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Coenzyme Q concentration and total antioxidant capacity of human milk at different stages of lactation in mothers of preterm and full-term infants

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Abstract

Coenzyme Q_{10}(CoQ_{10}) in human milk at different stages of maturity in mothers of preterm and full-term infants and its relation to the total antioxidant capacity of milk is described for the first time. Thirty healthy breastfeeding women provided colostrum, transition-milk and mature-milk samples. Coenzyme Q, α-, γ- and δ-tocopherol, fatty acids and the total antioxidant capacity of the milk were analyzed. Coenzyme Q_{10} was found at higher concentrations for colostrum (0.81 ± 0.06 vs. 0.50 ± 0.05 μmol/l) and transition milk (0.75 ± 0.06 vs. 0.45 ± 0.05 μmol/l) in the full-term vs. the preterm group (similar results were found for total antioxidant capacity). Concentrations of α- and γ-tocopherol were higher in the full-term group and decreased with time. In conclusion, CoQ_{10} is present in breast milk, with higher concentration in mothers of full-term infants. CoQ_{10} in breast milk decreases through lactation in mothers delivering full-term infants. Also, CoQ_{10}, α- and γ-tocopherol concentration in human milk directly correlates with the antioxidant capacity of the milk.

Keywords: *Tocopherol, ubiquinone, fatty acids, breast milk*

Introduction

The first food naturally ingested by newborn babies is breast milk. Breast milk from healthy women contains all the nutrients necessary for newborn infants and also contains a variety of growth and immune factors [1]. The composition of human milk may be influenced by different variables, such as genetic characteristics, dietary habits, nutritional and socio-economic state of the mother, duration of lactation and length of gestation [2,3]. Milk contains a plethora of antioxidant molecules that probably account for the vital antioxidant protection of the newborn at early stages of life. Buescher and McIlherhan [4] reported, for example, that colostrum manifests antioxidant properties, being capable of spontaneous reduction of cytochrome c, depletion of polymorphonuclear leukocyte-produced H_2O_2, etc. The complete list of active antioxidant components in human milk is not known, but α-tocopherol, cysteine, ascorbate or carotenoids have been found [1,5,6], as have antioxidant enzymes such as glutathione peroxidase, catalase and superoxide dismutase [7].

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Coenzyme Q_{10} (CoQ_{10}) or ubiquinone in human tissues comes from dietary intake and from endogenous biosynthesis. CoQ_{10} has a pivotal role as a redox link between flavoproteins and cytochromes in the mitochondrial respiratory chain [8] where it also displays important antioxidant properties under lipophilic conditions [9]. The CoQ_{10} content in food has been described [10,11] and the concentrations in different human tissues of this molecule are well documented [8]. However, there are no data on the CoQ_{10} concentration in human milk. Given that newborn infants undergo oxidative stress and that antioxidant content of human milk could partially account for newborn antioxidant defenses, it is of great interest to investigate the CoQ_{10} concentration of human milk.

Oxidative stress is critical at birth, both for the mediation of different physiologic processes involved towards the end of gestation and at delivery as well as for the change to the extra-uterine environment, with higher alveolar relative oxygen pressure [12–14]. Such changes prompt free-radical production, which must be buffered by the antioxidant system [15–16]. This oxidative stress appears to be exacerbated by the immaturity in enzymatic and non-enzymatic antioxidant mechanisms of premature infants [17,18]. Therefore, it is vital to establish whether the milk that the mother delivers to a preterm infant can balance possible deficiencies or whether, on the contrary, that milk contributes to differences and difficulties experienced as consequence of prematurity. As a marker of the susceptibility of milk to oxidative-stress-related lipid-peroxidation, it is necessary to test the fatty-acid profile, since it is well known that a greater number of double bonds in a fatty acid augments its oxidative potential and thus determines its antioxidant capacity [2]. Moreover, because some fatty acids are essential, their presence/absence needs to be investigated in breast milk [3].

The present study has a dual aim: on the one hand to describe, for the first time, the possible existence of CoQ_{10} in human milk and its concentration at different stages of maturity; and, on the other hand, to investigate possible differences in CoQ_{10} concentration between milk from mothers delivering preterm or full-term infants.

