

ORIGINAL COMMUNICATION

Fatty-acid composition of the colostrum and serum of fullterm and preterm delivering Iraqi mothers

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Objective: To determine the lipid components of colostrum and the fatty-acid (FA) composition of the colostrum and serum of Iraqi mothers, whether their delivery be fullterm (FT) or preterm (PT).

Design: A collection of colostrum and serum samples of FT and PT delivering Iraqi mothers.

Setting: Mosul province (in the north of Iraq).

Subjects: Colostrum and blood samples were obtained from FT and PT delivering mothers; their gestation periods were 39.2 and 32.7 weeks, respectively (age 20–40 y).

Procedures: Colostrum and serum samples were collected from each lactating mother. The nursing period was 3–5 days. The lipid components of colostrum, namely triglycerides (TGs) and cholesterol (C), were determined enzymatically and the phospholipids (PLs) were determined by using a colorimetric method based on the formation of a phosphomolybdate complex. The FA composition of colostrum and serum was determined by capillary gas chromatography.

Results: Compared to PT colostrum, FT colostrum exhibited a significant increase in lipid content, viz. TGs ($P=0.022$); a significant decrease in medium chain fatty acids (MCFAs), viz. C12 and C14 ($P=0.03$ and 0.005 , respectively); no significant differences in monounsaturated fatty acids and a significant increase in C20:5 n3 and C22:6 n3 ($P=0.001$ and 0.05 , respectively) and a slight increase in the level of n3/n6. The FA composition of the mother serum was found to mimic that of their colostrum, except for the level of MCFAs which was higher in the colostrum.

Conclusions: The lipid content, the percentage of C22:6 n3 (the most important FA) and the level of n3/n6 in PT colostrum were lower than those in FT colostrum. They may be affected by serum lipid and immaturity of the mammary gland. Generally, the level of n3/n6 for both groups (0.09 and 0.08) is lower than that recommended by WHO (0.1) for infants' optimum nutrition. The difference in the level of MCFAs between the mother serum and colostrum reflects their *de novo* synthesis in the mammary gland.

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Introduction

The first food naturally taken by newborn babies is breast milk. The human milk composition may be influenced by several factors, such as gestational age, genetic characteristics, dietary habits, nutritional and socioeconomic state of mothers, duration of lactation and length of gestation (Rueda *et al*, 1998; Beijers & Schaafsma, 1996;

Bunker *et al*, 1996; Yeh *et al*, 1996). Dietary (milk) lipids are very important for the normal growth and development of infants (Ambartsumyan, 1998; Giovannini *et al*, 1991). The fatty acids (FAs) present in various lipid molecules are the moieties of great human-health interest (Ambartsumyan, 1998). Linoleic acid (C18:2 n6) and α -linolenic acid (C18:3 n3), essential fatty acids, are the precursors to all n6 and n3 long-chain polyunsaturated fatty acids (LCPUFAs) (Morgan *et al*, 1998). LCPUFAs have critical functions in the brain and retina (Woltil *et al*, 1998; Beijers & Schaafsma, 1996).

The FAs of milk arise from lipids or from *de novo* synthesis in the mammary gland (Agostoni *et al*, 1999; Hurely,

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Internet). The high milk-serum ratio of Medium chain fatty acid (MCFAs) indicates their *de novo* synthesis in the mammary gland (Spear *et al*, 1992). The MCFAs are a good source of energy (Ambartsumyan, 1998; Giovannini *et al*, 1991). Owing to the importance of FAs, Genzel-Boroviczeny *et al* (1997), Beijers and Schaafsma (1996) investigated the change in FA profiles of fullterm and preterm milk. The differences in FA profiles are probably due to immaturity of the mammary gland and not due to a process resulting from evolution to provide necessary nutrients for the preterm infants; premature birth is an abnormal event (Jensen, 1999).

Conjugated linoleic acid (CLA), the anticarcinogenic and antiatherosclerotic agent (Wilson *et al*, 2000; Jensen *et al*, 1998), is found in the milk of mothers who consume ruminant products (Jensen *et al*, 1998).

The aim of this work is to determine the lipid components and FA composition of colostrum of Iraqi mothers, whether their delivery be fullterm (FT), or preterm (PT), and to compare the FA composition of colostrum and serum in FT and PT delivering mothers.

