Alpha-tocopherol is the most active form of vitamin E, while the other naturally occurring forms of vitamin E (beta-, gamma- and delta-tocopherols and the tocotrienols) do not contribute in meeting the vitamin E demands. The various forms of vitamin E are not interconvertible in humans and thus do not behave the same metabolically. Although absorbed by the intestine, they are not converted to alpha-tocopherol in the human body and are poorly recognised by the binding alpha-tocopherol transfer protein in the liver (Traber 2007).

Vitamin E activity in food is reported as alpha-tocopherol equivalents (α-TE). Factors for the conversion of the tocopherols and tocotrienols to α-TE units were based on the biological activity of the various forms as determined using the rat fetal resorption assay (Institute of Medicine 2000).

Infants have reduced antioxidant capacity and are often exposed to oxidative stress caused by infection, oxygen, mechanical ventilation, intravenous nutrition and blood transfusion (Hanna *et al*. 2004). Vitamin E deficiency may lead to haemolytic anaemia, retrolental fibroplasia, intraventricular haemorrhage, bronchopulmonary dysplasia and adversely affect the development of the central nervous system, especially in pre-term infants (Bell 1987). Deficiency of vitamin E is rarely reported from adults, and the dietary intake of tocopherols is adequate in most developed countries. However, recent studies show that the intake of vitamin E with diet decreases. According to surveys of the US Department of Agriculture (USDA 2010) and National Health and Nutrition Examination Survey (Gao *et al*. 2006), the intake of vitamin E by women between 19 and 50 years of age was lower than 90% of the Recommended Daily Allowance. While in men of the same age, the intake was close to 100% of the recommended dose. This is probably due to the change of eating habits such as decrease in dietary vegetable and fruit intakes and total fat restrictions in the diet (Institute of Medicine 2000; Gao *et al*. 2006; Traber 2007).

The main nutritional sources of tocopherols include plant-derived fats, such as wheat germ, sunflower seeds, corn seeds, nut and almond oils (Reboul *et al*. 2006). The Dietary Reference Intakes for vitamin E (alpha-tocopherol) is 15 mg day^{-1} for adults, 15 mg day^{-1} for pregnant, 19 mg day^{-1} for breastfeeding women, 4–5 mg day^{-1} for infants (0–12 months) and 6 mg day^{-1} for children between 1and 3 years of age (Institute of Medicine 2000).

The composition of human breast milk changes throughout lactation along with the requirements of growing child. Additionally, the breast milk content of some components is influenced by maternal diet (Lauwers & Swisher 2005). Available data on breast milk concentration of vitamin E are inconclusive, but the content of this compound was revealed to be higher in colostrum than in mature milk (Jansson *et al*. 1981; Macias & Schweigert 2001; Tokusoglu *et al*. 2008; Sziklai-Laszlo *et al*. 2009).

Tocopherols are added to infant formulas because they increase vitamin content and prevent lipid peroxidation during manufacture and storage, therefore, prolonging the product's shelf life. Another source of tocopherols is plant-derived oils included in the formulas (Chavez-Servin *et al*. 2006).

Key messages

- The content of alpha-tocopherol in human milk decreases with the time of lactation.
- There was no significant correlation between the dietary intake of vitamin E and its breast milk concentrations, also in women who declared vitamin supplementation.
- The content of alpha-tocopherol in infant formula is similar to the content in transitional and mature milk, while the content of gamma-tocopherol in infant formula is at least seven times higher than that in human milk.
- The results justify the need of additional vitamin E supplementation of bottle-fed infants during the initial 2–3 days of life.

The aim of this study was to determine the concentrations of alpha- and gamma-tocopherol in human breast milk samples from different periods of lactation and to compare them with tocopherol contents in commercially available formulas for infants of corresponding ages.

Materials and methods

Ethics

All procedures of this study were approved by the Local Ethics Committee of the Medical University of Gdansk. The subjects gave their informed consent before the start of any procedure.

Breast milk samples

This study included 93 breast milk samples from 48 lactating women living in Gdansk (Northern Poland). The age of participating mothers ranged from 21 to 39 years. The same woman provided more than one sample at different times of lactation. Other characteristics of study participants are summarised in Table 1.