Subjects and methods

Subjects

Thirty healthy breastfeeding women with healthy single, preterm or full-term newborns were recruited shortly after delivery at the Department of Pediatrics (University of Granada, Granada, Spain). The women chosen did not smoke, reported no clinical pathology during gestation, and followed a regular diet without supplements containing antioxidant vitamins or coenzyme Q. The university Ethics Committee approved the study protocol, and a written informed consent was obtained from all the participating women after a full explanation of the study. The infants were exclusively breast fed during the duration of the study. Regular contact was maintained with all the women throughout the study period and assistance was provided for milk sampling.

Experimental groups

The subjects were divided into two groups of 15 individual each, depending on the birth. Birth time was 32 ± 1 weeks of gestation (range: 31–34) in mothers from the preterm group, and 39 ± 1 weeks of gestation (range: 38–41) for the full-term group. The subjects provided three different milk samples: colostrum (milk provided at day 3 after birth), transition milk (at day 8 after birth) and mature milk (at day 30 after birth).

Dietary data and sample collections

Dietary intake of the mothers was recorded for four consecutive days (including one week-end day) using a four-day diet record, filled out by a pediatrician in presence of the subject. These records were kept at the start and the end of the study. Since no differences were found between the two periods, only the data from first 4 days of the recording period (at the start of the study) are presented here. All mothers were given standard written instructions on how to collect the samples in the morning at the same hour of the day. These instructions included the need to perform the milk collection before they had the breakfast, in order to avoid alterations in milk collection as a consequence of a close food intake. They used electric Medela Lactina Select breast pumps (Medela, USA) and the same breast was pumped for all the three samples. Subjects emptied one breast, gently mixed the contents, and transferred 10 ml into a plastic tube. Samples were immediately placed in ice, taken to the hospital laboratory, aliquoted in 1 ml tubes, and frozen at -80°C until processing (not more than 15 min elapsed from sampling to freezing). Moreover, samples were at all times protected from light.

Analyses

Records of the intake of energy, carbohydrate, protein, fat (and fat distribution into saturated, monounsaturated and polyunsaturated), cholesterol, fiber, vitamins A, C and E, were analyzed with Food and Health software [19], developed at the Institute of Nutrition, University of Granada, using a previously published Spanish food-composition database [20]. Intake of Coenzyme Q was calculated by using previously

published data on the coenzyme Q content in foods [10,11].

Coenzyme Q was assayed by HPLC-EC, following Battino et al. [21]. The HPLC system consisted of a Beckman System Gold 125 pump (Beckman Instruments, Fullerton, CA), a Water 717 plus autosampler (Milford, MA, USA), and a stainless-steel column (15 cm long and 4.6 mm I.D.) packed with 3 μm ODS Supelcosil, an ESA Coulochem III electrochemical detector, a model 5020 guard cell and a model 5011 analytical cell. Chromatograms were integrated using the System Gold Beckman system. The mobile phase consisted of 20 mM lithium perchlorate, 10 mM perchloric acid, 20% ethanol, 80% methanol at 1 ml/min; electrode 1 was set at −0.5 V, electrode 2 was set at +0.35 V. Briefly, 50 μl of the sample were precipitated with 150 μl of 1-propanol and vortexed for 60 s. After centrifugation at 11,200 *g* for 10 min in a bench-top centrifuge for Eppendorf vials, 30 μl of supernatant was injected into the HPLC. Tocopherols (α, γ and δ tocopherols) were also analyzed by HPLC-EC, using the same system and column as for coenzyme Q analysis. The mobile phase consisted of lithium 20 mM perchlorate, 10 mM perchloric acid, 10% purified water, 90% acetonitrile at 1.5 ml/min; electrode 1 was set at −0.5 V and electrode 2 was set at +0.40 V. Next, 100 μl of human milk was precipitated with 300 μl of 1-propanol, vortexed, and centrifuged as for coenzyme Q. Then, 50 μl of supernatant was injected into the HPLC for retinol detection and 15 μl for tocopherol detection. CoQ_{10}, α-, γ- and δ-tocopherol were identified by predetermining the retention times of individual standards. Since the method for analyzing coenzyme Q has not been previously used in human milk, we studied the recovery and precision of the method. The recovery analysis was based on a comparison between the peaks obtained by spiking samples with increasing concentrations of oxidized CoQ_9 and the corresponding peaks of the standard. Recovery was of 96.3%. Intra-assay precision showed a CV% of 4.7. Day-to-day precision showed a CV% of 2.26%.

