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Fatty acid composition of human milk triglyceride species

Possible consequences for optimal structures of infant formula triglycerides

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ABSTRACT

Human milk triglycerides (TGs) were separated into 14 fractions by silver ion high-performance liquid chromatography (HPLC) with light-scattering detection (LSD). Subsequent fractionation by reversed-phase HPLC–LSD resulted in 75 subfractions. The major 48 were analysed by gas chromatography for their intact TG and fatty acid (FA) compositions. Using a constrained non-linear optimization computer program, the FA compositions and abundances of 170 different TG combinations were calculated. The major two, 16:0/18:1/18:1 (11.8 mol%) and 16:0/18:1/18:2 (10.0 mol%), were almost twice as abundant as expected from random FA distribution. The apolarities of 16:0 and 18:0 seem to be modulated by unsaturated FAs (18:1, 18:2) or medium-chain FAs (MCFAs; 8:0–12:0). MCFAs were present in 19 mol%. Of these, 7.2 mol% are likely to harbour stereospecific structures with an MCFA at *sn*-3, 16:0 or 14:0 at *sn*-2, and 18:1 or 18:2 at *sn*-1. These structures are excellent substrates for lingual and gastric lipases, producing products that assist in duodenal TG lipolysis.

INTRODUCTION

Dietary fat is the major energy source of newborns [1]. During their first weeks of post-natal life preterm neonates exhibit a relatively low fat absorption coefficient from human milk, but especially infant formulas [2]. Contributing factors are the small bile acid pool and low lipolytic activity [1,3]. Higher bioavailability of human milk lipids is likely to be caused by the presence of bile salt-stimulated lipase (BSSL) and the specific milk triglyceride (TG) structures. Heat inactivation of BSSL diminished fat absorption from human milk by 33% [3]. Fat excretion by infants fed with an infant formula that contained natural lard was six times lower than in those fed with randomized lard [4].

Lipolysis in the human milk-fed newborn is accomplished by the concerted actions of BSSL and lingual, gastric and pancreatic lipases (for review see refs. 1, 3 and 5). The lipases have different pH optima, activators, substrate specificities and hydrolysis sites. Lingual and gastric lipases preferentially hydrolyse TGs that contain a medium-chain fatty acid (MCFA; 8:0–12:0) at the *sn*-3 position. Because of their broad pH optima of 3–6 they act in both stomach and duodenum. The produced diglycerides, monoglycerides and free fatty acids (FFAs) exhibit emulsifying properties. BSSL does not seem to require specific TG structures, but needs stimulation by bile salts or TG

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hydrolysis products. Its activity is confined to the duodenum by virtue of a pH optimum of 7.5–8.5. The products are glycerol and FFAs. Pancreatic lipase hydrolyses TGs at both *sn*-1 and *sn*-3 to form 2-monoglycerides and FFAs. For its action it needs colipase and the emulsifying properties of bile acids or TG hydrolysis products.

The structures of human milk TGs seem to be attuned to the subsequent steps in lipolysis during gastrointestinal passage. Predominant esterification of MCFAs at *sn*-3 [6,7] suggests predigestion by lingual and gastric lipases and subsequent activation of BSSL and pancreatic lipase by their hydrolytic products in the duodenum. This concept may have implications for optimal structures of TGs in infant formulas, which lack BSSL activity. Optimal lipolysis of infant formula TGs, prior to reaching the colon [8], may especially be dependent on adjustment of their structures to the substrate specificity of lingual and gastric lipases.

Little is known about the TG structures in human milk [7]. Breckenridge *et al.* [6] used preparative silver ion thin-layer chromatography followed by packed-column gas chromatographic (GC) analyses of intact TGs and fatty acids (FAs) in the isolated fractions. By combining the data with a stereospecific analysis of the total TG mixture they were able to identify and quantify the 13 major human milk TG species. In the present study we employed silver ion high-performance liquid chromatographic (Ag-HPLC) fractionation, followed by subfractionation with reversed-phase HPLC (RP-HPLC). Detection was done by light-scattering detection (LSD). Analyses comprised capillary GC assays of intact TGs and FAs in fractions and subfractions. The most likely FA compositions of human milk TGs were calculated with a constrained non-linear optimization computer program. Special attention was paid to comparison of experimental TG abundances with those calculated from random distribution and those structures that are potential substrates for lingual and gastric lipases.

EXPERIMENTAL

Materials

All organic solvents were HPLC grade, from either Merck (Darmstadt, Germany) or Rathburn (Walkerburn, UK). Butylated hydroxytoluene (BHT) was purchased from Fluka (Buchs, Switzerland). Odd-carbon-numbered FA methyl esters, used as internal calibration standards, were from Alltech (Deerfield, IL, USA).

A human milk pool was prepared by mixing equal volumes of mature (>15 days) milk samples from four healthy Dutch women. Milk samples were taken approximately halfway during a daytime feeding. The mothers were on typical western diets, characterized by a relatively high consumption of protein and fat, a low intake of carbohydrate and the use of vegetable oils. The pool contained 29.6 mmol/l TGs.

Preparation of a total lipid extract from human milk

A total lipid extract was prepared according to a modification of the method described by Radin [9]. To 1 ml of pooled milk were added 5 ml of hexane–isopropanol (3:2; v/v). The mixture was vortex-mixed and centrifuged for 5 min at 1500 *g*. The organic layer was collected and transferred to a tube containing 0.5 mg of BHT. After evaporation to dryness at 40°C under a stream of nitrogen the residue was redissolved in 1.5 ml of dichloromethane.

Separation of triglycerides by silver ion high-performance liquid chromatography

A 200 mm × 4 mm I.D. Nucleosil 5-SA column filled with 5-μm spherical sulphonic acid-containing particles (Macherey-Nagel, Düren, Germany) was loaded with silver ions according to Christie [10]. The HPLC system was a Perkin Elmer Series 410 LC solvent-delivery system (Perkin Elmer, Norwalk, CT, USA) equipped with a 50-μl Rheodyne injector (Rheodyne, Cotati, CA, USA) and coupled to a Varex ELSD II evaporative light-scattering detector (Varex, Rockville, MD, USA). A stream splitter (approximately one volume to detector and four to out-

let) was inserted between column and detector. Detector variables were: air pressure 150 kPa, heating temperature 58°C, and exhaust temperature 35°C. The gradient programme was: linear from 100% dichloromethane to 50% dichloromethane–50% acetone in 20 min, linear to 10% dichloromethane–70% acetone–20% acetone–acetonitrile (4:1, v/v) in 20 min and hold for 10 min [10–12]. Prior to each run the column was washed with dichloromethane for 10 min.

A 50-μl volume of pooled human milk total lipid extract (about 0.8 mg of TGs, see above) was injected with a 0.5-ml gas-tight syringe (Hamilton, Reno, NV, USA). Fractions were manually collected in tubes containing 0.2 mg of BHT via the stream splitter. For consecutive subfractionation with RP-HPLC, corresponding fractions obtained from five injections into the Ag-HPLC system were pooled.

