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ORIGINAL COMMUNICATION

Fatty acid and *sn*-2 fatty acid composition in human milk from Granada (Spain) and in infant formulas

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Objective: To investigate differences in fatty acid and *sn*-2 fatty acid composition in colostrum, transitional and mature human milk, and in term infant formulas.
Setting: Departament de Nutrició i Bromatologia, University of Barcelona, Spain and University Hospital of Granada, Spain.
Subjects: One-hundred and twenty mothers and 11 available types of infant formulas for term infants.
Design: We analysed the fatty acid composition of colostrum ($n=40$), transitional milk ($n=40$), mature milk ($n=40$) and 11 infant formulas. We also analysed the fatty acid composition at *sn*-2 position in colostrum ($n=12$), transitional milk ($n=12$), mature milk ($n=12$), and the 11 infant formulas.
Results: Human milk in Spain had low saturated fatty acids, high monounsaturated fatty acids and high linolenic acid. Infant formulas and mature human milk had similar fatty acid composition. In mature milk, palmitic acid was preferentially esterified at the *sn*-2 position (86.25%), and oleic and linoleic acids were predominantly esterified at the *sn*-1,3 positions (12.22 and 22.27%, respectively, in the *sn*-2 position). In infant formulas, palmitic acid was preferentially esterified at the *sn*-1,3 positions and oleic and linoleic acids had higher percentages at the *sn*-2 position than they do in human milk.
Conclusion: Fatty acid composition of human milk in Spain seems to reflect the Mediterranean dietary habits of mothers. Infant formulas resemble the fatty acid profile of human milk, but the distribution of fatty acids at the *sn*-2 position is markedly different.
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Keywords: colostrum; transitional milk; mature milk; infant formula; fatty acids; *sn*-2 position

Introduction

Human milk is considered the ideal food for full-term infants. Its special composition fulfils the new-born's nutritional and physiological needs during the first 6 months dof life (ESPGAN, Committee in Nutrition 1982, 1991; Department of Health and Social Security, 1988; American Academy of Paediatrics, 1982). Human milk is a very complex mixture of nutrients and non-nutritional factors which provide nourishment and aid the growth and development of the neonate (Jensen, 1995). Human milk can be divided according to the stage of lactation into colostrum (1–5 days post-delivery), transitional milk (6–15 days post-delivery) and mature milk (over 15 days post-delivery; WHO, 1985).

The fat content and fatty acid composition of human milk is variable. Milk's fatty acid composition is influenced by certain factors, ie diet, duration of pregnancy, maternal parity, stage of lactation, etc. Maternal diet appears to be the most important variable determining milk's fatty acid composition: differences observed in various geographic

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regions are primarily attributed to dietary differences (Rodriguez *et al*, 1999; Jensen *et al*, 1992). Fatty acids in milk lipids (90% of TG as esters) are one of the components that can be altered to some extent by maternal dietary manipulation (Jensen, 1999).

During the neonatal period the new-born has a high demand for essential nutrients as well as for an adequate energy supply. In human milk, and in most infant formulas, *ca* 50% of the dietary calories are supplied to the newborn as fat, and more than 98% of milk fat is in the form of triglycerides (TG), which contain saturated, monounsaturated and polyunsaturated fatty acids esterified to glycerol (Manson & Weaver, 1997; Giovannini *et al*, 1995; Small, 1991). Human milk contains both parent essential fatty acids (linoleic and α-linolenic acids) and very long-chain polyunsaturated fatty acids. A balanced amount of these fatty acids is required for the normal maturing and function of the nervous system (Agostoni *et al*, 1995; Makrides *et al*, 1995), as well as for adequate production of eicosanoids (Uauy *et al*, 2000; Sellmayer & Koletzko, 1999; Bjerve *et al*, 1987).

Most saturated fatty acid in human milk is palmitic acid ($C_{16:0}$), which represents about 20–25% of milk's total fatty acids. Triacylglycerols are stereo-specific and the three ester bonds are not equally susceptible to hydrolysis by lipase enzymes. Fatty acids are not randomly distributed among the three stereo-specific numbering (*sn*) positions, but are found selectively placed so as to provide the ideal mixture of fatty acids and monoacylglycerides for the neonate (Small, 1991; Jensen, 1989). In human milk, palmitic acid is esterified over 60% in the *sn*-2 position of the triglycerides (Jensen, 1995; Dotson *et al*, 1992; Tomarelli *et al*, 1968).

Vegetable oils and butterfat are the main constituents of infant formula fat. In these fats palmitic acid is predominantly esterified at the *sn*-1, 3 positions of the triglycerides (de Fown *et al*, 1994; Karleskind, 1992). Thus, while these formulas resemble the fatty acid profile of human milk, the percentage of palmitic acid in the *sn*-2 position is lower than in human milk. The distribution of the fatty acids between the outer and the *sn*-2 position in the glycerol molecule modifies the rate of intestinal fatty acid uptake (Decker, 1996; Innis *et al*, 1994; Bracco, 1994; Small, 1991).

Studies comparing palmitic acid absorption in human milk and formulas concluded that absorption of palmitic acid was higher in human milk (Chappel *et al*, 1986; Hanna *et al*, 1970; Tomarelli *et al*, 1968). Palmitic acid was also absorbed more with formulas rich in palmitic acid esterified at the *sn*-2 position of the triglycerides than with formulas containing palmitic acid largely esterified to the *sn*-1, 3 positions. These results were obtained in studies carried out on term infants (Carnielli *et al*, 1996; Filler *et al*, 1969), preterm infants (Carnielli *et al*, 1995) and rats (Aoyama *et al*, 1996; de Fown *et al*, 1994; Lien *et al*, 1993; Renaud *et al*, 1995; Tomarelli *et al*, 1968). Moreover, the location of palmitic acid in the *sn*-2 position in infant formulas improves the absorption of calcium in the small intestine (Lucas *et al*, 1997; Carnielli *et al*, 1996; Nelson *et al*, 1996).

The aim of this study is to find the fatty acid composition and the *sn*-2 fatty acid compositions of colostrum, transitional milk and mature milk from healthy mothers living in Granada, a Mediterranean area in the South of Spain. We also analysed fatty acid composition and *sn*-2 fatty acid composition in 11 infant formulas, in order to compare them with mature milk. Since, feeding with infant formula is necessary in circumstances where breast-feeding is impossible, insufficient or undesired, infant formulas should be as similar as possible to human milk.

Materials and methods

Subjects

One-hundred and twenty apparently healthy Spanish women from Granada, who gave birth a term infant (38–42 weeks of gestation) and breast-fed their babies, participated in the study. This voluntary study was explained to all mothers who gave their written consent. The project was approved by the Hospital Ethics and Scientific Committees. All mothers had similar educational background, dietary habits and were between 20 and 35 y old. The main characteristics of the mothers sampled are shown in Table 1.

Samples

Milk samples were collected from both breasts by means of an Ico® 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, for one day to minimize the effects of diurnal rhythm on the composition of the milk (Lammi-Keefe *et al*, 1990).

After extraction, samples were preserved at −20°C and the following day were taken to the hospital, where they were preserved at −70°C. Then, samples were sent by air in a cryogenic recipient with liquid nitrogen (−70°C) to our laboratory in Barcelona, where samples were stored at −70°C until analysis (0–1 month). Before analysis samples from each mother were thawed in a water bath at 37°C and pooled using a magnetic stirrer, at which point aliquots were separated for the different assays. Samples obtained between the 1st and 6th day post-delivery were assigned to the colostrum group ($n = 40$, 2.75 ± 1.10 days); samples obtained between the 7th and 15th day post-delivery were assigned to

Table 1 Characreristics of the sampled mothers

Type of delivery (vaginal/caesarean)	51/69
Gestational period (weeks)[a]	39.33 ± 0.97
Age (y)[a]	27.13 ± 3.21
Height (cm)[a]	159.31 ± 6.83
Weight (kg)[a]	58.74 ± 11.86
Parity[a]	1.56 ± 0.71

[a]Mean ± s.d.

the transitional group ($n=40$, 11.00 ± 1.87 days); and samples obtained after the 15th day post-delivery were assigned to the mature milk group ($n=40$, 23.94 ± 5.14 days). The *sn*-2 fatty acid composition of human milk was analysed in 12 samples of each human milk group.