Total antioxidant activity of human milk was measured by the 2,2′-azinobis(3-ethylbenzothiazoline-6-sulfonic acid) radical cation ($ABTS^{\cdot+}$) assay [22] adapted to a flow injection (FI) system as indicated by Bompadre et al. [23]. The method is based on the ability of antioxidant molecules to quench the long-lived $ABTS^{\cdot+}$, a blue–green chromophore with characteristic absorption at 734 nm, in comparison to that of Trolox, a water-soluble vitamin E analogue. The addition of antioxidants to the preformed radical cation reduces it to ABTS, determining a decolorization. The FI system consisted of a Beckman System Gold Programmable Solvent Module 126 pump, a rheodyne injection valve equipped with a 5 μl loop, a reaction coil (0.8 mm i.d. × 1.58 mm o.d. × 120 cm) (Supelco) and a Beckman System Gold Programmable Detector Module 166. The flow of the $ABTS^{\cdot+}$ solution was 1.2 ml/min at room temperature. Briefly, Trolox standard solutions (0.5–5 mM) were prepared in ethanol and the $ABTS^{\cdot+}$ working solution was prepared from an $ABTS^{\cdot+}$ stock solution obtained by reacting a 7 mM aqueous solution of ABTS with potassium persulfate 2.45 mM. The direct injection of standards and human-milk samples determined a decolorization peak having an area proportional to the loss of absorbance of the $ABTS^{\cdot+}$ working solution. The quantification of total antioxidant activity of human milk was made by comparing the area of the decolorization peak to the dose-response curve obtained with Trolox standards. Results are expressed as Trolox equivalent antioxidant-activity values, and TEAC is defined as mmol of Trolox per liter of human milk.

The fatty-acid profile of human milk was determined by gas–liquid chromatography as described by Lepage and Roy [24] in a Hewlett Packard HP 5890 Series II chromatograph (Hewlett Packard, Palo Alto, CA, USA) using a capillary column 60 m long, 32 mm i.d., and 20 mm thickness impregnated with Sp™ 2330 FS (Supelco Inc. Bellefonte, Palo Alto, CA, USA). Human milk (200 μl) was precisely weighed in glass tubes and dissolved in 2 ml of methanol/benzene (4:1, v/v); 100 μl of the fatty acid 13:0 (0.4 mg/dl) and 9μM of BHT were added to the samples as internal standard and antioxidant, respectively. To perform the quantitative determination of the different fatty acids the following formula was applied: $C = ((A_x \cdot RF_x)/(A_{is} \cdot RF_{is}))\ C_{is}$; where C is the concentration of a particular fatty acid, A_x is the area obtained in the chromatogram for this particular fatty acid, and RF_x its response factor; A_{is} is the area for the internal standard in the sample and RF_{is} its response factor; C_{is} is the concentration of the internal standard. The carrier gas was nitrogen, and individual fatty acids were identified by comparison with a mixture of fatty acid methyl esters (Sigma, St Louis, MO, USA). Increasing concentrations of fatty acid methyl esters mixture were used to correct the linearity of response for each individual fatty acid.

Statistical methods

Data for population characteristics and nutrient intakes were expressed as mean ± SD ($n = 15$). Data for milk analyses were expressed as mean ± SEM ($n = 15$). For each group, means within time were compared by using contrasts adjusted for a repeated-measures ANOVA and a Bonferroni correction. Means within groups for each type of milk were compared by using Student's *t*-test. Statistical significance was set at $P < 0.05$. Data were analyzed with SPSS, version 12 (SPSS Inc., Chicago, IL, USA).

