European Journal of Clinical Nutrition (2000) 54, 878–882

www.nature.com/ejcn

Triacylglycerol composition in colostrum, transitional and mature human milk

S Morera Pons[1], A Castellote Bargalló[1], C Campoy Folgoso[2], and MC López Sabater[1]*

[1]*Departament de Nutrició i Bromatologia-CeRTA, Facultat de Farmàcia Universitat de Barcelona, Barcelona, Spain; and* [2]*Neonatology Department, University Hospital of Granada, Granada, Spain*

Objective: Milk triglycerides from colostrum, transitional and mature human milk, were analyzed and compared in order to determine the differences in triacylglycerol composition throughout lactation.
Setting: Department of Food and Nutrition, University of Barcelona, Spain, and Neonatology Department of the University Hospital of Granada, Spain.
Subjects: Twenty-two healthy lactating women aged 21–35.
Design and interventions: The triacylglycerol profiles of 47 breast milk samples including colostrum (1–3 days), transitional milk (7–10 days) and mature milk (25–60 days) were analyzed by high-performance liquid chromatography (HPLC), with light-scattering detection (LSD).
Results: Significant differences regarding several triglycerides were found between three milk classes when the Kruskal–Wallis nonparametric test was applied to 47 human milk samples that had been compared using the complete chromatographic triacylglycerol profile. The ANOVAS for each equivalent carbon number (ECN) group of triglycerides revealed significant differences between colostrum, transitional milk and mature milk. By the discriminant analysis of triacylglycerol percentages, in 19 colostrum samples, 14 transitional milk samples and 14 mature milk samples, three milk types were distinguished, and three triglycerides (peak no. 4, LnOO and SOO) were found to be the most predictive variables over all the triacylglycerol profile or ECN groups.
Conclusions: Each state of lactation shows a specific profile of triacylglycerol composition in human milk. However the two most abundant triacylglycerides in colostrum, POO and POL, which account for more than 49% of the total, are also dominant in transitional (34%) and mature milk (42%).
Sponsorship: CeRTA (Centre de Referència en Tecnologia d'Aliments) supported this study.
Descriptors: milk; breast milk; human milk; colostrum; transitional milk; mature milk; triacylglycerols; triglycerides; HPLC; LSD
European Journal of Clinical Nutrition (2000) **54,** 878–882

Introduction

Human milk is considered the optimal form of nutrition for infants and is the main food for a healthy infant during the first 4–6 months of life (ESPGAN, 1991). Adequate growth and development of the newborn depend on the quantity and quality of available fetal stores and ingested milk, the efficiency of gastrointestinal absorption, and energy expenditure. With regard to macronutrients, the lipid fraction of human milk seems to be essential (Boersma *et al*, 1991).

Breast milk is a dynamic body fluid whose composition changes throughout lactation, providing the infant with the nutrients specifically needed at each age. There are three phases of milk: colostrum, transitional and mature milk, each with distinct characteristics.

Colostrum, present from delivery to approximately 5 days postpartum, contains the highest concentration of proteins, mainly immunoglobulins and lactoferrin. Its fat content is lower than that of mature milk (2% *vs* 3.5%).

Transitional milk is present between days 6–15 postpartum. The immunoglobulin levels decrease whereas those of lactose, fat and water-soluble vitamins increase. It shows the highest variability among mothers.

Mature milk is produced from day 15. Compared with colostrum, it is thin and watery. One-third is foremilk, which is thin and contains less fat. The rest is hindmilk, which comes at the end of feeding and contains about four times more fat than foremilk (Barry Lawrence, 1994).

Of all the nutrients in human milk, lipids have the greatest variability. It has long been known that milk fat content changes during each feeding and between feedings, according to the breast and to the stages of lactation. Moreover, recent studies have shown that milk fat composition may be influenced by the maternal diet (Harzer *et al*, 1993; Hamosh, 1997; Ruel *et al*, 1997; Emmett & Rogers, 1997). This dynamic state also hinders the determination of the exact composition of human milk (Ruel *et al*, 1997; Barry Lawrence, 1994).