LSD peak areas were calculated using a Nelson Analytical 3000 data system (Nelson Analytical, Cupertino, CA, USA). Peak-area percentages were considered to correspond with mass percentages (g/100 g). For conversion to mol percentages mean molecular masses (based on FA compositions, see Table II) were used.

Separation of silver ion fractions by reversed-phase high-performance liquid chromatography

A 220 mm × 4.6 mm I.D. Brownlee Spheri-5 RP-18 column filled with 5-μm C_{18}-containing spherical particles (Applied Biosystems, San Jose, CA, USA) was used in the same HPLC system as described above. The gradient programme was: 60% acetonitrile–40% dichloromethane for 15 min, then linear to 30% acetonitrile–70% dichloromethane in 45 min [13].

Pooled Ag-HPLC fractions were evaporated to dryness under a stream of nitrogen at 40°C. After redissolving the residue in 60 μl of acetonitrile–dichloromethane (3:2, v/v), 50 μl were injected. Subfractions were manually collected in tubes containing 0.2 mg of BHT. LSD peak areas were calculated and converted to mol percentages as described above.

Gas chromatographic analyses of the total lipid extract, fractions and subfractions

Parts of the total lipid extract from the human milk pool, Ag-HPLC fractions and RP-HPLC subfractions were evaporated to dryness at 40°C under a stream of nitrogen. Dependent on the estimated amount of TGs, they were redissolved in 50–200 μl of hexane and further analysed by high-temperature capillary GC of intact TGs and capillary GC of FA methyl esters following transmethylation (see below).

Gas chromatographic profiling of intact triglycerides

Analyses were done with high-temperature capillary GC with flame ionization detection (FID) [8]. Portions of 2 μl of the total lipid extracts from the human milk pool, Ag-HPLC fractions and RP-HPLC subfractions were injected into a Hewlett Packard Model 5890 gas chromatograph equipped with a Model 7673 automated injection system and a 25 m × 0.25 mm I.D., medium polar, 50% phenyl–50% methyl silicone-coated (film thickness 0.10 μm), wall-coated, open tubular, triglyceride analysis phase (TAP), fused-silica capillary column (Chrompack, Middelburg, Netherlands). Helium flow-rate was 1.26 ml/min, split ratio 1:45 and injection and detection temperatures were 350°C. Oven temperature programme was: initial temperature 275°C, hold for 5 min, increase of 10°C/min to 350°C, hold for 18 min.

Gas chromatographic profiling of fatty acid methyl esters

For transesterification [14], 2 ml of methanol–6 mol/l hydrochloric acid (5:1, v/v), 200 μl of chloroform and 2.5 μg each of a series of odd-carbon-numbered FA methyl esters (5:0, 7:0, 9:0, 11:0, 13:0, 15:0, 17:0) in 2.5 μl of chloroform were added to parts of the total lipid extract of human milk, Ag-HPLC fractions and RP-HPLC subfractions. The tubes were capped and heated at 90°C for 4 h. FA methyl esters were extracted into 500 μl of hexane. The hexane layer was carefully evaporated to 10–50 μl at room temperature under a stream of nitrogen.

Samples were analysed by capillary GC–FID, employing two temperature programmes. Portions of 2 μl were injected into a Hewlett Packard Model 5880 gas chromatograph equipped with a 50 m × 0.2 mm I.D. (apolar) Ultra 1 cross-linked methyl silicone column (film thickness 0.1 μm). Helium flow-rate was 0.52 ml/min, split ratio 1:20 and FID temperature 300°C. MCFA and 14:0 methyl esters were analysed at an injection temperature of 220°C and the following oven temperature programme: initial temperature 60°C, increase of 4°C/min to 215°C, increase of 20°C/min to 290°C and hold at 290°C for 20 min. For FA methyl esters with chain lengths >14:0 the injection temperature was set at 260°C. Oven temperature programme was: 160°C for 2 min, increase of 2°C/min to 240°C, increase of 10°C/min to 290°C and hold at 290°C for 15 min. FA methyl esters were identified by comparison of retention times with those of authentic standards.

Percentages (mol/100 mol) of MCFA and 14:0 methyl esters were calculated by the bracketing method [15,16], using 5:0–15:0 as internal calibration standards. Percentages of FA methyl esters with chain lengths >14:0 (mol/100 mol) were established on the basis of 17:0 as an internal calibration standard [14–17].

Calculation of triglyceride abundances in reversed-phase high-performance liquid chromatographic subfractions, and their contributions to the total milk pool

Abundances of TGs (in mol/100 mol) residing in RP-HPLC–LSD subfractions were calculated with a constrained non-linear optimization computer program [18]. Variables were: (1) degree of unsaturation (obtained from Ag-HPLC profiles); (2) carbon chain number (obtained from high-temperature GC analyses of intact TGs in RP-HPLC subfractions); and (3) FA composition of RP-HPLC subfractions (obtained from GC analyses of FA methyl esters in transmethylated RP-HPLC subfractions). A constrain was used to avoid negative outcomes of TG percentages.

The following data may serve as an example. With Ag-HPLC we collected an SSM fraction (see Table I). Subsequent fractionation with RP-HPLC gave a subfraction with presumably a carbon chain number of 50. This was confirmed with high-temperature GC analysis of that subfraction. The composition of FAs with abundances >0.5 mol/100 mol FAs, as determined by GC analysis following transmethylation, was: 12:0, 0.53; 14:0, 6.05; 16:0, 54.33; 18:0, 7.59; 16:1, 1.30; 18:1, 29.08; and 20:1, 0.62 mol/100 mol FAs. Based on the presence of one FA double bond per TG molecule (Ag-HPLC) and a carbon chain number of 50 (RP-HPLC, as confirmed with high temperature GC) the following FA combinations were possible:

20:1/18:0/12:0	*a* mol
20:1/16:0/14:0	*b* mol
18:1/18:0/14:0	*c* mol
18:1/16:0/16:0	*d* mol
16:1/18:0/16:0	*e* mol

Together with the FA composition (GC analysis of methyl esters) the following seven equations with five unknowns can be derived: for 12:0, $0.53 = a$; for 14:0, $6.05 = b + c$; for 16:0, $54.33 = b + 2d + e$; for 18:0, $7.59 = a + c + e$; for 16:1, $1.30 = e$; for 18:1, $29.08 = c + d$; and for 20:1, $0.62 = a + b$. The computer program solved the equations by taking the best fit with the constrain that TG values are ≥ 0. After normalization the solution was: $a = 0.80\%$; $b = 3.27\%$; $c = 13.77\%$; $d = 75.55\%$; $e = 6.60\%$.