Results

All 30 women who began the study completed it. Subjects were middle-class and well educated. There were no significant differences in body-mass index, age or parity between the two groups of women (Table I). Diet records were complete for the 30 women and nutrient intake was calculated as the average for the four days recorded (Table II). No significant differences were found in the mean intake of energy, carbohydrate, protein, fat (including different fatty acids classes and cholesterol), fiber, vitamins A, C, E, or coenzyme Q.

The results of HPLC analysis showed for the first time that human milk contains coenzyme Q_{10} (Figure 1). These concentrations were significantly higher for colostrums (mean; minimum; maximum; range of 0.81; 0.50; 1.38; 0.87 vs. 0.50; 0.26; 0.76; 0.50) and transition milk (mean; minimum; maximum; range of 0.75; 0.55; 1.20; 0.65 vs. 0.45; 0.28; 0.84; 0.56) in the full-term group vs. preterm group but similar for mature milk (mean; minimum; maximum; range of 0.54; 0.33; 0.87; 0.54 vs. 0.42; 0.25; 0.69; 0.44) in full-term vs. preterm group, respectively. No differences between colostrum, transition milk and mature milk were found in the preterm group concerning CoQ_{10}. The concentration of α- and γ-tocopherol was significantly higher for the three studied periods in the full-term group (Figure 2(a) and (b)), with decreasing concentrations in both groups with time. No differences were found between groups or with time in δ-tocopherol concentration (Figure 2(c)). The total antioxidant capacity of milk (Figure 3) registered significantly higher values for colostrum compared with mature milk in both groups and for transition milk compared with mature milk for the full-term group. However, the full-term group reached significantly higher antioxidant capacity for colostrum and transition milk than preterm group. A positive correlation between CoQ_{10} and total antioxidant capacity was found (0.338; $P < 0.05$). Also α-tocopherol (0.499; $P < 0.001$) and γ-tocopherol (0.325; $P < 0.05$) positively correlated with total antioxidant capacity.

Table I. Baseline characteristics of lactating women⋆.

	Preterm group ($n = 15$)	Full-term group ($n = 15$)
Delivery time (wk)	32 ± 1	39 ± 1†
BMI (kg/m^2)	26.1 ± 0.4	25.7 ± 0.5
Age (yr)	30.1 ± 6.3	29.8 ± 4.9
Parity	1.8 ± 1	1.9 ± 0.7

⋆Mean ± standard deviation. †Preterm vs. Full-term group ($P < 0.05$). Except for delivery time (higher in full-term group), there were no differences between experimental groups.

Table II. Estimated daily intakes in lactating women⋆.

	Preterm group ($n = 15$)	Full-term group ($n = 15$)
Energy (kJ)	11344 ± 1228	10224 ± 1232
Energy (kcal)	2714 ± 294	2446 ± 334
Carbohydrate (g)	395.7 ± 56.5	285.2 ± 80.4
Protein (g)	114.6 ± 13.1	103.4 ± 15.4
Fat (g)	113.9 ± 18.1	103.8 ± 22.2
Saturated (g)	27.5 ± 6.9	25.1 ± 5.6
Monounsaturated (g)	52.9 ± 8.8	48.2 ± 10.2
Polyunsaturated (g)	15.2 ± 2.1	13.9 ± 2.5
Cholesterol (mg)	422.6 ± 124.2	399.9 ± 138.1
Fiber (g)	19.3 ± 6.2	19.9 ± 5.4
Vitamin A (RE)	1012.1 ± 415.7	996.6 ± 395.5
Vitamin C (mg)	154.9 ± 81.4	148.3 ± 91.9
Vitamin E (mg)	6.1 ± 2.4	6.1 ± 0.9
Coenzyme Q (mg)	3.2 ± 1.1	2.7 ± 1.1

⋆Mean ± standard deviation. There were no differences between groups. RE, retinol equivalents.

Quantitative fatty-acid analysis (Figure 4) showed no differences for any type of milk between preterm and full-term group. However, differences were found concerning milk maturation. Thus, mature milk showed significantly higher concentrations of saturated, monounsaturated and polyunsaturated fatty acids when compared with transition milk and colostrum. A negative correlation was found between CoQ_{10} v. total fatty acids (-0.295; $P < 0.05$), total saturated fatty acids (-0.308; $P < 0.05$) and total monounsaturated fatty acids (-0.292; $P < 0.001$). When fatty acids were studied as qualitative (as a percentage of total fatty acids) profile (Figure 5), no significant differences were found concerning groups nor milk types.

