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SIMULTANEOUS QUANTIFICATION OF TOTAL MEDIUM- AND LONG-CHAIN FATTY ACIDS IN HUMAN MILK BY CAPILLARY GAS CHROMATOGRAPHY WITH SPLIT INJECTION

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SUMMARY

Four different quantification methods for the capillary gas chromatographic determination of medium-chain fatty acids (6:0–12:0) and myristic acid in human milk samples, using a split injector, were compared. Odd-carbon-numbered fatty acids (5:0–17:0) were added as internal standards. Each medium-chain fatty acid and myristic acid was calculated on the basis of: (1) the peak area of the internal standard with one methylene group less; (2) the peak area of the internal standard with one methylene group more; (3) half the sum of the peak areas of the internal standards with one methylene group less and more (bracketting method); (4) the peak area of 17:0. The peak-area ratio of each analyte and 17:0 in a standard was found to be subject to an unacceptably high coefficient of variation. From the methods using internal standards with one methylene group more and less, the bracketting method was found to be the best, resulting in recoveries close to 100%, with the lowest coefficients of variation. The method was applied for the determination of the fatty acid composition of mature milk samples of 47 Curaçaoan women.

INTRODUCTION

Mature human milk of well nourished mothers contains a rather uniform amount of 3–4 g fat per 100 ml (ca. 52 energy %), which contains ca. 98% triglycerides [1]. The fatty acid composition varies to a considerable extent with the maternal diet [2–7], the duration of lactation [8–10] and the degree of prematurity [9].

Knowledge of the composition of human milk is of importance to the under-

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standing of diseases that may occur in later life (e.g. atherosclerosis and obesity) and the nutrients that should be added to infant formulas. With respect to the fatty acid composition the relative amounts of the following (classes of) fatty acids seem to deserve special attention: (a) the long-chain C_{20}–C_{22} polyunsaturated fatty acids, including one of the precursors of the eicosanoids (arachidonic acid, 20:4c,ω6) and one of brains structural fatty acids (docosahexaenoic acid, 22:6c,ω3), which should be considered essential for the neonate [11]; (b) the medium-chain fatty acids (MCFAs; 6:0–12:0), which are exclusively synthesized in the mammary gland and represent an easily absorbable energy source for the breastfed infant [12]; (c) myristic acid (14:0), which is known to possess a hypercholesterolemic effect and may be part of the, still unproven, cholesterol challenge hypothesis [1].

There is a need for precise and standardized methods for the determination of the fatty acid composition of human milk. Fatty acid analyses are still predominantly performed by packed-column gas chromatography (GC). Besides their much lower separating power, compared with capillary columns, such methods may suffer from undesirable, concentration-dependent adsorption on the stationary phase-supporting solid. On the other hand, the most widely applied injection technique for routine capillary GC profiling methods, the split injection, suffers from split-dependent discrimination of the relatively low- and high-boiling components [13]. This problem, which may be overcome by the use of on-column injectors, will theoretically have a negative effect on the quality control.

We report here the simultaneous capillary GC determination of MCFAs and long-chain fatty acids (LCFAs), using the split injection mode. By the addition of odd-carbon-numbered MCFAs (5:0–13:0), 15:0 and 17:0 as internal standards, the quality-control data of several quantification methods for MCFAs were compared. The quantification and quality control of LCFA determinations has been described in a previous paper [14]. The finally selected method was used to establish the total fatty acid composition of 47 human milk samples of Curaçaoan mothers.

EXPERIMENTAL

Standards and reagents

Fatty acid and fatty acid methyl ester standards (for abbreviations see caption to Fig. 1) were obtained from Applied Science (Oud Beyerland, The Netherlands), United Technologies Packard (Downers Grove, IL, U.S.A.) and Chrompack (Middelburg, The Netherlands). All other reagents were from Merck (Darmstadt, F.R.G.).

Samples

Mature human milk samples (more than ten days after birth) were obtained from 47 well nourished mothers (ages 18–27 years), all born on Curaçao. None of these mothers was on a restricted diet. All samples were expressed halfway through a daytime feeding and collected in sterile plastic containers without preservative. The samples were deep frozen ($-20°C$) immediately until analysed.