Materials and Methods

Subjects

Colostrum and samples from both breasts and blood samples were obtained from 35 FT and 29 PT delivering mothers aged 20–40 y. The mean gestation period of the FT group was 39.2 ± 3.4 weeks and that of the PT group was 32.7 ± 2.9 weeks. A total of 25 of the FT group and 27 of the PT group had their first babies, seven of the FT group and two of the PT group had their second babies and three of the FT group had their third babies. All the mothers had singleton babies, and they were all residents of Al-Khansaa' Maternity Hospital (Mosul, Iraq).

Milk and blood sampling

At the time of sampling, mothers had been nursing for 3–5 days. They expressed 5–10 ml of breast milk (from both breasts), using a manual sterile glass sucker, and then total milk samples were immediately transferred into 15 ml sterile screw-capped glass vials containing 2 ml of ethanol to limit the lipolytic and oxidative degradation of milk lipids before extraction (Ali *et al*, 1984). The samples were collected in the mid morning when the lipid content is supposed to be highest (Rocquelin *et al*, 1998). Blood samples were drawn from all mothers, at the time of colostrum sampling, into sterile plastic tubes. Serum was separated 2 h after venipuncture by centrifugation. All the mothers were in fed state at the sampling time.

Lipid analysis

Milk lipids were solvent-extracted according to de Jong and Bading (1992) using ethanol, H_2SO_4 (0.25 M), and then a mixture of diethyl ether/hexane (1:1 V/V). Serum lipids were

solvent-extracted according to Folch *et al* (1957) using methanol then chloroform. The main lipid classes, namely cholesterol ester (CE), phospholipids (PLs) and triglycerides (TGs) were separated by thin-layer chromatography (Merck silica gel G 0.25 mm) with hexane:diethyl ether:formic acid (80:20:2 by volume). After drying, the plates were sprayed with 1% alcoholic solution of 2,7-dichlorofluorescein (a nondestructive reagent) to visualize each band under ultra-violet light. To avoid loss of volatile lipid components, extraction was performed in an ice-bath, and thin-layer chromatography in a refrigerated cabinet, and then each separated component was stored at -20°C . The TGs spot of the colostrum and the TGs, CE and PLs spots of serum were scraped into separate vials and their FAs moiety was converted to fatty acid-methyl esters (FA-Mes) by super dried—acidified methanol at $90\text{--}95^\circ\text{C}$ for 2 h (Al-Tamer, 1974). The proportional composition (%) of methylated fatty acids was determined by capillary gas chromatography. The samples were analysed in the National Centre for Scientific Research (CNRS), the Institute of Chemistry of Natural Substances (ICSN), France. The capillary gas chromatograph was CP-3800, Varian, USA. The capillary column was CP-Sil 8 CB, 0.25 mm ID \times 30 m with film thickness: 0.25 μm and helium as a carrier gas. The programme used a temperature gradient from 80 to 220°C , 5°C each min. The identity of 14 individual FA peaks was ascertained by comparing each peak's retention time relative to the retention times of FAs in synthetic standards. The relative amount of each FA (% of total FAs) was quantified by integrating the area under the peak and dividing the result by the total area for all FAs. To minimize transcription errors, the data from the gas chromatogram were electronically transferred to a computer for analysis.

With respect to the lipid components of colostrum, TGs and C were determined enzymatically using kits obtained from bioMeriux, France. The PLs were determined by a colorimetric method based on the formation of a phosphomolybdate complex (Tietz, 1986).

Statistical analysis

For comparison of means between two groups (lipid components and FA composition of serum and colostrum of FT and PT mothers), the *t*-test was used. All the data were expressed as mean \pm standard deviation of the mean. *P*-values ≤ 0.05 were considered significant.

Results

Mother-colostrum lipid components

The data on the general classes of lipids in the colostrum of FT and PT delivering mothers are shown in Table 1. In the FT and PT colostrum, the average amount of TGs, PLs and C were 3.1 and 2.6 g/100 ml, 30.0 and 24.5 mg/100 ml, and 28.3 and 40.2 mg/100 ml, respectively. These results indicate

Table 1 Colostrum lipid components (mean \pm s.d.)