The samples were obtained on the 2nd (colostrum, $n = 17$), 14th ($n = 30$), 30th ($n = 27$) and 90th day of lactation ($n = 19$). The mammary gland was evacuated completely with an aid of a manual lactator 2 h after the first morning feeding (between 5 and 7 am). The material was collected into sterile glass containers. Immediately after collection and careful mixing, 10 mL of samples (2 mL in the case of colostrums) was withdrawn and placed into another sterile container. The remaining milk was fed to the infants. In the case of breast milk obtained at home, the samples were frozen at –18°C, delivered to the laboratory within 6 h after collection and immediately frozen at –80°C until analysis.

Table 1. Characteristics of participating women ($n = 48$)

Parameter	Value
Age (years)	26.14 ± 5.18 (range 21–39)
Body weight at delivery (kg)	75.34 ± 12.06 (range 57–114)
Active smokers (%)	6.3 (frequencies 3/48)
Passive smokers (%)	10.4 (frequencies 5/48)
Vitamin supplementation (%)	
14th day	51.7 (frequencies 15/29)
30th day	51.9 (frequencies 14/27)
90th day	38.9 (frequencies 7/18)

Continuous variables were presented as mean ± standard deviation and range (in parentheses).

Dietary intake of vitamin E

Dietary intake of vitamin E was determined directly before milk sampling based on a 3-day nutritional diary reported by mothers and analysed with the use of Dieta 4.0 software (National Institute of Food and Nutrition, Warsaw, Poland).

Infant formulas

This study included two types of infant formulas: (i) initial formulas (IF) for infants between the first day and the end of the fifth month of life and (ii) follow-on formulas (FF) for 6-month and older babies. The five most frequently purchased brands of both types of formula were examined. For each formula, nine independent determinations were made, which corresponded to 90 samples analysed.

The formulas, in original commercially available packages, were obtained directly after manufacturing and stored at recommended conditions. The samples were taken in accordance with the respective Polish Standard PN-EN ISO 707:2008 (2008) and prepared along with manufacturer's instructions.

Analytical procedure

Total lipids were extracted from milk and ready-to-fed formula solutions with *n*-hexane by the method described by Romeu-Nadal *et al.* (2006). Five-hundred microlitres of analysed sample was poured into a tube. One millilitre of *n*-hexane was added, and the mixture was shaken for 1 min and centrifuged (10 min, 7000 rpm). The organic phase was poured into a new tube. Extraction was repeated using 0.5 mL of *n*-hexane. Combined organic phase was evaporated under nitrogen and made to the appropriate concentration of lipid with *n*-hexane (25 mg per 0.5 mL of *n*-hexane) for the purpose of normal-phase

high-performance liquid chromatography (NP-HPLC) assay.

Concentrations of alpha-, beta-, gamma- and delta-tocopherols were determined by NP-HPLC with UV detection (λ_{anal} = 295 nm), as described by the respective Polish Standard PN-EN 12822:2002 (2002) and Chavez-Servin *et al*. (2006). HPLC system was composed of Dionex P liquid chromatograph equipped with a 25-µL loop injector (Rheodyne 7725i, Rheodyne®, Rohnert Park, CA, USA) and SpectraSYSTEM UV3000HR detector (Thermo Electron Corporation, San Jose, CA, USA). The chromatographic separation was performed on a Separon™ SGX NH_2 column (150 × 3.0-mm inner diameter, 3-µm particle size; Tessek Ltd., Prague, Czech Republic) at 20°C. The isocratic elution technique with hexane/ethyl acetate, 85:15 (vol : vol), mobile phase was used. The flow rate of the mobile phase was 0.6 mL min^{-1}.