Standard solutions

The following standard solutions were prepared: a standard mixture containing 1 g each of the methyl esters of 6:0, 8:0, 10:0, 12:0 and 14:0, and 0.1 g of cholesterol per litre of chloroform; an internal standard mixture containing 1 g each of the methyl esters of 5:0, 7:0, 9:0, 11:0, 13:0, 15:0 and 17:0, and 0.1 g of 5β-cholestan-3α-ol per litre of chloroform; a solution containing 10 g of butylated hydroxytoluene (BHT) per litre of methanol.

Transesterification of fatty acids

A 100-μl volume of human milk, 200 μl of the internal standard mixture and 200 μl of the BHT- solution were added to a 15-ml Sovirel tube. After the addition of 2 ml of a methanol–hydrochloric acid solution, prepared by adding 50 ml of methanol to 10 ml of an aqueous solution of 6 *M* hydrochloric acid, the tube was flushed with a stream of nitrogen, tightly capped and heated at 90 °C for 4 h. After cooling, the sample was extracted with 1 ml of hexane. The hexane layer, containing the fatty acid methyl esters, was collected for GC analysis.

Gas chromatographic profiling of total fatty acids

Portions (2 μl) of the hexane layers were automatically injected into a Hewlett-Packard Model 5880 gas chromatograph equipped with a Model 7672 A automatic injection system and a 50 m $\times$ 0.20 mm I.D. apolar CP-Sil-5 cross-linked methyl silicone-coated column (Chrompack). The flow-rate of helium was 0.52 ml/min, the split ratio 1:20, the flame ionization detector temperature 300 °C, and the injector temperature 220 °C. The oven temperature programme was: 60 °C, 4 °C/min to 200 °C, 5 °C/min to 285 °C, 15 min at 285 °C.

Identification, quantification and quality control

Fatty acid methyl esters were identified by comparing their retention times with those of known standards. Peak areas were calculated using a Nelson Analytical 3000 data system (Cupertino, CA, U.S.A.).

Except for 14:0 and 14:1c,ω5, concentrations of LCFAs were determined by calculating the area ratio of each individual fatty acid and the internal standard 17:0. Assuming that, independent of the number of carbon atoms and double bonds, equal weights of LCFAs give rise to equal GC peak areas, the concentration (in g/l) was calculated by multiplying the above mentioned peak-area ratio by the ratio of the amount of added 17:0 and the volume of the milk sample [14].

For the MCFAs and 14:0 we compared the results of four quantification methods. For these methods we calculated the ratio of the peak area of each MCFA and 14:0 and (1) the peak area of the internal standard with one methylene group less ($n-1$ method), (2) the peak area of the internal standard with one methylene group more ($n+1$ method), (3) half the sum of the peak areas of the internal standards with one methylene group less and more (bracketting method) and (4) the peak area of 17:0. For the quantification (g/l) the respective response factors for each of the fatty acids and the internal standard(s) were taken into account. These were calculated from the GC peak areas of a standard containing equal weights of even- and odd-carbon-numbered saturated fatty acid methyl

esters (5:0–15:0, 17:0) that was prepared as follows: to a 15-ml Sovirel tube were added 200 μl of the standard mixture, 200 μl of the internal standard mixture and 200 μl of the BHT solution. Transesterification was done as described above.