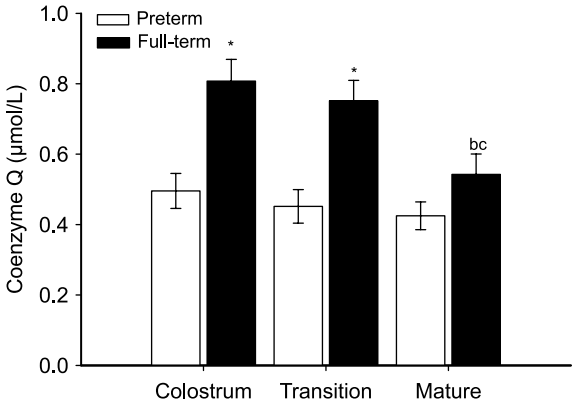

Figure 1. Mean (±SEM) breast-milk CoQ_{10} in mothers of preterm and full-term infants at different stages of lactation (colostrum, transition milk and mature milk). ⋆Differences between the preterm and full-term group for each stage of lactation ($P < 0.05$). Differences ($P < 0.05$) related to the lactation stage of each group: (a) transition milk vs. colostrum; (b) mature milk vs. colostrum; (c) mature milk vs. transition milk.

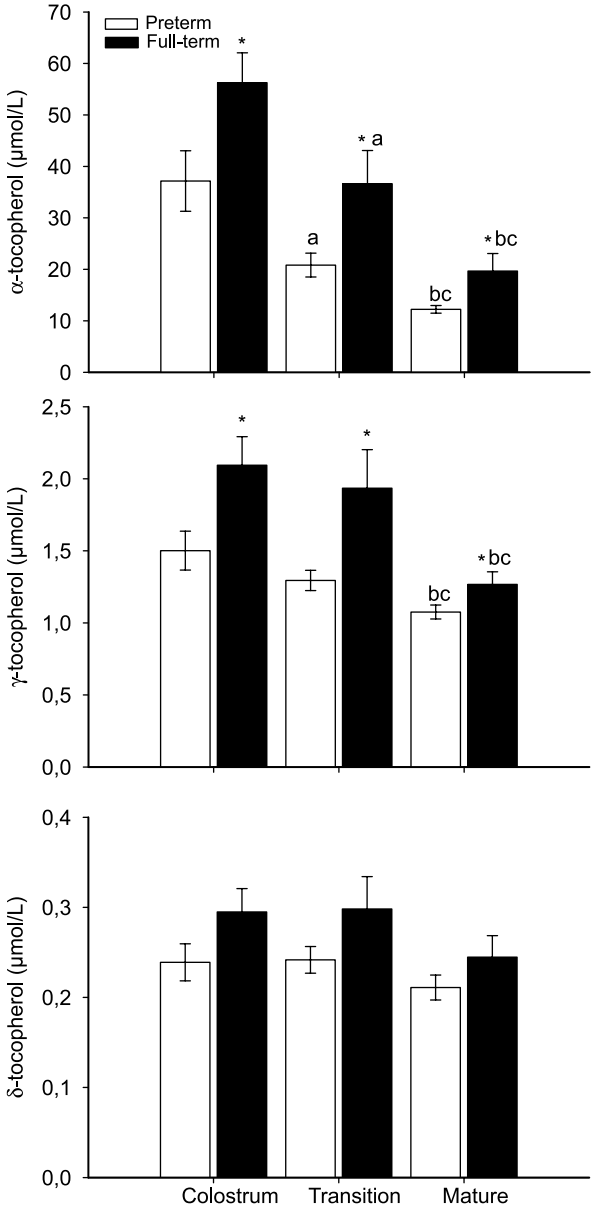

Figure 2. Mean (± SEM) breast milk α-, γ- and δ-tocopherol in mothers of preterm and full-term infants at different lactation stages (colostrum, transition milk and mature milk). ⋆ Differences between the preterm and full-term group for each lactation stage ($P < 0.05$). Differences ($P < 0.05$) related to the lactation stage of each group: (a) transition milk vs. colostrum; (b) mature milk vs. colostrum; (c) mature milk vs. transition milk.

