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Fatty Acid Composition of Human Breast Milk

Changes during the first week after delivery

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ABSTRACT

Breast milk was collected daily from 11 healthy Polish mothers during the first week after delivery. The method of Folch was found suitable for fat extraction from breast milk. The concentration of total fat and of triglycerides and the fatty acid composition of total fat were determined. The concentration of total fat was 2.4 g/100 ml on the second day after delivery and 4.4 g/100 ml on the seventh day. There was a similar rise in the triglyceride concentration. No relation was found between the concentration of total fat and the relative concentrations of fatty acids. The major fatty acids were 18:1 and 16:0, which comprised about 70 per cent of the fatty acids. The essential fatty acids comprised about 10 per cent of the total fatty acids. The fatty acid spectrum changed during the sampling period. The proportions of 10:0, 12:0, 14:0 and 16:1 increased, those of 16:0, 18:2, 20:0 and 22:0 were unchanged, and those of 18:0 and 20:4 decreased. Very long-chained polyunsaturated fatty acids, e.g. 22:6, were also present. All the polyenoic acids - except 18:2 - showed the highest concentrations on the first day after delivery.

INTRODUCTION

Newborn babies have low serum lipid concentrations at birth (10, 23, 24) and have a relatively low content of 18:2 (linoleic acid, with 18 carbon atoms and 2 double bonds) and a relatively high content of 20:4 (arachidonic acid) in cholesterol esters, phospholipids and triglycerides (11). During the perinatal period the demands for these essential fatty acids are probably high. An example of the high need for 20:4 is that this acid is a major substrate for prostaglandin synthesis, which has been shown to be intense in neonatal tissues (15). The essential fatty acids are also substrates for the synthesis of longer-chained and more polyunsaturated fatty acids, which are important for the growing baby (20). At birth the stores of the essential fatty acids in muscle (3) and adipose tissue (21), for example, are limited, and an early exogenous supply of these acids is therefore important in order to avoid a condition of essential fatty acid deficiency (8). The natural exogenous source of the essential fatty acids in newborns is breast milk.

Only few investigations have been made on the fatty acid pattern in human breast milk during the first week after delivery (2, 4, 9, 14, 16, 19). Regional differences in the composition of breast milk have been observed (2, 13, 17, 19). The existing reports, however, give very little information about normal individual variations and changes during the initial period of lactation. Furthermore, knowledge about long-chained fatty acids is very sparse.

The purpose of this study was to investigate the total amount of fat and triglycerides and the relative concentrations of fatty acids in the total fat in breast milk from individual mothers, and to follow the changes in the fatty acid composition during the first week after delivery.

MATERIAL AND METHODS

Subjects

Eleven healthy mothers, delivered in March-August 1977 at the Clinic of Obstetrics, National Institute of Mother and Child, Warsaw, Poland, were studied. Their pregnancies passed without complications and the deliveries were normal. All newborns were healthy and had 5-minute Apgar scores of 8-10.

Breast milk was collected each day during the first week after delivery. The first portion of the second meal was always used for the analyses and this portion was expressed manually by the mothers before the baby was fed.

All mothers began to secrete milk on the first day after delivery. In three mothers the amounts were minute on the first two days and too small to allow duplicate estimations of the total fat and triglyceride concentrations. These data are therefore not included.

Methods

The specimens (1-10 ml) were frozen and stored at -20°C until analysed. All analyses were performed within three days after collection of the samples. The concentration of total fat and of triglycerides, and the fatty acid composition of the total fat, were determined.

Total fat was extracted by the method of Folch (7) and its concentration was determined as described by Woodman and Price (22). Duplicate estimations were always made and the difference between the two values never exceeded 2.5 per cent.

In thirty samples the total fat was determined both in whole breast milk diluted 1:5 with 0.9 per cent NaCl, and in the extract. A high correlation was found between the values obtained by these two methods, as shown in Figure 1.