Lipid class	Fullterm	Preterm
TGs (g/100 ml)*	3.09 \pm 0.39	2.61 \pm 0.36
TC (mg/100 ml)**	28.29 \pm 4.17	40.19 \pm 0.06
% TC/TGs***	0.92 \pm 1.12	1.53 \pm 0.06
PLs (mg/100 ml)****	29.78 \pm 2.29	24.48 \pm 3.21
%PLs/TGs	0.97 \pm 0.11	0.94 \pm 0.11

P-values: *0.022, **0.0001, ***0.007, ****0.002.

that, in contrast to the FT colostrum, the PT colostrum showed a significantly lower level of TGs and PLs, the amounts of C and the C/TGs ratio were higher and there was no significant difference in the PLs/TGs ratio.

Mother-colostrum fatty acid composition

The data on FA composition of the colostrum of FT and PT delivering mothers (listed in Table 2) show that SFAs represented the major FAs of breast colostrum (more than 50% for both groups) and C16 had the highest level. The PT group showed a higher amount of MCFAs (C10–C14) but total SFAs were lower. More than 30% of FA contents in both groups were monounsaturated fatty acids (MUFAs) (with C18:1 as the major component) and they were higher in the PT group.

As regards polyunsaturated fatty acids (PUFAs) in both groups, represented about 13% with C18:2 n6 as a major component. With respect to the PT group, the amount of C18:3 n3 and C20:4 n6 was higher, and that of C20:5 n3, and C22:6 n3, but a slight decrease in (CLA C18:2 ct) and the n3/n6 ratio were lower.

Mother-serum fatty acid composition

The details of FA composition of the serum lipid components (CE, PLs and TGs) of FT and PT delivering mothers are as follows (see Table 3a, b and c):

(a) **Serum CE fatty acids:** Compared to the FT group, the PT group showed a lower amount of SFAs. Both groups, however, showed a low level of MCFAs (C10–C14) but a high level of C16. In the PT group, MUFAs were higher, while PUFAs (mainly C18:3 n3 and C22:6 n3) and the n3/n6 ratio were lower (Table 3a).

(b) **Serum PL fatty acids:** The amount of SFAs in the PT group was lower than that in the FT group. Both groups, however, showed a low level of MCFAs (C10–C14) but a high level of C16 and C18. The MUFAs, and PUFAs (C18:2 n6 and C18:3 n3) for the PT group were higher. On the other hand, CLA and the n3/n6 ratio were slightly lower in the PT group (Table 3b).

(c) **Serum TG fatty acids:** TGs exhibited a nonsignificant increase in SFAs for the PT group compared with the FT group as shown in Table 3c and the MUFAs were, to some

Table 2 Mother-colostrum (3–5 days postpartum) fatty acid composition (wt%)

Fatty Acid Saturated	Mean \pm s.d.	
	Fullterm	Preterm
10:0	2.02 \pm 0.90	1.71 \pm 0.63
12:0**	3.67 \pm 1.33	4.79 \pm 1.06
14:0***	7.14 \pm 2.46	9.11 \pm 1.04
16:0	33.97 \pm 5.31	31.94 \pm 1.51
18:0***	7.02 \pm 1.56	5.28 \pm 1.21
Total	53.82 \pm 6.15	52.83 \pm 3.95
Monounsaturated		
16:1***	2.23 \pm 0.69	2.51 \pm 0.38
18:1	29.09 \pm 5.08	29.57 \pm 5.11
Total*	31.32 \pm 5.29	32.08 \pm 5.22
Polyunsaturated		
18:2 n6*	10.61 \pm 1.21	10.88 \pm 0.60
18:3 n6*	0.11 \pm 0.03	0.09 \pm 0.02
18:3 n3**	0.60 \pm 0.15	0.63 \pm 0.16
20:4 n6*	1.00 \pm 0.21	1.13 \pm 0.17
20:5 n3***	0.02 \pm 0.009	0.009 \pm 0.004
22:6 n3**	0.44 \pm 0.04	0.40 \pm 0.07
18:2 ct	0.11 \pm 0.02	0.10 \pm 0.02
n3*	1.06 \pm 0.058	1.04 \pm 0.051
n6*	11.72 \pm 1.27	12.2 \pm 1.05
n3/n6*	0.09 \pm 0.030	0.08 \pm 0.024

P-values \leq *0.1, **0.05, ***0.005.

extent, higher in the PT group. All PUFAs (except CLA) and the n3/n6 ratio were significantly lower for the PT group; the level of CLA was similar in both groups.

