

Maternal Supplementation With Natural or Synthetic Vitamin E and Its Levels in Human Colostrum

**Heleni A. Clemente, [†]Heryka M.M. Ramalho, *Mayara S.R. Lima,
*Evellyn C. Grilo, and *Roberto Dimenstein*

ABSTRACT

Objectives: Newborns are considered a high-risk group for vitamin E deficiency. Breast milk is a source of alpha-tocopherol (α -TOH), a form of vitamin E that prevents deficiency. The present study aimed to assess whether supplementation with a natural or synthetic form of α -TOH, in addition to maternal sources of vitamin E, would increase the concentration of α -TOH in colostrum.

Methods: A total of 109 healthy lactating women were recruited from a Brazilian public maternity clinic and randomized into 3 groups: control without supplementation ($n = 36$), natural α -TOH supplementation ($n = 40$), and synthetic α -TOH supplementation ($n = 33$). Blood and colostrum samples were collected before and after supplementation to check the nutritional status of these women by high-performance liquid chromatography. The Kruskal-Wallis test was applied for independent samples, and Tukey test was used for 2-way analysis of the averages of the groups. The baseline nutritional status of vitamin E of all of the lactating women enrolled in the trial was considered adequate.

Results: Women who received supplementation had higher concentrations of α -TOH in colostrum than the control group, with 57% and 39% increases in women supplemented with the natural and synthetic forms of α -TOH, respectively.

Conclusions: Supplementation with both forms of α -TOH increased vitamin E concentrations in colostrum; however, the natural form was more efficient in increasing the levels.

Key Words: all-rac- α -tocopherol, maternal supplementation, *RRR*- α -tocopherol, vitamin E

(*JPGN* 2015;60: 533–537)

Vitamin E is naturally found in various foods and has 8 stereoisomers, each comprising a 2-methyl-6-chromanolic ring; the isomers can be differentiated based on the methylation status of the α , β , γ , δ tocopherols and a phytyl side chain (1,2).

Received March 27, 2014; accepted November 13, 2014.

From the *Department of Biochemistry, Federal University of Rio Grande do Norte, and the [†]Department of Biotechnology, Potiguar University, Natal, Rio Grande do Norte, Brazil.

Address correspondence and reprint requests to Roberto Dimenstein, PhD, UFRN, Biosciences Center, Department of Biochemistry, Laboratory of Biochemistry of Food and Nutrition, Federal University of Rio Grande do Norte—UFRN, Avenue Senador Salgado Filho, no. 3000, Bairro, Lagoa Nova, Natal, CEP 59072-970, Rio Grande do Norte, Brazil (e-mail: rdimension@gmail.com).

This study was supported by funding from Conselho Nacional de Desenvolvimento Científico e Tecnológico (CNPq).

The authors report no conflicts of interest.

Copyright © 2015 by European Society for Pediatric Gastroenterology, Hepatology, and Nutrition and North American Society for Pediatric Gastroenterology, Hepatology, and Nutrition

DOI: 10.1097/MPG.0000000000000635

Commercially, vitamin E is available both as natural *RRR*- α -tocopherol and as the synthetic form all-rac- α -tocopherol. Synthetic vitamin E is generated from the reaction of trimethyl hydroquinone with synthetic isophytol, resulting in a mixture of 8 optical isomers, half with chiral centers in the 2*R* form and the other half in 2*S* (3–6).

The most abundant form of vitamin E found in human tissues is alpha-tocopherol (α -TOH), its most common form being *RRR*- α -TOH in healthy adults. This is attributable to the presence of methyl groups in a 2*R* configuration in the chromanol ring, which results in additional hydrophobic interactions between the tocopherol and α -TOH transfer protein (α -TTP) (7). This occurs in the liver, where α -TTP has a higher affinity for *RRR*- α -TOH stereoisomers, followed by the 2*R*-*RSR*-, *RRS*-, and *RSS*- α -TOH forms. 2*S* forms have not been found in plasma or tissues, and no differences between the activity of natural and synthetic forms have been demonstrated (8–11).

Newborns are considered a high-risk group for vitamin E deficiency and are recommended an intake of 4 mg/day (4). Insufficient consumption of vitamin E at this phase of life can compromise the immune system and affect lung development (12). Premature newborns are more susceptible to vitamin E deficiency, and low levels of α -TOH in this group may lead to complications such as thrombocytosis, hemolytic anemia, retrolental fibroplasia, and intraventricular hemorrhage (13).