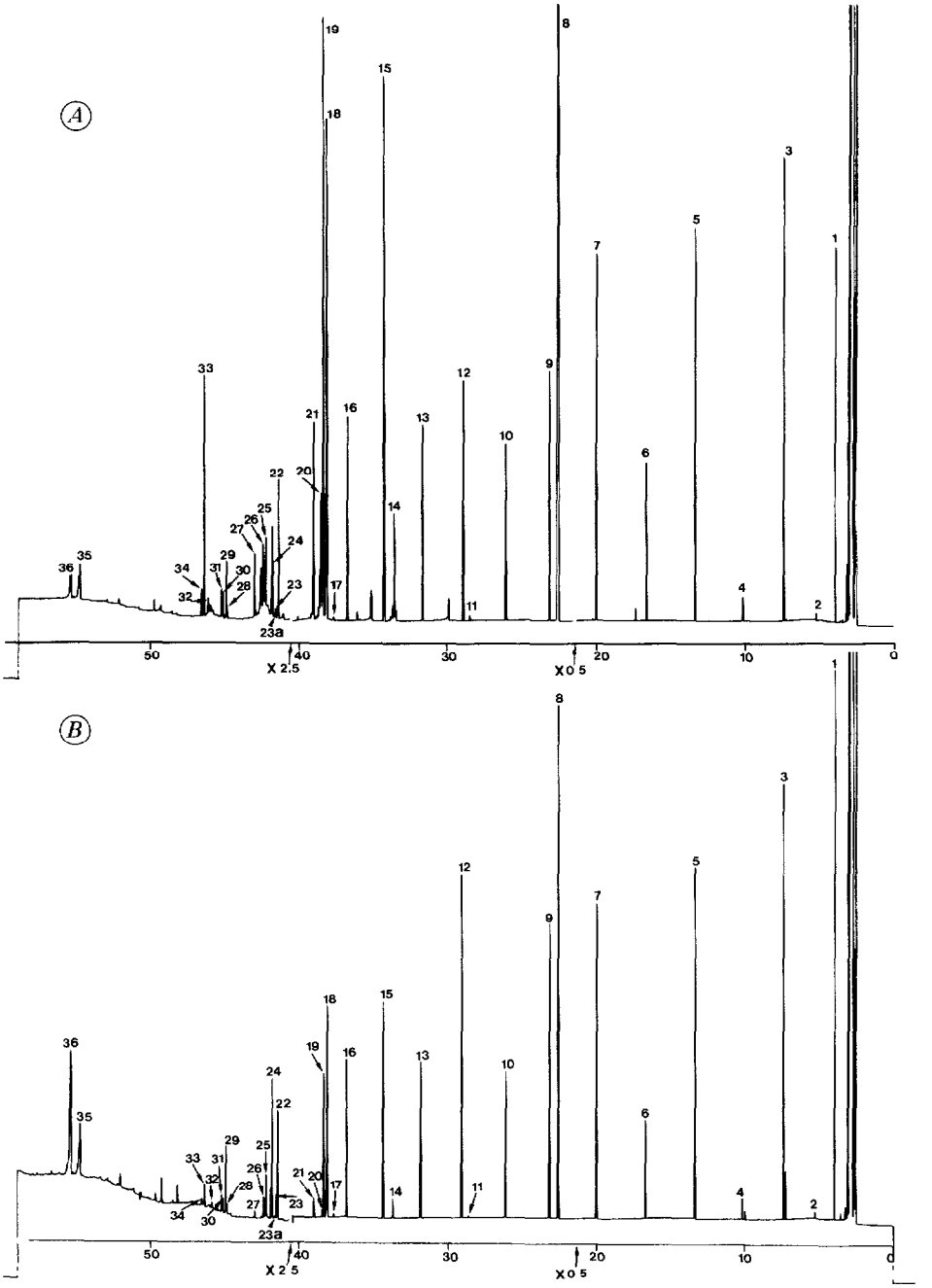
Within-series and series-to-series quality control was studied using the data for a pooled human milk sample of Curaçaoan origin. Recoveries of MCFAs and 14:0 were calculated by analysing 100 μl of the pooled milk sample enriched with 200 μl of the standard mixture.

RESULTS AND DISCUSSION

Fig. 1 shows typical gas chromatograms of methylated total fatty acids in a human milk sample of Curaçaoan origin containing a low percentage of MCFAs (10.56 g per 100 g) and in a Tanzanian sample containing a high percentage of MCFAs (26.90 g per 100 g). The difference between the relatively low and high percentages of MCFAs is amongst others reflected by the peak-height ratio of lauric acid (12:0; peak 9) and palmitic acid (16:0; peak 15), a fatty acid with a rather constant relative amount (see below).

Table I shows the response factors of MCFAs and 14:0 as calculated on the basis of four different internal standards. Relative to the response factors calculated by using the odd MCFAs as internal standards, the peak-area ratios of MCFAs and 17:0 were found to be subject to high coefficients of variation (C.V.). This phenomenon is caused by variation in the previously mentioned split-dependent discrimination between components with clear differences in their boiling points. (For instance the boiling points [15] of the methyl esters of 6:0, 10:0, 14:0, 16:0 and 18:0 (stearic acid) are ca. 151, 224, 295, 418 and 443°C, respectively; note an increase of the boiling temperature of ca. 30°C per ethylene unit, with a sudden step of ca. 120°C between 14:0 and 16:0.) Based on these high coefficients of variation an unacceptably poor precision of MCFA and 14:0 data