Since breast milk fat is the natural source of fat for the new-born and triacylglycerides (TAGs) account for 98% of the lipids in human milk, the structure of those TAG may be used as a biological reference (Martin *et al*, 1993; Jensen *et al*, 1995).

The study of this structure may influence the design of milk fat for infant formulas so that it resembles human milk as far as possible (Gresti *et al*, 1993).

*Correspondence: MC López Sabater, Departament de Nutrició i Bromatologia, Facultat de Farmàcia, Universitat de Barcelona, Avda. Joan XXIII s/n 08028 Barcelona, Spain.
E-mail: mclop@farmacia.far.ub.es
Received 25 February 2000; revised 20 April 2000; accepted 18 July 2000

Some works report the differences between the fatty acid composition of human milk at each stage of lactation (Jansson *et al*, 1981; Gibson & Kneebone, 1981; Guesnet *et al*, 1993; Martin *et al*, 1993; Boroviczény *et al*, 1997). However, few reports on differences of milk TAGs between colostrum and mature human milk, without any reference to transitional milk, have been published (Lyapkov & Kiseleva, 1992; Martin *et al*, 1993).

The present study was designed to provide information on the changes in the TAG composition of human milk from day 1 to the establishment of mature milk.

Methods

Multivariate analysis (stepwise discriminant analysis) from SSPS was performed to determine the variables (individual triglycerides and ECN groups) that best discriminate milk classes. The chromatographic methods of separation and identification of human milk TAGs used in the study have been published (Morera *et al*, 1998b). The ECN for each individual TAG can be calculated as follows: (ECN = CN−2 ND), where CN is the number of carbon atoms, and ND is the number of double bonds.

Experimental design and subjects

Samples of breast milk were from volunteers who delivered at the University Hospital of Granada. The main characteristics of the mothers sampled are presented in Table 1. All donors were apparently healthy, non-diabetic and with no pregnancy complications. Women taking drugs, alcohol or cigarettes were not included in the study. The study protocol was approved by the Hospital ethics committee.

Samples

Milk samples were collected from both breasts by means of an Ico^{R} mechanical breast pump (Ico, Spain) following the manufacturer's instructions. The milk from each breast was obtained at both the beginning and the end of each feed of the day. Samples were stored at −80°C until lipid extraction, to inactivate lipases and avoid TAG hydrolysis (Morera *et al*, 1998a). To make our study as homogeneous as possible all mothers provided milk from the three stages of lactation; nevertheless samples with minimum traces of hydrolysis were rejected. In all, 19 samples were obtained between 1 and 3 days postpartum (colostrum), 14 between 7 and 10 days postpartum (transitional milk) and 14 between 25 and 60 days postpartum (mature milk). There was a sample of 11 women in each period of lactation (33/47).

Milk lipid extraction

Lipid extraction was performed following a modification of the method described by Chen *et al* (1981). Dichloromethane−methanol (2 : 1), 25 ml, were added to 1.5 ml of mature human milk contained in a centrifuge tube. The mixture was shaken mechanically for 15 min and centrifuged at 3000 *g* for 8 min. Approximately 8 ml of distilled water were pipetted into a tube and, after shaking for 15 min, the sample was centrifuged (8 min, 3000 *g*). As much of the upper aqueous fraction as possible was removed. The organic layer was washed in a saturated solution of NaCl (Panreac, Barcelona, Spain) and finally mixed (15 min) and centrifuged (8 min, 3000 *g*). The organic fraction was carefully transferred to a separating funnel and filtered through 1PS paper (Whatman, Maidstone, UK) containing anhydrous sodium sulphate (Panreac, Barcelona, Spain).

The extract was concentrated by removing the solvent in a rotary evaporator and dried under a gentle stream of nitrogen. The residue was stored at −20°C and redissolved in HPLC-grade dichloromethane (5% w/v) immediately before HPLC analysis.