Contributions of identified TGs to the total TG composition of the milk pool (in mol/100 mol) were calculated from their contributions to the RP-HPLC subfraction, the contribution of the RP-HPLC–LSD subfraction to the corresponding Ag-HPLC–LSD fraction and the contribution of the Ag-HPLC–LSD fraction to the total human milk lipid extract.

RESULTS

Analyses

Fractionation of the total lipid extract of the human milk pool by Ag-HPLC–LSD resulted in 14 fractions (Fig. 1A; Table I). Tentative identification of FA combinations (based on total num-

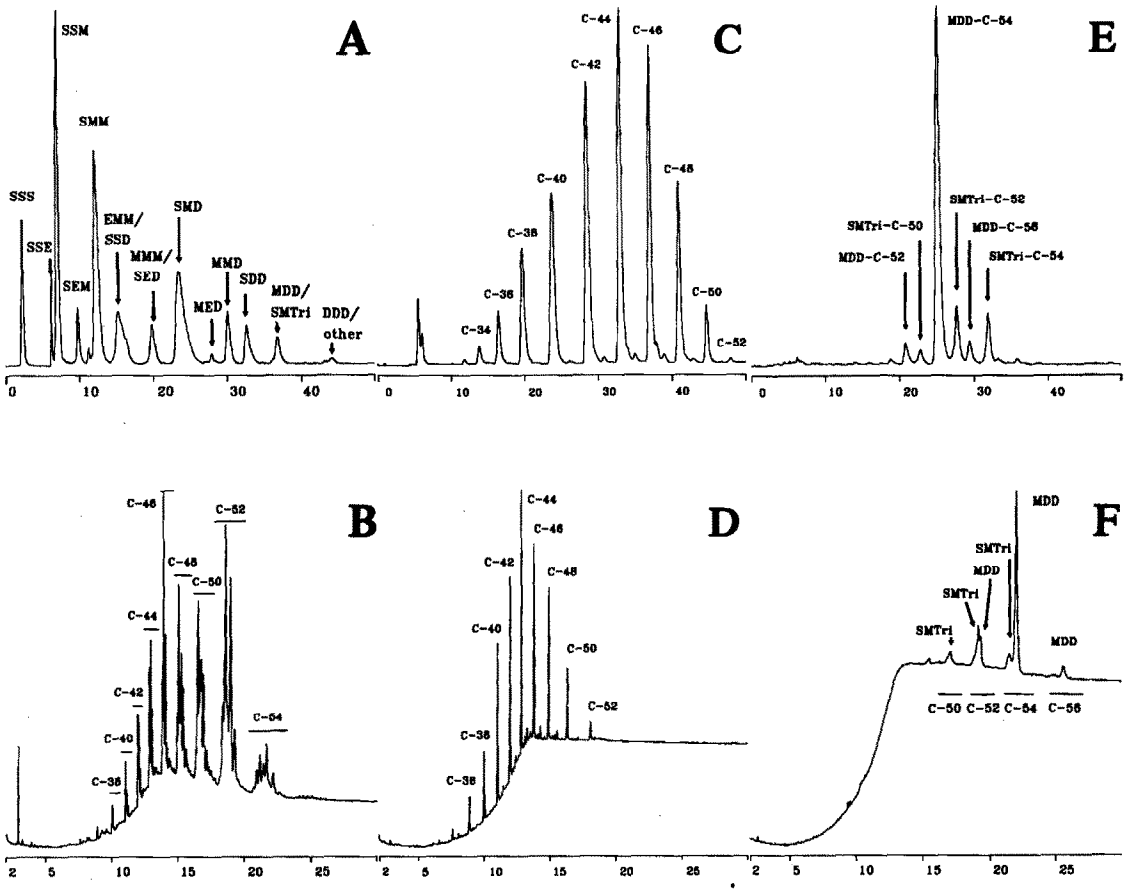

Fig. 1. Profiles of human milk TGs and human milk TG fractions by Ag-HPLC–LSD, RP-HPLC–LSD and GC–FID. (A) Ag-HPLC–LSD of total lipids in human milk. (B) GC–FID of total lipids in human milk. (C) RP-HPLC–LSD of the SSS Ag-HPLC–LSD fraction. (D) GC–FID of the SSS Ag-HPLC–LSD fraction. (E) RP-HPLC–LSD of the MDD/SMTri Ag-HPLC–LSD fraction. (F) GC–FID of the MDD/SMTri Ag-HPLC–LSD fraction. For abbreviations, see legend to Table I. Units of *x*-axis are minutes. SMC/CHOL is the small peak between SEM and SMM. Note the almost Gaussian distribution of abundances as a function of carbon number in the SSS fraction (C and D).

ber of double bonds) in their TGs was done by comparison with results from Christie [10]. Each fraction was subjected to RP-HPLC–LSD, giving rise to a median of 5.5 (range 0–11) subfractions per fraction and a total of 75 subfractions. Two fractions (SSE and SMC/CHOL) did not give detectable RP-HPLC–LSD subfractions. RP-HPLC–LSD profiles of the SSS and MDD/SMTri fractions are presented in Fig. 1C and E, respectively. Fig. 1B, D and F shows GC–FID profiles of the total lipid extract of the human milk pool and the SSS and MDD/SMTri fractions, respectively.

Table I shows mol percentages of Ag-HPLC fractions and RP-HPLC subfractions, together with retention times on HPLC and GC–FID (subfractions only). RP-HPLC subfractions gave rise to single peaks on GC–FID with retention times consistent with the tentative carbon numbers assigned to them on the basis of retention behaviour on the RP-HPLC column. In a minority of cases (indicated by ?) no positive GC–FID identification could be made. Contributions of RP-HPLC subfractions to the total TG composition of the milk pool ("mol% of total HM" in Table I) were calculated from their RP-HPLC and Ag-HPLC mol percentages.

TABLE I

HPLC–LSD MOL PERCENTAGES AND HPLC–LSD AND GC–FID RETENTION TIMES OF HUMAN MILK POOL TG FRACTIONS AND SUBFRACTIONS OBTAINED BY Ag-HPLC AND RP-HPLC, RESPECTIVELY

Identity is defined as some combination of indicated three fatty acids (FAs; for abbreviations see below) at the glycerol moiety for Ag-HPLC fractions and extended by its carbon number (total number of FA carbon atoms; as confirmed by GC–FID) for RP-HPLC subfractions; Mol%, mol of Ag-HPLC–LSD fractions and RP-HPLC–LSD subfractions (these data are assumed to represent, in mol/100 mol, the contribution of each Ag-HPLC fraction to total human milk TGs and the contribution of each RP-HPLC subfraction to its parent Ag-HPLC fraction); Mol% of total HM, mol percentages of RP-HPLC–LSD subfractions multiplied by the corresponding mol percentages of the parent Ag-HPLC–LSD fractions (these data represent the calculated contribution of each RP-HPLC subfraction, in mol/100 mol, to total human milk TGs); t_R, HPLC, retention times (in min) as determined for fractions and subfractions on Ag-HPLC–LSD and RP-HPLC–LSD, respectively; t_R, GC, retention times (in min) as determined for RP-HPLC–LSD subfractions on GC–FID. Abbreviations and symbols: S = saturated FA; E = elaidic (*trans*-monoenoic) FA; M = monoenoic (*cis*) FA; C = conjugated dienoic FA; D = dienoic FA; Tri = trienoic FA; Tetra = tetraenoic FA; CHOL = cholesterol; ? = unidentified component; * = subfractions subjected to further analysis of their FA compositions by GC–FID; SUM* = sum of the fractions indicated with *.