Fig. 1. Typical gas chromatograms of methylated total fatty acids in (A) a human milk sample of Curaçaoan origin containing a low percentage of MCFA (10.56 g per 100 g) and (B) a human milk sample of Tanzanian origin containing a high percentage of MCFA (26.90 g per 100 g). Time axis in minutes. Relative recording attenuation values are indicated on the time axis. Internal standards 5:0 (peak 1), 7:0 (peak 3), 9:0 (peak 5), 11:0 (peak 7), 13:0 (peak 10), 15:0 (peak 13) and 17:0 (peak 16). 5β-Cholestan-3α-ol (peak 35) and antioxidant BHT (peak 8) were added to the samples before transesterification. Peaks: 1 = valeric acid, 5:0; 2 = caproic acid, 6:0; 3 = enanthic acid, 7:0; 4 = caprylic acid, 8:0; 5 = pelargonic acid, 9:0; 6 = capric acid, 10:0; 7 = undecylic acid, 11:0; 8 = butylated hydroxy-toluene, BHT; 9 = lauric acid, 12:0; 10 = tridecylic acid, 13:0; 11 = myristoleic acid, 14:1c,ω5; 12 = myristic acid, 14:0; 13 = pentadecylic acid, 15:0; 14 = palmitoleic acid, 16:1c,ω7; 15 = palmitic acid, 16:0; 16 = margaric acid, 17:0; 17 = γ-linolenic acid, 18:3c,ω6; 18 = linoleic acid plus linolenic acid, 18:2c,ω6 + 18:3c,ω3; 19 = oleic acid, 18:1c,ω9; 20 = vaccenic (*cis*) acid, 18:1c,ω7; 21 = stearic acid, 18:0; 22 = arachidonic acid, 20:4c,ω6; 23 = all-*cis*-5,8,11,14,17-eicosapentaenoic acid, 20:5c,ω3; 23a = all-*cis*-5,8,11-eicosatrienoic acid, 20:3c,ω9; 24 = dihomo-γ-linolenic acid, 20:3c,ω6; 25 = *cis,cis*-11,14-eicosadienoic acid, 20:2c,ω6; 26 = *cis*-11-eicosenoic acid, 20:1c,ω9; 27 = arachidic acid, 20:0; 28 = all-*cis*-4,7,10,13,16-docosapentaenoic acid, 22:5c,ω6; 29 = all-*cis*-4,7,10,13,16,19-docosahexaenoic acid, 22:6c,ω3; 30 = all-*cis*-7,10,13,16-docosatetraenoic acid, 22:4c,ω6; 31 = all-*cis*-7,10,13,16,19-docosapentaenoic acid, 22:5c,ω3; 32 = behenic acid, 22:0; 33 = nervonic acid, 24:1c,ω9; 34 = lignoceric acid, 24:0; 35 = 5β-cholestan-3α-ol, CHOLA; 36 = cholesterol, CHOLE.


A
B
X 2.5
X 0.5
50
40
30
20
10
0

calculated by using 17:0 as an internal standard can be expected. Comparing all data of the response factors calculated by the use of the odd MCFAs as internal standards, the bracketting method was found to result in response factors that were closest to 1.000 with the lowest coefficients of variation. It may therefore be expected that data calculated by the bracketting method will be the most precise, and the use of the response factor in the final calculation seems unnecessary.

TABLE I

MEAN PEAK-AREA RATIOS OF FIVE STANDARDS DETERMINED IN A PERIOD OF FIVE WEEKS

Fatty acid	Peak-area ratio							
	$n-1$*		Bracketting**		$n+1$***		17:0§	
	Mean	C.V. (%)	Mean	C.V. (%)	Mean	C.V. (%)	Mean	C.V. (%)
6:0	1.352	1.5	1.081	0.7	0.900	0.6	0.974	21.8
8:0	1.161	2.5	1.065	0.7	0.984	2.1	1.253	19.7
10:0	1.070	2.6	1.043	0.6	1.019	2.7	1.359	17.5
12:0	0.972	2.9	1.002	0.8	1.033	2.2	1.294	16.0
14:0	0.995	2.5	1.026	1.0	1.060	2.7	1.243	13.6

*Area ratio of fatty acid and its internal standard containing one methylene group less.
**Area ratio of fatty acid and half of the sum of the internal standards containing one methylene group less and one methylene group more.
***Area ratio of fatty acid and its internal standard containing one methylene group more.
§Area ratio of fatty acid and internal standard 17:0.