The main source of α -TOH for newborns is breast milk. α -TOH prevents possible damage from oxidative stress generated by the transition from a prenatal intrauterine environment relatively poor in oxygen to a postnatal extrauterine environment considerably richer in oxygen (14,15).

Studies have shown that serum levels of α -TOH increase significantly in neonates fed colostrum (16). Maternal supplementation with α -TOH can be an efficient way to boost vitamin E levels in neonates. Most studies evaluating α -TOH supplementation have, however, been conducted in animals, and little is known about the effect of this supplementation in humans, or the influence of supplementation with natural or synthetic forms of α -TOH on the levels of α -TOH in human breast milk (16–19). Thus, the aim of this study was to evaluate levels of vitamin E in human colostrum when lactating mothers were given supplements of either natural or synthetic forms of α -TOH.

METHODS

Study Design

A randomized clinical study was performed at the Januário Cicco public maternity clinic located in Natal, RN, Brazil, from June 2012 to December 2013. To calculate the minimum sample size required, a power analysis using the software GPower (version 3; Shimadzu Corporation, Kyoto, Japan) was conducted. The parameters considered were effect size $d = 0.5$, probability of error $\alpha = 0.05$, test power $\beta = 0.95$, and a confidence interval of 95%.

Using these parameters, the required minimum sample size was 22 subjects per group.

The study was a randomized double-blind clinical trial with 3 treatment arms: a control group (CG) without treatment, a group receiving an acetate capsule with natural *RRR*- α -TOH (G_{NAT}), and a group receiving an acetate capsule with synthetic all-rac- α -TOH (G_{SINT}). All of the participants and researchers were blinded to the treatment groups. Unblinding was performed only after the statistical analysis was completed.

Enrolled participants included healthy women ages 18 to 40 years without signs of infections, syphilis, HIV, gastrointestinal tract and liver diseases, heart disease, diabetes, hypertension, or cancer. Only women who had full-term, healthy deliveries, no history of miscarriage, and no vitamin supplement use during pregnancy were included in the study.

Ethical Approval

The study was approved by the ethics in research committee—ethics of the Universidade Federal do Rio Grande do Norte, according to protocol 234/11—CEP/UFRN CAAE 0260.0.051.294-11. The clinical trial was registered under number U1111-1144-1649. All of the women formalized their participation by signing the informed consent form.

Sample Collection and Preparation

Blood and milk colostrum were collected from participants after an overnight fast, approximately 12 hours postpartum. The supplemented groups (G_{SINT} and G_{NAT}) received a capsule containing 400 IU of *RRR*- α -TOH or 400 IU of all-rac- α -TOH following the first milk collection. Milk was collected 24-h after supplementation.

For serum analysis, 5 mL of blood was collected by venipuncture into light-protected polypropylene tubes and transported to a laboratory under refrigeration. The blood was centrifuged, and 1 mL of serum was transferred to a new tube and stored at -18°C .

Colostrum was obtained by manual expression at the end of breast-feeding to prevent fluctuations in fat and tocopherol contents. The foremilk was discarded, and 2 mL of colostrum was collected before and after supplementation. Following collection, colostrum was incubated at 37°C and homogenized, after which a 500- μL aliquot was removed and stored at -18°C .

Sample Analysis

The technique used to extract serum tocopherol was adapted from Ortega et al (20). Briefly, 1 mL of 95% ethanol was added to an equal volume of serum, and after stirring, 2 mL of hexane was stirred into this mixture. The final mixture was centrifuged for 10 minutes at $500 \times g$. This step was repeated thrice, amounting to 3 extraction steps, and yielding a total extraction volume of 6 mL.

A similar method to extract tocopherol from colostrum was adapted from Romeu-Nadal et al (21). Equal volumes of colostrum and 95% ethanol were combined and subjected to mechanical agitation for 1 minute, following which extraction was performed in 2 steps with 2 mL of hexane, resulting in a total extraction of 4 mL from the hexane phase. Serum (2 mL) and colostrum (2 mL) extracts were dried and dissolved in 500 μL of absolute ethanol for further analysis.