Eleven different infant formulas for term infants were analysed. The formulas were Blemil 1 Plus and Blemil 1 AE (Ordesa S.L., St Boi del Llobregat, Spain), Adapta 1 (Novartis Nutrition S.A., Barcelona, Spain), Almiron 1 and Almiron Omneo (Nutricia S.A., Madrid, Spain), Conformil 1 (Milupa S.A., Madrid, Spain), Miltina 1 (Milte, Barcelona, Spain), Modar 1 (Novartis Nutrition S.A., Barcelona, Spain), Nativa 1 (Nestlé S.A., Esplugues de Llobregat, Spain), Nenatal (N. V. Nutricia, Zoetermeer, Holland) and Nutriben Natal SMA (Alter Farmacia S.A., Madrid, Spain). The formulas were purchased in pharmacies and analysed prior to their expiry date. Infant formulas were randomly codified as IF 1–11.

Determination of human milk's fatty acid composition

The fatty acid composition of the milk was analysed by capillary gas chromatography with split injection and flame ionization detection (López-López *et al*, 2001a). In brief, 100 µl of human milk with the internal standards were placed in test tubes closed with teflon-lined caps. Then, we first used a basic catalyst in the presence of anhydrous methanol (sodium methylate 0.5% w/v) because under these conditions milk tri-, di- and monoglycerides are completely transesterified in a few minutes (15 min). Subsequently, we used an acidic catalyst in the presence of anhydrous methanol (boron trifluoride in methanol 20%) as a rapid (15 min) esterification mean of milk's free fatty acids (Christie, 1992). Then, 400 µl of *n*-hexane were added to the tube. The clear *n*-hexane top layer, containing the FAMEs, was transferred with a micropipette into an automatic injector vial equipped with a volume adapter of 300 µl. The vial was stored at -20°C until injection into the gas chromatograph.

Fatty acid composition of human milk in *sn*-2 position

A lipid extract from human milk was obtained by extraction with organic solvents (Morera *et al*, 1998). We then followed the AOCS official method (1997) to determine fatty acids at the *sn*-2 position. In brief, this method consists of hydrolysis of the triglycerides with pancreatic lipase, and subsequent separation of the *sn*-2 monoglycerides by TLC. Fatty acids at the *sn*-2 position of the monoglycerides were then methylated and analysed by capillary gas chromatography with flame ionisation detection (López-López *et al*, 2001a).

Determination of infant formula fatty acid composition

The fatty acid composition of the infant formula was analysed by capillary gas chromatography with split injection and flame ionization detection (Park & Goins, 1994). In brief, 100 µg of infant formula with the internal standards were placed in test tubes closed with teflon-lined caps. We then used a basic catalyst with anhydrous methanol (sodium methylate 0.5% w/v). Under these conditions, milk tri-, di- and monoglycerides are completely transesterified in a few minutes (15 min). Subsequently, we used an acidic catalyst in the presence of anhydrous methanol (boron trifluoride in methanol 20%) as a rapid (15 min) esterification mean of milk's free fatty acids (Christie, 1992). After that, 400 µl of *n*-hexane were added to the tube. The clear *n*-hexane top layer, containing the FAMEs, was transferred with a micropipette into an automatic injector vial equipped with a volume adapter of 300 µl. The vial was stored at -20°C until injection into the gas chromatograph.

Fatty acid composition of infant formula in *sn*-2 position

The lipid fraction from infant formulas was extracted by using dichloromethane/methanol (2:1, v/v), in line with a modification of the method proposed by Folch *et al* (1957). Dichloromethane was chosen instead of chloroform due to its lower toxicity and equal extracting capacity (Chen *et al*, 1981).

The fatty acid composition in the *sn*-2 position was then determined by HPLC-ELSD (López-López *et al*, 2001b). Briefly, this method consists of purifying the fat through alumina, then hydrolysing the triglycerides with pancreatic lipase. After this, the hydrolysis products were dissolved in 3 ml of HPLC-grade acetone. The samples were then filtered through a 0.45 µm filter and stored at -20°C until injection into the HPLC-ELSD. The chromatographic separation of *sn*-2 monoglycerides was carried out using an isocratic elution with acetonitrile-acidified water, with the flow-rate of the eluent at 1 ml/min and the column temperature 30°C. The volume of sample injected was 5 µl. The mass detector oven was at 55°C and the gas flow (from air compressor) was 10 l/min.

Statistical analysis

All determinations were made in duplicate. The results of colostrum, transitional milk, mature milk and infant formulas are reported as means (wt%) and standard deviations (s.d.). Results were evaluated with Statgraphics Plus for Windows 1.4 (Statistical Graphics Corporation, USA). The statistical analysis included one-way analysis of variance (ANOVA) for differences between human milk groups. The level of statistical significance was set at 5% for all analyses. Infant formula data were considered different if they fell outside the mean ± 2 s.d. range of corresponding mature milk data, as proposed by Huisman *et al*, (1996).

Table 2 Fatty acid composition of colostrum, transitional and mature milk (% wt/wt)