TABLE II

RECOVERIES OF MCFAs AND 14:0 FOR A POOLED HUMAN MILK SAMPLE CALCULATED BY THREE DIFFERENT QUANTIFICATION METHODS

Fatty acid	Recovery* (%)					
	$n-1$**		Bracketting***		$n+1$§	
	Mean	C.V. (%)	Mean	C.V. (%)	Mean	C.V. (%)
6:0	97.40	1.5	100.11	0.7	102.09	0.5
8:0	98.12	2.4	100.42	0.6	102.45	2.6
10:0	97.01	3.1	100.47	1.2	104.02	3.7
12:0	111.91	8.3	113.40	6.1	115.27	7.4
14:0	125.86	12.1	118.58	9.6	111.55	8.6

*Mean recoveries of five determinations equally divided over a period of five weeks.
**Quantification of fatty acid using the peak area of the internal standard with one methylene group less.
***Quantification of fatty acid using half of the sum of the peak areas of the internal standards with one methylene group less and one methylene group more.
§Quantification of fatty acid using the peak area of the internal standard with one methylene group more.

Table II shows the series-to-series recovery data of MCFAs added to a pooled milk sample as calculated by the three methods in which the odd-carbon-numbered MCFAs were used as internal standards. The respective response factors were taken into account. As expected, the data show recoveries for the bracket-

TABLE III

QUALITY CONTROL DATA FOR THE DETERMINATION OF THE TOTAL FATTY ACID COMPOSITION OF A POOLED HUMAN MILK SAMPLE OF CURAÇAOAN ORIGIN

MCFAs, 14:0 and 14:1c,ω5 were determined by the bracketting method, taking the response factors of the standard into account. Except for 14:0 and 14:1c,ω5, LCFAs were determined using 17:0 for quantitative purposes.

Fatty acid	Relative amount (g per 100 g)					
	Within-series precision			Series-to-series precision		
	Mean*	C.V. (%)	Range	Mean**	C.V. (%)	Range
6:0	0.08	18.6	0.07- 0.11	0.08	10.6	0.07- 0.09
8:0	0.33	10.0	0.30- 0.40	0.35	9.1	0.31- 0.39
10:0	2.10	6.2	1.98- 2.37	2.24	7.6	2.04- 2.44
12:0	8.99	4.1	8.63- 9.70	9.38	4.7	8.85- 9.88
14:0	10.05	1.9	9.90-10.33	10.15	1.3	9.99-10.29
14:1c,ω5	0.26	4.4	0.25- 0.28	0.27	4.8	0.26- 0.29
16:1c,ω7	2.52	1.7	2.46- 2.59	2.59	3.0	2.52- 2.72
16:0	21.72	1.5	21.12-22.03	21.41	3.4	20.84-22.53
18:3c,ω6	0.11	4.3	0.11- 0.12	0.13	24.8	0.11- 0.19
18:2c,ω6***	15.71	1.3	15.41-15.96	15.94	1.6	15.66-16.25
18:1c,ω9	24.22	1.2	23.75-24.50	24.14	1.3	23.61-24.42
18:1c,ω7	3.83	17.8	3.38- 4.82	3.68	12.6	3.36- 3.83
18:0	6.03	4.2	5.63- 6.35	5.69	6.7	5.17- 6.09
20:4c,ω6	0.73	3.8	0.70- 0.78	0.74	4.4	0.70- 0.79
20:5c,ω3	0.06	6.2	0.06- 0.07	0.06	11.8	0.05- 0.07
20:3c,ω9	0.09	8.7	0.08- 0.10	0.06	26.1	0.05- 0.09
20:3c,ω6	0.59	0.8	0.58- 0.59	0.57	4.5	0.55- 0.61
20:2c,ω6	0.56	1.3	0.55- 0.57	0.55	9.1	0.50- 0.63
20:1c,ω9	0.47	2.9	0.45- 0.49	0.42	9.2	0.37- 0.47
20:0	0.25	8.7	0.22- 0.28	0.22	12.5	0.18- 0.25
22:5c,ω6	0.08	4.8	0.07- 0.08	0.08	0.0	0.08- 0.08
22:6c,ω3	0.45	11.6	0.34- 0.49	0.46	3.6	0.45- 0.49
22:4c,ω6	0.23	10.5	0.18- 0.25	0.23	3.1	0.22- 0.24
22:5c,ω3	0.22	13.3	0.16- 0.25	0.22	2.4	0.22- 0.23
22:0	0.11	13.1	0.10- 0.14	0.11	5.2	0.10- 0.11
24:1c,ω9	0.11	17.6	0.08- 0.14	0.11	16.0	0.09- 0.13
24:0	0.11	14.3	0.08- 0.13	0.11	4.1	0.10- 0.11
ΣFA	100.01			100.00		
ΣMCFA	11.50	4.7	10.98-12.58	12.06	5.4	11.27-12.80
ΣMCFA+14:0	21.55	3.3	20.79-22.91	22.20	3.5	21.26-23.09