The concentrations of α -TOH were determined by HPLC (LC-20AT; Shimadzu). Chromatographic separation was performed with a reverse-phase column (CLC-ODS; LC Shim-pack; Shimadzu): 4.6 mm \times 25 cm with UV detection ($\lambda_{\text{max}} = 292 \text{ nm}$). The elution was isocratic, with a mobile phase consisting of

methanol/ultrapure water (Milli-Q; Millipore Corporation, Darmstadt, Germany) 97:3 (v:v) at 1.0 mL/min flow rate). The chromatograms were integrated using the LC-solution program.

The α -TOH was identified and quantified in the samples by comparing the peak area obtained in the chromatogram with the area of the respective standard of α -TOH (Sigma, St Louis, MO) at a retention time of 12.5 minutes. The standard concentration was confirmed by the α -TOH-specific extinction coefficient (ϵ 1%, 1 cm = 75.8) in absolute ethanol (22). Participants who presented values lower than 499.6 $\mu\text{g/dL}$ (11.6 $\mu\text{mol/L}$) of α -TOH were considered α -TOH deficient (23).

Quantification of α -TOH in Capsules

Solutions of α -TOH acetate from capsules were prepared and compared with a standard solution of commercially available α -TOH acetate (Sigma). α -TOH in the capsules was identified and quantified by comparing the peak area obtained in the chromatogram (UV detection at $\lambda_{\text{max}} = 286 \text{ nm}$) of the test sample with the corresponding area obtained from a standard solution of α -TOH acetate, as well as by the α -TOH acetate-specific extinction coefficient (ϵ 1%, 1 cm = 40) in absolute ethanol (23). The concentration values of *RRR*- α -TOH and all-rac- α -TOH were accurate to 98% and 99% of the values reported on the capsules, respectively.

Validation of the Method

For the quantification of α -TOH, calibration curves were established as follows. A stock solution of the standard α -TOH (Sigma) was prepared, and 6 dilutions were made with concentrations ranging between 1.2 and 41.3 $\mu\text{g/mL}$. The method demonstrated excellent linearity, and the linearity coefficient of the curves always presented values higher than 0.9998. To determine the precision and accuracy, recovery and repeatability tests were performed and expressed as relative standard deviation (RSD), with 3 concentrations (4, 8, and 16 $\mu\text{g/mL}$) covering the range of variation within the linearity curve when α -TOH was always detected in serum and colostrum. The average recovery rate of α -TOH in serum was 100% and in colostrum, 98.80%. The results showed satisfactory reproducibility, with RSD values of 7.70% for serum and 4.22% for milk.

To determine the limit of detection (LD) and limits of quantification (LQ), colostrum and serum samples from known concentrations were diluted. A sample of each dilution was applied, and the LD of the observed peak was determined when there was no longer a distinction between noise and the analytical signal at a concentration of 0.48 $\mu\text{g/mL}$. The LQ was determined as the point at which the analytical signal was detected at a dilution equivalent to 0.97 $\mu\text{g/mL}$.

Statistical Analysis

SPSS version 19 (IBM SPSS Statistics, Armonk, NY) was used for statistical analysis. The concentration data for serum and colostrum are presented as means and standard errors. A preliminary comparison of the average α -TOH concentration before supplementation satisfied the basic normality assumptions of analysis of variance (Kolmogorov-Smirnov test), as well as homoscedasticity (Levene test). The response to treatment was calculated as a percentage increase in the concentration of α -TOH postsupplementation, and the treatment response data were converted into percentage variation (% var). To determine which capsule elicited the best response to the supplementation, the Kruskal-Wallis test for independent samples and Tukey test with 2-way analysis of group

averages were performed. The scatter graph and Pearson correlation coefficient were used to determine the correlation between α -TOH levels in serum and milk at 0 hour and those after supplementation.

