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Lipid composition in human breast milk from Granada (Spain): Changes during lactation

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Abstract

Objective: To determine possible differences of composition in the course of lactation, phospholipid (PL) classes (phosphatidylethanolamine, phosphatidylinositol, phosphatidylserine, phosphatidylcholine, and sphingomyelin) and fatty acid composition of PL and triacylglycerol (TGs) fractions of milk fat were analyzed in 66 samples from mothers from Granada (Spain) who gave birth to full-term infants. Analyses included colostrum, transitional milk, and mature milk.

Methods: After milk fat extraction, PLs and TGs were separated by thin-layer chromatography and fatty acids of each fraction were converted into their methyl esters, which were analyzed by gas chromatography. PL classes were determined by high-performance liquid chromatography using an evaporative light-scattering detector.

Results: Mature human milk showed a lower content ($P = 0.020$) of PLs than did the other milks. Percentage of sphingomyelin was constant for all stages of lactation, whereas the percentage of phosphatidylcholine in mature milk was significantly lower ($P < 0.05$) than in colostrum and transitional milk. TGs in mature human milk contained lower percentages ($P < 0.001$) of arachidonic acid, docosahexaenoic acid, and nervonic acid. Docosahexaenoic acid and nervonic acid also showed a significant decrease ($P < 0.001$) in total PLs from colostrum and mature milk.

Conclusions: The composition of PL classes and fatty acids in PLs and TGs in milk of mothers in Granada (Southern Europe) is different from that in milk from mothers in other parts of the world. In addition, the ratio of long-chain polyunsaturated fatty acids delivered in the form of PLs to long-chain polyunsaturated fatty acids delivered in the form of triacylglycerols diminishes as lactation proceeds.

Keywords: Choline; LC-PUFA; Nervonic acid; Phospholipids; Triacylglycerols

Introduction

Human milk is considered the optimal form of nourishment for infants during the first 6 mo of life [1,2]. In terms of its macronutrients, the lipid fraction is crucial in fulfilling a newborn's nutritional needs because almost 50% of dietary calories are supplied to the newborn infant as fat [3]. Numerous studies have demonstrated that lipids are also involved in several structural and physiologic functions in the organism [4–7].

The main compounds of milk fat are fatty acids. These are esterified mainly in the form of triacylglycerols (TGs), which account for 98% of milk fat [3]. A smaller portion of fatty acids is esterified in the form of phospholipids (PLs), which are included in a membrane that surrounds and stabilizes the lipidic core of the fat globule of milk [8]. PLs also perform a nutritional function as suppliers of long-chain polyunsaturated fatty acids (LC-PUFA), nervonic

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acid (NA; C24:1 ω-9), and choline. These nutrients are needed to achieve optimal development and function in the newborn.

The most relevant LC-PUFA (arachidonic acid [AA], C20:4 ω-6; and docosahexaenoic acid [DHA], C22:6 ω-3) are incorporated into the PL membranes of the retina and brain during the third trimester of pregnancy and continue to accumulate during the first 2 y of life [9,10]. In mature human milk, approximately 85% of LC-PUFA is in the form of TGs, and 15% of LC-PUFA is in the form of PLs [11]. Due to their different chemical structures, LC-PUFAs delivered by PLs or TGs have different metabolic pathways, mainly due to the processes of enzymatic hydrolysis, absorption, and incorporation into lipoproteins [12]. Recently, several investigators have studied whether this involves differences in plasma accretion and, hence, uptake of LC-PUFA into developing tissues [13,14].

NA is a major compound of myelin, a structural lipid incorporated in the developing central nervous system up to age 2 y [15,16]. Several studies performed on mammals have demonstrated that NA does not cross the placental barrier in rats, but it can cross the mammary epithelial and intestinal barriers [17]. This suggests that the NA supply in the first months of life is essential because NA content in cerebellar white matter increases with age in human newborns [18].

Choline is another PL-related compound involved in several biological processes, mainly in metabolism and construction of the membranes of the neonate [19–21]. Maternal reserves of choline are depleted during pregnancy and lactation, suggesting that the neonate requires large amounts of this compound [22]. For this reason, it has been postulated to be an essential nutrient [23], mainly in preterm newborns, but the metabolic pathways of choline synthesis are not fully developed [24]. Although approximately 17% of total choline is supplied to the neonate by PLs (sphingomyelin [SM] and phosphatidylcholine [PC]) [25,26], this is metabolized by pathways different from the main choline compounds found in milk [27].

Human milk is a dynamic system whose fat composition is influenced by factors such as maternal diet, duration of pregnancy, or stage of lactation [28]. There are three phases of milk production: colostrum (1 to 5 d postpartum), transitional milk (6 to 15 d postpartum), and mature milk (after 15 d). Recent works have reported differences between the total fatty acid composition of human breast milk [29–32] and its TG composition [30,33,34] at different stages of lactation. Nevertheless, we found no recent studies that focused on the influence of stage of lactation in the esterification of LC-PUFA and NA into PLs or TGs or of composition of choline-containing PLs.

In the present work, the main PL classes (phosphatidylethanolamine [PE], phosphatidylinositol, phosphatidylserine, PC, and SM) and the fatty acid compositions of PL and TG were determined in samples from colostrum to fully established mature milk in mothers who gave birth to full-term infants. This provides information on the changes that occur in the composition of human milk as lactation proceeds. This greater knowledge should contribute to a better design of infant formulas.

Materials and methods

Experimental design and subjects

Thirty apparently healthy Spanish women from Granada who had given birth to term infants (38 to 42 wk of gestation) and breast-fed their babies participated in the study. This voluntary study was explained to all the mothers who gave their written consent. The hospital ethics and scientific committees approved the project. All mothers had similar educational backgrounds and were 17 to 36 y of age. The main characteristics of the sampled mothers are presented in Table 1. All were non-vegetarian and had similar dietary habits over the duration of the study, as determined by a questionnaire of feeding frequency. No changes in the dietary habits of mothers were observed during the study. All mothers exclusively breast-fed their infants at all studied time points.

Samples

Sixty-six milk samples were collected from both breasts with an Ico mechanical breast pump (Ico, Barcelona, Spain) according to the manufacturer's instructions. Subsamples from each breast were obtained at the beginning of each feeding throughout the day (foremilk) and then were pooled. Samples were stored at −80°C until lipid extraction to inactivate lipases and avoid TAG hydrolysis [35]. Thirty samples were collected over the first 1 to 5 d postpartum (colostrum), 17 at 6 to 15 d postpartum (transitional milk), and 19 at 16 to 30 d postpartum (mature milk). Of 30 women enrolled in the study, 17 of them delivered samples at each sampling time. We included samples from all mothers in the statistical analysis of results.

Analysis of phospholipid classes

Milk samples were thawed, warmed to 37°C, and vortexed before analysis. The chromatographic method of separation and identification of human milk PLs used in this study were presented previously [36]. The chromatographic equipment consisted of a pump system (model 1050, Hewlett-Packard, Waldbroom, Germany), an autosampler (717 Plus Autosampler, Waters, Milford, MA, USA), an evaporative light-scattering detection (ELSD) detector (model 750/14, ACS, Macclesfield, UK), and a 3365 series II Chemstation (Hewlett-Packard) that acquired data from the ELSD detector. The analytical column used was an Extrasil silica (150 × 4.0 mm inner diameter, 3-μm particles) with a precolumn (2 × 4.0 mm) from Tracer Analitica