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Lipid composition and vitamin E content in human colostrum and mature milk

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Lipidic components, as well as fatty acid composition and vitamin E content were determined in colostrum (days 3-5 of postpartum) and mature milk (day 21) in 8 women from Murcia (Spain). Triglycerides concentration was higher and cholesterol and esterified cholesterol were lower in mature milk than in colostrum, whereas phospholipid content was similar. These differences indicate that the diameter of milk fat globules increases in mature milk. The percentage of medium-chain fatty acids (12:0 and 14:0) increased in mature milk as compared to colostrum, reflecting *de novo* synthesis of fatty acids. With the only exception of stearic acid which was lower in mature milk than in colostrum, the remaining long-chain fatty acids was similar. The proportion of both linoleic (18:2) and eicosapentaenoic (20:5) acids found in mature milk and colostrum is higher than in studies from other countries and may reflect the intake of high proportions of polyunsaturated fat from vegetable oils and fish in the studied women. Both vitamin E content and vitamin E/linoleic acid ratio in mature milk are lower than in colostrum, evidencing the efficient mechanism of mammary gland vitamin E uptake around parturition.

Key Words: Fatty acids, Human milk, Vitamin E, Lipids.

The increasing popularity of breast-feeding has encouraged the analysis of the composition of breast milk in various countries (5, 6, 32, 35). Fat constitutes about 50 % of the total calorie value of human milk (18), and its composition varies with the diet. Thus, the increased consumption of vegetable oils, rich in polyunsaturated lipids, has been associated to an increase in polyunsaturated fatty

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acids (PUFA) in breast milk (15). The most abundant essential fatty acid in human milk is linoleic acid (C18:2), and recent studies report higher concentrations (15, 20) than older studies (36), probably as a result of changes in the diet.

An increase in *PUFA* content in the milk may cause excessive peroxidation and, therefore, it may increase vitamin E requirements (8, 37). Thus, breast milk lipid analysis should also give information on vitamin E content in order to establish the proper dietary recommendations and attain the appropriate vitamin E/linoleic acid ratio requirements.

In order to know the lipidic composition of breast milk in women from an area where the regular diet is abundant in vegetable oils and fish, the present study was addressed to determine the concentration of lipid fractions, fatty acids composition and vitamin E content in colostrum (3 to 5 days postpartum) and mature breast milk (21 days) from healthy mothers living in Murcia, a Mediterranean area South East of Spain.

Materials and Methods

Materials.– Standards p.a. were from Sigma (USA), solvents were HPLC quality grade from Scharlau (Spain) and perchloric acid p.a. from Merck (Germany).

Human milk samples.– Milk samples were collected with an electric breast pump from eight 22-30 year old healthy female volunteers living in Murcia (Spain), with similar educational background and dietary habits. At morning nursing (9 to 10 am), milk samples were put into plastic containers, and immediately frozen at -80 °C. They consumed ad libitum diets. Samples were taken from the same women on days 3-5 of postpartum (colostrum) and on day 21.

This study was approved by the Ethical Committee of the Universidad San Pablo-CEU.

Lipid extraction and fractionation.– Immediately after thawing, aliquots of milk were extracted with chloroform-methanol (2:1) according to FOLCH *et al.* (11). Lipid fractions were quantified following an image analysis after separation by one-dimensional thin-layer chromatography (TLC), by the method of Ruiz and Ochoa (33), with few modifications. In short, aliquots of the lipid extracts were spotted on Merck Silica gel $60F_{254}$ plates and developed first in chloroform/ methanol/ formic acid (1:2:1, by vol.), a second time in heptane/diisopropyl ether/acetic acid (70:30:2) and a third time in heptane. After TLC, lipids were charred and spots were quantitatively determined by spectrodensitometry in situ using the G5-700 BIOIMAGE TLC scanner of Bio-Rad (Hercules, CA, USA). Optical density of the spots were compared to standards on each plate and curves were drawn from 2nd order least square regression equations on the standards.