	Colostrum (n = 40)		*Transitional* (n = 40)		*Mature* (n = 40)	
Fatty acids	*Mean ± s.d.*	*Range*	*Mean ± s.d.*	*Range*	*Mean ± s.d.*	*Range*
C8:0	0.07[a,b] ± 0.04	0.00–0.21	0.19 ± 0.07	0.06–0.35	0.21 ± 0.06	0.11–0.36
C10:0	0.66[a,b] ± 0.37	0.15–1.87	1.66 ± 0.57	0.55–3.10	1.63 ± 0.47	0.85–3.08
C12:0	3.49[a,b] ± 1.77	1.40–8.76	6.97 ± 2.21	3.00–11.31	6.28 ± 1.33	4.05–9.35
C14:0	4.75[a,b] ± 1.65	2.46–8.93	6.94 ± 1.88	4.03–11.63	6.00 ± 1.44	3.60–9.13
C14:1 *n*-5	0.14 ± 0.07	0.00–0.35	0.16 ± 0.08	0.06–0.44	0.20 ± 0.10	0.00–0.53
C15:0	0.23 ± 0.05	0.16–0.39	0.25 ± 0.17	0.09–1.11	0.23 ± 0.07	0.11–0.48
C15:1	0.03 ± 0.03	0.00–0.12	0.04 ± 0.03	0.00–0.10	0.04 ± 0.03	0.00–0.12
C16:0	21.17[a,b] ± 1.60	17.68–23.94	19.35 ± 2.03	15.64–23.33	19.48 ± 2.06	15.43–24.46
C16:1 *n*-9	0.62[a,b] ± 0.32	0.42–2.17	0.43 ± 0.08	0.27–0.61	0.42 ± 0.07	0.29–0.58
C16:1 *n*-7	1.36[b] ± 0.46	0.00–2.46	1.52 ± 0.48	0.92–2.90	1.78 ± 0.52	1.10–2.81
C17:0	0.35[a,b] ± 0.04	0.28–0.44	0.31 ± 0.07	0.18–0.43	0.30 ± 0.05	0.19–0.44
C17:1	0.19 ± 0.03	0.13–0.26	0.18 ± 0.04	0.11–0.28	0.19 ± 0.05	0.10–0.34
C18:0	6.29 ± 0.80	4.66–8.20	6.20 ± 0.85	4.27–7.49	6.25 ± 0.99	4.60–8.13
C18:1 *n*-9	38.83[a,b] ± 3.76	28.54–45.88	35.48 ± 2.95	29.34–39.77	36.35 ± 3.46	28.30–43.83
C18:2 *n*-6	16.10 ± 2.73	11.19–22.80	15.74 ± 3.73	8.80–24.10	16.59 ± 4.10	10.61–25.30
C18:3 *n*-6	0.06[b] ± 0.06	0.00–0.18	0.07[c] ± 0.04	0.00–0.18	0.13 ± 0.07	0.00–0.27
C20:0	0.19 ± 0.06	0.00–0.31	0.19 ± 0.04	0.07–0.29	0.20 ± 0.05	0.13–0.35
C18:3 *n*-3	0.41[a,b] ± 0.07	0.23–0.56	0.58 ± 0.13	0.31–0.79	0.75 ± 0.32	0.41–1.68
C20:1 *n*-9	0.94[a,b] ± 0.19	0.64–1.50	0.68 ± 0.18	0.36–1.19	0.60 ± 0.13	0.34–0.82
C20:2 *n*-6	0.86[a,b] ± 0.15	0.54–1.19	0.56[c] ± 0.15	0.34–0.94	0.40 ± 0.06	0.28–0.55
C20:3 *n*-6	0.65[a,b] ± 0.22	0.38–1.57	0.49 ± 0.13	0.26–0.80	0.48 ± 0.14	0.25–0.73
C22:0	0.08 ± 0.11	0.00–0.67	0.04 ± 0.04	0.00–0.10	0.05 ± 0.03	0.00–0.09
C20:4 *n*-6	0.74[a,b] ± 0.14	0.46–1.12	0.67[c] ± 0.14	0.50–0.97	0.53 ± 0.12	0.23–0.75
C22:1 *n*-9	0.18[a,b] ± 0.11	0.00–0.66	0.10[c] ± 0.06	0.00–0.27	0.06 ± 0.03	0.00–0.10
C23:0	0.02 ± 0.03	0.00–0.11	0.04 ± 0.03	0.00–0.11	0.05 ± 0.03	0.00–0.08
C20:5 *n*-3	0.19[a,b] ± 0.03	0.13–0.24	0.11 ± 0.05	0.05–0.23	0.10 ± 0.05	0.00–0.24
C24:0	0.09 ± 0.05	0.00–0.22	0.05 ± 0.06	0.00–0.20	0.03 ± 0.02	0.00–0.07
C22:4 *n*-6	0.44[a,b] ± 0.16	0.24–0.80	0.23[c] ± 0.17	0.10–0.88	0.15 ± 0.04	0.09–0.24
C22:4 *n*-3	0.08[b] ± 0.03	0.00–0.14	0.07[c] ± 0.03	0.00–0.12	0.04 ± 0.03	0.00–0.08
C22:5 *n*-6	0.04[b] ± 0.04	0.00–0.13	0.03 ± 0.04	0.00–0.11	0.01 ± 0.05	0.00–0.22
C22:5 *n*-3	0.20[b] ± 0.06	0.09–0.36	0.16[c] ± 0.06	0.08–0.33	0.12 ± 0.02	0.08–0.16
C22:6 *n*-3	0.56[b] ± 0.16	0.39–0.92	0.50[c] ± 0.15	0.29–0.79	0.36 ± 0.12	0.15–0.56
SFA	37.37[a,b] ± 3.72	30.06–46.24	42.15 ± 3.44	35.86–52.40	40.66 ± 3.87	34.18–47.48
MUFA	42.29[a,b] ± 4.02	31.14–49.71	38.59 ± 3.24	32.05–43.31	39.63 ± 3.57	32.08–47.34
PUFA	20.34 ± 2.73	16.05–27.00	19.26 ± 3.73	11.63–27.98	19.71 ± 4.38	13.26–29.15
PUFA *n*-3	1.44 ± 0.19	0.93–1.74	1.42 ± 0.37	0.86–2.44	1.38 ± 0.45	0.81–3.06
PUFA *n*-6	18.89 ± 2.68	14.81–25.77	17.79 ± 3.70	10.61–26.00	18.33 ± 4.22	12.10–27.77
LC-PUFA *n*-3	1.03[b] ± 0.19	0.58–1.44	0.84[c] ± 0.29	0.53–1.60	0.63 ± 0.18	0.34–0.89
LC-PUFA *n*-6	2.73[a,b] ± 0.38	1.98–3.43	1.98[c] ± 0.47	1.39–3.50	1.61 ± 0.26	1.17–2.16
n-6/*n*-3	14.70 ± 2.68	10.26–22.85	13.08 ± 4.34	6.63–22.20	13.99 ± 4.02	7.31–21.13
LA/LnA	42.02[a,b] ± 7.84	26.14–59.70	30.56 ± 8.62	18.19–50.96	24.84 ± 6.57	14.41–39.49
AA/DHA	1.48 ± 0.36	1.07–2.39	1.41 ± 0.44	0.58–2.25	1.61 ± 0.56	0.61–2.55

[a]Denotes significant differences ($P < 0.05$) between colostrum and mature milk groups.
[b]Denotes significant differences ($P < 0.05$) between colostrum and transitional milk groups.
[c]Denotes significant differences ($P < 0.05$) between transitional and mature milk groups.
ND, not detectable.
LA/LnA, linoleic acid/linolenic acid; AA/DHA, arachidonic acid/docosahexaenoic acid.

Results

Fatty acid composition of human milk

Table 2 shows the fatty acid composition of colostrum, transitional and mature milk. Thirty-two fatty acids were identified and quantified. A comparison between values obtained in the various stages of lactation showed significant differences in fatty acid composition from colostrum to mature milk. Significant differences ($P < 0.02$) in total fatty acid amount were found between colostrum (20.2 mg/dl), transitional milk (25.9 mg/dl) and mature milk (32.8 mg/dl).

Saturated fatty acids (SFA) in human milk

We observed a significant increase ($P < 0.0001$) for $C_{8:0}$, $C_{10:0}$, $C_{12:0}$ and $C_{14:0}$ between the colostrum group and the transitional and mature milk groups. Palmitic acid ($C_{16:0}$), which is the major SFA, showed a significant decrease ($P < 0.001$) from the colostrum group to the transitional and mature milk groups. The other saturated fatty acids did not show significant differences. The transitional and mature milk groups showed no significant differences for saturated fatty acids. Total SFA showed

Table 3 Fatty acid composition of infant formulas (% wt/wt)