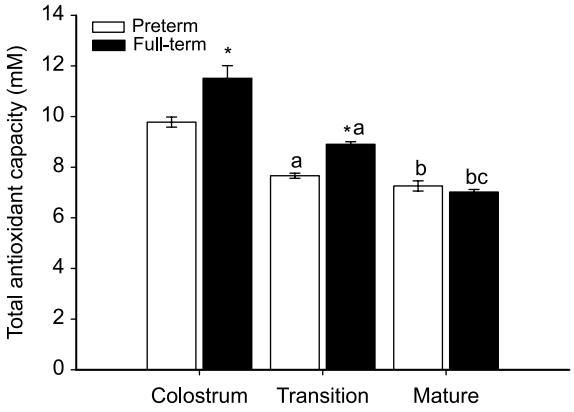

Figure 3. Mean (± SEM) breast milk total antioxidant capacity in mothers of preterm and full-term infants at different lactation stages (colostrum, transition milk and mature milk). ⋆ Differences between the preterm and full-term group for each stage of lactation ($P < 0.05$). Differences ($P < 0.05$) related to stage of lactation for each group: (a) transition milk vs. colostrum; (b) mature milk vs. colostrum; (c) mature milk vs. transition milk.

Discussion

The aim of the present study was to investigate the presence of CoQ_{10} in human milk and possible differences concerning that molecule between milk from mothers of preterm and full-term infants. Additionally, as far as we know, this is the first longitudinal study to report milk CoQ_{10} concentrations and to estimate CoQ_{10} intake during the first month of lactation. Data available in the literature refer only to cow milk, with concentrations ranging from 6 to 11.6 μmol/l [10,25]. In the present work, concentrations in humans were found to be around 0.4 μmol/l in preterm group and 0.7 μmol/l for the full-term group. These differences do not appear to come from variability in the CoQ_{10} intake concentration (CoQ_{10} may come from dietary sources or from endogenous biosynthesis), since both groups of mothers reported similar intake (3.2 ± 1.1 mg/day and 2.7 ± 1.1 for mothers of preterm and full-term infants, respectively). These concentrations are similar to those reported by Mattila and Kumpulainen [11] for Finnish women around 3.8 mg/day. The rest of the average dietary intake nutrients were comparable to those published for similar groups elsewhere [1].

The data indicate that in the full-term group, CoQ_{10} decreases with milk maturity. Coenzyme Q_{10} and vitamin E are lipophilic in nature, and decreasing lipophilic vitamins with increasing lactation time have been previously reported [26]. These authors have described α-tocopherol concentrations of 51.1 μmol/l in colostrum and 13.2 μmol/L in mature milk from full-term mothers. Also in the present work, similar trends were found in the preterm and full-term groups for α- and γ-tocopherol, but not for δ-tocopherol. Here, concentrations of α-tocopherol decreased in the full-term group from 57 μmol/l in colostrum to 38 μmol/l in transition milk and 20 μmol/l in mature milk. A similar trend was found in the preterm group, but starting with lower concentrations. Thus, although starting concentrations were similar to those reported by Schweigert et al. [26], final concentrations tended to be higher in our study. Other antioxidants such as β-carotene, lutein and zeaxanthin have also shown a rapid postpartum fall [1,27,28]. However, although for these studies retinol also fell with milk maturity, we found no changes over time neither for that molecule nor for ascorbic or uric acid (data not shown). A noteworthy finding is that