Identity	Mol%	Mol% of total HM	t_R, HPLC (min)	t_R, GC (min)
Fractions (Ag-HPLC)				
SSS	7.6		2.6	
SSE	2.2		7.0	
SSM	21.0		7.5	
SEM	3.1		10.0	
SMC/CHOL	0.7		11.6	
SMM	20.3		12.4	
SSD/EMM	10.7		15.8	
MMM/SED	3.9		20.0	
SMD	17.1		23.4	
MED	0.6		27.5	
MMD	4.3		28.5	
SDD/SSTri	4.4		29.4	
MDD/SMTri	2.9		30.9	
DDD/other	1.2		33.1	
Subfractions (RP-HPLC)				
SSS-C32	0.3	0.02	11.8	?
SSS-C34*	1.1	0.08	13.9	7.92
SSS-C36*	3.5	0.26	16.5	9.17
SSS-C38*	8.3	0.63	19.7	10.28
SSS-C40*	14.2	1.08	23.8	11.27
SSS-C42*	21.4	1.63	28.5	12.18
SSS-C44*	22.2	1.69	32.9	13.08
SSS-C46*	16.5	1.25	36.9	14.05
SSS-C48*	9.9	0.75	40.9	15.24
SSS-C50*	2.6	0.20	44.7	16.75
SSS-C52*	0.2	0.01	48.0	18.68
SSM-C40	0.7	0.15	18.1	11.33
SSM-C42*	3.5	0.73	21.7	12.23
SSM-C44*	9.8	2.06	26.4	13.12
SSM-C46*	20.5	4.30	31.2	14.13
SSM-C48*	21.2	4.44	35.4	15.38
SSM-C50*	27.4	5.76	39.5	16.92
SSM-C52*	16.2	3.41	44.2	18.84
SSM-C54	0.7	0.15	48.2	21.36
SEM-C48	6.3	0.20	29.9	15.54
SEM-C50	21.7	0.67	34.3	17.02
SEM-C52*	67.9	2.10	38.1	18.98
SEM-C54	4.1	0.13	42.4	21.58
SMM-C46*	1.6	0.33	30.5	14.15
SMM-C48*	9.4	1.90	34.0	15.47
SMM-C50*	23.8	4.83	37.2	17.03
SMM-C52*	59.5	12.07	40.6	19.12
SMM-C54*	5.7	1.16	44.7	21.54
SSD-C40	0.4	0.04	14.5	11.43
SSD-C42*	2.3	0.24	17.3	12.35
SSD-C44*	6.5	0.70	20.9	13.24
SSD-C46*	16.7	1.79	25.3	14.30
SSD-C48*	19.7	2.10	30.1	15.54
SSD-C50*	29.5	3.15	34.4	17.39
SSD-C52*	18.4	1.97	38.4	19.51
SSD-C54	0.7	0.08	42.8	22.23
EMM-C52	1.9	0.20	33.0	?
EMM-C54	4.0	0.43	36.8	?
MMM-C48	1.2	0.05	21.0	15.87
MMM-C50	3.3	0.13	25.7	17.51
MMM-C52*	19.3	0.75	29.0	19.60
MMM-C54*	54.4	2.12	33.5	21.78
MMM-C56	1.4	0.06	37.4	25.83
SED-C50	0.6	0.02	24.2	17.53
SED-C52*	19.4	0.76	30.8	19.65
SED-C54	0.3	0.01	35.4	21.93
SMD-C46*	1.1	0.19	18.0	14.55
SMD-C48*	8.0	1.38	21.5	15.89
SMD-C50*	18.6	3.18	25.9	17.59
SMD-C52*	61.2	10.47	30.2	19.83
SMD-C54*	10.4	1.79	34.6	22.53
SMD-C56	0.6	0.10	37.9	26.22
MED-C52	22.0	0.13	27.4	19.86
MED-C54	73.0	0.44	31.6	22.68
MED-C56	5.0	0.03	35.4	?
MMD-C52*	16.6	0.71	25.2	19.97

TABLE I (*continued*)

Identity	Mol%	Mol% of total HM	t_R, HPLC (min)	t_R, GC (min)
MMD-C54*	80.1	3.45	29.7	22.80
MMD-C56*	3.3	0.14	33.6	26.90
SDD-C48*	5.5	0.24	17.8	16.07
SDD-C50*	11.4	0.50	21.3	17.81
SDD-C52*	73.3	3.22	25.7	20.11
SDD-C54*	9.7	0.43	30.3	22.91
MDD-C52	4.0	0.12	20.8	20.35
MDD-C54*	67.7	1.96	25.1	23.83
MDD-C56*	4.7	0.14	29.4	27.13
SMTri-C50	3.0	0.09	22.8	17.88
SMTri-C52*	12.2	0.35	27.7	20.08
SMTri-C54*	8.4	0.24	31.9	22.74
DDD-C54*	30.1	0.36	19.2	24.78
?-C52	11.0	0.13	21.1	20.50
?	7.5	0.09	22.7	?
?-C54	13.2	0.16	25.1	23.20
SMTetra-C54	24.6	0.30	27.6	?
?	4.5	0.05	30.0	?
?	9.1	0.11	32.0	?
SUM*		93.00		

FA compositions of Ag-HPLC fractions are presented in Table II. In the majority of cases percentages of saturated FAs (SAFAs), monounsaturated FAs (MUFAs), polyunsaturated FAs (PUFAs) and double-bond index proved to be in excellent agreement with their tentative identification according to Christie [10]. Of the 75 RP-HPLC subfractions, 48 (see Table I) were subjected to further GC–FID analyses of their FA compositions (data not shown).

Using the constrained non-linear optimization computer program, the most likely FA compositions and abundances of TGs in each RP-HPLC subfraction were calculated. Table III shows the resulting FA compositions of 170 human milk pool TGs in order of abundance. Enumeration of abundances revealed that the two quantitatively most important TGs accounted for about 22 mol/100 mol total human milk TGs, the major 12 for 50 mol/100 mol and the major 45 for 80 mol/100 mol.

Since the abundances in Table III result from multiplication of three data, each of which suffers from analytical errors, an accumulation of errors may be expected. Table IV gives results of the FA composition of the milk pool obtained by direct transmethylation (from Table II) and the calculated FA composition from TG abundances and compositions given in Table III. There was reasonable agreement between the quantitatively most important FAs. However, the contents of notably the quantitatively minor SAFAs (8:0, 10:0 and 20:0) and PUFAs (18:3, 20:4 and 20:3) proved to be underestimated.