Fatty acids	IF1 Mean ± s.d.	IF2 Mean ± s.d.	IF3 Mean ± s.d.	IF4 Mean ± s.d.	IF5 Mean ± s.d.	IF6 Mean ± s.d.	IF7 Mean ± s.d.	IF8 Mean ± s.d.	IF9 Mean ± s.d.	IF10 Mean ± s.d.	IF11 Mean ± s.d.
C4:0	0.05 ± 0.00	0.16 ± 0.00	0.09 ± 0.00	0.03 ± 0.00	0.00 ± 0.00	0.00 ± 0.00	0.03 ± 0.00	0.02 ± 0.00	0.00 ± 0.00	0.00 ± 0.00	0.05 ± 0.00
C6:0	0.10 ± 0.00	0.24 ± 0.01	0.19 ± 0.00	0.07 ± 0.00	0.06 ± 0.00	0.03 ± 0.00	0.18 ± 0.00	0.07 ± 0.00	0.19 ± 0.01	0.07 ± 0.00	0.07 ± 0.00
C8:0	0.56[a] ± 0.01	0.75[a] ± 0.01	0.55[a] ± 0.00	1.20[a] ± 0.05	0.80[a] ± 0.02	0.81[a] ± 0.04	0.51[a] ± 0.01	1.00[a] ± 0.02	0.86[a] ± 0.02	0.97[a] ± 0.02	0.93 ± 0.01
C10:0	0.74 ± 0.02	1.14 ± 0.01	0.84 ± 0.01	1.24 ± 0.04	0.89 ± 0.05	1.10 ± 0.05	0.81 ± 0.00	0.99 ± 0.02	0.89 ± 0.01	0.97 ± 0.01	0.98 ± 0.03
C12:0	7.74 ± 0.16	5.57 ± 0.02	5.65 ± 0.07	10.87[a] ± 0.32	11.50[a] ± 0.15	11.21[a] ± 0.30	5.19 ± 0.07	8.52 ± 0.08	12.64[a] ± 0.07	8.38 ± 0.04	12.22[a] ± 0.10
C14:0	4.39 ± 0.04	5.11 ± 0.01	4.48 ± 0.02	5.01 ± 0.11	4.84 ± 0.02	5.25 ± 0.07	4.14 ± 0.04	4.34 ± 0.02	5.91 ± 0.13	4.06 ± 0.01	4.79 ± 0.01
C14:1 *n*-5	0.09 ± 0.00	0.27 ± 0.00	0.21 ± 0.00	0.00 ± 0.00	0.07 ± 0.00	0.01 ± 0.00	0.20 ± 0.00	0.01 ± 0.00	0.02 ± 0.00	0.02 ± 0.00	0.05 ± 0.00
C15:0	0.16 ± 0.00	0.35 ± 0.00	0.28 ± 0.00	0.05[a] ± 0.00	0.10 ± 0.00	0.06[a] ± 0.00	0.25 ± 0.00	0.06[a] ± 0.00	0.07[a] ± 0.00	0.06[a] ± 0.00	0.13 ± 0.00
C16:0	23.87[a] ± 0.25	23.86[a] ± 0.08	26.75[a] ± 0.04	17.96 ± 0.04	18.97 ± 0.11	23.60 ± 0.05	27.42[a] ± 0.14	23.83[a] ± 0.02	22.98 ± 0.06	21.90 ± 0.01	18.18 ± 0.07
C16:1 *n*-7	0.52[a] ± 0.00	0.78 ± 0.00	0.53[a] ± 0.01	0.16[a] ± 0.00	0.16[a] ± 0.01	0.15[a] ± 0.00	0.51[a] ± 0.00	0.16[a] ± 0.00	0.14[a] ± 0.00	0.15[a] ± 0.00	0.14[a] ± 0.01
C17:0	0.17[a] ± 0.00	0.30 ± 0.00	0.22 ± 0.00	0.07[a] ± 0.00	0.09[a] ± 0.00	0.08[a] ± 0.00	0.22 ± 0.00	0.08[a] ± 0.00	0.08[a] ± 0.00	0.10[a] ± 0.00	0.08[a] ± 0.00
C17:1	0.07[a] ± 0.00	0.14 ± 0.00	0.09 ± 0.00	0.04[a] ± 0.00	0.04[a] ± 0.00	0.03[a] ± 0.00	0.09 ± 0.00	0.03[a] ± 0.00	0.04[a] ± 0.00	0.04[a] ± 0.00	0.04[a] ± 0.00
C18:0	4.55 ± 0.07	6.72 ± 0.02	4.82 ± 0.02	3.33[a] ± 0.02	3.51[a] ± 0.00	3.69[a] ± 0.07	4.95 ± 0.07	3.66[a] ± 0.01	3.05[a] ± 0.02	4.40 ± 0.01	3.24[a] ± 0.00
C18:1 *n*-9	40.40 ± 0.20	38.09 ± 0.17	36.59 ± 0.06	44.69[a] ± 0.34	42.09 ± 0.16	34.34 ± 0.25	36.24 ± 0.08	37.34 ± 0.08	41.52 ± 0.13	37.94 ± 0.10	41.44 ± 0.12
C18:2 *n*-6	13.88 ± 0.08	13.55 ± 0.10	16.42 ± 0.02	11.06 ± 0.04	12.67 ± 0.01	16.50 ± 0.10	17.02 ± 0.19	16.76 ± 0.05	8.93 ± 0.07	18.43 ± 0.09	13.35 ± 0.04
C20:0	0.26 ± 0.00	0.38[a] ± 0.00	0.31[a] ± 0.00	0.40[a] ± 0.01	0.60[a] ± 0.00	0.35[a] ± 0.00	0.29 ± 0.00	0.36[a] ± 0.00	0.22 ± 0.00	0.30[a] ± 0.00	0.62[a] ± 0.00
C18:3 *n*-3	1.25 ± 0.02	1.23 ± 0.00	1.25 ± 0.00	2.78[a] ± 0.00	2.69[a] ± 0.00	1.96[a] ± 0.01	1.45 ± 0.03	2.05[a] ± 0.01	0.67 ± 0.00	1.56 ± 0.10	2.83[a] ± 0.00
C20:1 *n*-9	0.45 ± 0.02	0.45 ± 0.00	0.21[a] ± 0.00	0.62 ± 0.01	0.53 ± 0.00	0.40 ± 0.00	0.17[a] ± 0.00	0.39 ± 0.00	0.47 ± 0.01	0.20[a] ± 0.00	0.49 ± 0.00
C20:2 *n*-6	0.06[a] ± 0.00	0.06[a] ± 0.00	0.06[a] ± 0.00	0.05[a] ± 0.00	ND ± 0.00	0.11[a] ± 0.00	0.04[a] ± 0.00	0.04[a] ± 0.00	0.41 ± 0.01	0.03[a] ± 0.00	ND ± 0.00
C20:3 *n*-6	0.03[a] ± 0.00	0.04[a] ± 0.00	0.01[a] ± 0.00	ND ± 0.00	ND ± 0.00	0.01[a] ± 0.00	0.02[a] ± 0.00	ND ± 0.00	0.02[a] ± 0.00	ND ± 0.00	ND ± 0.00
C22:0	0.15[a] ± 0.00	0.25[a] ± 0.00	0.14[a] ± 0.00	0.25[a] ± 0.01	0.34[a] ± 0.00	0.20[a] ± 0.00	0.12[a] ± 0.00	0.19[a] ± 0.00	0.17[a] ± 0.01	0.30[a] ± 0.00	0.32[a] ± 0.00
C20:4 *n*-6	0.22[a] ± 0.00	0.22 ± 0.00	0.03[a] ± 0.00	ND ± 0.00	ND ± 0.00	ND ± 0.00	0.02[a] ± 0.00	ND ± 0.00	0.35 ± 0.00	ND ± 0.00	ND ± 0.00
C20:5 *n*-3	0.04 ± 0.00	0.05 ± 0.00	0.05 ± 0.00	0.00 ± 0.00	0.00 ± 0.00	0.00 ± 0.00	0.02 ± 0.00	0.00 ± 0.00	0.07 ± 0.00	0.00 ± 0.00	0.00 ± 0.00
C24:0	0.08 ± 0.00	0.11[a] ± 0.09	0.09[a] ± 0.00	0.11[a] ± 0.00	0.05 ± 0.00	0.10[a] ± 0.00	0.08 ± 0.00	0.09[a] ± 0.00	0.09[a] ± 0.01	0.12[a] ± 0.00	0.05 ± 0.00
C22:5 *n*-3	0.03[a] ± 0.00	0.04[a] ± 0.00	0.03[a] ± 0.00	ND ± 0.00	ND ± 0.00	ND ± 0.00	0.02[a] ± 0.00	ND ± 0.00	ND ± 0.00	ND ± 0.00	ND ± 0.00
C22:6 *n*-3	0.14 ± 0.00	0.14 ± 0.00	0.13 ± 0.01	ND ± 0.00	ND ± 0.00	ND ± 0.00	ND ± 0.00	ND ± 0.00	0.20 ± 0.01	ND ± 0.00	ND ± 0.00
Total	100.00 ± 0.00	100.00 ± 0.00	100.00 ± 0.00	100.00 ± 0.00	100.00 ± 0.00	100.00 ± 0.00	100.00 ± 0.00	100.00 ± 0.00	100.00 ± 0.00	100.00 ± 0.00	100.00 ± 0.00
SFA	42.82	44.94	44.40	40.60	41.75	46.47	44.20	43.22	47.16	41.64	41.66
MUFA	41.53	39.73	37.62	45.50	42.89	34.94	37.21	37.93	42.19	38.35	42.16
PUFA	15.65	15.33	17.97	13.89	15.36	18.59	18.59	18.85	10.65[a]	20.02	16.18
PUFA *n*-3	1.46	1.46	1.45	2.78[a]	2.69[a]	1.96	1.49	2.05	0.94	1.56	2.83[a]
PUFA *n*-6	14.19	13.87	16.52	11.11	12.67	16.63	17.09	16.80	9.71	18.46	13.35
LA/LnA	11	11	13	4[a]	5	8	12	8	13	12	5
AA/DHA	1.57	1.57	0.23[a]						1.77		

ND, not detectable.
LA/LnA, linoleic acid/Linolenic acid; AA/DHA, arachidonic acid/docosahexaenoic acid.
[a]Denotes differences with mature milk (values were considered different if they fell outside the mean ± 2 s.d. of corresponding mature milk).

significant differences between the colostrum (37.37%) group and the transitional (42.15%) and mature (40.66%) milk groups.

Monounsaturated fatty acids (MUFA) in human milk

Significantly lower percentages were found in transitional and mature milk groups for $C_{16:1}$ *n*-9, $C_{18:1}$ *n*-9 ($P < 0.002$), $C_{20:1}$ *n*-9 ($P < 0.0001$) and $C_{22:1}$ *n*-9 ($P < 0.0001$) than in colostrum group. Moreover, we obtained a significant increase for $C_{16:1}$ *n*-7 ($P < 0.01$) between colostrum and mature milk, and a significant decrease for $C_{22:1}$ *n*-9 ($P < 0.001$) between the transitional and mature milk groups. Total MUFA decreased significantly from the colostrum group (42.29%) to the transitional milk (38.59%) and mature milk (39.63%) groups.