Fatty acid analysis in neutral lipid fractions by gas liquid chromatography.– Parallel TLC plates from each sample were processed as described above. After development the plates were sprayed with 0.1 % 2',7'-dichlorofluorescein in 95 % methanol, and spots visualized under ultraviolet lamp. Spots were eluted with 2 ml of methanol/toluene (4:1) and after adding 200 µl of acetyl chloride, they were kept at 80 °C for 2.5 h. An internal standard of methylheptadecanoate (10 nmols in toluene) was added to each tube and the

reaction was stopped with 5 ml of 6 % potassium carbonate. Then, 0.5 ml of toluene were added and tubes were centrifuged for 10 min at 800 g. The organic phase was evaporated under vacuum and the residue dissolved in 40 µl of toluene, then the mixture of methyl esters was ready for injection into the gas chromatograph. Chromatographic analysis was performed with a gas chromatograph (Autosystem from Perkin-Elmer, St. Louis, MO, USA), equipped with a fused-silica (30 m x 0.25 mm ID, 0.25 µm film) capillary column (Supelco, Bellefonte, PA, USA), under the following conditions: oven temperature, 200 °C; flame ionization detector temperature, 260 °C; injector temperature, 250 °C; nitrogen was used as a carrier gas; air and hydrogen flows were adjusted to give maximal detector response; Split ratio was 1:5100; sample size injected, 0.3 µl.

Peak identification was made based on relative retention times of external standards and results are expressed as weight percentage of all fatty acids measured.

Vitamin E determination.– Vitamin E in milk samples was determined by HPLC (Gold System, Beckman) following the method described by CUESTA and CASTRO (9). Chromatography was carried out using methanol/water 97.5: 2.5 (v/v) as eluent at a flow rate of 2 mL/min with a C-18 Nucleosil column. Detection was at 325 nm for retinyl acetate and 295 nm for vitamin E with a Diode Array detector. α-tocoferol standard solution was subjected to the same procedure and used as external standard for quantification.

Statistics.– The differences in the average values between milk and colostrum samples were tested by paired Student's *t* test.

Results and Discussion

Lipid components.– Table I shows the lipid class components of colostrum (3-5 days postpartum) and mature milk (21 days). Since lypolisis occurred in our samples, values have been corrected for artifacts as previously proposed by BITMAN *et al.* (2). This artifact was shown by the presence of a considerable quantity of free fatty acids (3.2 % in colostrum and 18.5 % in mature milk), monoglycerides (0.16 % in colostrum and 0.87 % in mature milk) and diglycerides (1.39 % in colostrum and 4.06 % in mature milk), indicating a substantial hydrolysis of triglycerides. Since the major lipidic component in human milk is known to be triglycerides (2, 4, 19), individual data were recalculated by the addition of free fatty acids, monoglycerides and diglycerides to the triglycerides fraction. As shown in table I, triglycerides in colostrum and mature milk gave values of 96.6 and 98.9 % of total lipids, respective-

Table I. *Concentration of lipid fractions in human colostrum and mature milk in Murcia (Spain).* Samples were extracted with chloroform-methanol and lipid fractions were quantified following an image analysis after separation by one-dimensional thin layer chromatography. Mean ± SE (n = 8). Paired statistical comparisons between colostrum and mature milk.

	Colostrum	Mature milk
Triglycerides (g/dl)	1.04 ± 0.23	2.90 ± 0.48**
Cholesterol (mg/dl)	13. 9 ± 1.2	8.28 ± 0.62***
Phospholipids (mg/dl)	9.69 ± 3.12	8.91 ± 1.70
Cholesterol esters (mg/dl)	12.1 ± 3.3	0.81 ± 0.53**