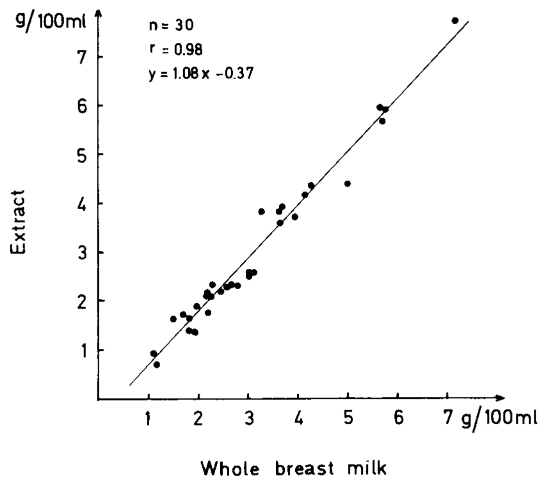

Fig. 1. A comparison between estimations of total fat in whole breast milk and in extract of the same breast milk.

The method of Folch was thus accepted in this study, though it was primarily described as a method of fat extraction from serum. Standards were prepared from pooled breast milk. As different methods of extraction and determination of total fat have been used both in this and in other laboratories previously, extractions of fat were performed both by the method of Folch (7) and by that of Röse-Gottlieb (18) on a smaller material. In these two extracts the total fat was determined both gravimetrically (12) and as described by Woodman and Price (22). The differences between these four estimations never exceeded ±4 per cent.

Triglycerides in the extract were determined according to the method of Eggstein (5).

Fatty acids. After evaporation under N_2 at 60^oC, the extracted acids were transesterified with 2 ml of BF_3 in methanol (14 per cent by weight). Methyl esters were extracted with N-hexane (2 x 1 ml), evaporated under N_2 and dissolved with 20 µl of n-hexane.

1-µl samples were analysed in a PYE 104 gas-liquid chromatograph which was equipped with paired 1.52 glass columns (Φ = 4 mm) of 10 per cent polyethylene glycol adipate on Gas-Chrom Z, 80/100 mesh dual flow rate of 40 ml/min. The column temperature was maintained at 189^oC and the temperature of the detectors was 220^oC. Standard mixtures of fatty acid methyl esters (NIH standards, KE, KF, KD, methyl 5, 8, 11, 14, 17 eicosaphentaenoate, methyl 4, 7, 10, 13, 16, 19 docosahexaenoate, and methyl erucate $C_{22:1}$) and Sigma Chemical Corporation fatty acid standards Stock No. 189-1 and methyl arachidonate $C_{20:4}$ were used for idenfication of the fatty acids. Peak areas were calculated by multiplication of the absolute retention volumes by the peak height. Values are thus given as area percentages.

In a smaller material the fatty acids were analysed after extraction of fat both by the method of Folch (7) and by the method of Röse-Gottlieb (18). Only minor differences were found and no systematic differences in fatty acid composition attributable to the method of fat extraction were found.

RESULTS

Concentrations of total fat and triglycerides

The concentrations of total fat and triglycerides in breast milk in the first seven days after delivery are given in Figure 2.

Fig. 2. Total fat and triglycerides in human breast milk during the first week of lactation. (Mean ± SEM. n=8 on days 1, 2, n=11 on days 3-7 in all figures.)