*Mean of seven determinations.

**Mean of five determinations equally divided over a period of five weeks.

***Sum of 18:2c,ω6 and 18:3c,ω3.

ting method to be closest to 100.00%, with the lowest coefficients of variation. We therefore conclude that from the four calculation methods investigated in the present study for MCFAs the bracketting method leads to results with both highest precision and accuracy.

In Table III the within-series and series-to-series quality-control data on the fatty acid composition of the pooled milk sample are depicted. MCFAs, 14:0 and 14:1c,ω5 were determined with the bracketting method, taking the respective response factors into account, whereas the LCFAs were calculated on the basis

TABLE IV

MEAN FATTY ACID COMPOSITION OF 47 MATURE HUMAN MILK SAMPLES OF CURAÇAOAN ORIGIN

MCFAs, 14:0 and 14:1c,ω5 were determined by the bracketting method, taking the response factors of the standard into account. Except for 14:0 and 14:1c,ω5, LCFAs were determined using 17:0 for quantitative purposes.

Fatty acid	Composition (g per 100 g)		
	Mean	C.V. (%)	Range
6:0	0.07	35.4	0.04- 0.17
8:0	0.36	22.9	0.19- 0.53
10:0	2.40	23.6	0.40- 3.37
12:0	10.71	29.4	4.71-17.09
14:0	10.47	34.0	3.90-18.62
14:1c,ω5	0.20	45.2	0.05- 0.45
16:1c,ω7	1.97	33.6	0.89- 4.25
16:0	20.21	11.3	14.17-25.93
18:3c,ω6	0.09	57.3	0.00- 0.24
18:2c,ω6*	16.06	22.7	9.61-27.25
18:1c,ω9	23.40	15.1	16.30-30.83
18:1c,ω7	3.83	30.1	1.78- 8.20
18:0	6.23	15.2	4.87- 8.90
20:4c,ω6	0.71	23.6	0.36- 1.13
20:5c,ω3	0.05	60.3	0.00- 0.14
20:3c,ω9	0.09	45.6	0.04- 0.23
20:3c,ω6	0.50	24.0	0.25- 0.78
20:2c,ω6	0.58	24.3	0.36- 1.15
20:1c,ω9	0.47	23.0	0.30- 0.82
20:0	0.27	24.9	0.17- 0.47
22:5c,ω6	0.08	42.5	0.05- 0.22
22:6c,ω3	0.43	32.0	0.12- 0.83
22:4c,ω6	0.24	51.7	0.09- 0.62
22:5c,ω3	0.21	29.3	0.09- 0.39
22:0	0.14	31.1	0.09- 0.26
24:1c,ω9	0.12	47.7	0.05- 0.37
24:0	0.14	40.6	0.07- 0.38
ΣFA	100.03		
ΣMCFA	13.54	27.1	6.42-20.88
ΣMCFA + 14:0	24.01	29.0	10.63-39.39

*Sum of 18:2c,ω6 and 18:3c,ω3.

of the peak area of 17:0. For each fatty acid, relatively small differences in the mean within-series and series-to-series amounts were noted. The coefficient of variation seems strongly dependent on the abundancy of the fatty acid in question.