Triacylglycerol analysis

For the triacylglycerol analysis, one aliquot of 200 µl was transferred to a conical flask containing 0.5 mg of triundecanoin (C33 : 0) as internal standard (IS). The chromatographic equipment consisted of a Hewlett-Packard model 1050 pump system (Waldbronn, Germany), a Rheodyne model 7125 injector (Cotati, CA, USA) with a 20 µl sample loop, a mass detector (model 750/14, ACS, Macclesfield, UK), and an HP 3365 series II Chemstation which used data acquired from the mass detector. The analytical column used was a Spherisorb ODS-2 (250 × 4.6 mm IDS, 5 µm particle size) Tracer Analitica (Barcelona, Spain).

The chromatographic separation was carried out using a linear gradient of acetonitrile : dichloromethane : acetone from 80 : 15 : 5 (v/v/v) to 10 : 80 : 10 (v/v/v) in 60 min and, after 2 min of isocratic elution with 95% dichloromethane, the initial conditions were reached in 5 min. The flow-rate of the eluent was 1 ml/min and the column temperature was 30°C. The volume of the sample injected was 10 µl. The mass detector oven was 55°C and the gas flow (from an air compressor) was 10 l/min.

Triacylglycerides were identified as described previously (Goiffon *et al*, 1981; Parcerisa *et al*, 1994). TAGs were quantified by normalization, assuming that the detector response was the same for all molecules.

Statistical analysis

Statistical comparison of TAG composition data was performed by ANOVA to reveal several significant differences for each equivalent carbon number group of triglycerides (ECN) among different milk samples (colostrum, transitional milk and mature milk) using the Scheffé test. However, the differences between the three milk phases for each triglyceride were analysed with the Kruskal−Wallis nonparametric one-way test, since the variances for each variable are not always homogeneous. Thereafter, we used multiple comparison procedures following Dunn analysis (Dunn, 1964). Multivariate analysis (stepwise discriminant analysis) was then performed to determine the variables that best discriminate milk types.

Table 1 Characteristics of the sampled mothers

Type of delivery (vaginal/caesarean)	9/13
Gestational period (weeks)[a]	39.69 ± 1.33
Age (y)[a]	28.09 ± 3.98
Height (cm)[a]	161.26 ± 5.8
Weight (kg)[a]	58.14 ± 10.68
Parity[a]	1.69 ± 0.52

[a]Mean ± s.d.

Results and discussion

The concentration of each TAG in colostrum, transitional milk and mature milk are shown in Table 2. The differences between mature and transitional milk were significant only

Table 2 Triacylglyceride percentages in milk samples at different stages of lactation