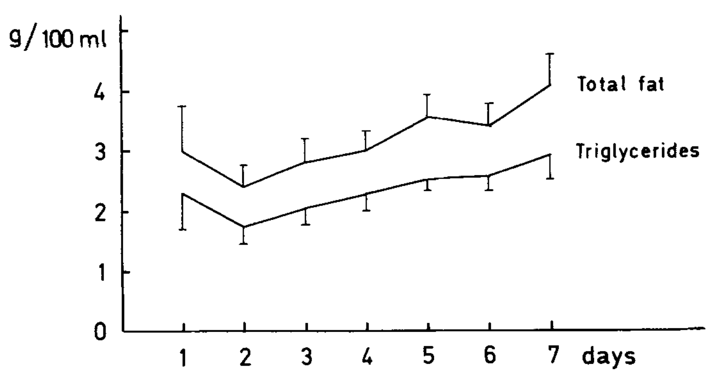

Table 1. The relative concentrations of fatty acids in human colostrum obtained in the 1st, 3rd and 7th days after delivery (mean ± SD, per cent of the total amount). x $p < 0.05$, xx $p < 0.01$, xxx $p < 0.001$ indicate the degree of significance for the difference between the concentration in question and that on the 7th day (paired t-test).

	1st day	3rd day	7th day
C 8:0	-	-	0.08±0.03
C10:0	0.10±0.06	0.16±0.10	0.44±0.21
C12:0	0.92±0.22^{xx}	1.65±0.93^{xxx}	2.79±1.05
C14:0	3.91±0.52^{xxx}	5.38±1.69^{xx}	5.15±1.43
C14:1	0.29±0.08^{x}	0.39±0.09	0.40±0.08
C15:0	0.43±0.11^{xx}	0.54±0.10	0.51±0.10
Unidentified	0.21±0.09	0.20±0.04	0.18±0.04
C16:0	24.13±1.58	24.85±2.02	23.98±1.73
C16:1	2.68±0.26^{xx}	3.02±0.42^{xx}	3.58±0.62
C17:0	0.62±0.10	0.63±0.06	0.61±0.07
Unidentified	0.44±0.07	0.47±0.08	0.52±0.08
C18:0	9.02±0.71^{xx}	7.91±0.82	7.48±0.85
C18:1	38.56±4.37	36.56±3.01^{x}	38.07±3.07
C18:2	6.70±1.59	6.94±0.97	7.60±1.14
C18:3	0.65±0.12	0.68±0.10	0.73±0.11
C20:0	0.85±0.23^{x}	0.88±0.19	0.93±0.21
C20:1	2.56±0.50^{xxx}	2.30±0.55^{xxx}	1.62±0.40
C20:2	0.85±0.26^{xxx}	0.68±0.20^{xxx}	0.50±0.14
C20:3	0.84±0.30^{xx}	0.67±0.17^{xx}	0.50±0.13
C20:4	1.37±0.37^{x}	1.05±0.27^{x}	0.87±0.17
C22:0	0.45±0.23	0.40±0.23	0.29±0.12
C22:1+20:5	1.78±0.52^{xx}	1.64±0.69^{xx}	1.01±0.33
Unidentified	0.59±0.22^{x}	0.53±0.18^{x}	0.37±0.12
C24:0	0.86±0.42^{xx}	0.83±0.36^{xx}	0.50±0.31
C24:1	0.93±0.53^{x}	0.86±0.34^{xx}	0.55±0.20
C22:6	0.90±0.39	0.77±0.21	0.70±0.16

The total fat concentration increased from the second to the seventh day in all mothers except one ($p < 0.05$). The triglyceride concentration comprised about 75% of the total fat and increased in all mothers during this period.

Fatty acid composition of total fat

The fatty acid composition was determined each day during the first week after delivery. The complete results of the analyses for the first, third and seventh days are summarized in Table 1. More than 20 different fatty acids, including odd-numbered ones, were detected. A few of these detected fatty acids could not be identified. Table 2. shows the corresponding values calculated only for the eight acids 14:0, 16:0, 16:1, 18:0, 18:1, 18:2, 18:3 and 20:4. The fatty acids 16:0, 18:0, 18:1 and 18:2 were predominant throughout the first week after delivery.

Table 2. The relative concentrations of fatty acids in human colostrum recalculated from the sum of the concentrations of 14:0, 16:0, 16:1, 18:0, 18:1, 18:2, 18:3 and 20:4 representing the total amount of fatty acids (mean ± SD, per cent).

