

Fatty acid composition of mature breast milk of Saudi lactating mothers

Abdullah A. Al-Othman,* Hanan A. El-Fawaz, Fawzy M. Hewdy & Nadia M. Abdullah

Department of Food Science and Human Nutrition, College of Agriculture, King Saudi University, Riyadh, Saudi Arabia

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The fatty acid compositions of 54 Saudi mothers' mature breast milk samples were examined. Fatty acid composition was determined by gas-liquid chromatography. Oleic acid (18:1) was the major unsaturated fatty acid, while palmitic acid (16:0) was the major saturated fatty acid. The major fatty acids are 14:0, 16:0, 18:0, 18:1 and 18:1. These fatty acids accounted for 83.4% of the total fatty acids. Mature breast milk was characterized by a higher percentage of saturated fatty acids (> 50% of total fatty acids), and a moderate level of linoleic acid. The P/S ratio was approximately 0.17. We observed higher total saturated fatty acids than those published for Western countries. Milk from younger mothers (under 34 years) had greater polyunsaturated fatty acid (PUFA) concentrations than that from older mothers. Milk from mothers over 34 years had higher saturated fatty acids than those from younger mothers. Total saturated fatty acids in the milk increased with parity (number of times mother has given birth) increased, while total unsaturated and monounsaturated fatty acids decreased as parity increased. Polyunsaturated fatty acid concentration from the milk of mothers with more than eight children was higher (about two-fold) than those from mothers with less than four children. Copyright © 1996 Elsevier Science Ltd

INTRODUCTION

Human milk is considered to be the optimal source of nutrition for the infant (American Academy of Pediatrics, 1978). An increasing number and percentage of American women are choosing to breast feed their infants, and more of these women are breast feeding for longer periods (Martinez & Nalezienski, 1979, 1981). In contrast, a growing accumulation of literature suggests a decline in breast feeding in Saudi Arabia as a result of economic development in the country and cultural transformation in the nutritional status of the Saudi population (Sawaya *et al.*, 1978).

Since breast feeding is acknowledged as providing important benefits to the infant, a large number of studies have been published concerning several aspects of the lipid and mineral composition of milk from mothers of term and pre-term infants (Belavady, 1978).

Lipids constitute about 50% of the total caloric value of human milk (Hambraeus, 1978). Milk lipid constituents play other important roles in the metabolism of mammals as well. For instance, linoleic acid (C18:2, ω -6) and linolenic acid (C18:3, ω -3) are converted to long-chain polyunsaturated fatty acids (long-chain PUFA). These metabolites of the ω -6 and ω -3 series have been shown to affect the fluidity of biological membranes (Olegard & Svennerholm, 1971; Sinclair & Crawford, 1976) and to be important precursors for prostaglandins (Van Dorf *et al.*, 1964). The high need for both phospholipids (PL) and long-chain PUFAs during post-natal myelinization and their presence in visual cells may suggest their importance for the development of the newborn (Sinclair & Crawford, 1976).

The composition of milk lipids varies to some extent with maternal diet; an increased consumption of dietary polyunsaturated fats, for example, leads to an increase in the PUFA content of milk (Soderhjelm, 1953; Insull & Ahrens, 1959). In American mothers, Guthrie et al. (1977) demonstrated that breast milk contains more PUFA than it has in the past. This change coincides with increased ingestion of vegetable oils, rich in polyunsaturated lipids. Borschel et al. (1986) carried out a comparative study on the fatty acid composition of mature human milk of Egyptian and American women. The study showed that breast milk from Egyptian -women contained a significantly higher percentage of capric, lauric, myristic, linoleic and arachidonic acids. Conversely, milk of American women contained higher percentages of stearic and oleic acids.

^{*}To whom correspondence should be addressed.

There are many reports on the total lipid and fatty acid composition of breast milk concentration from different countries. In contrast, little is known about the fatty acid composition of breast milk of Saudi nursing mothers, and, therefore, this study was designed to provide more information about the fatty acids of their breast milk.