**$p < 0.01$; ***$p < 0.001$.

ly. Total triglyceride content in mature milk was significantly higher than in colostrum whereas the content of phospholipids was similar and both cholesterol and cholesterol esters were significantly lower in milk than in colostrum. An increase in triglyceride and a decrease in cholesterol content in mature milk as compared to colostrum has been consistently found by several groups (1-3, 13, 14, 19) whereas phospholipids have been found either to decrease (2) or not to change (19), as it was the case in present study. However, the marked decrease of both phospholipid/triglyceride (ranging from $7.4 \times 10^{-3} \pm 0.5 \times 10^{-3}$ to $5.7 \times 10^{-3} \pm 0.4 \times 10^{-3}$, $p < 0.05$) and cholesterol/triglycerides (ranging from $13.6 \times 10^{-3} \pm 2.1 \times 10^{-3}$ to $3.4 \times 10^{-3} \pm 0.7 \times 10^{-3}$, $p < 0.001$) detected, agrees with previous reports (19). Since most phopholipids and free cholesterol are associated with milk fat globules, the proportional decrease in these membrane components is consistent with the reported finding that, as lactation proceeds, the diameter of these globules increases and their membrane becomes thinner (23, 31).

Fatty acid composition.– As shown in table II, fatty acid composition of milk changes from colostrum to mature milk. From over 21 identifiable fatty acids, only 13 were quantified corresponding to more than 99 % of the total fatty acids. Medium chain saturated fatty acids, 12:0 and 14:0 were higher in mature milk than in colostrum, which is consistent with similar findings in other Spanish reports from different geographical areas (2, 14, 19). These changes in milk composition may indicate variations in mammary gland biosynthetic capability, being an expression of its functional maturity. On the other hand, since these medium-chain fatty acids are easily absorbed by the digestive system of the newborn infant (25, 30), their presence in the milk may also represent an adaptation of mammary gland to secrete these lipids in parallel to the development of the digestive system. Palmitic acid (16:0), stearic acid (18:0), oleic acid (18:1), linoleic acid (18:2) and eicosapentaenoic acid (20:5) are the most abundant long-chain fatty acids in colostrum and mature milk (table II). With the only exception of stearic acid (18:0) which is significantly lower in mature milk than in colostrum, neither of the remaining long-chain fatty acids changed from colostrum to mature milk. This stability of fatty acids composition has been previously reported by other investigators (2, 20, 24). Although the proportional level of the most abundant fatty acids, palmitic acid (22-24 %) and oleic acid (33-37 %) were very similar to those reported ones (2, 4, 13, 15, 16, 24, 28, 34), the proportion of linoleic acid (18:2) found in mature milk (13.7 %) and colostrum (13.3 %) is higher than that in some other studies, which range from 6 to 8 % in Swiss (4), British (16) or Ivory Coast women (22), or 9 to 10 % in Malaysia (21) and Italy (35), but similar to the 11 to 16 % range reported in Australian (13), Swedish (20), and American (2, 15) women as well as those from Northern Spain (28). Since the composition of human milk depends on the diet, as shown by changes in dietary interventions (12, 26, 34), the high levels of linoleic acid found in the studied Spanish women must reflect high proportions of polyunsaturated fat intake from vegetable oils.

Eicosapentaenoic acid (20: 5) was the other polyunsaturated fatty acid found in substantial amounts (4-5 %) in both colostrum and mature milk (table II). These amounts are almost ten times high-

Table II. *Fatty acid composition (g/100 g) of human colostrum and mature milk samples in Murcia (Spain).*
After thin layer chromatography, spots corresponding to triglycerides, diglycerides, monoglycerides and fatty acids were eluted and, after methylation, fatty acids were measured by gas chromatography. Means ± SE (n = 8). Paired statistical comparison between colostrum and mature milk.