	1st day	3rd day	7th day
14:0	4.48±0.50	6.23±1.96	5.90±1.71
16:0	27.76±2.02	28.78±2.23	27.37±2.00
16:1	3.08±0.22	3.50±0.44	4.07±0.66
18:0	10.39±1.02	9.17±1.03	8.54±1.04
18:1	44.24±3.94	42.35±3.24	43.40±2.75
18:2	7.79±1.94	8.04±1.14	8.65±1.18
18:3	0.75±0.15	0.79±0.12	0.83±0.11
20:4	1.58±0.45	1.21±0.31	0.99±0.19

A change in the fatty acid spectrum was noted during the observation period. The relative concentrations of the saturated fatty acids with a chain length of 10-14 carbon atoms increased. The concentrations of 16:0, 20:0 and 22:0 remained essentially unchanged, while that of 18:0 decreased. There was an increase in 16:1 and a decrease in the monounsaturated fatty acids with more than 18 carbon atoms. Of the polyunsaturated fatty acids, 18:2 showed no significant change, but the relative concentration of most of the longer-chained fatty acids including 20:4 decreased. Figure 3 shows the changes in the relative amounts of some of the saturated fatty acids and Figure 4 the changes in some of the polyunsaturated fatty acids.

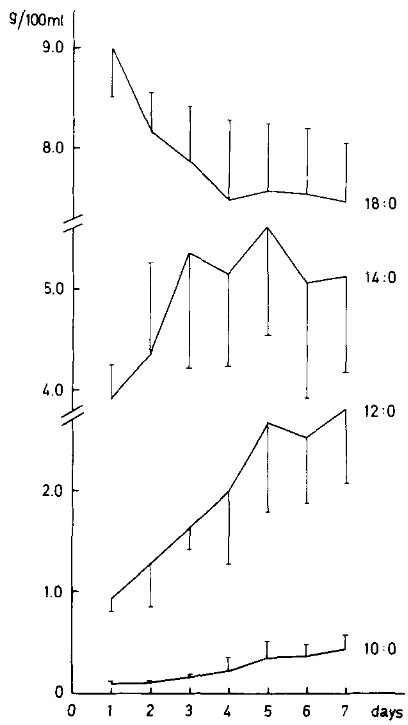

Fig. 3. Changes in the relative amounts of some saturated even-numbered fatty acids in human breast milk during the first week of lactation. (Mean ± SEM).

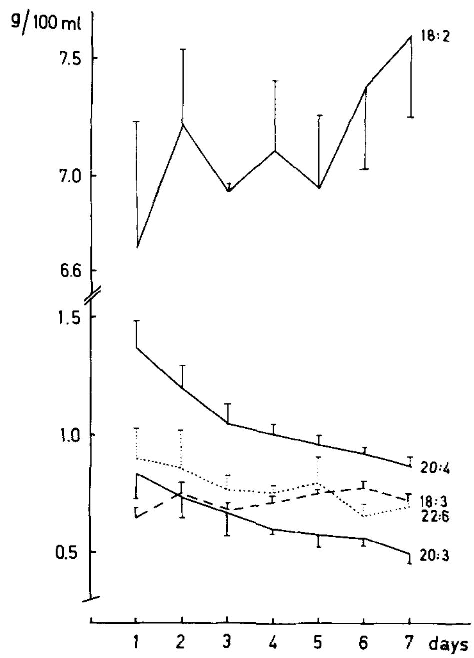

Fig. 4. Changes in the relative amounts of polyunsaturated long-chained fatty acids in human breast milk during the first week of lactation. (Mean ± SEM).

Relationship between the fatty acid composition and the concentration of total fat

No relationship was found between any of the fatty acids and the concentration of total fat on any of the days after delivery. Figure 5 shows the concentrations of 20:4 in relation to the concentration of total fat on the seventh day after delivery.