Polyunsaturated fatty acids (PUFA) in human milk

The total PUFA, PUFA *n*-3 and PUFA *n*-6 content of milk did not significantly vary with the stage of lactation. Linoleic ($C_{18:2}$ *n*-6, LA) and linolenic ($C_{18:3}$ *n*-3, LnA) acids are the most important PUFA: both these fatty acids are essential, that is, they must be supplied in the diet because they cannot be synthesized in humans. LA was the only PUFA that significantly increased ($P < 0.0001$) with the stage of lactation, while LnA did not change. The ratio LA/LnA was significantly higher in colostrum than in transitional and mature milk.

Arachidonic acid ($C_{20:4}$ *n*-6, AA) significantly decreased with the stage of lactation ($P < 0.01$). Docosahexaenoic acid ($C_{22:6}$ *n*-3, DHA) percentages in colostrum and transitional milk were significantly higher ($P < 0.001$) than in mature milk. AA and DHA are derived from LA and LnA, respectively. The ratio AA/DHA did not vary with maturation of milk.

The percentage in long-chain polyunsaturated fatty acids *n*-3 (LC-PUFAs *n*-3) in human milk lipids was significantly higher in colostrum and transitional milk than in mature milk, and LC-PUFA *n*-6 decreased significantly from colostrum to transitional and mature milk.

Fatty acid composition of infant formulas

Table 3 shows the fatty acid composition of infant formulas. We identified and quantified 26 fatty acids. We compared the fatty acid composition of mature milk with infant formulas. We considered significant differences when the percentages of infant formulas were out of the range of mature milk mean ± 2 s.d. The percentages of fatty acids determined in the mature milk and infant formulas were similar.

The contribution of the SFAs in the infant formulas was comparable to that in mature milk. However, some infant

Table 4 *Sn*-2 fatty acid composition (% wt/wt) of colostrum, transitional, mature milk and infant formulas (IF1-IF11)

Fatty acids	*Colostrum Mean ± s.d. (n = 12)*	*Transitional Mean ± s.d. (n = 12)*	*Mature Mean ± s.d. (n = 12)*	*IF1 Mean*	*IF2 Mean*	*IF3 Mean*	*IF4 Mean*	*IF5 Mean*	*IF6 Mean*	*IF7 Mean*	*IF8 Mean*	*IF9 Mean*	*IF10 Mean*	*IF11 Mean*
C8:0	ND	ND	ND	ND	0.07	0.05	0.05	0.04	0.05	0.03	0.03	0.09	0.05	0.04
C10:0	$0.21^{a,b} \pm 0.07$	$0.29^{c} \pm 0.05$	0.36 ± 0.10	0.16	1.54^{d}	0.41	0.44	0.26	0.37	0.37	0.31	0.36	0.35	0.27
C12:0	$2.41^{a,b} \pm 0.83$	4.61 ± 1.57	4.81 ± 0.81	8.11^{d}	9.85^{d}	14.67	5.01	10.58^{d}	15.32^{d}	4.68	11.62^{d}	11.45^{d}	11.50^{d}	11.92^{d}
C14:0	$6.69^{a,b} \pm 1.33$	$10.74^{c} \pm 1.48$	9.66 ± 1.61	3.62^{d}	7.10	2.64^{d}	4.53^{d}	3.86^{d}	3.14^{d}	4.57^{d}	2.23^{d}	5.58^{d}	2.57^{d}	4.05^{d}
C14:1 *n*-5	ND	ND	ND	ND	0.05	0.03	ND	ND	0.02	0.34	0.03	0.04	0.05	ND
C15:0	0.51 ± 0.08	0.46 ± 0.11	0.53 ± 0.11	ND	0.02^{d}	0.03^{d}	0.02	0.05^{d}	0.04^{d}	0.31	0.04^{d}	0.12^{d}	0.05^{d}	0.05^{d}
C16:0	52.23 ± 2.72	51.17 ± 4.91	52.30 ± 4.44	32.67^{d}	15.05^{d}	5.88^{d}	12.41^{d}	22.08^{d}	8.67^{d}	13.23^{d}	6.22^{d}	43.01^{d}	8.11^{d}	22.57^{d}
C16:1 *n*-7	1.97 ± 0.49	1.72 ± 0.32	1.88 ± 0.51	0.21^{d}	0.17^{d}	0.11^{d}	0.08^{d}	0.09^{d}	0.11^{d}	0.52^{d}	0.12^{d}	0.09^{d}	0.12^{d}	0.09^{d}
C17:0	0.38 ± 0.02	0.37 ± 0.06	0.37 ± 0.06	ND	ND	0.02^{d}	0.13^{d}	0.05^{d}	0.03^{d}	0.14^{d}	0.03^{d}	0.11^{d}	0.04^{d}	0.05^{d}
C18:0	1.68 ± 0.21	1.80 ± 0.39	1.71 ± 0.29	1.45	2.05	0.68^{d}	1.55	1.67	0.91^{d}	1.39	0.56^{d}	2.38^{d}	0.99	1.35
C18:1 *n*-9	$17.43^{a,b} \pm 2.20$	14.41 ± 2.43	13.97 ± 2.74	34.85^{d}	40.93^{d}	52.37^{d}	48.68^{d}	37.57^{d}	43.47^{d}	46.99^{d}	49.06^{d}	26.33^{d}	49.40^{d}	35.31^{d}
C18:2 *n*-6	11.58 ± 2.89	10.32 ± 3.22	10.95 ± 2.75	17.05^{d}	20.59^{d}	19.41^{d}	25.71^{d}	18.92^{d}	24.44^{d}	25.95^{d}	26.69^{d}	8.14	25.23^{d}	19.33^{d}
C20:0	0.16 ± 0.04	0.16 ± 0.05	0.13 ± 0.04	0.18^{d}	0.50^{d}	0.67^{d}	0.16	0.68^{d}	0.17	0.14	0.15	0.08	0.15	0.59^{d}
C18:3 *n*-3	$0.28^{a,b} \pm 0.07$	$0.41c \pm 0.11$	0.59 ± 0.10	1.67^{d}	1.92^{d}	2.84^{d}	1.03^{d}	4.03^{d}	3.19^{d}	1.25^{d}	2.82^{d}	0.91^{d}	1.32^{d}	4.31^{d}
C20:1 *n*-9	0.51 ± 0.11	0.40 ± 0.09	0.41 ± 0.11	ND	0.13^{d}	0.13^{d}	0.14^{d}	0.08^{d}	0.04^{d}	0.07^{d}	0.06^{d}	0.20	0.04^{d}	0.04^{d}
C20:2 *n*-6	$0.40^{b} \pm 0.08$	$0.31^{c} \pm 0.12$	0.21 ± 0.05	ND	ND	ND	ND	ND	ND	ND	ND	0.35^{d}	ND	ND
C20:3 *n*-6	0.34 ± 0.05	0.32 ± 0.09	0.25 ± 0.09	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND
C22:0	ND	ND	ND	0.02	0.04	0.02	0.02	0.05	0.02	0.02	ND	0.03	0.03	0.03
C20:4 *n*-6	$1.16^{a,b} \pm 0.18$	$0.88^{c} \pm 0.12$	0.67 ± 0.15	ND	ND	ND	ND	ND	ND	ND	ND	0.40	ND	ND
C24:0	ND	ND	ND	ND	ND	0.03	0.03	ND	ND	ND	0.02	0.05	ND	ND
C22:4 *n*-6	$0.84^{a,b} \pm 0.07$	$0.55^{c} \pm 0.09$	0.29 ± 0.06	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND
C22:5 *n*-3	0.33 ± 0.06	0.28 ± 0.06	0.27 ± 0.05	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND
C22:6 *n*-3	$0.93^{b} \pm 0.11$	$0.81^{c} \pm 0.13$	0.64 ± 0.10	ND	ND	ND	ND	ND	ND	ND	ND	0.28	ND	ND
TOTAL	100	100	100	100	100	100	100	100	100	100	100	100	100	100

[a]Denotes significant differences ($P < 0.05$) between colostrum and mature milk groups.
[b]Denotes significant differences ($P < 0.05$) between colostrum and transitional milk groups.
[c]Denotes significant differences ($P < 0.05$) between transitional and mature milk groups.
[d]Denotes differences with mature milk (values were considered different if they fell outside the mean ± 2 s.d. of corresponding mature milk).
ND, not detectable.

formulas (IF4, IF5, IF6, IF9, and IF11) had higher amounts of $C_{12:0}$ and lower amounts of $C_{18:0}$ than mature milk. High percentages of $C_{16:0}$ were found in five infant formulas (IF1, IF2, IF3, IF7 and IF8), but in three of these the percentages were only slightly higher than in mature milk (IF1, IF2 and IF7; see Table 3 for details). MUFA percentages in infant formulas and in mature milk were similar. The percentages found for the major MUFA, oleic acid, in infant formulas was similar to that found in mature milk, except in IF4 which was slightly higher than in mature milk.