MATERIALS AND METHODS

Subjects and milk samples collection

Fifty-four samples of fresh human milk were obtained from healthy Saudi mothers at 3–4 months of lactation. All mothers ranged in age from 19 to 39 years. Twenty mothers were between the ages of 19 and 24 years, 15 between the ages of 25 and 29 years, and 10 between 30 and 34 years, while nine were over 34 years. Information on the health of mother and infant, vitamin and mineral supplementation, and previous history of lactation were obtained through personal interviews. The mothers expressed one (right) breast totally with a manual pump at 09.00–11.00 a.m. Using this pump, the milk is only in contact with the glass surface. All samples were transported to the laboratory on ice and frozen at -20° C immediately on arrival until analysed.

Lipid analysis

Lipid extraction

Milk lipids were extracted as described by Gibson & Kneebone (1981). A 5 ml sample of milk was diluted with 5 ml water and shaken with 70 ml chloroformmethanol (2:1), the mixture centrifuged for 10 min at 1000g, and the upper phase removed. The lower phase was shaken with a further 20 ml of water and recentrifuged. The lower phase was removed and evaporated to dryness under nitrogen. Total fat was determined gravimetrically on residues of the chloroform extract. All milk samples were extracted within 2 days of collection.

Preparation of fatty acid methyl esters

Methylation of fatty acid was performed by the boron trifluoride (BF_3) -methanol method as described by Harzer *et al.* (1983).

Aliquots of lipid extract were hydrolysed in methanolic KOH (0.5 N) solution by refluxing for 15 min at 90°C in tightly sealed Pyrex tubes. After the addition of BF₃-methanol reagent (14% BF₃ in methanol), the samples were incubated for another 15 min at 19°C and fatty acid methyl esters were extracted into 4 ml of hexane.

Gas-liquid chromatography

The fatty acid composition was determined by gas chromatography using a Shimadzu GC-14A gas chromatograph. The chromatograph was fitted with a glass column $(2 \text{ m} \times 1/8')$ packed with 10% SP-2300 on

100/120 Chromosorb WAW, temperature programmed from 130 to 200°C at 11.3 °C/min; the final oven temperature was maintained for 40 min, the nitrogen carrier gas was set at a flow rate of 40 ml/min. The injection port was maintained at 250°C and the flame ionization detector at 300°C. Aliquots of the derivatized extracts were injected into the column. Identification of the individual fatty acid methyl esters was achieved by comparison with authentic reference standards.

Statistical analysis

All data were stored and analyses were performed using the Statistical Analysis System (SAS Institute Inc., 1988). A *P* value of 5% was chosen to indicate significance. Data are presented as means \pm SD for the number of assays shown. Means were tested by one-way analysis of variance (ANOVA).

RESULTS AND DISCUSSION

Data for fatty acid composition of mature breast milk are shown in Table 1. No short chain fatty acids were noted. The concentration of medium-chain saturated fatty acids (SFAs) (lauric and myristic) comprised 14.9% of the total fatty acids. Saturated fatty acids constitute 56.1% of the total fatty acids in the lipids of mature breast milk. Pacid (16:0) contributes 52.4% of total saturated fatty acids of mature breast milk. Table 1 reveals that fatty acids 14:0, 16:0, 18:0, 18:1 and 18:2 accounted for 83.4% of total fatty acids. Oleic acid (18:1) was the major unsaturated fatty acid, while palmitic acid (16:0) was the major saturated fatty acid.

Table 1. Fatty acid composition of mature breast milk*

Fatty acid	% of total fatty acid (mean + SD)		
Saturated total	56.2		
12:0	6.1 ± 1.8		
14:0	8.9 ± 1.5		
16:0	29.4 ± 3.2		
17:0	0.6 ± 0.2		
18:0	8.3 ± 0.9		
20:0	0.8 ± 0.3		
21:0	0.6 ± 0.4		
22:0	0.8 ± 0.7		
24:0	0.7 ± 0.7		
Unsaturated total	43.9		
Monounsaturated total	34.1		
14:1	0.4 ± 0.1		
16:1	3.7 ± 0.9		
17:1	0.3 ± 0.2		
18:1	28.2 ± 5.5		
18:2	8.7 ± 2.8		
18:3	1.1 ± 0.5		
20:1	0.7 ± 0.4		
22:1	0.8 ± 0.4		
Polyunsaturated total	9.8		

*Number of subjects is 54.

Total unsaturated fatty acids of mature breast milk accounted for 43.9% of the total fatty acids. Furthermore, most of the unsaturates are monounsaturated fatty acids which contribute 34.2% of the total fatty acids.