RESULTS

Of 151 women initially recruited, 109 completed the study. The participants were randomized into 3 arms, namely, a CG receiving no treatment (CG, $n=36$), a treatment group receiving natural vitamin E supplementation (G_{NAT} , $n=40$), and a second treatment group receiving synthetic vitamin E supplementation (G_{SINT} , $n=33$). Sixty-five percent of the women were married, with an average age of 24 ± 6 years (Table 1). The level of education varied: 36% of women had completed primary education, and only 0.9% had attended university. In 81.4% of the women, the monthly family income ranged between US\$ 264.08 and 1056.34. Regarding obstetric variables, 53.1% women were multiparous, 58.1% had undergone cesarean section, and the average gestation period was 39.1 ± 1.4 weeks. The women in CG, G_{NAT} , and G_{SINT} had average serum α -TOH concentrations of 1016 ± 52 , 1236 ± 51 , and 1083 ± 61 $\mu\text{g/dL}$, respectively. There were no significant differences among the groups ($P=0.546$). Of the participants, 6.6% had low α -vitamin E levels, with α -TOH serum concentrations ranging from 499.6 to 697.7 $\mu\text{g/dL}$. None of the participants had α -TOH deficiency. The analysis of variance of α -TOH concentrations in colostrum before supplementation revealed no difference in the mean ($P=0.253$) or the variance ($P=0.767$) among the 3 treatment arms. A comparison of α -TOH concentrations (Fig. 1) in colostrum at 0 and 24 hours demonstrated that women who received no treatment (CG) had similar concentrations at both periods. Nevertheless, the average concentration of α -TOH in the milk collected at 24 hours from women supplemented with natural (G_{NAT}) and synthetic (G_{SINT}) α -TOH increased by 57.6% and 39.0%, respectively.

The average %var before and after supplementation (Fig. 2) were statistically different in the 2 experimental groups. The G_{NAT} group presented the highest percentage of variation as confirmed by a Kruskal–Wallis test for independent samples ($P=0.000$). The average concentration for each group was also compared using Tukey test and revealed that the average of CG differed from those of G_{NAT} ($P=0.000$) and G_{SINT} ($P=0.040$), and that the average of G_{NAT} differed from that of G_{SINT} ($P=0.040$). When comparing the %var of CG with that of the supplemented groups, the variation after supplementation with the natural capsule was 101% and 51.6% higher than CG and G_{SINT} , respectively, when compared with the same group without supplementation.

TABLE 1. Characteristics of the pregnant women participating in the study according to maternal and obstetric characteristics

Characteristics	CG (36)	G_{NAT} (40)	G_{SINT} (33)	Total (n = 109)
Age, y	$23.4 \pm 5.0^*$	$23.6 \pm 5.7^*$	$24.5 \pm 6.1^*$	$24.1 \pm 5.6^*$
Gestational age, wk	$39.2 \pm 1.2^*$	$38.9 \pm 1.6^*$	$38.9 \pm 1.6^*$	$39.1 \pm 1.4^*$
No. children	$1.9 \pm 1.3^*$	$1.8 \pm 1.2^*$	$2.1 \pm 0.85^*$	$2.0 \pm 1.4^*$
Primiparous, n (%)	20 (55.6)	23 (57.5)	18 (54.5)	45 (46.8)
Multiparous, n (%)	16 (44.4)	17 (42.5)	15 (45.5)	51 (53.1)
Type of delivery, n (%)				
Natural	15 (41.6)	18 (45)	13 (39.3)	44 (41.9)
Cesarean	21 (58.3)	22 (55)	20 (60.6)	61 (58.1)

Maternidade Escola Januário Cicco (Natal-RN) from June 2012 to December 2013. CG = control group; G_{NAT} = group receiving an acetate capsule with natural RRR - α -TOH; G_{SINT} = group receiving an acetate capsule with synthetic all- α -TOH; α -TOH = alpha-tocopherol.

* Mean \pm standard deviation.

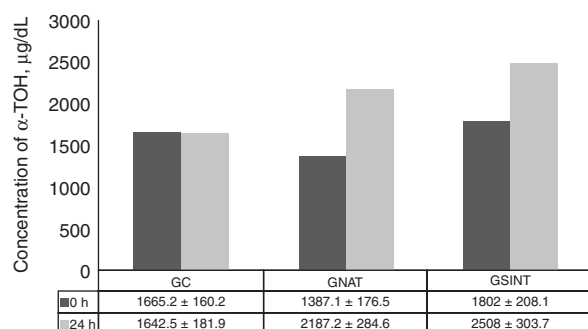


FIGURE 1. Alpha-tocopherol concentration in colostrum at 0 and 24 hours in the 3 experimental groups.