Validation and linearity

The method was validated for recovery (internal standard method), repeatability (RSD – relative standard deviation for six independent measurements at three analyte dilutions), limit of detection (LOD), limit of quantification (LOQ) and linearity. LOD was calculated in view of the standard deviation of signals and the slope of the calibration curve, where LOQ is three times the LOD. Only alpha- and gamma-tocopherols were detected in human milk and infant formulas. The average recovery rate of alpha- and gamma-tocopherol exceeded 95% (97.92% for human milk and 95.17% for infant formula) and 96% (97.73% for human milk and 96.87% for infant formula), respectively. The measurements were characterised by satisfactory repeatability, with RSD amounting to 6.23% and 6.03% for alpha- and gamma-tocopherol, respectively. LOD was 3.4 and 10.7 ng mL^{-1} for alpha- and gamma-tocopherol, respectively, and LOQ was 10.2 and 32.1 ng mL^{-1}, respectively (for ingestion). Method detection limit was 5.5 mg L^{-1} of milk for alpha-tocopherol and 17.4 mg L^{-1} of milk for gamma-tocopherol.

Calibration curves were plotted using standard solutions of tocopherols (Merck, Darmstadt, Germany). The linearity of the analytical method for each tocopherol was confirmed by determining the statistical significance of calibration curve coefficients (>0.998). The calibration curve for alpha-tocopherol was linear within 0.9–10.6 µg mL^{-1} (r = 0.999), while the calibration curve for gamma-tocopherol was linear over the range 0.9–10.4 µg mL^{-1} (r = 0.998).

The amounts of native tocopherols were calculated on the basis of their peak area and percentage recovery. Based on alpha- and gamma-tocopherol contents, the concentration of alpha-tocopherol equivalents (TE) was estimated with the following formula (Olafsdottir *et al*. 2001): TE (mg) = alpha-tocopherol (mg) + 0.25 × gamma-tocopherol (mg).

Statistical analysis

Normal distribution of continuous variables was tested with the Kolmogorov–Smirnov test. The results were presented as arithmetic means and their standard deviations. Dietary intake of vitamin E, and breast milk and infant formula concentrations of tocopherols and vitamin E were compared with one-way analysis of variance and Tukey's post-hoc test. Additionally, Pearson's linear coefficient of correlation was calculated between the dietary intake of vitamin E and the breast milk concentration of this vitamin. Calculations were performed using Statistica 8 (StatSoft®, Warsaw, Poland) software, with statistical significance defined as $P \leq 0.01$.

Results

Dietary intake of vitamin E

Women at various periods of lactation (14th, 30th and 90th day) did not differ significantly in terms of dietary intake of vitamin E (Table 2). Mean daily intake of vitamin E was 14.9 ± 8.3 TE mg (higher than the recommended dose for breastfeeding women), but almost 35% of mothers consumed less than 10 mg vitamin E per day. These were women who did not enrich their diet and, in this group, the average daily intake of vitamin E was 4.3 mg day^{-1} (among the mothers taking vitamin supplements about 19.4 mg day^{-1}). The main declared dietary sources of this vitamin included supplements and fats

Table 2. Dietary daily intake of vitamin E in participating women

Source	Day of lactation	Content (TE mg day^{-1})
Food	14th	8.20 ± 3.40 (4.24–16.67)
	30th	8.41 ± 3.48 (3.43–16.08)
	90th	9.33 ± 3.80 (4.00–15.76)
Supplementation	14th	7.32 ± 8.34 (0–30.00)
	30th	6.69 ± 7.19 (0–20.10)
	90th	7.62 ± 3.02 (0–20.10)

TE, alpha-tocopherol equivalents. Values were presented as mean ± standard deviation and range (in parentheses).

(margarine and vegetable oils). Supplements containing vitamin E received approximately 52% of women in the first period of lactation and only 39% after 90 days.

Breast milk concentration of total lipids

The fat content in the milk increases during the first month of lactation. The average total fat content in the samples was 2.71 ± 0.17% for colostrums, 3.23 ± 0.44% for transitional milk, 3.68 ± 0.52% for mature milk in the 30th day post-partum and 3.87 ± 0.40% in the 90th day.

Breast milk concentrations of tocopherols and vitamin E

Colostrum was characterised by the highest concentrations of alpha-tocopherol and vitamin E. Significant reduction of these two parameters were observed on the 14th and 30th day of lactation with no further marked changes noted in the 90th day of lactation. Significant differences in gamma-tocopherol concentration were observed only between the 14th and 30th day of lactation (Table 3).