Comparison of experimental results with random distributions

In Fig. 2 experimental percentages of TGs in the Ag-HPLC fractions (in mol/100 mol total human milk TGs; Table I) are compared with percentages of total human milk TGs that would have been expected in case of random FA distribution. It should be noted that calculation of random FA distribution was based on the 1,2,3-random hypothesis and not the 1-random, 2-random, 3-random hypothesis. The former uses the total FA composition to calculate possible combinations, whereas the latter uses data from actually measured FA compositions of the *sn*-1, -2 and -3 positions for the same purpose. Apart from the *trans*-FA-containing TG fractions, the SMM, SMD and SDD fractions were found to be more abundant than would have been expected in case of random FA distribution. The fractions SSS, SSM, MMM, MMD, SST and SMT were clearly less abundant.

Fig. 3 shows that the various SAFA species were not equally distributed amongst the SAFA-containing Ag-HPLC fractions. With increasing TG desaturation (from left to right) 16:0 had a tendency to become more abundant, whereas 12:0 and 18:0 became less abundant. Myristic acid (14:0) was about equally distributed amongst these fractions.

TGs that merely contained the six quantitatively most important FAs (12:0, 14:0, 16:0, 18:0, 18:1 and 18:2; see Table II, "Total HM") were selected and regrouped according to number of SAFA per TG molecule. Data were obtained from Table III. Fig. 4 shows the ratios between experimental and random abundances for each

TABLE II

FATTY ACID COMPOSITION (in mol/100 mol FAs) OF THE TOTAL HUMAN MILK POOL AND ITS Ag-HPLC–LSD FRACTIONS

MCL = mean chain length; DBI = double-bond index, mean number of double bonds per FA; mean MW = mean molecular mass of TGs in the Ag-HPLC fraction. For other abbreviations see legend to Table I. FA compositions of the 48 analysed RP-HPLC subfractions are not given.

Identity	Total HM	SSS	SSM	SEM	SMM	SSD/ EMM	MMM/ SED	SMD	MED	MMD	SDD/ SSTri	MDD/ SMTri	DDD/ other
8:0	0.36	0.96	0.75	0.55	0.23	0.87	0.69	0.21	1.59	0.57	0.43	0.21	0.43
10:0	2.56	5.42	3.88	1.20	1.02	3.21	0.91	0.70	1.48	0.43	0.80	0.83	1.76
12:0	6.86	22.01	13.58	4.79	5.03	11.71	2.45	4.15	1.50	0.59	4.08	1.75	3.13
14:0	8.01	21.08	13.21	6.85	6.48	11.77	2.70	5.69	2.71	0.88	5.63	2.79	4.68
16:1c,ω7	2.29	0.00	1.90	4.10	4.67	0.00	6.89	2.31	3.00	6.20	0.45	2.50	1.15
16:0	22.70	36.26	30.69	26.67	23.72	28.22	9.43	21.19	7.92	2.97	21.86	10.47	18.05
18:3c,ω6	0.05	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.14	0.34	0.26
18:2c,ω6	14.10	0.00	0.00	0.00	0.00	25.84	11.28	30.88	31.88	31.11	53.70	35.73	24.75
18:3c,ω3	0.70	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	3.27	5.91	4.82
18:1c,ω9	26.70	0.00	24.53	28.76	50.84	8.67	51.84	27.25	29.49	48.64	4.69	25.17	14.71
18:1c,ω7	3.84	0.00	3.00	20.19	4.38	0.00	9.13	2.38	14.56	4.97	0.00	3.08	1.83
18:0	5.79	13.77	7.26	5.09	2.27	8.02	2.08	2.71	2.72	0.70	0.00	2.66	6.72
20:4c,ω6	0.95	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.42	9.00
20:5c,ω3	0.07	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00
20:3c,ω6	0.79	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	1.69	4.38	3.66
20:2c,ω6	1.45	0.00	0.00	0.00	0.00	1.50	0.73	1.77	2.01	1.66	3.14	1.89	1.37
20:3c,ω3	0.24	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	1.00	0.74
20:1c,ω9	1.08	0.00	0.50	0.82	1.02	0.00	1.37	0.57	0.82	1.14	0.00	0.63	0.34
20:1c,ω7	0.27	0.00	0.10	0.75	0.22	0.00	0.36	0.11	0.33	0.13	0.00	0.05	0.08
20:0	0.30	0.38	0.20	0.22	0.12	0.19	0.14	0.10	0.00	0.00	0.11	0.07	0.22
22:4c,ω6	0.39	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.07	2.28
22:1c,ω9	0.30	0.00	0.33	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.05	0.00
22:0	0.19	0.11	0.06	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00
SAFA	46.76	100.00	69.64	45.37	38.87	63.99	18.40	34.74	17.91	6.13	32.92	18.78	35.00
SAFA ⩽ 12:0	9.78	28.39	18.22	6.54	6.29	15.79	4.05	5.06	4.57	1.59	5.31	2.79	5.32
SAFA > 12:0	36.99	71.60	51.42	38.84	32.58	48.20	14.35	29.68	13.34	4.54	27.61	15.99	29.68
MUFA	34.49	0.00	30.36	54.63	61.13	8.67	69.59	32.61	48.19	61.09	5.13	31.48	18.11
PUFA	18.75	0.00	0.00	0.00	0.00	27.34	12.01	32.65	33.89	32.78	61.94	49.74	46.89
MCL	16.67	14.59	15.65	16.71	16.79	15.95	17.33	17.03	17.37	17.71	17.08	17.61	17.46
DBI	0.77	0.00	0.30	0.55	0.61	0.63	0.94	0.98	1.16	1.27	1.34	1.44	1.44
Mean MW	832	747	791	835	838	803	860	847	861	875	848	871	864

TG in the resulting SSS (Fig. 4A), SSX (Fig. 4B) and SXX and XXX (Fig. 4C) groups, in which X represents either 18:1 or 18:2. The data show that in the quantitatively minor (6.03 mol/100 mol TGs) SSS fraction (Fig. 4A) the combination of three long-chain SAFAs (LCSAFAs, either 16:0 or 18:0) was considerably less abundant than might have been expected if all FAs were randomly distributed. There was clear preference for the combination of three medium-chain SAFAs (MCSAFAs, either 12:0 or 14:0). The combination of two LCSAFAs with one MCSAFA was less abundant, whereas in combinations of one LCSAFA and two MCSAFAs there was preference for the LCSAFA to be 18:0.