The means and %var in response to supplementation showed a significant difference between the G_{NAT} and G_{SINT} groups ($P=0.04$). The women receiving the natural vitamin E supplement had on average a 49.6% higher concentration in the milk of α -TOH than those receiving the synthetic capsule, indicating that the natural form results in a greater increase in α -TOH in colostrum.

Concentrations of α -TOH in serum were compared with those of colostrum (0 hour) for all groups, and no correlation between these variables ($r=0.073$, $P=0.440$) was found. No correlations were observed in the α -TOH serum biochemical analysis between the G_{SINT} and G_{NAT} groups, and concentrations of α -TOH in colostrum after supplementation.

DISCUSSION

The inclusion criteria established in the study allowed us to compare maternal characteristics such as age, parity, weight, and type of delivery among the groups. Maternal serum α -TOH levels less than 499.6 $\mu\text{g/dL}$ suggest vitamin E deficiency; values between

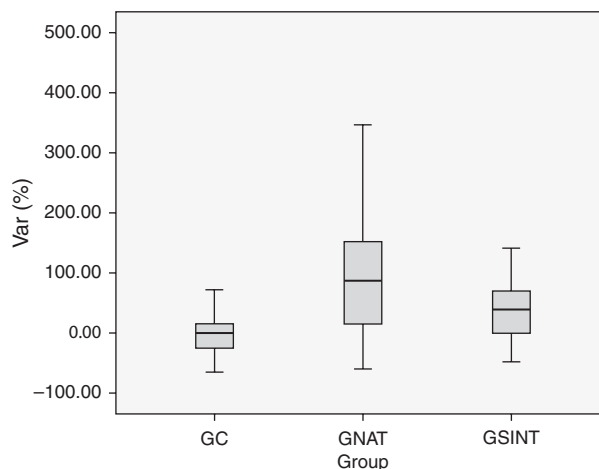


FIGURE 2. Comparison of mean percentage variation (%var). For comparison of the groups for 24 hours, an exploratory analysis was performed by observing the graph, assuming no difference among the means of groups, and that group G_{NAT} had a higher percentage of variation than the other groups. *Significantly different between groups CG and G_{NAT} ($P=0.000$). **Significantly different between groups GC and G_{SINT} ($P=0.000$). ***Significantly different between groups G_{NAT} and G_{SINT} ($P=0.04$). CG = control group; G_{NAT} = group receiving an acetate capsule with natural RRR - α -TOH; G_{SINT} = group receiving an acetate capsule with synthetic all- α -TOH.

499.6 and 697.7 $\mu\text{g/dL}$ indicate some risk of deficiency, while values more than 697.7 $\mu\text{g/dL}$ are considered healthy (23). The mean concentrations of α -TOH in the serum of the studied groups were as follows: CG (1016 ± 52 $\mu\text{g/dL}$), G_{NAT} (1236 ± 51 $\mu\text{g/dL}$), and G_{SINT} (1083 ± 61 $\mu\text{g/dL}$). These values are acceptable, based on reference values for serum α -TOH concentrations. Similar concentrations were found in other studies (24–26).

When individual breastfeeding women were assessed, we found that 6.6% had a low nutritional status for α -TOH. This value is lower than the 12% previously reported by Lira et al, which can be explained by the participation of adolescents in their study.

The concentration of α -TOH in colostrum at 0 hour is within the expected range when compared with studies conducted in Brazil: Dimenstein et al (24) reported 1363.2 ± 727.9 $\mu\text{g/dL}$; Garcia et al (27), 1206 ± 858.5 $\mu\text{g/dL}$; and Campos (28), 1313.9 ± 798.7 $\mu\text{g/dL}$. These values are compatible with the present findings. Studies performed in the United States and Turkey also showed similar concentrations, that is, 1335.2 ± 198.1 and 1326.6 ± 68.9 $\mu\text{g/dL}$, respectively (29,30).