Discussion

Mother-colostrum lipid components

In the present study, the level of TGs was higher and the levels of C and C/TGs were lower in FT-colostrum than in PT-colostrum. This seems to be in harmony with what Bitman *et al.* (1983) state. But the level of PLs was lower in the PT colostrum, which does not agree with what was stated by Bitman *et al.* (1983). On the other hand, the mean lipid content of PT- and FT-colostrum was, to some extent, higher (2.6 and 3.0 g/100 ml, respectively) than that reported by Ehrenkranz *et al.* (1984) and Bitman *et al.* (1983) for human PT and FT-colostrum (2.0 and 2.5 g/100 ml, respectively). It is worth noting that breast milk lipid content varies according to the dietary profile. High-carbohydrate and low-fat diets, commonly consumed in many developing countries, lead to higher breast-milk lipid content than do low-carbohydrate and high-fat diets (Rocquelin *et al.*, 1998; Harzer *et al.*, 1984). In general, and because of the economic sanctions (imposed on Iraq since 1990), the diet of Iraqi people was poor as it included a limited variety of foods (poor in animal protein and rich in carbohydrate and lipid).

Table 3 Serum^{*}-lipid fatty acid composition of fullterm and preterm, delivering mothers

Fatty Acid	Mean \pm s.d.	
	Fullterm	Preterm
(a) Serum CE fatty acid composition (wt.%)		
<i>Saturated</i>		
10:0	0.00	0.00
12:0 ^{***}	0.024 \pm 0.01	0.01 \pm 0.008
14:0	0.61 \pm 0.10	0.65 \pm 0.16
16:0 ^{**}	19.78 \pm 1.94	17.52 \pm 2.01
18:0	1.12 \pm 0.36	1.11 \pm 0.38
Total [*]	21.53 \pm 0.73	19.29 \pm 1.55
<i>Monounsaturated</i>		
16:1	1.22 \pm 0.34	1.28 \pm 0.47
18:1 ^{**}	20.91 \pm 5.38	24.76 \pm 6.23
Total [*]	22.13 \pm 3.51	26.04 \pm 4.47
<i>Polyunsaturated</i>		
18:2 n6 [*]	40.49 \pm 6.40	39.33 \pm 7.67
18:3 n6 ^{**}	1.38 \pm 0.21	1.10 \pm 0.19
18:3 n3 ^{**}	0.52 \pm 0.16	0.38 \pm 0.16
20:4 n6 [*]	9.51 \pm 1.93	9.37 \pm 1.79
20:5 n3 ^{**}	0.70 \pm 0.23	0.63 \pm 0.14
22:6 n3 ^{***}	0.69 \pm 0.16	0.52 \pm 0.16
18:2 ct	0.035 \pm 0.028	0.032 \pm 0.028
n3 ^{***}	1.92 \pm 0.34	1.53 \pm 0.31
n6	51.42 \pm 7.59	49.83 \pm 8.64
n3/n6 ^{**}	0.041 \pm 0.014	0.031 \pm 0.006
(b) Serum PL fatty acid composition (wt%)		
10:0	0.00	0.00
12:0	0.15 \pm 0.048	0.14 \pm 0.048
14:0 [*]	0.42 \pm 0.084	0.44 \pm 0.089
16:0 ^{**}	28.35 \pm 5.35	27.51 \pm 5.81
18:0 ^{**}	11.34 \pm 4.24	10.03 \pm 3.23
Total [*]	40.26 \pm 4.78	38.12 \pm 6.33
<i>Monounsaturated</i>		
16:1 ^{***}	3.25 \pm 1.21	1.62 \pm 0.73
18:1 ^{**}	13.55 \pm 2.51	17.39 \pm 4.83
Total [*]	16.80 \pm 4.70	19.01 \pm 3.03
<i>Polyunsaturated</i>		
18:2 n6 ^{**}	13.93 \pm 2.41	15.24 \pm 3.66
18:3 n6	0.73 \pm 0.22	0.70 \pm 0.22
18:3 n3 ^{**}	0.30 \pm 0.091	0.38 \pm 0.10
20:4 n6 [*]	8.83 \pm 2.67	9.03 \pm 2.23
20:5 n3	0.80 \pm 0.25	0.77 \pm 0.29
22:6 n3 [*]	6.49 \pm 1.75	6.31 \pm 1.96
18:2 ct	0.10 \pm 0.04	0.09 \pm 0.04
n3 [*]	7.59 \pm 1.05	7.06 \pm 1.85
n6 [*]	23.49 \pm 4.78	24.98 \pm 3.51
n3/n6 [*]	0.34 \pm 0.10	0.30 \pm 0.13
(c) Serum TG fatty acid composition (wt%)		
10:0	0.85 \pm 0.28	0.87 \pm 0.34
12:0	6.27 \pm 2.12	5.21 \pm 1.76
14:0	3.36 \pm 1.08	3.03 \pm 1.00
16:0 ^{***}	26.43 \pm 6.57	28.07 \pm 5.46
18:0 ^{***}	3.64 \pm 1.11	5.55 \pm 1.16
Total	40.55 \pm 6.75	42.73 \pm 4.78
<i>Monounsaturated</i>		
16:1 ^{***}	1.10 \pm 0.33	1.70 \pm 0.41
18:1	28.96 \pm 4.68	29.84 \pm 4.58
Total [*]	30.06 \pm 2.69	31.54 \pm 2.33