Dietary and breast milk contents of vitamin E

No significant correlation was observed between the dietary intake of vitamin E and the breast milk concentration of this vitamin ($r = 0.034$, $P = 0.22$). Women who declared vitamin supplementation at the time of sampling did not differ significantly from non-supplemented individuals in terms of mean breast

Table 3. Concentration of alpha-tocopherol, gamma-tocopherol and vitamin E in human milk (concentration per litre of milk and per gram of total lipid)

Component	Units	Type of milk		
		2nd day ($n = 17$)	14th day ($n = 30$)	30th day ($n = 27$)	90th day ($n = 19$)
Alpha-tocopherol	mg L^{-1} milk	9.99^{a} ± 1.51 (7.18–12.13)	4.45^{a,b} ± 0.95 (2.23–6.47)	2.92^{b} ± 0.84 (1.71–4.28)	2.07 ± 0.66 (0.94–2.80)
	μg g^{-1} total lipid	352.4^{a} ± 53.2 (253.4–427.9)	133.6^{a,b} ± 28.5 (67.1–194.6)	81.6^{b} ± 23.6 (62.5–11.9)	55.1 ± 17.5 (25.1–74.5)
Gamma-tocopherol	mg L^{-1} milk	0.57 ± 0.21 (0.18–0.79)	0.60^{c} ± 0.21 (0.18–0.86)	0.30^{c} ± 0.14 (0.07–0.54)	0.22 ± 0.10 (0.07–0.38)
	μg g^{-1} total lipid	20.0 ± 7.4 (6.4–27.9)	17.9^{c} ± 6.5 (5.4–25.8)	8.5^{c} ± 3.8 (2.0–15.1)	5.9 ± 2.6 (1.9–10.1)
Vitamin E	TE mg L^{-1} milk	10.13^{d} ± 1.50	4.59^{d,e} ± 0.93	3.00^{e} ± 0.85	2.13 ± 0.67
	TE μg g^{-1} total lipid	357.4^{d} ± 52.8	138.0^{d,e} ± 28.0	83.8^{e} ± 23.7	56.6 ± 17.9

TE, alpha-tocopherol equivalents. Values were presented as mean ± standard deviation and range (in parentheses). ^{a,b,c,d}Significantly different compared with previous period of lactation ($P < 0.001$; Tukey's post-hoc test).

Table 4. Concentration of alpha-tocopherol, gamma-tocopherol and vitamin E in infant formulas (IF – initial formula; FF – follow-on formula)

Sample ($n = 9$)*	Alpha-tocopherol (mg L^{-1})	Gamma-tocopherol (mg L^{-1})	Vitamin E (TE mg L^{-1})
IF A	5.88 ± 0.17	5.01 ± 0.21	7.14 ± 0.21
IF B	5.50 ± 0.19	3.74 ± 0.13	6.44 ± 0.17
IF C	3.07 ± 0.06	4.53 ± 0.23	4.20 ± 0.11
IF D	2.51 ± 0.09	2.33 ± 0.05	3.09 ± 0.09
IF E	2.73 ± 0.17	4.67 ± 0.04	3.90 ± 0.16
IF average	3.94 ± 1.47	4.06 ± 0.98	4.95 ± 1.58
FF A	6.22 ± 0.08	4.34 ± 0.28	7.31 ± 0.12
FF B	5.31 ± 0.08	2.47 ± 0.19	5.93 ± 0.07
FF C	5.03 ± 0.10	2.99 ± 0.13	5.78 ± 0.12
FF D	3.96 ± 0.08	3.02 ± 0.05	4.71 ± 0.09
FF E	4.38 ± 0.14	4.07 ± 0.05	5.40 ± 0.14
FF average	4.98 ± 0.80	3.38 ± 0.73	5.82 ± 0.87

TE, alpha-tocopherol equivalents. Values were presented as mean ±standard deviation. *Number of sample for each kind of infant formula.

milk concentration of vitamin E (3.46 ± 1.36 TE mg L^{-1} vs. 3.35 ± 1.25 TE mg L^{-1}; 95% confidence interval, –0.56 to 0.78, $P = 0.33$).