Other studies have reported varying levels of α -TOH in colostrum. Schweigert et al (31) and Quiles et al (32) reported colostrum α -TOH values of 2200.9 and 2454.9 $\mu\text{g/dL}$, which are higher than the ones reported in this study, whereas Ahmed et al (33) reported values lower than 919.1 $\mu\text{g/dL}$. The higher concentrations found in studies conducted by Schweigert et al (31) and Quiles et al (32) may be owing to the Mediterranean diet being rich in almonds, olives, nuts, and fish, which are rich sources of α -TOH. The below-average colostrum values found in the study by Ahmed et al (33) may be attributed to the low socioeconomic status of the population, which may possess a very low diversified supply of the vitamin (31–33).

In the present study, women treated with both the natural and the synthetic forms of α -TOH demonstrated an increase in concentration of α -TOH in colostrum. To date, similar studies with postpartum maternal supplementation with α -TOH have not been performed. Comparisons with studies in other mammals such as mares, pigs, rats, and cows, however, showed a significant increase after supplementation (16–18,34). Results demonstrating an increased efficacy of the natural form of α -TOH to improve maternal concentrations of vitamin E over the synthetic form have been reported in animal studies, wherein investigators supplemented cows with natural and synthetic forms and showed results similar to those found in humans (17,35,36). Cows fed diets supplemented with all-rac- α -TOH preferentially use the 2R- α -TOH stereoisomer, and such discrimination may occur in humans.

Slots et al (16) observed differing proportions of 2R-stereoisomers of α -TOH in cow milk. They reported that RSS, RRS, and RSR- α -TOH accounted for 2.4%, 2.9%, and 2.1%, respectively, in milk, which is considered low. The natural form represents 92.6% of total α -TOH in milk. None of the 2S stereoisomers was detected in the milk before or after supplementation with the synthetic form (16). The 2S-stereoisomers are not retained in the plasma or in tissue, and the relative activity of the synthetic form compared with the natural form is 50% (4). Similar values were observed in this study, as the natural capsule increased α -TOH concentrations in milk 49.6% more than the synthetic capsule. This suggests that there may be a transport mechanism of α -TOH to the mammary gland in humans, who depend on the activity of α -TTP. The stereoisomer selection mechanism works continuously from the liver into the plasma after birth, and according to Lauridsen et al (37), the presence of an α -TTP-dependent mechanism in the mammary gland cannot be excluded because the expression of α -TTP can facilitate α -TOH secretion into milk (37). Another alternative mechanism for transporting α -TOH has been suggested, wherein α -TOH can reach the breast tissue by means of low-density

lipoprotein receptors and/or be transported by SR-B1 receptors, possibly involving intracellular membrane receptors for α -TTP in the mammary epithelium (38,39).

The lack of correlation between α -TOH in maternal serum with colostrum ($r = 0.073$, $P = 0.440$) has been reported (24,27,30,34,40). Dimenstein et al (24) proposed that the absence of correlation between these variables preclude the existence of passive transfer mechanisms during the passage of vitamin E from the mammary gland to the milk, thereby indicating the existence of a transport mechanism in the breast that is independent of plasma concentration. This could be valid because in late pregnancy and at the beginning of lactation, there is a gradual decrease in serum levels of α -TOH, which may explain the considerable increase in α -TOH in colostrum (37,41,42). This phenomenon may be because of an increased activity of low-density lipoprotein receptors by the mammary gland around parturition. The concentration of α -TOH has been found to depend on the plasma lipid fraction, that is, the ability of plasma lipoproteins to incorporate α -TOH, which ultimately results in the absorption of α -TOH by the mammary gland (2). Therefore, α -TTP would have an important role in the absorption of vitamin E to the mammary gland because its function is to transfer α -TOH to the lipoproteins.

Supplementation with natural and synthetic forms promoted α -TOH increase in colostrum when compared with the group without supplementation. Interestingly, the natural form of α -TOH was more efficient in increasing the preexisting levels than the synthetic form.