TAGs	*Colostrum* ($n = 19$)	*Transitional milk* ($n = 14$)	*Mature milk* ($n = 14$)
LLL	0.12 ± 0.03^{A}	1.24 ± 0.19^{B}	0.73 ± 0.08^{B}
LnLO	0.12 ± 0.03^{A}	1.03 ± 0.16^{B}	0.46 ± 0.05^{B}
NI (peak no. 3)[a]	0.66 ± 0.12^{A}	2.31 ± 0.36^{B}	1.91 ± 0.22^{B}
NI (peak no. 4)[a]	0.12 ± 0.03^{A}	0.37 ± 0.03^{B}	0.40 ± 0.02^{B}
NI (peak no. 5)[a]	0.52 ± 0.09^{A}	1.82 ± 0.28^{B}	1.11 ± 0.13^{B}
LLO	0.62 ± 0.12^{A}	3.49 ± 0.40^{B}	2.20 ± 0.23^{B}
NI (peak no. 7)[a]	2.11 ± 0.25^{A}	2.06 ± 0.38^{A}	2.45 ± 0.40^{A}
LnOO	0.66 ± 0.07^{A}	1.96 ± 0.19^{B}	1.28 ± 0.07^{B}
LLP	2.02 ± 0.25^{A}	2.07 ± 0.42^{A}	2.40 ± 0.32^{A}
LnOP	2.05 ± 0.14^{A}	2.98 ± 0.26^{B}	2.97 ± 0.18^{B}
NI (peak no. 11)[a]	1.03 ± 0.09^{A}	2.61 ± 0.21^{B}	2.19 ± 0.14^{B}
MOL	0.81 ± 0.10^{AC}	1.58 ± 0.17^{B}	1.13 ± 0.09^{BC}
NI (peak no. 13)[a]	2.35 ± 0.27^{A}	6.56 ± 0.58^{B}	4.25 ± 0.34^{B}
LOO	6.31 ± 0.33^{AC}	4.69 ± 0.48^{B}	5.71 ± 0.57^{BC}
SLL/PaOP	1.68 ± 0.11^{A}	2.89 ± 0.21^{B}	2.30 ± 0.09^{B}
POL	20.11 ± 0.92^{A}	14.97 ± 1.19^{B}	18.84 ± 1.01^{A}
PaOP	3.34 ± 0.14^{A}	3.96 ± 0.32^{A}	3.82 ± 0.25^{A}
PPL	1.99 ± 0.14^{A}	1.89 ± 0.14^{A}	1.77 ± 0.11^{A}
MOP	2.70 ± 0.23^{AC}	4.20 ± 0.38^{B}	2.97 ± 0.24^{BC}
OOO	6.03 ± 0.38^{AC}	4.99 ± 0.45^{BC}	4.46 ± 0.32^{B}
SLO	1.41 ± 0.12^{A}	1.57 ± 0.18^{A}	1.46 ± 0.16^{A}
POO	29.07 ± 1.50^{AC}	19.23 ± 1.50^{B}	23.73 ± 1.37^{BC}
SLP	1.92 ± 0.15^{A}	1.62 ± 0.17^{A}	1.64 ± 0.10^{A}
PPO	5.66 ± 0.28^{AC}	4.71 ± 0.52^{BC}	4.54 ± 0.46^{B}
PPP/PaPS	0.44 ± 0.04^{A}	0.51 ± 0.06^{A}	0.32 ± 0.05^{B}
SOO	1.31 ± 0.08^{A}	0.92 ± 0.12^{B}	0.76 ± 0.05^{B}
SOP	4.27 ± 0.37^{A}	3.33 ± 0.29^{A}	3.47 ± 0.27^{A}
SPP	0.31 ± 0.03^{AC}	0.27 ± 0.03^{BC}	0.21 ± 0.03^{B}
SOS	0.12 ± 0.01^{A}	0.09 ± 0.01^{A}	0.06 ± 0.01^{B}
SSP	0.12 ± 0.01^{AC}	0.10 ± 0.01^{BC}	0.07 ± 0.01^{B}

Mean $\pm$ standard error of the mean (s.d./$n^{1/2}$). Means in the same row with different superscripts differ significantly; $P < 0.05$. Abbreviations: M = myristin; Pa = palmitin; S = stearin; L = linolein; Ln = linolenin; O = olein; P = palmitin; Pa = palmitolein. NI = not identified. [a]Number of the peak in a chromatographic elution.

for a few TAGs ($P < 0.05$) and not in the same way. Mature milk contained about 25% more POL (the second most abundant TAG in human milk) than transitional milk. However, PPP and SOS, which are considered minor TAGs during lactation, are more abundant in transitional than in mature milk. The differences between colostrum and transitional milk and between colostrum and mature milk are more significant. Several TAGs (LLL, LnLO, LLO, LnOO, LnOP, MOL, SLL/PaOP, MOP and the nonidentified peaks, nos 3, 4, 5, 11 and 13) showed colostral levels significantly lower than those found in transitional milk, whereas LOO, POL, POO and SOO were significantly lower ($P < 0.05$) in transitional milk than in colostrum. The contents of LLL, LnLO, LLO, LnOO, LnOP, SLL/PaOP and the nonidentified peaks, nos 3, 4, 5, 11 and 13, were lower in colostrum than in mature milk ($P < 0.05$), while the contents of OOO, PPO, PPP/PaPS, SOO, SPP, SOS and SSP were higher in colostrum than in mature milk at levels of significance.

The levels of the TAGs that elute before LOO in chromatographic analysis were, in general, lower in colostrum than in mature and transitional milk. However, the concentrations of LOO and the majority of the TAGs that elute behind this peak, such as POL, OOO, POO, SPL, PPO, SOO and SOP, were higher in colostrum than in mature and transitional milk.