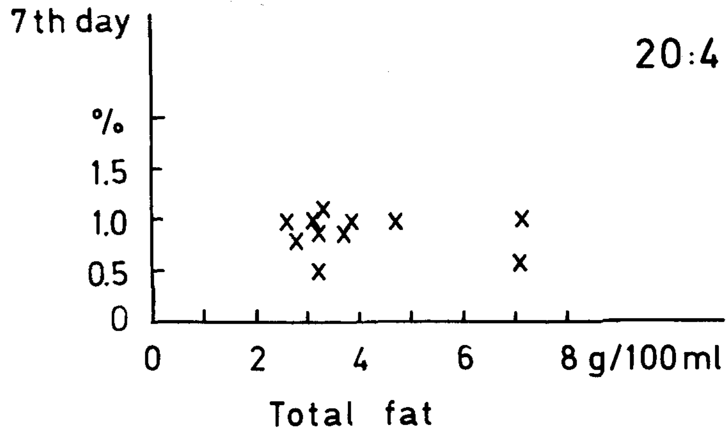

Fig. 5. Relation between the relative concentration of 20:4 and the concentration of total fat on the seventh day after delivery.

DISCUSSION

There are regional differences in the concentration of total fat and the composition of fat in human breast milk (2, 13, 17, 19) but there are probably no age-related or seasonal differences in these respects (19). In the present study no regard was taken to the age or parity of the mothers.

As the production of breast milk during the first days after delivery is small and removal of relatively large amounts of milk can easily disturb lactation, the method of Folch (7) (which requires only 0.5 ml of the sample) was used. This method, which from the beginning was intended for extraction of fat from serum, was found to be valid for fat extraction from breast milk. This is in agreement with a previous report (12). As the concentration of fat increases during the meal (6), the first portions of milk collected in this study would not reflect the mean concentration of total fat in the whole meal. The fat content seemed to be higher on the first day, but almost the entire volume of this meal was used for the analysis, which may explain this finding. The individual variations were also large. Nevertheless, an increase in the mean concentration of total fat was observed during the first week after delivery. In this study of the first portion of milk the triglycerides comprised a smaller proportion of the total fat than has been observed in studies on whole-meal milk. Despite the wide range of the concentration of total fat, the proportions of fatty acids were remarkably constant and were not at all correlated to the concentration of total fat. Therefore, the fatty acid spectrum in the first portion of milk is probably representative of that in the whole meal, as has been reported previously (6).

As expected, there were both similarities and dissimilarities in the fatty acid spectrum of breast milk from Polish mothers as against that observed in mothers of other countries. One major difference was in the concentration of 18:2. Japanese (19) and Ugandian (2) mothers have been found to secrete colostrum with a higher content of 18:2 (14% and 12% respectively) than Polish mothers (8%). The differences are probably due to environmental factors. Information about fatty acids with longer chain lenghts is sparse, and comparisons with other reports are therefore difficult and there are few reports with which the present findings can be compared.

The fatty acid spectra of serum lipids in newborn babies are different from those in adults (10). The most impressive findings at birth are the much lower contents of 18:2 in cholesterol esters, phospholipids and triglycerides and the higher contents of 20:4 in all serum lipid fractions. The stores of the essential fatty acids are limited at birth, and if the exogenous supply is inadequate essential fatty acid deficiency may develop as early as during the first weeks postnatally (8). For prevention of this deficiency 20:4 is about three times more effective than 18:2 (1). The former fatty acid is one of the main substrates for prostaglandin synthesis, which is intense during the perinatal adaptation period (14). Furthermore, 20:4 is a substrate for the synthesis of longer-chained and more polyunsaturated fatty acids - important substances for organ development in the rapidly growing baby (20). Consequently the demands for essential fatty acids in the neonate seem to be high. These high requirements are covered by the large content of 20:4 in the circulating lipids in the newborn and the content of this fatty acid in the colostrum. The presence of 20:4 and the extremely long-chained polyunsaturated fatty acids in human colostrum means that the newborn baby obtains the necessary substrate for immediate use in many important metabolic pathways.

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