When we compared PUFA, we found comparable results between infant formulas, except IF9, and mature milk. LA, an essential fatty acid, showed similar percentages in infant formulas and in mature milk. In five infant formulas (IF4, IF5, IF6, IF8 and IF11), LnA, the other essential fatty acid, was higher than in mature milk. The ratio LA/LnA in infant formulas was within the margin between 1:5 and 1:15 suggested by the ESPGAN Committee on Nutrition (1991), except in one infant formula (IF4) that had lower than recommended ratios.

Sn-2 fatty acid composition of human milk and infant formulas

Table 4 shows the *sn*-2 fatty acid composition of colostrum, transitional milk, mature milk and infant formulas. For the benefit of other authors these results are shown in Table 4. However, we think this is not the most suitable way of presenting data of *sn*-2 fatty acid composition (see Discussion).

Table 5 shows the percentage of each fatty acid that is *sn*-2 position (relative fatty acid in *sn*-2 position; *sn*-2 fatty acid/3) ∗ 100/total fatty acid in human milk), in *sn*-2 position in colostrum, transitional milk, mature milk and infant formulas.

We only found significant differences for relative $C_{10:0}$ in the *sn*-2 position ($P<0.02$) between colostrum with transitional and mature milk, and for relative $C_{22:4}$ *n*-6 in the *sn*-2 position ($P<0.02$) between colostrum and mature milk and between transitional and mature milk. All the other fatty acids showed no significant differences between colostrum, transitional milk and mature milk.

In Table 5 we also shows the values for infant formulas. There were differences between mature human milk and infant formulas when the figures for infant formulas did not fall within the range of the mature human milk mean ± 2 s.d. The percentages of fatty acids differed considerably between mature and infant formulas. Palmitic acid was lower in all infant formulas than in mature milk. Oleic and linoleic acids were higher in all infant formulas than in mature milk, and similar results were obtained for linolenic acid, except for IF4, IF7 and IF10 that were within the mature milk range.

Table 5 Relative percentage of each fatty acid in *sn*-2 position in colostrum, transitional, mature and infant formulas (IF1-IF11)

Fatty acids	*Colostrum Mean ± s.d. (n = 12)*	*Transitional Mean ± s.d. (n = 12)*	*Mature Mean ± s.d. (n = 12)*	*IF1 Mean*	*IF2 Mean*	*IF3 Mean*	*IF4 Mean*	*IF5 Mean*	*IF6 Mean*	*IF7 Mean*	*IF8 Mean*	*IF9 Mean*	*IF10 Mean*	*IF11 Mean*
C8:0	ND	ND	ND	ND	2.61	3.05	1.41	1.60	1.97	1.90	1.03	3.58	1.86	1.46
C10:0	12.28[a,b] ± 3.59	8.25 ± 3.05	8.26 ± 3.25	6.31	40.49[d]	16.48	11.84	9.73	11.09	15.19[d]	10.30	13.31	11.90	9.33
C12:0	29.24 ± 9.99	25.04 ± 5.37	24.61 ± 4.28	31.45	53.03[d]	86.62[d]	15.35	30.68	45.58[d]	30.07	45.43[d]	30.19	45.73[d]	32.52
C14:0	55.32 ± 7.53	52.88 ± 6.43	52.77 ± 6.21	24.73[d]	41.66	19.64[d]	30.10[d]	26.59[d]	19.93[d]	36.84[d]	17.14[d]	31.47[d]	21.08[d]	28.19[d]
C14:1 *n*-5	ND	ND	ND	ND	5.89	4.35	ND	ND	56.76	57.36	67.92	60.31	72.48	ND
C15:0	76.55 ± 6.29	72.09 ± 5.82	78.47 ± 9.02	ND	2.12[d]	3.87[d]	14.99[d]	18.21[d]	21.25[d]	40.74[d]	19.81[d]	51.62[d]	27.00[d]	11.83[d]
C16:0	80.30 ± 4.63	86.25 ± 7.73	87.86 ± 6.67	41.06[d]	18.92[d]	7.33[d]	23.03[d]	38.80[d]	12.25[d]	16.09[d]	8.71[d]	62.37[d]	12.35[d]	41.36[d]
C16:1 *n*-7	37.86 ± 3.07	37.60 ± 5.09	38.99 ± 6.42	12.29[d]	6.41[d]	6.97[d]	17.93[d]	19.95[d]	24.79[d]	34.25	25.19[d]	22.00[d]	26.98	20.91[d]
C17:0	36.44 ± 4.17	37.55 ± 4.46	40.48 ± 4.64	ND	ND	3.67[d]	61.06[d]	17.77[d]	12.18[d]	20.68[d]	10.83[d]	43.19	12.68[d]	19.24[d]
C18:0	8.98 ± 0.99	9.05 ± 1.50	9.04 ± 1.58	9.54	9.16	4.68[d]	15.53[d]	15.81[d]	8.26	9.34	5.12[d]	25.96[d]	7.52	13.93[d]
C18:1 *n*-9	14.10 ± 0.85	13.03 ± 1.74	12.22 ± 2.01	25.88[d]	32.24[d]	47.71[d]	36.31[d]	29.75[d]	42.19[d]	43.22[d]	43.80[d]	21.14[d]	43.41[d]	28.40[d]
C18:2 *n*-6	23.84 ± 3.00	23.54 ± 4.36	22.07 ± 2.60	36.86[d]	45.58[d]	39.40[d]	77.50[d]	49.76[d]	49.36[d]	50.82[d]	53.09[d]	30.39[d]	45.64[d]	48.25[d]
C20:0	27.03 ± 9.86	24.35 ± 7.83	21.47 ± 9.55	21.12	39.74	72.78[d]	13.28	37.40	16.36	15.87	13.95	12.16	15.97	31.82
C18:3 *n*-3	26.30 ± 5.04	25.76 ± 3.80	24.68 ± 5.47	40.17[d]	46.78[d]	76.03[d]	12.36	50.06[d]	54.28[d]	28.79	45.86[d]	45.52[d]	28.33	50.79[d]
C20:1 *n*-9	17.41 ± 1.62	20.34 ± 3.61	22.06 ± 5.34	ND	8.47[d]	20.33	7.81[d]	4.71[d]	3.68[d]	13.62	5.49[d]	14.41	7.43[d]	2.99[d]
C20:2 *n*-6	15.35 ± 1.19	16.86 ± 6.70	16.92 ± 3.48	ND	ND	ND	ND	ND	ND	ND	ND	28.38[d]	ND	ND
C20:3 *n*-6	20.28 ± 2.20	19.83 ± 5.42	16.66 ± 2.14	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND
C22:0	ND	ND	ND	3.16	4.81	5.01	3.00	5.01	3.36	4.17	ND	5.87	3.40	3.55
C20:4 *n*-6	47.85 ± 7.93	43.66 ± 5.81	43.27 ± 7.67	ND	ND	ND	ND	ND	ND	ND	ND	38.63	ND	ND
C24:0	ND	ND	ND	ND	ND	10.96	7.86	ND	ND	ND	7.65	20.70	ND	ND
C22:4 *n*-6	70.96[b] ± 7.14	73.48[c] ± 9.70	62.79 ± 8.07	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND
C22:5 *n*-3	66.92 ± 9.24	68.13 ± 7.43	72.29 ± 8.19	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND
C22:6 *n*-3	52.63 ± 7.18	56.8 ± 7.18	61.39 ± 7.76	ND	ND	ND	ND	ND	ND	ND	ND	48.17	ND	ND
TOTAL	33.33	33.33	33.33	33.33	33.33	33.33	33.33	33.33	33.33	33.33	33.33	33.33	33.33	33.33

[a]Denotes significant differences ($P<0.05$) between colostrum and mature milk groups.
[b]Denotes significant differences ($P<0.05$) between colostrum and transitional milk groups.
[c]Denotes significant differences ($P<0.05$) between transitional and mature milk groups.
[d]Denotes differences with mature milk (values were considered different if they fell outside the mean ± 2 s.d. of corresponding mature milk).
ND, not detectable.