Changes in the ECN levels of milk samples at different stages of lactation are shown in Table 3. The differences between colostrum and mature milk were significant ($P < 0.005$) for ECN 42, ECN 44, ECN 48, ECN 52 and ECN 50 ($P < 0.05$). For ECN 46, no significant differences were found between the two types of milk. Colostrum was significantly different from transitional milk at $P < 0.005$ for ECN 42, ECN 44, ECN 48 and ECN 50 and at $P < 0.05$ for ECN 52. ECN 46 data were not available, which prevented us from distinguishing the two milk stages. Finally, the Scheffé test did not reveal any significant difference when mature milk was compared with transitional milk.

Table 3 Equivalent carbon number percentages in milk samples at different stages of lactation

TGs	*Colostrum* ($n = 19$)	*Transitional milk* ($n = 14$)	*Mature milk* ($n = 14$)
ECN42	1.62 ± 0.28^{A}	6.33 ± 0.95^{B}	4.60 ± 0.41^{B}
ECN44	11.51 ± 0.98^{A}	22.70 ± 1.54^{B}	18.87 ± 1.00^{B}
ECN46	35.37 ± 0.68^{A}	33.75 ± 1.45^{A}	35.42 ± 1.09^{A}
ECN48	45.12 ± 1.61^{A}	32.73 ± 2.16^{B}	36.16 ± 1.78^{B}
ECN50	6.12 ± 0.37^{A}	4.33 ± 0.44^{B}	4.45 ± 0.30^{B}
ECN52	0.26 ± 0.02^{A}	0.18 ± 0.03^{B}	0.13 ± 0.02^{B}

Mean $\pm$ standard error of the mean (s.d./$n^{1/2}$). Means in the same row with different superscripts differ significantly; $P < 0.05$. Abbreviations: ECN = equivalent carbon number (ECN = CN−2ND); CN = number of carbon atoms; ND = number of double bonds.

Table 4 Discriminant analysis classification of results

				Total cases correctly classified		
Discriminant analysis	*Selected variables*	*Groups*[b]	*Cases*[a]	*Group*[b]	*No.*	*%*
1	31 TAGs	3	47	1	13	68.4
				2	5	35.7
				3	9	64.3
2	Peak no. 4, LnOO, SOO	3	47	1	17	89.5
				2	9	64.3
				3	14	100
3	ECN (42–52)	3	47	1	16	84.2
				2	10	71.2
				3	12	85.7

[a]19 = Colostrum; 14 = transitional milk; 14 = mature milk. [b]1 = Colostrum; 2 = transitional milk; 3 = mature milk.

To identify the discriminating variables, we used a linear discriminator. Table 4 shows the number of milk samples classified into each milk type (colostrum, transitional milk and mature milk) and the percentage of successful classifications after three discriminant analyses. The discriminant functions in the first analysis were all the 30 TAGs separated in the chromatographic elution, which provides a correct classification of 13 of 19 colostrums (68.4%), five of 14 transitional milk samples (35.7%) and nine of 14 mature milk samples (64.3%). In the second analysis, three TAGs were selected by stepwise analysis and used in the discriminant functions (LnOO, SOO and nonidentified peak no. 4). This selection allows improvement of the milk sample classification, with 100% certainty for the 14 mature milk samples, 89.5% for colostrum (17 of 19) and 64.3% for transitional milk (9 of 14). The third analysis involved the ECN groups of TAGs (ECN 42–52) and provided a correct classification of 16 of the 19 colostrum samples (84.2%), 10 of the 14 transitional milk samples (71.4%) and 12 of the 14 mature milk samples (85.7%). Figures 1–3, respectively, illustrate the results of three discriminant analyses on the space defined by values of the appropriate discriminant functions. The structure matrix or pooled within-groups correlation between discriminating variables and discriminating functions denotes the largest absolute correlation between each variable and any discriminant function for all the analyses. The results are satisfactory, especially in the second analysis. However, in the three cases the best differentiating percentages were for colostrum and mature milk. The major similarities between transitional and mature milk with respect to colostrum agree with the study reported by Jansson *et al* (1981) on the fatty acid composition in human milk. Nevertheless, the two most abundant triacylglycerides in colostrum, POO and POL, which account for more than 41% of total triglycerides, also prevail throughout milk maturation, constituting over 34% and 42% of total transitional milk and mature milk, respectively.