Table 3 (Continued)

Fatty Acid	Mean \pm s.d.	
	Fullterm	Preterm
<i>Polyunsaturated</i>		
18:2 n6 [*]	22.05 \pm 3.17	19.81 \pm 4.09
18:3 n6 ^{***}	1.31 \pm 0.32	0.73 \pm 0.31
18:3 n3	0.69 \pm 0.29	0.66 \pm 0.29
20:4 n6 ^{**}	1.57 \pm 0.50	1.15 \pm 0.52
20:5 n3 ^{***}	0.29 \pm 0.10	0.13 \pm 0.05
22:6 n3 ^{***}	0.87 \pm 0.22	0.43 \pm 0.12
18:2 ct	0.004 \pm 0.002	0.004 \pm 0.003
n3 [*]	1.85 \pm 0.27	1.22 \pm 0.52
n6	24.93 \pm 3.27	21.69 \pm 3.97
n3/n6 ^{**}	0.075 \pm 0.023	0.056 \pm 0.02

P values \leq *0.1, ** 0.05, ***0.005.

Mother colostrum fatty-acid composition

During late fetal and early postnatal life, the fetus and infant are optimally supplied with FAs from the mother's placenta and breast milk. Previous studies indicate that colostrum FA composition is influenced by the length of gestation (Genzel-Boroviczeny *et al*, 1997; Gibson & Makrides, 1998). As shown in Table 2, total SFAs, which are the main source of energy for infants, exhibited (in both the FT and PT groups) the highest level compared with MUFAs and PUFAs. It has been reported that human milk provides 50–60% of its energy as lipid, only 5% of which is generated by essential FAs and 1% by LCPUFAs (FAO/WHO, 1994). PT-colostrum contained a higher proportion of MCFAs (C10–C14) than FT-colostrum (viz. 15.6 vs 12.8%, respectively). This higher proportion of MCFAs might be advantageous for the fat and calcium absorption of PT infants (Genzel-Boroviczeny *et al*, 1997).

The amount of C18:2 n6 was found to be slightly larger in the PT group, which was the same for most colostrum samples from developed countries, but lower than that in developing countries (Ogunleye *et al*, 1991; Jensen, 1999). On the other hand, the amount of C18:3 n3 was found to be lower, for both groups (0.6% for the FT group and 0.63% for the PT group), than that reported for mothers from developed countries (more than 1%). The levels of both C18:2 n6 and C18:3 n3 reflect their level in the mothers diet.

The relative amount of C18:3 n3/C18:2 n6 was about 0.06 for both the FT and the PT groups. It was smaller than the value recommended for optimum infant nutrition, about 0.09 (Gerster, 1998).

Arachidonic acid (AA) (C20:4 n6) and Docosahexaenoic acid (DHA) (C22:6 n3) are the most representative LCPUFAs as they affect infant growth and development and they are found abundantly in the brain, in the retina and in other nervous tissues. In PT human infants, adding DHA to milk formula seems to improve the delayed recovery of dark-adapted electroretinogram and visual acuity