Tocopherols and vitamin E concentrations in infant formulas

The FF type was characterised by significantly higher concentration of alpha-tocopherol and vitamin E and significantly lower gamma-tocopherol content than IF (Table 4, detailed statistics not shown). Mean concentration of alpha-tocopherol in initial infants' formula was 3.94 mg L^{-1} (ready-to-feed solution), gamma-tocopherol was 4.06 mg L^{-1}. The FF contained an average of 4.98 mg of alpha-tocopherol and 3.38 mg of gamma-tocopherol per litre. These concentrations corresponded to 4.95 and 5.82 mg vitamin E (TE mg) L^{-1}, respectively, of infant formula and FF. More interestingly, various brands of IF- and FF-type formulas differed significantly in terms of mean values of analysed parameters (Table 4).

Comparison between breast milk and infant formulas

Mean colostrum concentrations of alpha-tocopherol and vitamin E were markedly higher than in IF-type formulas ($P < 0.001$ for each parameter). However, no significant differences in alpha-tocopherol and vitamin E content were noted when 14-day milk was compared with the IF-type formulas ($P = 0.364$ and $P = 0.765$, respectively), and the levels of these parameters in 30- and 90-day milk samples were significantly lower than in the formulas (alpha-tocopherol: $P = 0.002$ and $P < 0.001$; vitamin E: $P < 0.001$ for both analysed periods).

Throughout the entire analysed period of lactation, IF-type formulas were characterised by markedly higher levels of gamma-tocopherol than those determined in breast milk ($P < 0.001$ for each analysed period). Additionally, tocopherols and vitamin E contents in 90-day milk were compared with those in the FF-type formulas. Concentrations of alpha- and gamma-tocopherol and vitamin E content in the formulas were significantly higher than in both breast milk ($P < 0.001$ for each comparison).

Discussion

This study revealed that the breast milk concentrations of alpha-tocopherol decreased with the time of lactation (Table 3). This relation can also be seen by expressing the content of alpha-tocopherol per 100 g of total fat. In this case, content of alpha-tocopherol varies from 35.24 mg per 100 g total fat in colostrum by 13.36 mg per 100 g total fat in the 14th day and 7.6 mg in the 30th day to about 5.4 mg per 100 g of total fat in the 90th day of lactation.

The most pronounced drop off was observed during the initial 2 weeks of lactation when the levels of alpha-tocopherol decreased by more than half of its colostrum content. After 3 months of lactation, breast milk concentration of alpha-tocopherol reached about 2.1 mg L^{-1}. Our findings concerning breast milk content of alpha-tocopherol is consistent with those from Sweden (Jansson *et al.* 1981), Cuba (Macias & Schweigert 2001), Brazil (de Azeredo & Trugo 2008) and Hungary (Sziklai-Laszlo *et al.* 2009). However, the values observed in our study were lower than those reported from Iceland (Olafsdottir *et al.* 2001), Turkey (Tokusoglu *et al.* 2008) and Greece (Antonakou *et al.* 2010). More interestingly, alpha-tocopherol concentration in breast milk of Turkish