In order to study the effect of maternal age on fatty acid composition of mature breast milk, the mothers were classified into four age groups. Twenty mothers were between the ages of 19 and 24 years, 15 between the ages of 25 and 29 years, and 10 between the ages of 30 and 34 years, while nine were between 35 and 39 years. On the other hand, to investigate a possible influence of maternal parity upon fatty acid compositions of mothers mature breast milk, the 54 women were divided into four parity groups of low (one-three births), medium (four-six births), high (seven-eight births) and very high (nine-twelve births) parity.

The effect of maternal age on fatty acid composition of mature breast milk is listed in Table 2. Milk from younger mothers (under 34 years of age) had greater PUFA concentrations than that from older mothers. Mothers over 34 years had increased saturated fatty acids. The fatty acid 22:1 concentration was lower in older (over 39 years) than in younger (under 30 years) women. Maternal age had no significant influence on the concentrations of 12:0, 14:0, 17:0, 18:0, 24:0, 16:1, 18:1 and 18:3.

The influence of maternal parity on fatty acid composition of mature breast milk is presented in Table 3. Statistical analysis showed that the maternal parity had no significant effect on the concentrations of 14:0, 17.0, 18:0, 20:0, 21:0, 210, 16:1, 18:3 and 22:1. Milk from mothers with more children (more than eight children) had higher concentrations of 16:0, 24:0, 18:1 and 18:2 than those from mothers with less children. PUFA concentrations from the milks of mothers with more than eight children were higher (about two-fold) than those from mothers with less than four children. Total saturated fatty acids in the milk increased as parity increased, while total unsaturated and monosaturated fatty acids decreased as parity increased.

Fatty acids appear in milk as a result of dietary intake, mobilization from fat depots and endogenous synthesis by the mammary gland. The concentrations of medium-chain saturated fatty acids in the present study are similar to those reported by Borschel *et al.* (1986) in the milk of rural Egyptian women, and lower than that reported by the same authors in the milk of American women. It has been established that medium-chain SFAs are well absorbed by the infant (Bitaman *et al.*, 1983; Fomon, 1974). Furthermore, medium-chain triglycerides, from which medium-chain SFA are derived, are more readily hydrolysed than long-chain triglycerides (Bitaman *et al.*, 1983).

Saturated fatty acids accounted for 56.1% of the total fatty acids in mature breast milk. These findings are contrary to those of Gibson & Kneebone (1981) who reported that saturated fatty acids comprised 46% of the total fatty acids of mature breast milk. Lower values for saturated fatty acids have been reported by Borschel *et al.* (1986) for both American and Egyptian mothers.

Oleic acid represented the largest percentage of total unsaturated fatty acid in Saudi mothers milk. The level of oleic acid reported in the present study is lower than that observed in the milk of American women and higher than that found in the milk of Egyptian women (Borschel *et al.*, 1986). Moreover, Gibson & Kneebone (1981) obtained higher values for oleic acid than those reported in the present study.