REFERENCES

1. Clarker MW, Burnett JR, Croft KD. Vitamin E in human health and disease. *Crit Rev Clin Lab Sci* 2008;45:417–50.
2. Debier C, Larondelle Y. Vitamins A and E: metabolism, roles, and transfer to offspring. *Br J Nutr* 2005;93:153–74.
3. Eitenmiller RR, Ye L, Landen Junior WO. *Vitamins Analysis for the Health and Food Sciences*. 2nd ed. Boca Raton, FL: CRC Press; 2008.
4. Institute of Medicine. *Dietary Reference Intakes for Vitamin C, Vitamin E, Selenium, and Carotenoids*. Washington, DC: National Academy Press; 2000.
5. Acuff RV, Thedford SS, Hidioglou NN, et al. Relative bioavailability of RRR- and all rac-alpha-tocopheryl acetate in humans: Studies using deuterated compounds. *Am J Clin Nutr* 1994;50:397–402.
6. Jensen SK, Lauridsen C. Alpha-tocopherol stereoisomers. *Vitam Horm* 2007;76:281–308.
7. Meier R, Tomizaki T, Brieze CS, et al. The molecular basis of vitamin E retention: structure of human alpha-tocopherol transfer protein. *J Mol Biol* 2003;331:725–34.
8. Aggarwal BB, Sundaran C, Prasad S, et al. Tocotrienols, the vitamin E of the 21st century: its potential against cancer and other chronic diseases. *Biochem Pharmacol* 2010;80:1613–31.
9. Gagné A, Wei SQ, Fraser WD, et al. Absorption, transport, and bioavailability of vitamin E and its role in pregnant women. *J Obstet Gynaecol* 2009;31:210–7.
10. Traber MG. Vitamin E. In: Erdman JW, Macdonald IA, Zeisel SH, eds. *Present Knowledge in Nutrition*. Washington, DC: ILSI Press; 2007: 211–9.
11. Kiyose C, Saito H, Kaneko K, et al. Alpha-tocopherol affects the urinary and biliary excretion of 2,7,8-trimethyl-2 (2'-carboxyethyl)-6-hydroxyxanthroman, γ -tocopherol metabolite in rats. *Lipids* 2001;36:467–72.
12. Antonakou A, Chiou A, Andrikopoulos NK, et al. Breast milk tocopherol content during the first six months in exclusively breastfeeding Greek women. *Eur J Nutr* 2011;50:195–202.
13. Brion LP, Bell EF, Raghuvier TS. Vitamin E supplementation for prevention of morbidity and mortality in preterm infants. *Cochrane Database Syst Rev* 2003;4:CD003665.
14. Robles R, Palomino N, Robles A. Oxidative stress in the neonate. *Early Hum Dev* 2001;65:75–81.
15. Gomes M, Saunders C, Accioly E. Vitamin A role preventing oxidative stress in newborns. *Rev Bras Saude Matern Infant* 2005;5:275–82.