Table 5. Literature evidence of tocopherol concentration in human milk

References	Country/year	Type of milk (days post-partum)	No. of samples	Alpha-tocopherol (mg L^{-1})	Gamma-tocopherol (mg L^{-1})
Jansson *et al.*	Sweden/1981	Colostrum (4 days)	6	9.91 ± 5.43	1.68 ± 0.43
		Transitional (6–10 days)	10	4.48 ± 1.80	1.03 ± 0.60
		Mature (12 day–5 months)	24	3.10 ± 1.68	0.90 ± 0.56
Olafsdottir *et al.*	Iceland/2001	After 2 vs. 4 months	77	4.14 ± 0.20	1.03 ± 0.06
Macias & Schweigert	Cuba/2001	Colostrum (24–48 h)	21	11.8 ± 0.63	–
		Transitional (7 days)	21	5.00 ± 0.30	–
		Mature (15 days)	21	2.7 ± 0.11	–
Tokusoglu *et al.*	Turkey/2007	Mature (65 ± 0.8 day)	92	9.84 ± 2.13	–
de Azeredo & Trugo	Brazil/2007	Mature (30–120 days)	72	2.70 ± 0.21	0.37 ± 0.02
Sziklai-Laszlo *et al.*	Hungary/2008	Transitional (5–10 days)	12	4.14 ± 2.17	0.53 ± 0.28
		Mature (2–40 weeks)	18	3.00 ± 1.16	1.17 ± 0.44
Antonakou *et al.*	Greek/2009	Mature, 1st month	64	3.57 ± 1.46	0.25 ± 0.14
		3rd month	39	3.49 ± 1.81	0.32 ± 0.20
		6th month	23	3.66 ± 2.02	0.42 ± 0.48

Values were presented as mean ± standard deviation.

women was even threefold higher than that observed in our study (Tokusoglu *et al.* 2008). We can only hypothesise that this difference resulted from different dietary contents of tocopherols in Turkish women. Evidently, Mediterranean diet that predominates in Turkey will markedly differ qualitatively and quantitatively from that of Polish women – also in terms of tocopherol content.

The other tocopherol detected in human breast milk in this study (gamma-tocopherol) reached its peak level – 0.6 mg L^{-1} (1.79 mg per 100 g total fat) on the 14th day of lactation and then dropped off to 0.3 mg L^{-1} (0.80 mg per 100 g total fat) on the 30th day and next slightly above 0.2 mg L^{-1} (0.58 mg per 100 g total fat) on the 90th day of lactation day. The research results about the dynamics of changes in the concentration of the gamma-tocopherol in breast milk presented by other authors are inconclusive. Similar to our study, Jansson *et al.* (1981) observed a decline in gamma-tocopherol content of human milk progressing with the time of lactation. In contrast, Sziklai-Laszlo *et al.* (2009) reported a nearly twofold increase in the breast milk concentration of gamma-tocopherol during consecutive stages of lactation. Also, Antonakou *et al.* (2010) observed a slight increase in the gamma-tocopherol content between the 30th and 90th day of lactation. These differences can be attributed to a variety of factors. One possible explanation includes dietary differences between populations of studied countries – noticeably, both countries with increasing content of gamma-tocopherol (Hungary and Greece) are located south from Poland which probably is reflected in the diet of the studied women. One should note that the Swedish women studied by Jansson *et al.* (1981) were characterised by similar dynamics of gamma-tocopherol levels as those in our study. Literature evidence on breast milk concentrations of tocopherols at various stages of lactation is summarised in Table 5.

Biological function of vitamin E is mostly determined by alpha-tocopherol. Alpha-tocopherol represents primary lipophilic antioxidant of mammalian and plant cells. It is located in cell membranes and protects cellular lipoproteins. It functions as antioxidant scavenging peroxide free radicals. Protection of polyunsaturated fatty acids located in membrane phospholipids is possible due to higher affinity of lipid-generated free radicals to alpha-tocopherol than to membrane lipids. Alpha-tocopherol inhibits the activity of protein kinase C, an enzyme involved in cell proliferation and differentiation in smooth muscle cells, platelets and monocytes (Institute of Medicine 2000).

The mechanisms of gamma-tocopherol biological function are not fully understood thus far. However, it is established as an antioxidant of foods where it

corresponds to approximately 70% of total vitamin E content. Valuable sources of gamma-tocopherol include plant-derived oils such as corn, soybean, sesame and nut oil (Chun *et al*. 2006; Reboul *et al*. 2006).

In addition, vitamin E is involved in immune function, cell signalling, regulation of gene expression and other metabolic processes (Meydani *et al*. 2005; Debier 2007; Antonakou *et al*. 2010). Vitamin E also increases the expression of two enzymes that suppress arachidonic acid metabolism, thereby increasing the release of prostacyclin from the endothelium, which, in turn, dilates blood vessels and inhibits platelet aggregation (Institute of Medicine 2000).