In the SSX group (25.51 mol/100 mol TGs) there was a preference for combination of 16:0/18:0 with X. With decreasing chain length of the

TABLE III

ABUNDANCES OF HUMAN MILK POOL TGs WITH ESTABLISHED FATTY ACID COMPOSITIONS

Data for 170 TGs are listed in order of abundance (in mol/100 mol TGs). Abundance of TGs with indicated FA composition was calculated by multiplying the contribution of the RP-HPLC subfraction in which it resides (Table I) by the contribution of the TGs to that subfraction (as derived from the constrained non-linear FA optimization method). Indicated FA sequences in TGs do not necessarily correspond with sequences at glycerol moieties. TGs that contain three different FAs (A/B/C) are theoretically composed of three components differing in the position of FA at the glycerol moiety (ABC; BAC; BCA), of which each may harbour two enantiomers (*sn*-ABC, *sn*-CBA; *sn*-BAC, *sn*-CAB; *sn*-BCA, *sn*-ACB). Abbreviations: 18:1 = 18:1c,ω9, 18:1c,ω7, or both; 18:3 = 18:3c,ω6, 18:3c,ω3, or both; 18:1t = *trans*-monoenoic FA; 20:1 = 20:1c,ω9, 20:1c,ω7, or both; 20:3 = 20:3c,ω6, 20:3c,ω3, or both.

% of total HM	Type of TG	% of total HM	Type of TG	% of total HM	Type of TG
11.799	16:0/18:1/18:1	0.390	12:0/12:0/16:0	0.122	18:0/16:1/18:2
10.045	16:0/18:1/18:2	0.388	14:0/18:0/18:2	0.119	12:0/18:1/20:1
4.351	16:0/16:0/18:1	0.386	12:0/14:0/18:2	0.119	10:0/18:1/18:1
3.342	18:1/18:1/18:2	0.381	16:0/18:0/16:1	0.119	10:0/18:0/18:1
3.261	14:0/16:0/18:1	0.367	12:0/14:0/14:0	0.116	10:0/18:1/18:2
3.220	16:0/18:2/18:2	0.360	18:2/18:2/18:2	0.116	10:0/12:0/14:0
3.143	12:0/16:0/18:1	0.352	12:0/12:0/18:1	0.107	12:0/14:0/20:2
3.057	16:0/18:0/18:1	0.338	12:0/12:0/14:0	0.106	12:0/18:1/20:2
2.787	14:0/18:1/18:1	0.324	10:0/16:0/16:0	0.095	14:0/14:0/20:2
2.385	16:0/16:0/18:2	0.303	14:0/16:0/20:2	0.094	18:0/18:0/16:1
2.279	14:0/18:1/18:2	0.301	12:0/14:0/18:0	0.090	10:0/12:0/18:0
2.100	16:0/18:1t/18:1	0.298	16:0/16:0/16:0	0.088	12:0/14:0/20:1
2.056	18:1/18:1/18:1	0.296	14:0/16:0/16:1	0.087	16:0/16:1/20:3
1.940	18:1/18:2/18:2	0.279	12:0/18:0/18:2	0.079	18:1/18:1/20:2
1.924	16:0/16:1/18:1	0.275	14:0/18:1/20:2	0.078	10:0/14:0/14:0
1.707	16:0/18:0/18:2	0.271	18:0/16:1/18:1	0.078	12:0/12:0/12:0
1.476	14:0/16:0/18:2	0.271	14:0/14:0/18:2	0.075	12:0/18:0/20:2
1.398	12:0/18:1/18:1	0.251	12:0/12:0/18:0	0.071	12:0/18:2/20:2
1.309	12:0/16:0/18:2	0.250	12:0/16:0/20:2	0.066	8:0/16:0/18:1
1.220	12:0/18:1/18:2	0.248	10:0/16:0/18:2	0.065	16:1/18:1/20:1
1.103	12:0/14:0/18:1	0.240	12:0/18:2/18:2	0.063	16:1/20:1/18:2
0.978	18:0/18:1/18:2	0.236	16:0/18:1/18:3	0.061	18:1/20:1/18:2
0.793	14:0/18:0/18:1	0.226	16:0/16:0/20:1	0.061	12:0/14:0/16:1
0.787	12:0/14:0/16:0	0.224	12:0/16:0/16:1	0.060	16:0/16:1/16:1
0.765	16:0/16:1/18:2	0.218	18:0/18:2/18:2	0.058	10:0/18:0/18:2
0.760	16:0/18:1t/18:2	0.214	16:0/18:1/20:3	0.056	10:0/14:0/18:2
0.750	16:1/18:1/18:1	0.212	16:0/18:2/20:2	0.054	8:0/16:0/16:0
0.750	12:0/16:0/16:0	0.212	16:0/20:1/18:2	0.054	12:0/16:1/18:2
0.710	16:1/18:1/18:2	0.211	16:0/16:0/20:2	0.052	8:0/16:0/18:0
0.644	18:0/18:1/18:1	0.211	12:0/16:1/18:1	0.051	14:0/18:0/20:2
0.644	10:0/16:0/18:1	0.208	14:0/14:0/14:0	0.051	10:0/16:0/16:1
0.634	14:0/14:0/18:1	0.194	10:0/16:0/18:0	0.049	14:0/14:0/20:1
0.591	16:0/18:1/20:2	0.188	14:0/16:0/20:1	0.046	12:0/18:0/20:1
0.549	14:0/16:0/16:0	0.186	10:0/14:0/18:1	0.045	10:0/16:0/20:2
0.545	12:0/16:0/18:0	0.179	10:0/14:0/16:0	0.044	16:1/18:1/20:2
0.516	16:0/18:1/20:1	0.177	12:0/16:0/20:1	0.041	10:0/12:0/12:0
0.494	12:0/18:0/18:1	0.170	16:0/16:0/18:0	0.037	8:0/12:0/16:0
0.429	14:0/18:2/18:2	0.164	10:0/12:0/16:0	0.035	12:0/12:0/20:2
0.424	14:0/16:1/18:1	0.156	14:0/16:1/18:2	0.034	14:0/18:0/16:1
0.424	16:0/16:0/16:1	0.140	18:1/18:2/20:2	0.031	8:0/16:0/20:1
0.413	14:0/14:0/16:0	0.127	14:0/14:0/18:0	0.030	12:0/18:0/18:0
0.402	14:0/16:0/18:0	0.125	12:0/12:0/18:2	0.030	10:0/12:0/20:2

(*Continued on p. 18*)

TABLE III (*continued*)