Discussion

Fatty acid composition of human milk

Saturated fatty acids (SFA) in human milk. We observed a significant increase for $C_{8:0}$-$C_{14:0}$ between the colostrum group and the transitional and mature milk groups, which is consistent with similar findings reported from Spain (Barbas & Herrera, 1998) and different geographic areas (Pago-Gunsam *et al*, 1999; Boersma *et al*, 1991). These changes in milk composition may indicate variations in mammary gland biosynthetic capacity.

The SFA mean in colostrum (37.37%) was very similar to the lowest value in the European Range (ER 46.88 – 37.24%; Fidler & Koletzko, 2000) and the values observed 38.54, 37.24 and 37.68% in other Spanish (Dominguez Ortega *et al*, 1997; Pita *et al*, 1985) and Slovenian (Fidler *et al*, 2001) studies, respectively. The SFA mean found in mature milk (40.66%) fell within the European Range (39.0 – 51.3%; Koletzko *et al*, 1992). This figure, as with colostrum, was closer to the lowest figures reported in mature milk and confirmed the figures of 41.09 and 41.39% in other Spanish studies (de la Presa-Owens *et al*, 1996; Barbas & Herrera, 1998), respectively. These low SFA levels could be due to the dietary habits in the Mediterranean countries, which contains a low proportion of SFA.

Monounsaturated fatty acids (MUFA) in human milk. As we saw above, oleic acid ($C_{18:1}$ *n*-9), the main fatty acid, decreased as lactation progressed, which was consistent with similar findings reported from different geographic areas (Pago-Gunsam *et al*, 1999; Serra *et al*, 1997; Boersma *et al*, 1991). Although oleic acid is not an essential fatty acid, it is very important because, in addition to the usual functions of fatty acids (source of energy and structural components), it reduces the melting point of triacylglycerides, thus providing the liquidity required for the formation, transport and metabolism of milk fat globules (Jensen, 1999).

Our result for MUFA in colostrum was in the middle of the European Range (ER 39.11 – 45.19%; Fidler & Koletzko, 2000) and agree with the figures in other Spanish (Rueda *et al*, 1998; Pita *et al*, 1985) studies of 42.00 and 41.46%, respectively. We found similar MUFA percentages in a German study (Genzel-Boroviczeny *et al*, 1997), but the percentage of oleic acid was only 32.16%, whereas in our study oleic acid accounted for 38.83%. The MUFA mean value in mature milk (39.63%) was in the middle of the European Range (ER 34.42 – 44.90%; Koletzko *et al*, 1992). Our mean value agree with other Spanish studies (Barbas & Herrera, 1998; de la Presa-Owens *et al*, 1996), 40.20 and 41.97%.

Polyunsaturated fatty acids (PUFAs) in human milk. The LA percentage in colostrum (16.10) was similar to the highest figure on the ER (7.86 – 15.30; Fidler & Koletzko, 2000). Our LA results agree with those obtained in other Spanish (Pita *et al*, 1985) and Slovenian (Fidler *et al*, 2001) studies, 15.30% and 15.25%. The LA mean value found in mature milk was, as occurred in colostrum, a little higher than the ER (6.9 – 16.4; Koletzko *et al*, 1992). The percentage of LnA in colostrum was similar to the lowest figures on the ER (0.35 – 1.09), and was similar to other Spanish (Rueda *et al*, 1998; Dominguez Ortega *et al*, 1997) and Italian (Serra *et al*, 1997) studies, with figures of 0.45, 0.51 and 0.35, respectively. In mature milk, the percentage of LnA was similar to the lowest values in the ER (0.7 – 1.3; Koletzko *et al*, 1992). The LA/LnA ratio was higher in colostrum and in mature milk than in their respective European ranges. In our study, the high ratios in colostrum (42.02%) and mature milk (24.84%) were due to the high LA.

Our results confirm the trend in two Spanish studies reported with an interval of 13 y (Rueda *et al*, 1998; Pita *et al*, 1985). The latter study showed a considerably lower linolenic acid content (0.41 *vs* 1.09%), accompanied by a remarkable increase of the LA/LnA ratio, from 19.1 (1985) to 27.5 (1998). A desirable ratio of 5:1 to 15:1 was established (ESPGAN committee in nutrition 1991), even though the ratio in many milks is greater than 15:1 (Jensen, 1999). This ratio is of importance because both essential fatty acids compete with each other for the same enzyme during the synthesis of long-chain polyunsaturated fatty acids (Hernell, 1990). However, some recent studies (Sheaff *et al*, 1995; Sauerwald *et al*, 1996) indicated that high levels of LA did not inhibit the conversion of LnA to DHA in rats or term infants.

In colostrum, LC-PUFA accounted for 3.76% of the total fatty acids, of which 1.03% were represented by the *n*-3 series and 2.73% by the *n*-6 series, the LC-PUFA *n*-6/LC-PUFA *n*-3 ratio (2.65) was within the ER (2.23 – 3.19%, Fidler & Koletzko, 2000). In mature milk, LC-PUFA represented 2.24% of total fatty acids, of which LC-PUFA *n*-3 were 0.63% and LC-PUFA *n*-6 were 1.61%, and the LC-PUFA *n*-6/LC-PUFA *n*-3 ratio (2.55%) was within the ER (1.6 – 4.0%; Koletzko *et al*, 1992).

Fatty acid composition of human milk.
The fatty acid composition of human milk obtained in this study seems to reflect dietary habits in the Mediterranean countries, where diets contain a high percentage of MUFA and low SFA. When we compared our mature milk results with those from another Mediterranean country, Italy (Serra *et al*, 1997), we found higher percentages of MUFA in the Italian study (42.69%) than in our study (39.63%). The major MUFA is oleic acid; in the Italian study oleic acid was 39.93%, whereas in our study it was 36.35%. We observed that differences between MUFA in the studies were due, basically, to oleic acid. In the Italian study PUFA represented 11.82% of all fatty acids, with the major fatty acid linoleic acid accounting for 9.79%, while in our study PUFA were 19.71% and linoleic acid was 16.59%. We found that the major contribution to the differences between both studies in PUFA was made by linoleic acid.

Even though both studies were conducted in Mediterranean countries with similar dietary habits, there are some major differences in the fatty acid composition of mature milk. However, Serra-Majem *et al*, (1997) showed differences in fats between Spain and Italy's dietary habits, which could explain the differences found in the fatty acid composition of mature milk. Serra-Majem *et al*, showed higher levels of olive oil (rich in oleic acid) in the Italian diet (32.1 g/person/day) than in the Spanish (29.6 g/person/day). This could be the reason for higher levels of oleic acid in Italian than in Spanish mature milk. Whereas Spanish diets had higher sunflower seed and soybean oils (27.9 g/person/day) than Italian diets (18.6 g/person/day), these oils are rich in linoleic acid, which could be the reason for higher percentages of linoleic acid in Spanish mature milk than in Italian mature milk.

The Western lifestyle has been held to be responsible for the increasing susceptibility to atopic sensitization (Black & Sharpe, 1997). One explanation is the dietary hypothesis, according to which the increased prevalence of atopic diseases has been linked to a increase in PUFA consumption (Simopoulos, 1991; Black & Sharpe, 1997, Duchén *et al*, 1998). Western diets contain between 10 and 25 times more LA than LnA (Black & Sharpe, 1997). LA and LnA are precursors of longer chain PUFA, but are in continuous competition for the same desaturation and elongation enzymes. Eicosanoids derived from AA, a *n*-6 fatty acid, are important factor promoting atopic inflammation (Chan *et al*, 1993; Ohtsuka *et al*, 1997). In contrast, eicosanoids derived from *n*-3 fatty acids have been demonstrated to possess anti-inflammatory properties (Alexander, 1998; Calder, 1998).

Breast-feeding has been demonstrated to protect against the development of atopic diseases (Oddy *et al*, 1999), although infants may have atopic diseases even during exclusive breast-feeding (Isolauri *et al*, 1999). Since mother's diet influences the fatty acid composition of human milk, variations in the PUFA proportion of breast milk may explain why breast-feeding has a variable influence on the prevention of atopic diseases.

Breast milk obtained from healthy mothers of infants with newly developed atopic dermatitis had more LA and decreased proportion of *n*-3 PUFA than the milk of healthy mothers of non-atopic infants (Businco *et al*, 1993; Yu *et al*, 1998). Hodge *et al*, (1996) and Kankaapää *et al* (2001) suggest that the excessive dietary supply of *n*-6 PUFA or reduced proportion of *n*-3 PUFA, may be a risk factor for the development of atopic disease, even though relationship between PUFA and atopic diseases is still controversial (Koletzko, 2000; Duchén, 2001). So we recommend further research with respect to high *n*-6/*n*-3 ratio in human milk and atopic diseases in infants.