Fatty acid	Age (years)				
	19-24 (n=20)	25-29 (n=15)	30-34 (n=10)	35-39 (n=9)	
	% of total fatty acid				
Saturated total	56.5	55.4	57.0	59.7	
12:0	6.4 ± 1.41^{a}	6.5 ± 2.09^{a}	6.6 ± 2.40^{a}	5.5 ± 1.25^{a}	
14:0	$8.8 \pm 1.62^{\mathrm{a}}$	8.0 ± 0.96^{a}	9.7 ± 1.71^{a}	9.5 ± 1.45^{a}	
16:0	29.2 ± 2.00^{a}	29.2 ± 3.36^{a}	30.2 ± 4.14^{a}	32.9 ± 2.17^{b}	
17:0	0.85 ± 0.21^{a}	0.81 ± 0.17^{a}	0.61 ± 0.1^{a}	$0.75\pm0.12^{\rm a}$	
18:0	$8.7 \pm 1.1.0^{a}$	7.8 ± 0.73^{a}	7.6 ± 0.63^{a}	$0.68\pm0.20^{\mathrm{a}}$	
20:0	0.54 ± 0.08^{ab}	0.6 ± 0.32^{a}	0.39 ± 0.14^{b}	0.68 ± 0.20^{a}	
21:0	$0.86\pm0.30^{\mathrm{a}}$	0.9 ± 0.29^{a}	0.65 ± 0.22^{ab}	0.53 ± 0.09^{b}	
22:0	0.70 ± 0.18^{ab}	1.0 ± 0.52^{a}	0.49 ± 0.32^{b}	0.76 ± 0.39^{ab}	
24:0	0.39 ± 0.09^{a}	$0.6\pm0.01^{\mathrm{a}}$	0.80 ± 0.18^{b}	$0.55\pm0.09^{\rm a}$	
Unsaturated total	43.5	44.6	43.01	40.3	
Monounsaturated total	33.6	34.2	312.61	32.9	
16:1	3.5 ± 0.98^{a}	$3.6\pm0.90^{\rm a}$	3.9 ± 0.78^{a}	3.9 ± 1.20^{a}	
17:1	0.55 ± 0.19^{a}	0.6 ± 0.17^{a}	0.48 ± 0.17^{ab}	0.50 ± 0.17^{b}	
18:1	28.5 ± 8.51^{a}	28.9 ± 2.06^{a}	27.8 ± 2.84^{a}	28.1 ± 1.52^{a}	
18:2	8.93 ± 2.44^{a}	$9.2 \pm 2.48^{\rm a}$	9.3 ± 3.51^{a}	6.4 ± 2.32^{b}	
18:3	0.97 ± 0.49^{a}	1.2 ± 0.63^{a}	1.8 ± 0.53^{a}	0.99 ± 0.15^a	
22:1	$1.02 \pm 0.29^{\rm a}$	$1.0 \pm 0.49^{\rm a}$	0.5 ± 0.18^{b}	0.43 ± 0.18^{b}	
Polyunsaturated total	9.9	10.4	10.4	7.4	

Table 2. Effect of maternal age on fatty acid composition of mature breast milk*

*Mean \pm SD; the number (n) of subjects is in parentheses.

Means within the same row sharing the same superscript(s) are not significantly different using one-way ANOVA test (P < 0.05).

Fatty acid	Parity					
	1-3 (n=21)	4-6 (n=13)	7-8 (n=12)	9-12 (n=8)		
	% of total fatty acid					
Saturated total	55.7	65.2	57.0	64.8		
12:0	7.0 ± 0.96^{a}	5.0 ± 1.47^{b}	6.4 ± 2.29^{ab}	4.9 ± 1.44^{b}		
14:0	$8.5 \pm 1.39^{\mathrm{a}}$	8.4 ± 1.18^{a}	9.5 ± 2.09^{a}	9.7 ± 1.01^{a}		
16:0	28.6 ± 2.17^{a}	32.4 ± 3.37^{b}	31.3 ± 2.69^{ab}	$36.8 \pm 0.12^{\circ}$		
17:0	$0.89 \pm 0.19^{\rm a}$	0.65 ± 0.20^{a}	0.72 ± 0.13^{a}	0.89 ± 0.14^{a}		
18:0	8.1 ± 1.25^{a}	$8.06\pm0.53^{\rm a}$	8.2 ± 0.90^{a}	8.7 ± 0.84^{a}		
20:0	0.32 ± 0.33^{a}	0.32 ± 0.09^a	$0.72\pm0.13^{\mathrm{a}}$	0.7 ± 0.16^{a}		
21:0	$0.88\pm0.27^{\mathrm{a}}$	$0.52\pm0.23^{\rm a}$	0.78 ± 0.26^{a}	0.60 ± 0.22^{a}		
22:0	$0.85 \pm 0.48^{\rm a}$	0.45 ± 0.22^{a}	0.82 ± 0.47^{a}	0.53 ± 0.23^a		
24:0	0.44 ± 0.14^{a}	0.48 ± 0.1^{a}	0.53 ± 0.06^{a}	0.82 ± 1.01^{b}		
Unsaturated total	44.3	43.8	40.69	36.2		
Monounsaturated total	34.0	33.8	32.88	30.1		
16:1	3.7 ± 0.99^{a}	$4.0 \pm 0.67^{\mathrm{a}}$	3.8 ± 0.91^{a}	$4.1\pm1.34^{\rm a}$		
17:1	$0.61\pm0.18^{\mathrm{a}}$	0.44 ± 0.19^{ab}	0.5 ± 0.16^{ab}	0.35 ± 0.165^{b}		
18:1	29.0 ± 2.38^{a}	28.7 ± 1.56^{a}	28.2 ± 2.32^{a}	25.5 ± 1.21^{b}		
18:2	$9.2 \pm 2.89^{\rm a}$	9.0 ± 2.43^{a}	$7.0 \pm 1.82^{\rm a}$	4.9 ± 0.52^{b}		
18:3	$1.1 \pm 0.63^{\rm a}$	0.90 ± 0.30^{a}	1.4 ± 0.51^{a}	0.91 ± 0.33^{a}		
22:1	0.73 ± 0.43^{a}	0.69 ± 0.43^{a}	$0.40\pm0.21^{\rm a}$	0.59 ± 0.30^{b}		
Polyunsaturated total	10.3	9.9	8.1	5.74		