% of total HM		Type of TG	% of total HM		Type of TG	% of total HM		Type of TG
	0.028	8:0/14:0/16:0		0.016	10:0/16:0/20:0		0.008	18:0/16:1/20:2
	0.026	14:0/18:1/20:3		0.016	12:0/12:0/20:1	160	0.008	12:0/20:1/18:2
	0.026	18:0/18:1/18:3		0.015	12:0/20:1/20:2		0.008	14:0/16:0/20:0
130	0.024	8:0/16:0/18:2		0.014	8:0/16:0/20:2		0.008	10:0/14:0/18:0
	0.023	10:0/20:1/20:2		0.014	6:0/18:0/18:1		0.007	8:0/10:0/18:0
	0.023	8:0/12:0/18:0		0.013	16:0/16:1/20:2		0.006	8:0/14:0/20:2
	0.021	14:0/20:0/18:1		0.013	10:0/14:0/20:2		0.006	10:0/10:0/14:0
	0.021	10:0/14:0/20:1	150	0.013	12:0/12:0/20:0		0.004	10:0/18:1/20:2
	0.021	10:0/16:0/20:1		0.012	10:0/18:0/18:0		0.004	8:0/8:0/18:0
	0.020	16:1/18:2/20:2		0.012	10:0/10:0/16:0		0.003	10:0/18:0/16:1
	0.020	14:0/18:0/18:0		0.011	10:0/18:0/20:0		0.003	12:0/18:0/20:0
	0.020	8:0/20:1/18:2		0.011	14:0/18:0/20:1	170	0.002	8:0/18:0/18:2
	0.020	8:0/18:0/18:0		0.011	8:0/12:0/14:0			
140	0.019	8:0/10:0/16:0		0.010	16:0/18:0/18:0		92.999	SUM
	0.018	10:0/18:1/20:1		0.010	12:0/16:0/20:0		6.990	UNKNOWN
	0.018	14:0/14:0/16:1		0.009	8:0/14:0/14:0		99.989	SUM

TABLE IV

FA COMPOSITION OF THE MILK POOL OBTAINED BY DIRECT TRANSMETHYLATION, COMPARED WITH THAT RECONSTITUTED FROM THE EXPERIMENTAL FA COMPOSITIONS OF TGs

Data (in mol/100 mol FAs) are obtained from Table II (total HM) and Table III (by reconstituting the total FA composition of the TGs from their abundances and FA compositions).

FA	Direct trans-methylation	Calculated from TGs
8:0	0.36	0.14
10:0	2.56	1.02
12:0	6.86	5.93
14:0	8.01	7.57
16:1	2.29	2.46
16:0	22.70	24.24
18:3	0.76	0.09
18:2	14.10	14.41
18:1	30.54	30.30
18:0	5.79	4.11
20:4	0.95	0.00
20:3	1.03	0.11
20:2	1.45	0.95
20:1	1.35	0.67
20:0	0.30	0.03
Minor	0.95	0.96
Total	100.00	92.99

SS part (Fig. 4B, from left to right), combination of SS with X became less preferred. Within this decreasing preference there was a greater decrease in the combination with 18:2 than with 18:1.

The SXX group (35.26 mol/100 mol TGs) was the quantitatively most important one. In contrast to 18:0 and 12:0, combination of XX with 16:0 was more abundant than expected from random distribution. The resulting three TGs (Fig. 4C, second group of blocks from left) accounted for 25.95 mol/100 mol total human milk TGs. Combinations of XX with 14:0 were about as expected from random distribution.

The XXX group (7.70 mol/100 mol TGs) showed increasing preference of 18:2 to combine with 18:2, and decreasing preference of 18:1 to combine with 18:1 (Fig. 4C). As a whole the data were not much different from random distribution.

DISCUSSION

Analyses

The combination of three different chromatographic systems (*i.e.* Ag-HPLC–LSD, RP-

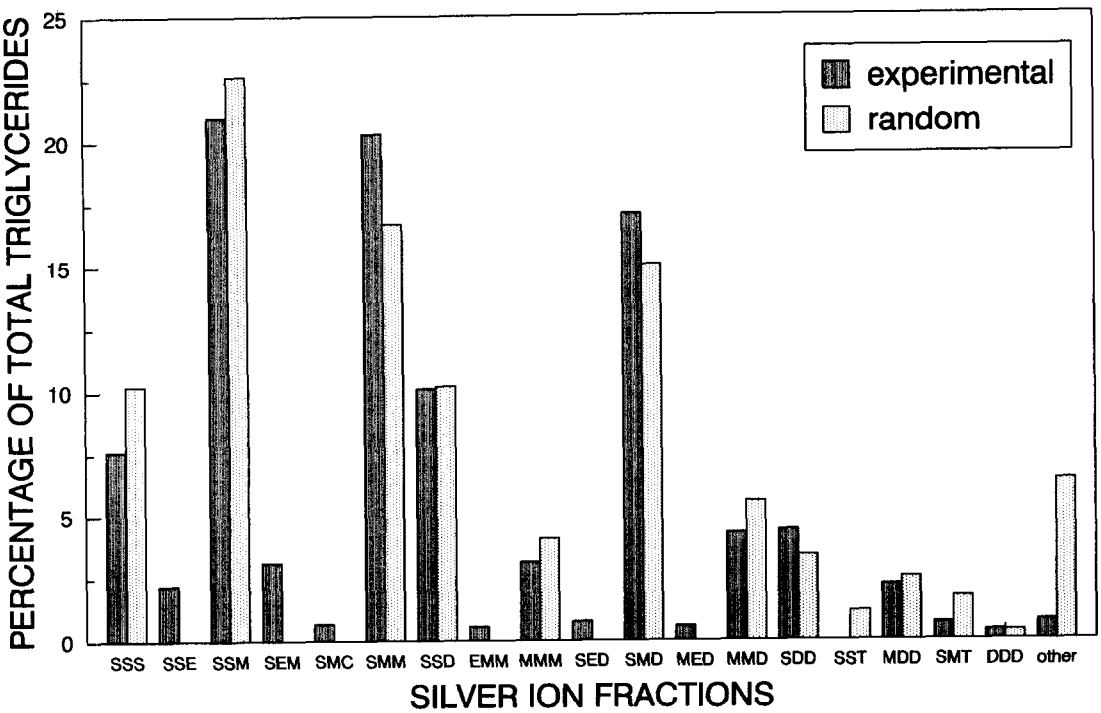

Fig. 2. Experimental and theoretical abundances (in mol/100 mol TGs) of human milk Ag-HPLC fractions. Theoretical values (right bars) were calculated by taking the sum of the TGs that would have been expected in case of random FA distribution. For abbreviations, see legend to Table I.