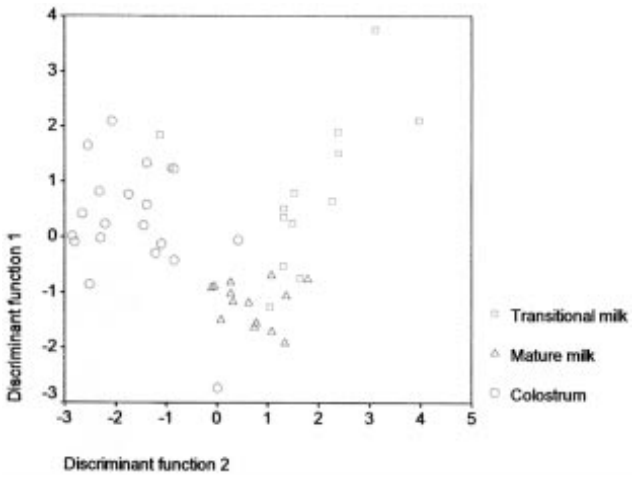

Figure 1 Discriminant analysis using all TAGs as variables.

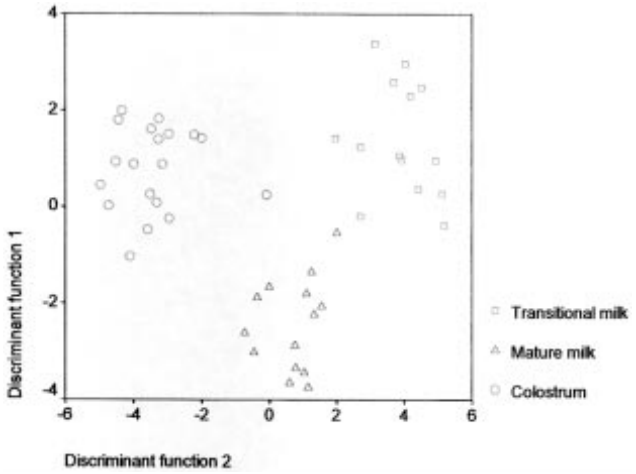

Figure 2 Discriminant analysis using three TAGs as variables.

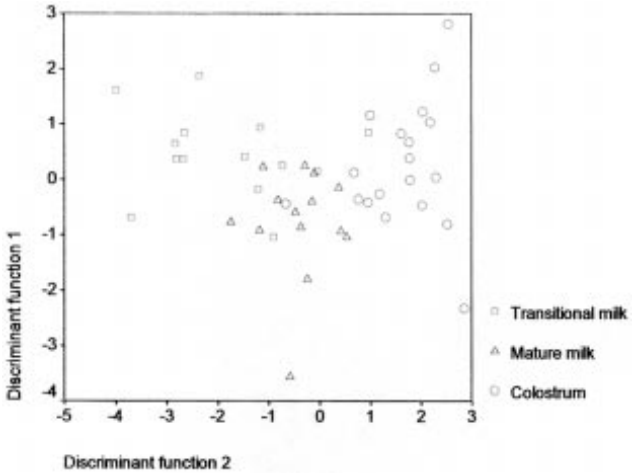

Figure 3 Discriminant analysis using ECN groups as variables.

In conclusion, the results show that, over interindividual differences, a specific profile of this compound is maintained in each different stage of lactation and suggest that the maternal factors which could influence the lipid content, ie weight, age, height, gestational period, parity and also maternal diet, do not affect the triacylglycerol profile of human milk classes, meeting all the needs of the infants at each age.

Acknowledgements—We thank the mothers for their help and co-operation, and the staff of the University Hospital of Granada for their assistance. We also wish to thank Dr MC Ruiz de Villa, who provided valuable help with the statistical analysis and Professor Robin Rycroft for the English revision of the manuscript.