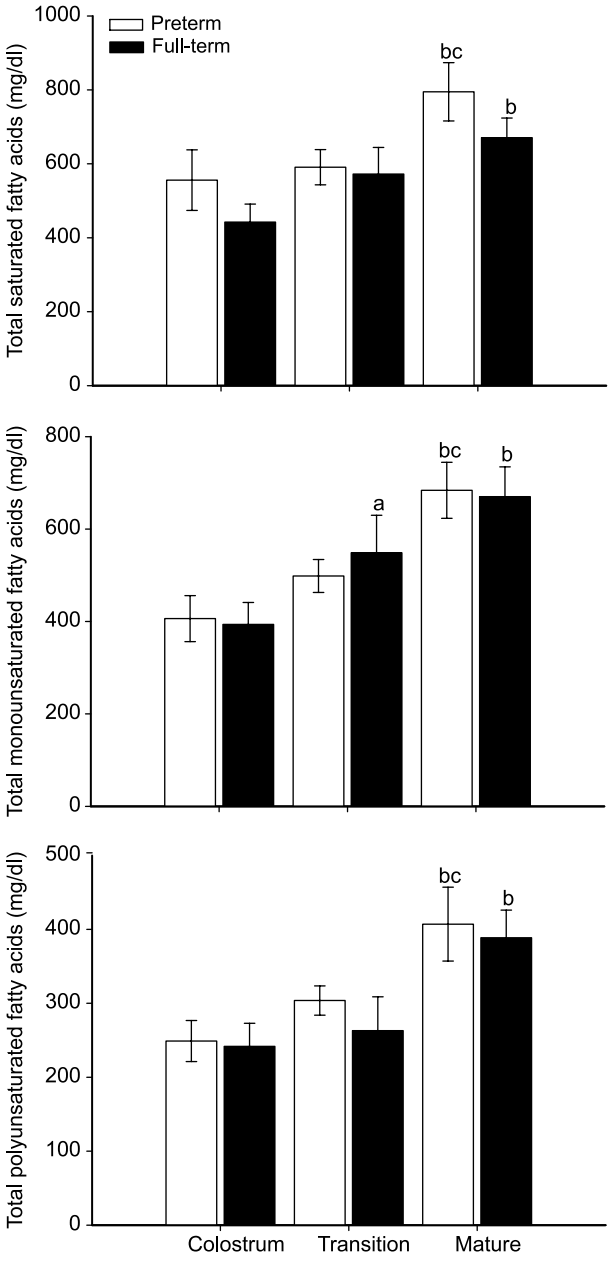

Figure 4. Mean (±SEM) breast milk fatty-acid content (mg/dl) in mothers of preterm and full-term infants at different lactation stages (colostrum, transition milk and mature milk). Differences ($P < 0.05$) related to lactation stage for each group: (a) transition milk vs. colostrum; (b) mature milk vs. colostrum; (c) mature milk vs. transition milk.