Fatty acid	Colostrum	Mature milk
Saturates		
12:0	2.59 ± 0.35	4.20 ± 0.40*
14:0	4.75 ± 0.57	7.67 ± 0.59*
15:0	0.37 ± 0.05	0.41 ± 0.05
16:0	23.67 ± 1.33	22.13 ± 1.34
18:0	11.48 ± 1.03	7.74 ± 0.56***
22:0	1.31 ± 1.13	0.24 ± 0.05
Monounsaturates		
15:1	0.12 ± 0.03	0.08 ± 0.01
16:1(w-7)	2.11 ± 0.41	2.04 ± 0.36
18:1(w-9)	33.91 ± 2.35	37.51 ± 1.56
20:1(w-9)	0.58 ± 0.13	0.58 ± 0.12
Polyunsaturates		
18:2(w-6)	13.27 ± 2.06	13.73 ± 0.69
20:4(w-6)	0.11 ± 0.07	0.21 ± 0.10
20.5(w-3)	4.03 ± 1.01	4.88 ± 0.83

*$p < 0.05$; ***$p < 0.001$.

er than those reported by others (2, 13, 21, 28, 35), which never reached 0.5 %. Since this essential fatty acid originates in fish and greatly increases in milk when the diet is supplemented with fish oil (12, 18), the high levels detected in the milk of the studied women may reflect a high consumption of fish. Eicosapentaenoic acid is a much closer metabolic precursor of docosahexaenoic acid (C22:6) (DHA) than is linoleic acid (27) and thus its presence in milk may warrant the availability of DHA in the suckling newborn for appropriate brain development.

Vitamin E.– Colostrum samples had a vitamin E content of 1.44 ± 0.23 mg/dl, a value that is of the same range as previously reported (20). Also in agreement with previous reports (3, 20), vitamin E content in mature milk was significantly lower ($p < 0.001$) than in colostrum (0.31 ± 0.05 mg/dl).

Tissue delivery of α-tocopherol has been shown to be promoted by the action of lipoprotein lipase (LPL) on triglyceride-rich lipoproteins (7), and although not studied directly, the induction of LPL activity in mammary gland occurring around parturition (29) would enhance the uptake of the α-tocopherol associated to these lipoproteins in this organ. Throughout this mechanism, the newformed colostrum becomes inundated with vitamin E, which is needed by the newborn infant during the first week of life, when the infant's tissue stores of vitamin E are low and need supplementation.

Since enhanced level of polyunsaturated fatty acids may produce excessive peroxidation, and thus increase vitamin E requirements (8, 10, 37), vitamin E levels in milk must be evaluated on a relative basis. Consistent with previous findings (20), α-tocopherol/linoleic ratio was higher in colostrum (89.8 ± 14.7 μg/g) than in mature milk (25.9 ± 3.49, $p < 0.001$) and evidences the efficient mechanism of mammary gland vitamin E uptake around parturition.

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Se determinan los componentes lipídicos, la composición en ácidos grasos y el contenido de vitamina E en calostro (días 3-5 posparto) y en leche (día 21) en 8 mujeres de Murcia (España). La concentración de triglicéridos es mayor y la de colesterol y colesterol esterificado es menor en leche que en calostro, mientras que la de fosfolípidos es similar. Estas diferencias sugieren que el diámetro de los gránulos de grasa aumenta en la leche madura en relación al calostro. El aumento en el porcentaje de ácidos grasos de cadena media (12:0 y 14:0) en la leche en relación al calostro, refleja la síntesis *de novo* de los ácidos grasos. Con excepción del ácido esteárico, que se encuentra en niveles menores en la leche, los demás ácidos grasos están en proporciones similares en ambas preparaciones. La proporción de los ácidos linoleico (18:2) y eicosapentaenoico (20:5) encontrada en leche y calostro es superior a la observada en otros trabajos, y puede reflejar una elevada ingesta de ácidos poliinsaturados procedentes de aceite vegetal y pescado en las mujeres estudiadas. Tanto el contenido de vitamina E como el cociente vitamina E/ácido linoleico es mayor en calostro que en leche madura, lo que refleja el eficaz mecanismo de captación de vitamina E por la glándula mamaria alrededor del parto.

Palabras clave: Ácidos grasos, Leche humana, Vitamina E, Lípidos.

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