In Table IV the data obtained from the analysis of 47 mature human milk samples from Curaçaoan origin are shown. Taking into account the series-to-series analytical and apparent biological coefficients of variation in Tables III and IV, respectively, the biological variations for the quantitatively most important fatty acids were calculated. These amounted to: MCFAs (26.6%), MCFAs+14:0 (28.8%), 16:0 (10.8%), 18:0 (13.6%), 18:1c,ω9 (oleic acid) (15.0%) and 18:2c,ω6 (linoleic acid) plus 18:3c,ω3 (linolenic acid) (22.6%). The lowest biological variation was found for 16:0, which may be related to the special function of 16:0 in human milk triglycerides. Palmitic acid (16:0) accounts for ca. 62% of the fatty acids on the *sn* 2-position of human milk triglycerides, which configuration is connected with their high absorbability from human milk [1].

In Table V we compare the relative amounts of the quantitatively most important fatty acids in the milk of Curaçaoan and Tanzanian mothers with some data obtained from the recent literature. The composition for Tanzanian samples [18] was determined in our laboratory with the above-described capillary GC method, whereas, except for Harris et al. [3], all other data from the literature were obtained by packed-column GC methods. Theoretically, the much lower relative amounts for the MCFAs and MCFAs+14:0 found in "Western" countries may be caused by differences in: analytical methods, race, climate, the composition of the diet [2–7] and probably the amount of fat accumulated in the maternal body

TABLE V

COMPARISON BETWEEN THE MEAN RELATIVE AMOUNTS OF THE QUANTITATIVELY MOST IMPORTANT FATTY ACIDS IN MATURE HUMAN MILK OF MOTHERS IN DIFFERENT COUNTRIES

n = number of samples; Σ = total amount of fatty acids considered

Reference	Year	n	Country	Relative amount (g per 100 g)						Σ
				MCFA	MCFA +14·0	16:0	18·0	18:1c,ω9	18:2c,ω6	
Jansson et al. [16]	1981	24	Sweden	4.4	10.7	23.0	8 4	38 0	12.9	93.0
Gibson and Kneebone [10]	1981	61	Australia	5.31	10.94	22.44	9.20	35.00	10 75	88.33
Harzer et al. [8]*	1983	14	F.R.G.	6 46	13.77	23.05	8.55	35.69	10.48	91.54
Bitman et al. [9]**	1983	6	U.S A.	5.43	11.11	22.20	7.68	35.51	15.58	92.08
Harris et al [3]	1984	8	U.S.A.	4.2	10 1	22.8	8.2	32 6	15 3	89 0
Lepage et al[17]**	1984	8	Canada	8 16	15 26	20.3	7.8	36.6	9 7	89.66
This study	1985	47	Curaçao	13.54	24 01	20.21	6.23	23.40	16 06***	89.91
Muskiet et al. [18]	1985	11	Tanzania	19 99	34 70	19.98	3.65	19 42	13.88	91 63

*Mean of days 15, 22, 29 and 36.
**Mean of mothers who delivered at term.
***Sum of 18:2c,ω6 and 18·3c,ω3.

during pregnancy [18]. With respect to possible methodological differences it is interesting to note that the MCFA and MCFA + 14:0 contents reported by Lepage et al. [17] seem the highest of all presented data for the Western countries. These authors calculated the relative amounts of 6:0, 8:0 and 10:0 on the basis of added 9:0; 12:0 and LFCAs up to 18:3c,ω3 on added 13:0; and LCFAs from 18:3c,ω3 on added 23:0. This methodological approach is intended to correct for losses based on differences in volatility [17]. Recently two capillary GC methods with split injection for quantification of both MCFAs and LCFAs in human milk were reported [3,19]. One of these methods was found to give similar results using a packed-column GC method [19]. In both reports quantification seems to be performed by assuming that equal weights of MCFAs and LCFAs give rise to equal peak areas. Theoretically, however, the high injector temperatures (250°C) and the high split ratios (1:100 and 1:60, respectively) used in the above-mentioned methods may lead to an underestimation of the relative amounts of MCFAs. This may notably be the case for 6:0, 8:0 and 10:0. We conclude that, as no quality-control data are given by the others, no definitive statements concerning the actual differences in the relative amounts of MCFAs and MCFAs + 14:0 in human milk samples of Western and developing countries can be made, and that speculations concerning the physiological background of these differences seem premature.

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