16. Slots T, Skibsted LH, Nielsen JH. The difference in transfer of all-rac- α -tocopherol stereoisomers to milk from cows and the effect on its oxidative stability. *Int Dairy* 2007;17:737–45.
17. Lindqvist H, Nadeau E, Waller KP, et al. Effects of RRR- α -tocopheryl acetate supplementation during the transition period on vitamin status in blood and milk of organic dairy cows during lactation. *Livestock Sci* 2011;142:155–63.
18. Bondo T, Jensen SK. Administration of RRR- α -tocopherol to pregnant mares stimulates maternal IgG and IgM production in colostrum and enhances vitamin E and IgM status in foals. *J Anim Physiol Anim Nutr* 2008;95:214–22.
19. Nucci LC, Schmidt LI, Duncana BB. Nutritional status of pregnant women: prevalence and associated pregnancy outcomes. *Rev Salud Publ* 2001;35:502–7.
20. Ortega RM, López-Sobaler AM, Martínez RM, et al. Influence of smoking on vitamin E status during the third trimester of pregnancy and on breast-milk tocopherol concentrations in Spanish women. *Am J Clin Nutr* 1998;68:662–7.
21. Romeu-Nadal M, Morena-Pons S, Castellote AI, et al. Determination of γ - and α -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* 2006;114:132–7.
22. Nierenberg DW, Nann SL. A method for determining concentrations of retinol, tocopherol, and five carotenoids in human plasma and tissue samples. *Am J Clin Nutr* 1992;56:417–26.
23. Sauberlich HE, Dowdy RP, Skala JH. *Laboratory Tests for the Assessment of Nutritional Status*. Cleveland, OH: CRC Press; 1974:74–80.
24. Dimenstein R, Pires JF, Garcia LRS, et al. Concentração de alfa-tocoferol no soro e colostro materno de adolescentes e adultas. *Rev Bras Ginecol Obstetr* 2010;32:267–72.
25. Papas A, Stacewicz-Sapuntzakis M, Lagiou P, et al. Plasma retinol and tocopherol levels in relation to demographic, lifestyle and nutritional factors of plant origin in Greece. *Br J Nutr* 2003;89:83–7.
26. Rodríguez GP, Alonso DP, Sintés GS, et al. Vitaminas antioxidantes en un grupo de embarazadas y recién nacidos durante un año de estudio. *Revista Cubana Aliment Nutr* 2002;16:85–94.
27. Garcia LRS, Ribeiro KDS, Araújo KF, et al. Níveis de alfa-tocoferol no soro e leite maternos de puérperas atendidas em maternidade pública de Natal, Rio Grande do Norte. *Rev Bras Saúde Matern Infant* 2009;9:423–8.
28. Campos JM. *Perfil dos níveis de vitaminas A e E no leite de doadoras primíparas e múltiparas em bancos de leite humano* [Dissertation]. Recife, Brazil: Universidade Federal de Pernambuco; 2005.
29. Gossage CP, Deyhim M, Yamini S, et al. Carotenoid composition of human milk during the first month postpartum and the response to β -carotene supplementation. *Am J Clin Nutr* 2002;76:193–7.
30. Orhon FS, Ulukol B, Kahya D, et al. The influence of maternal smoking on maternal and newborn oxidant and antioxidant status. *Eur J Nutr* 2009;168:975–81.
31. Schweigert FJ, Bathe K, Chen F, et al. Effect of the stage of lactation in humans on carotenoid levels in milk, blood plasma, and plasma lipoprotein fractions. *Eur J Nutr* 2004;43:39–44.
32. Quiles JL, Ochoa JJ, Ramirez-Tortosa MC, et al. Coenzyme Q concentration and total antioxidant capacity of human milk at different stages of lactation in mothers of preterm and full-term infants. *Free Radic Res* 2006;40:199–206.
33. Ahmed L, Nasrul Islam SK, Mni K, et al. Antioxidant micronutrient profile (vitamin E, C, A, copper, zinc, iron) of colostrum: association with maternal characteristics. *J Trop Pediatr* 2004;50:357–8.
34. Martinez S, Herrera E, Barbas C. Uptake of alpha-tocopherol by the mammary gland but not by white adipose tissue is dependent on lipoprotein lipase activity around parturition and during lactation in the rat. *Metabolism* 2002;51:1444–51.
35. Horn MJ, Van Emon ML, Gunn PJ, et al. Effects of natural (RRR α -tocopherol acetate) or synthetic (all-rac α -tocopherol acetate) vitamin E supplementation on reproductive efficiency in beef cows. *J Anim Sci* 2010;88:3121–7.
36. Meglia GE, Jensen PK, Lauridsen C, et al. Alpha tocopherol concentration, and stereoisomer composition in plasma and milk from dairy cows fed natural or synthetic vitamin E around calving. *J Dairy Res* 2006;73:227–34.
37. Lauridsen C, Engel H, Jensen SK, et al. Lactating sows and suckling piglets preferentially incorporate RRR- over all-rac-a-tocopherol into milk, plasma, and tissues. *J Nutr* 2002;132:1258–64.
38. Mardones P, Rigotti A. Cellular mechanisms of vitamin E uptake: relevance. *J Nutr Biochem* 2004;15:252–60.
39. Azeredo VB, Trugo NMF. Retinol, carotenoids, and tocopherols in the milk of lactating adolescents and relationships with plasma concentrations. *Nutrition* 2008;24:133–9.
40. Lira LQ, Ribeiro PPC, Grilo EC, et al. Níveis de alfa-tocoferol no soro e colostro de lactantes e associação com variáveis maternas. *Rev Bras Ginecol Obstetr* 2012;34:362–8.
41. Debier C, Pomeroy PP, Baret PV, et al. Vitamin E status and the dynamics of its transfer between mother and pup during lactation in grey seals (*Halichoerus grypus*). *Can J Zool* 2002;80:727–37.
42. Gay S, Kronfeld DS, Grimsley-Cook JJ, et al. Retinol, beta-carotene and beta-tocopherol concentrations in mare and foal plasma and in colostrums. *J Equine Vet Sci* 2004;24:115–20.