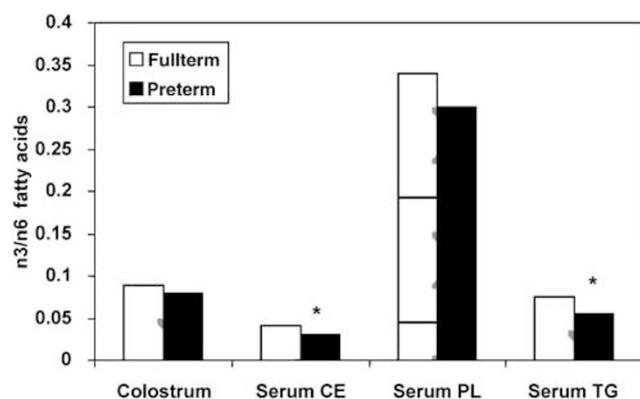
(Suh *et al*, 1994; Agostoni *et al*, 1999; Ambartsumyan, 1998). In the present study, the level of arachidonic acid, C20:4 n6, was slightly lower in the FT-colostrum than in the PT-colostrum, but its level was higher than that reported in other studies (Jensen, 1999). The level of DHA, the most important FA, was lower in the PT group (viz. 0.4%) than in the FT group (viz. 0.44%). Although dihomogammalinoleic acid may coelute with AA, its concentration is comparatively low. Similarly, C24:1 may coelute with DHA.

The balance between total n6 and n3 FAs in the diet is important because of their competitive nature. For optimum nutrition, the ratio of n3/n6 must not be less than 0.1 (Gerster, 1998; FAO/WHO, 1994), but in the present study it was lower for both the FT- and PT-colostrum (about 0.09 and 0.08, respectively).

An almost similar amount of CLA for both groups was observed, which was lower than that reported for mothers from developed countries (eg Canadian milk), but higher than that reported for mothers from developing countries (eg Sudanese milk). The level of CLA in the mother's milk reflects the amount of consumption of ruminant products, namely, beef, milk and dairy products (Jensen, 1999; Jensen *et al*, 1998).

Relation of colostrum fatty acids to mother-serum fatty acids

Tables 2 and 3 show that there are marked differences between the percentages of colostrum MCFAs (viz. 12.9% for FT-colostrum and 15.61% for PT-colostrum) and the percentages of serum MCFAs in PLs, CE and TGs (viz 0.58, 0.66 and 9.11% for FT-mothers and 0.57, 0.63 and 10.48% for PT-mothers, respectively). The higher level of colostrum MCFAs, compared with that of serum lipid, indicates their *de novo* synthesis in the mammary gland (Spear *et al*, 1992; Gunstone *et al*, 1994).



* Significant difference at $p \leq 0.05$

Figure 1 Comparison between n3/n6 fatty acids of FT- and PT-mother serum and colostrum.

The level of total SFAs in all serum components for both the FT- and PT-colostrum was almost the same and the level of MUFAs was lower in the FT-colostrum for all mothers serum components as shown in Table 3. In addition, the levels of the n3 FAs and the n3/n6 ratio (Figure 1) were higher in all serum components of FT-mothers than those of PT-mothers. Finally, the table shows that the level of CLA in all serum components for the two groups was almost the same. All these observations are in harmony with those obtained for FT- and PT-colostrum.

From what has preceded, the mother's colostrum FA composition seems to mimic that for serum lipids, except for the *de novo* synthesis of MCFAs in the mammary gland (Agostoni *et al*, 1999). With respect to this, it is worth mentioning that the diet of Iraqi mothers is poor in n3 FAs which is reflected in the FA composition of their colostrum and serum.

In conclusion, mother's milk is the most obvious source of infant nutrition and its lipid provides 50–60% of the energy needed for optimum growth. In the present study, it has been observed that the lipid content of colostrum of FT delivering mothers is higher than that of PT delivering mothers. In addition, the percentage of DHA and the ratio of n3/n6 in PT colostrum are lower than in FT colostrum. This indicates that there is a decrease in the absolute and relative amounts of DHA, the most important FA for infant growth (Wu *et al*, 2002). Generally, it has been observed that the level of n3/n6 for both groups (mainly PT-group) is lower than that recommended by WHO for infant optimum nutrition (0.1). It was also observed that the FA composition of the serum of FT and PT mothers mimicked the FA composition of their colostrum except for the level of MCFAs in both groups, which was higher in the colostrum. This reflects their biosynthesis in the mammary gland. Since colostrum FA composition reflects the serum FA composition of mothers that, in turn, depends on their nutrition (in addition to hormonal factors), there is a need for reinforcing educational messages for mothers to take a suitable diet with a relatively high amount of n3 FAs, found mainly in fish (Nordiy *et al*, 2001; Minihaane *et al*, 2002), in order to obtain milk with the recommended FA composition. Furthermore, it is also recommended to add physiological concentrations of nutrients (eg DHA) to milk substitute formula (in addition to breast-feeding).

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