Table 3. Effect of maternal parity on fatty acid composition of mature breast milk*

*Mean \pm SD, the number of subjects (*n*) in is parentheses.

Means within the same row sharing the same superscript(s) are not significantly different using one-way ANOVA test (P < 0.05).

Total unsaturated fatty acids comprised less than 50% of total fatty acids of Saudi mothers' milk fat and most of the unsaturates are monounisaturated fatty acids which contribute 34.2% of the total fatty acids of mature breast milk. Several investigators (Borschel et al., 1986; Gibson & Kneebone, 1981; Bitaman et al., 1983) reported that unsaturated fatty acids accounted for more than 50% of total fatty acids; such levels are higher than those of our findings. The lower levels of unsaturated fatty acids in the present study undoubtedly reflect diets with higher proportions of saturated fat. The obtained data indicated that the most abundant essential fatty acid in Saudi mothers mature breast milk was linoleic acid (18:2). The linoleic acid level obtained in the present study was in agreement with the findings of Hall (1979) for British mothers. On the other hand, the level of linoleic acid reported in our study was higher than the findings of Bracco et al. (1972) for pooled milk from a Swiss milk bank. The level of linoleic acid in Saudi mothers' milk was substantially lower than reported by some other investigators. Linoleic acid values of 14-16% were reported in previous studies (Bitaman et al., 1983; Jansson et al., 1981) for American women and 11% in mature breast milk of Australian women (Gibson & Kneebone, 1981).

Studies by several investigators have demonstrated that the composition of human milk fat readily changes with diet. Sanders *et al.* (1978) found that breast milk of 22 English vegans contains 32% linoleic acid (18:2) as compared to 7% in omnivore controls. Mellies *et al.* (1979) reported that linoleic acid levels of 14% in the milk of American women could be increased to 24% by *ad libitum* consumption of a diet rich in polyunsaturated fat and, conversely, decreased to 10% by consumption of a diet rich in saturated fat. The linoleic acid level in the present study falls in the range of that stated by the American Academy of Pediatrics Committee on Nutrition (American Academy of Pediatrics, 1976) who reported that linoleic acid comprises 8–10% of the fat in human milk. One could conclude that the moderate level of linoleic acid in Saudi mothers' mature breast milk reflects the typical dietary habits of the Saudi people who consume foods derived from animals sources as the main part of their daily meals. It is well established that infants have an urgent need for linoleic and linolenic acids in order to synthesize long-chain PUFAs which are important for cell membrane and prostaglandin synthesis (Van Dorf *et al.*, 1964).

The P/S ratio (total of polyunsaturated fatty acids with more than one double bond divided by total saturated fatty acids) was approximately 0.17. This P/S ratio is remarkably lower than that obtained by Harzer *et al.* (1983) who reported a value of approximately 0.32.

The GLC analysis indicated that Saudi mothers' mature breast milk contained 18:1, 16:0, 18:2, 18:0 and 14:0 as major fatty acids. These acids accounted for 83.4% of the total fatty acids; such values are slightly lower than those found previously by Clark *et al.* (1982) who noted that 18:1, 16:0, 18:2, 18:0 and 14:0 accounted for 86.7–88% of the total fatty acids, and that the stage of lactation from 2 to 16 weeks had little influence on the levels of these fatty acids.

The current study has examined the fatty acid composition of mature breast milk in 54 lactating mothers. The general lipid classes, in particular cholesterol, the triglycerides, the steroids and the phospholipids, need to be investigated. They should be quantified and the influence of maternal diet measured. The fat-soluble vitamins should also be determined. There is a definite need for a systematic determination of the effect of stage of lactation on all these vitamins.

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