References

Barry Lawrence P (1994): Breast milk. *Pediatr. Clin. N. Am.* **41**, 925–941.

Boersma ER, Offringa JP, Muskict AJ, Chase MW & Simmons JI (1991): Vitamin E, lipid fractions, and fatty acid composition of colostrum, transitional milk, and mature milk: an international comparative study. *Am. J. Clin. Nutr.* **53**, 1197–1204.

Boroviczény OG, Wahle J & Koletzco B (1997): Fatty acid composition of human milk during the 1st month after term and preterm delivery. *Eur. J. Pediatr.* **156**, 142–147.

Chen SC, Shen J & Sheppard AJ (1981): Comparison of methylene chloride and chloroform for the extraction of fats food products. *J. Am. Oil. Chem. Soc.* **58**, 599–601.

Dunn OJ (1964): Multiple comparison using rang sums. *Technometrics* **6**, 241–252.

Emmet P & Rogers IS (1997): Properties of human milk and their relationship with maternal nutrition. *Early Hum. Devl.* **49**, S7–S28.

ESPGAN, Committee on Nutrition (1991). *Acta Paediatr. Scand.* **80**, 887–896.

Gibson AR & Kneebone MG (1981): Fatty acid composition of human colostrum and mature breast milk. *Am. J. Clin. Nutr.* **34**, 252–257.

Goiffon JP, Reminiac C & Furon D (1981): Application de la chromatographie haute performance à l'analyse des triglycride des corps gras. Il Grandeurs de rétention des triglycérides. *Rev. Franç. Corps. Gras.* **28**, 199–206.

Gresti J, Bugaut M, Maniguioni C & Bezard J (1993): Composition of molecular species of triacylglycerols in bovine milk fat. *J. Dairy Sci.* **76**, 1850–1869.

Guesnet P, Antoine JM, Rochette de Lempdes JB, Galent A & Durand G (1993): Dependence of human milk essential fatty acids on adipose stores during lactation. *Am. J. Clin. Nutr.* **58**, 653–659.

Hamosh M (1997): Lipid metabolism in paediatric nutrition. *Pediatr. Clin. N. Am.* **42**, 839–859.

Harzer G, Maug M, Dieterich I & Gentner RP (1938): Changing patterns of human milk lipids in the course of lactation and during the day. *Am. J. Clin. Nutr.* **37**, 612–621.

Jansson L, Akesson B & Holmberg L (1981): Vitamin E and fatty acid composition of human milk. *Am. J. Clin. Nutr.* **34**, 8–13.

Jensen GR, Bitman J, Carlson SE, Couch CS, Hamosh M & Newburg DS (1995): Milk lipids. In: *Handbook of Milk Composition*, pp 495–542. London: Academic Press.

Lyapkov BG & Kiseleva TV (1992): Triglycerides molecular types composition in human milk: influence of lactation stage. *Vopr. Pitan.* **5–6**, 59–61.

Martin ChJ, Bougnoux Ph, Antoine JM & Larson M (1993): Triacylglycerol structure of human colostrum and mature milk. *Lipids* **28**, 637–643.

Morera S, Castellote AI & López-Sabater MC (1998a): Evaluation by high-performance liquid chromatography of the hydrolysis of human milk triacylglycerides during storage at low temperatures. *J. Chromatogr. A.* **823**, 467–474.

Morera S, Castellote AI & López-Sabater MC (1998b): Analysis of human milk triacylglycerol by high-performance liquid chromatography with light-scattering detection. *J. Chromatogr. A.* **823**, 475–482.

Parcerisa J, Rafecas M, Castellote AI, Codony R, Farran A, Garcia J, Lopez A, Romero A & Boatella J (1994): Influence of variety and geographical origin on the lipid fraction of hazelnuts (*Coryllus avellana* L) from Spain. 2. Triglyceride composition. *Food Chem.* **50**, 245–249.

Ruel TM, Dewey GK, Martinez C, Flores R & Brown K (1997): Validation of single daytime samples of human milk to estimate the 24th concentration of lipids in urban Guatemalan mothers. *Am. J. Clin. Nutr.* **65**, 439–444.