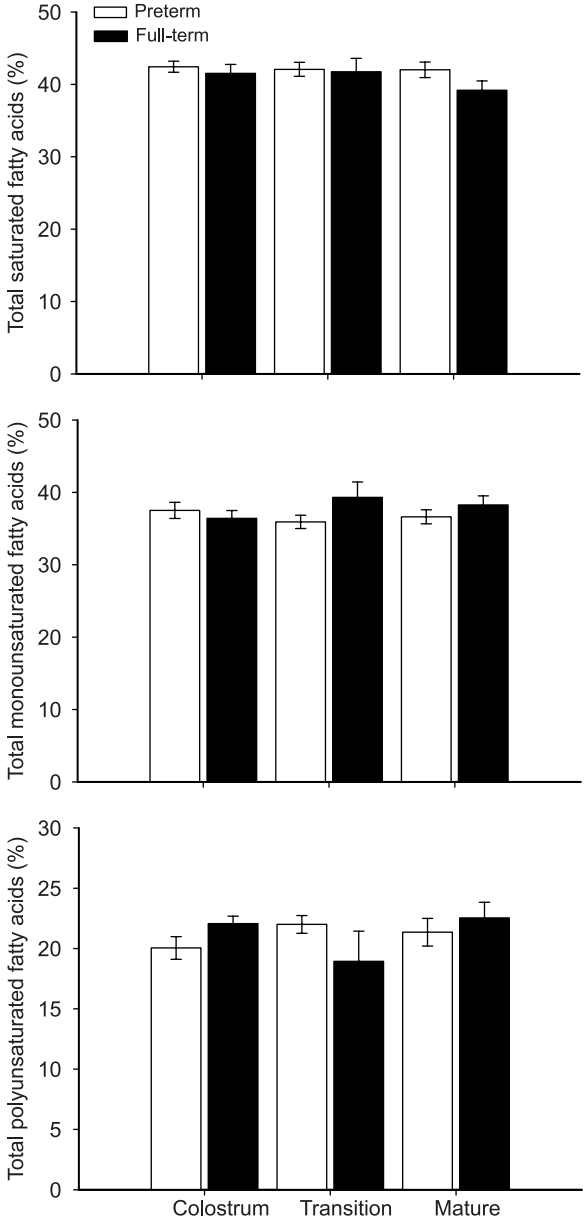

Figure 5. Mean (±SEM) breast milk fatty-acid profile (percentage) in mothers of preterm and full-term infants at different lactation stages (colostrum, transition milk and mature milk).

both CoQ_{10} and tocopherols (α and γ) in milk from mothers delivering premature infants registered lower values at the different stages of lactation (except for CoQ_{10} in mature milk, which showed similar concentrations). Differences between milk from mothers delivering preterm infants and milk from mothers delivering full-term infants in terms of antioxidants have been previously described. Thus, Jewell et al. [28] have reported lower concentrations of lutein in the milk from mothers delivering preterm infants and the zeaxanthin concentration was higher in the milk from mothers delivering full-term infants.

According to the concentrations of tocopherols and CoQ_{10}, we could hypothesize that milk from mothers of preterm infants would have worse antioxidant capacity than that from full-term infants and that the total antioxidant capacity would decrease over time until milk reaches maturity. After analyzing the total antioxidant capacity, we confirm this hypothesis. However, our results disagree with those from Friel et al. [6], who have reported similar antioxidant capacity of human milk from mothers of preterm and full-term infants. Nevertheless, in our study, total antioxidant-capacity values agreed reasonably well

with the tocopherols and CoQ_{10} concentrations. Also, the results from the fatty-acid profile agree with data for total antioxidant capacity. In fact, although no changes were found when fatty acids were analyzed as a percentage, we found a net increase in all types of fatty acids (saturated, monounsaturated and polyunsaturated) in the mature milk from both groups of mothers when expressed in mg/dl. Overall, a higher fatty-acid concentration in the milk represents a higher need for antioxidants for preservation, so that a higher fatty-acid content could correlate with a lower antioxidant capacity, as in fact occurred in the present study.

Birth implies strong oxidative stress, both for the rapid change from relatively hypoxic intra-uterine to the extra-uterine environment, where alveolar pO_2 is almost five times higher, as for the mediation of several physiologic processes involved in the finalization of the gestation and delivery [12–14]. These changes and processes greatly increase the newborn's oxidative stress, which must be controlled by the antioxidant defense system, the maturation of which follows the course of the gestation [15,16]. Birth-related oxidative stress, which increases in premature infants from birth before week 37 of gestation, appears to generate an immaturity in enzymatic and non-enzymatic antioxidant mechanisms [17,18,29]. Moreover, both in preterm and in full-term infants, we have reported [30] that oxidative stress begins to diminish at 72 h after birth (apparently, as the infant grows, less antioxidant protection is needed). Findings from the present study could explain, at least in part, some of the differences between preterm and full-term infants regarding a higher hydroperoxide concentration and a lower level of α-tocopherol and lower SOD and cGPx activity found at birth in preterm infants compared with full-term infants [30]. It may also explain certain changes over time in oxidative-stress status in newborn infants, such as the decreased hydroperoxide concentration in the first 72 h of life in full-term infants [30].

In summary, we found that: (i) CoQ_{10} is present in the milk of mothers delivering preterm and full-term infants, (ii) the CoQ_{10} concentration decreases with the stage of lactation in mothers of full-term infants, and (iii) mothers of full-term infants have higher concentrations of CoQ_{10} than do those of preterm infants (at least for colostrum and transition milk). Finally, (iv) CoQ_{10} as well as α- and γ-tocopherols concentration in human milk are directly correlated with the antioxidant capacity of the milk.

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