Alpha-tocopherol is the predominant form of vitamin E in most human and animal tissues, including blood plasma. In humans, plasma alpha-tocopherol concentrations are 4–10 times higher than those of gamma-tocopherol. Also, breast milk content of gamma-tocopherol is reported to be approximately one-fourth that of alpha-tocopherol level (Jansson *et al*. 1981).

In our study, we did not observe significant correlation between vitamin E content in the diet of lactating women and the estimated concentration of this vitamin in breast milk. Also, some previous studies did not confirm significant association between dietary intake of tocopherols and their concentrations in human breast milk (Chappell *et al*. 1985; Tokusoglu *et al*. 2008). Additionally, Garg *et al*. (1988) found no differences in vitamin E concentrations in breast milk from well- and undernourished Indian women. However, a study of breastfeeding mothers from Bangladeshi revealed that individuals with extremely low socio-economic status were characterised by very low breast milk levels of vitamin E, which is insufficient to cover requirements of their infants (Barua *et al*. 1997). Also, Olafsdottir *et al*. (2001) suggested that potential relationship exists between the contents of vitamin E in maternal diet and human milk. This relationship was previously confirmed by a single case report, which has documented that high maternal intake of vitamin E (approximately 27 mg day^{-1}) was reflected by elevated level of this vitamin in human milk (Anderson & Pittard 1985).

This study revealed that the average content of vitamin E in the infant formulas intended for children under 6 months of age amounts to 4.34 mg (including 3.94 mg of alpha-tocopherol) per 1 L of ready-to-feed solution. This concentration did not differ significantly from that of human milk from the 14th day of lactation and was higher than the breast milk concentrations determined on the 30th and 90th day of lactation. In contrast, mean concentrations of gamma-tocopherols in analysed infant formulas were approximately 5- to 18-fold higher than in human milk. This disproportion in gamma-tocopherol concentration results from the technological aspects of infant formula manufacturing. During processing, components of the formula are exposed to high temperatures at aerobic conditions. These conditions promote oxidation of polyunsaturated fatty acids included in formulas, and gamma-tocopherols are added to prevent this oxidative degradation.

Noticeably, none of infant formulas analysed in this study contained a quantity of alpha-tocopherol similar to that found in colostrum. Consequently, one may argue whether these formulas really cover nutritional needs of neonates during their first days of life. Therefore, we postulate that neonates should be breastfed at least during the initial 2–3 days of life, and vitamin E supplementation should be implemented in cases when formula feeding is the only option.

Conclusions

Depending on the stage of lactation, human breast milk contained 2.07–12.13 mg L^{-1} of alpha-tocopherol (from 35.24 to 5.51 mg per 100 g of total fat) and 0.22–0.60 mg L^{-1} (0.59–2.0 mg per 100 g total fat) of gamma-tocopherol. The content of alpha-tocopherol in human milk decreases with the time of lactation. While the content of gamma-tocopherol in human milk is stable to 14th day, next it decreases until the 30th day of lactation and remains unchanged afterwards. The content of alpha-tocopherol in infant formula is similar to the content in transitional and mature milk, while the content of gamma-tocopherol in infant formula is at least seven times higher than that in human milk. There was no significant correla-

tion between the dietary intake of vitamin E and its breast milk concentrations, also in women who declared vitamin supplementation. Compared with human colostrum, infant formulas were characterised by significantly lower concentrations of alpha-tocopherol and vitamin E. This finding suggests the need of additional vitamin E supplementation of bottle-fed infants during the initial 2–3 days of life.

Acknowledgements

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

Source of funding

This research was funded by Gdańsk University of Technology and Medical University of Gdańsk.

Conflicts of interest

The authors declare that they have no conflicts of interest.

Contributions

DM-Ż analysed and interpreted the data and wrote the initial draft of the manuscript, and provided statistical guidance in data analyses. AS-S and MZ assisted in the interpretation of results. All co-authors participated in manuscript preparation and critically reviewed all sections of the text for important intellectual comment.

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