Fatty acid composition of infant formulas

The lower LA/LnA ratio of IF4 is of importance because both essential fatty acids compete with each other for the same enzyme during the synthesis of long-chain polyunsaturated fatty acids (Hernell, 1990).

LA and LnA acids are the metabolic precursors of AA and DHA, respectively, which are the most important fatty acids utilised by the brain, retina and are important structural components of the membrane systems of all tissues (Giovannini *et al*, 1998; Emmett & Rogers, 1997; Makrides *et al*, 1995). Therefore, the lower ratio in IF4 could affect the levels of AA in the newborn, and even more when, as we can see in Table 3, IF4 was not supplemented in AA. We observed that only four infant formulas (IF1, IF2, IF3 and IF9) were supplemented with AA and DHA. In these formulas, the ratio AA/DHA was comparable with that obtained in mature milk, except for IF3 that showed a low ratio, because IF3 had low supplementation with AA. We can conclude that the fatty acid composition of infant formulas is comparable to mature milk, though one infant formula (IF4) does not have an adequate LA/LnA ratio.

Supplementation of infant formulas for term infants with DHA and AA is still controversial (Gibson *et al*, 2001; Gibson & Makrides, 2000). Studies assessing the relationship between brain fatty acids and diet in infancy have demonstrated that breast-fed infants have higher concentrations of DHA in their cerebral cortex compared with infants fed formula without AA and DHA (Farquharson *et al*, 1992; 1995), but there were no differences in the level of AA. Makrides *et al*, (1994) and Decsi *et al*, (1995) observed that infants fed with formula without AA and DHA develop AA and DHA depletion of structural lipids.

Visual benefits of adding AA and DHA to formulas have been reported in some studies (Carlson *et al*, 1996; Birch *et al*, 1998; Jorgensen *et al*, 1998), whereas other studies have failed to detect a benefit of AA and DHA supplementation (Makrides *et al*, 2000; Auestad *et al*, 1997; Innis *et al*, 1997). There is also mixed evidence for the support of an effect of dietary AA and DHA on more global measures of development, Birch *et al*, (2000) and Willats *et al*, (1998) reported benefits of dietary AA and DHA, whereas Makrides *et al*, (2000) and Lucas *et al*, (1999) failed to detect benefits of dietary AA and DHA.

Sn-2 fatty acid composition of human milk and infant formulas

Table 4 shows the *sn*-2 fatty acid composition of colostrum, transitional milk, mature milk and infant formulas; however, we think this is not the most suitable way of presenting data of *sn*-2 fatty acid composition. The reason for this can be explained easily through an example. If we compared our result of *sn*-2 palmitic acid in mature milk (52.30%) with other studies (Martin *et al*, 1993; Nelson & Innis, 1999), our result was consistent with that of Martin *et al*, (51.22%), but was different from the result of Nelson and Innis (56.4%). The error is that we were comparing percentages of *sn*-2 palmitic acid, but without taking into account the total percentage of palmitic acid in human milk, which varies in the studies. When we expressed palmitic acid in *sn*-2 position as the percentage of the palmitic acid that is in *sn*-2 position (relative palmitic acid in *sn*-2 position; *sn*-2 palmitic acid/3) $*$ 100/total palmitic acid in human milk), we found that 87.86% of the palmitic acid was in the *sn*-2 position, whereas in Martin *et al*, this figure was 71.08% and in Nelson and Innis it was 81.38%. Now, our result is closer to Nelson and Innis than to Martin *et al*. We think it is better to express the results as 'relative fatty acid in *sn*-2 position' because the total percentage of each fatty acid is included. It is wrong to say that two samples have 30% palmitic acid in *sn*-2 position if the percentage of palmitic acid in one sample is 20% and in the other 30%, because in the first sample the relative palmitic acid in *sn*-2 position is 50% and in the second it is only 33.33%. This can be extended to studies that work with infant formulas with different percentages of palmitic acid in the *sn*-2 position. In some studies authors only detailed the percentage of palmitic acid in the *sn*-2 position, but this is only useful when the total amount of palmitic acid in the formulas compared is the same, because if it is not the same, as described above, this value is much less important than the relative palmitic acid in the *sn*-2 position. What is being studied is how different percentages of palmitic acid in the *sn*-2 position affect absorption of palmitic acid. In conclusion, we recommend authors to express all *sn*-2 values as relative fatty acid in *sn*-2 position (Table 5).

Comparison with Table 4 shows that only a few fatty acids had significant differences between colostrum, transitional milk and mature milk. This is because, even though fatty acids in Table 4 showed differences, when we expressed these results as each fatty acid percentage in *sn*-2 position, these results were not significant as the fatty acid composition of human milk changes with lactation.

We found (Table 5) that the proportion of relative saturated fatty acids in the *sn*-2 position increased as the carbon chain lengthened from 10 to 16 carbons. Therefore, the carbon chain length (between 10 and 16 carbons) seems to be a discriminating factor in the distribution of these saturated fatty acids in the *sn*-2 position of the triacylglycerides. Similar results were found by Martin *et al*, (1993). Moreover, this increase was observed in C_{18} fatty acids, in which we found that relative percentages of these fatty acids in the *sn*-2 position increased from C18:0 to C18:3 *n*-3. Martin *et al*, (1993) did not find this because they did not work with relative fatty acid in the *sn*-2 position percentages.

The similarities of the relative fatty acids in the *sn*-2 position in colostrum, transitional milk and mature milk indicate that the acylation of fatty acids to glycerol *sn*-2 position in the mammary gland is not affected by the stage of lactation.

In infant formulas, we did not find that the proportion of relative saturated fatty acids in the *sn*-2 position increased as the carbon chain lengthened from 10 to 16 carbons, as we did in human milk. Moreover, the relative palmitic acid in the *sn*-2 position is lower than in mature milk, even though some formulas (IF1, IF5, IF9 and IF11) had higher amounts than others.

The distribution of palmitic acid in the triacylglycerides has special interest, because studies carried out on term infants with infant formulas rich in palmitic acid esterified at the *sn*-2 position of the triglycerides showed higher levels of palmitic acid absorption than when palmitic acid is principally in the *sn*-1, 3 positions (López-López *et al*, 2001c; Nelson & Innis, 1999; Kennedy *et al*, 1999; Carnielli *et al*, 1996; Filler *et al*, 1969). Similar results had been obtained in preterm infants (Carnielli *et al*, 1995) and rats (Aoyama *et al*, 1996; de Fown *et al*, 1994; Lien *et al*, 1993; Renaud *et al*, 1995; Tomarelli *et al*, 1968).

In the gut, pancreatic lipase releases the fatty acids in the *sn*-1, 3 positions of the triacylglycerides to produce free fatty acids and 2-monoacylglycerides. When palmitic acid is in *sn*-2 position it is well absorbed as 2-monoacylglyceride, while when palmitic acid is in sn-1, 3 positions it is released as free fatty acid and forms insoluble calcium soaps (Small, 1991). Several studies (Quinlan *et al*, 1995; Carnielli *et al*, 1996) have observed that calcium soaps produce harder stools and constipation in infants.

Moreover, the location of palmitic acid in the *sn*-2 position in infant formulas improve the absorption of calcium in the small intestine (Lucas *et al*, 1997; Carnielli *et al*, 1996; Nelson *et al*, 1996) and results in a significantly higher whole-body mass (Kennedy *et al*, 1999).

If we look at the major fatty acids in human milk (palmitic, oleic and linoleic acids), in mature milk palmitic acid is preferentially esterified at the *sn*-2 position (86.25%) and oleic and linoleic acids are predominantly esterified at the *sn*-1,3 positions (12.22 and 22.27%, respectively, at the *sn*-2 position). However, in infant formulas palmitic acid is preferentially esterified at the *sn*-1,3 positions and oleic and linoleic acids have higher percentages in the *sn*-2 position than human milk (see Table 5 for details). Even though infant formulas and mature milk had similar fatty acid profiles, as we have seen above, the distribution of fatty acids in the triacylglycerides is different.

We think that formulas should resemble palmitic acid distribution of human milk, because the distribution of palmitic acid in the triacylglyceride is of great importance.

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