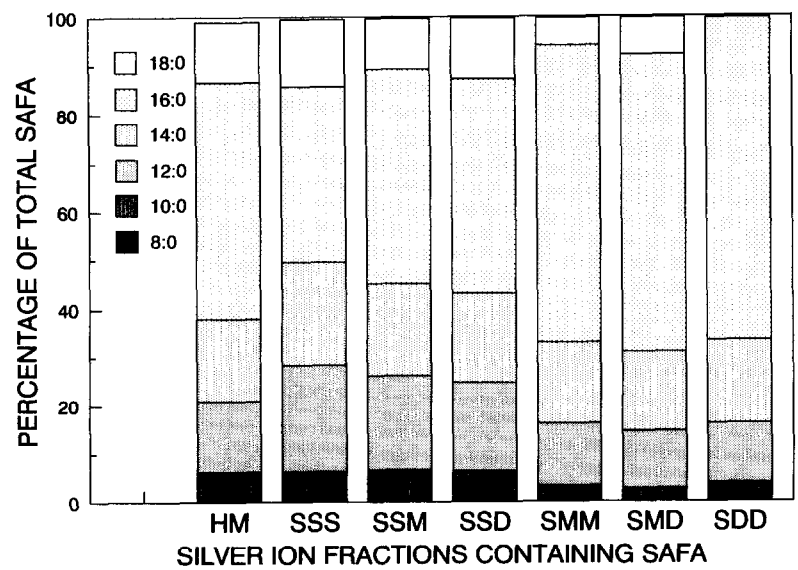

Fig. 3. Relative amounts of SAFA species in the main SAFA-containing Ag-HPLC fractions. Percentages of total SAFAs are in mol/100 mol SAFAs. For abbreviations, see legend to Table I.

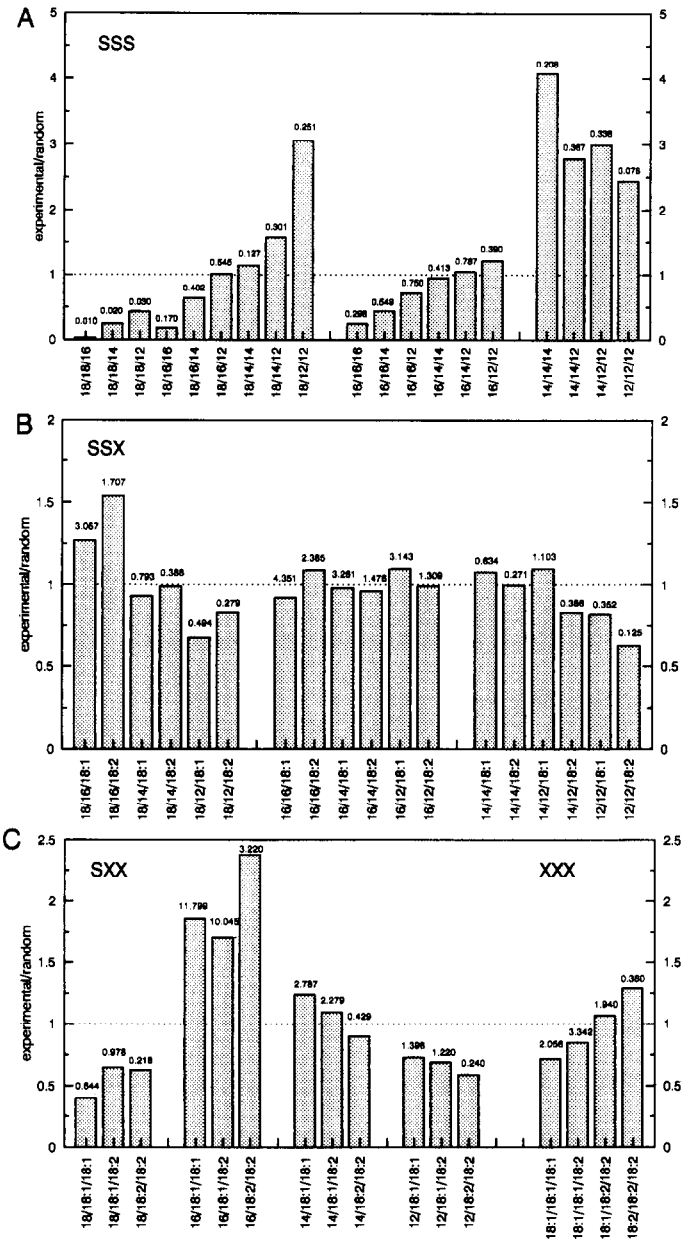

Fig. 4. Ratios between experimental and theoretical abundances for human milk TGs, grouped according to number of SAFAs per TG molecule. Experimental data were obtained from Table III (in mol/100 mol TGs). Theoretical values were obtained from calculation of random FA distribution (for this FA data were obtained from Table II "Total HM"). Only TGs that contained the six quantitatively most important FAs (12:0, 14:0, 16:0, 18:0, 18:1 and 18:2) were taken into account. Numbers on top of the bars represent abundances of TGs as given in Table III (in mol/100 mol TGs). (A) SSS Ag-HPLC fraction. (B) SSX group (TGs in the SSM and SSD Ag-HPLC fractions). (C) SXX group (TGs in the SMM, SMD and SDD) and XXX group (TGs in the MMM, MMD, MDD and DDD). S denotes SAFAs (12:0, 14:0, 16:0 or 18:0); X is MUFA (18:1) or PUFA (18:2). Indicated FA sequences in TGs do not necessarily correspond with sequences at glycerol moieties.

HPLC–LSD and GC–FID) will undoubtedly have introduced inaccuracies in both qualitative (contamination with extraneous FAs) and quantitative (analytical errors) results. LSD is linear over only a small range and its response is influenced by the type of component and the changing liquid composition during gradient elution [13,19]. Exposure of PUFAs to oxidative stress in the HPLC systems may have reduced the quantities of TGs that notably contain PUFAs with three or more double bonds. It may explain their underestimation (Table IV). From the data in Table I it can be deduced that the total lipid extract contained 7.4 mol/100 mol TGs with one *trans*-monoene FA. Others have reported a total of about 4 mol/100 mol *trans*-FAs in human milk from mothers on a western diet [7]. We were unable to identify these FAs in the FA methyl ester analysis on an apolar stationary phase. The quantitatively most important *trans*-FA (E, elaidic acid, 18:1t,ω9) was not resolved from 18:1c,ω7. As a consequence, only two TGs containing elaidic acid are included in Table III. However, for the six quantitatively most important FAs in human milk the results of direct transmethylation were in reasonable agreement with data obtained by reconstituting the total FA profile from FA compositions and abundances of experimental TGs (Table IV).

Comparison with previously reported human milk triglyceride compositions

Studies on the FA compositions of human milk TGs have previously been described [6,20–22]. Myher *et al.* [20] studied milk TGs from a type I hyperlipidaemic woman. Weber *et al.* [21] fractionated TGs on an RP-HPLC column with a temperature programme and refractive index detection. TGs in isolated fractions were analysed by high-temperature GC and their FAs quantified by GC–FID. Dotson *et al.* [22] separated TGs by RP-HPLC with refractive index detection. Tentative identification was performed on the basis of theoretical considerations and comparison of retention times with TG mixtures from natural sources. We compared our results with those of Breckenridge *et al.* [6]. They fractionated human milk TGs from the second (sample 1) and seventh (sample 3) days of lactation by silver ion thin-layer chromatography. This technique has a lower resolving capacity than the presently used Ag-HPLC method. The 6–7 fractions isolated per sample by thin-layer chromatography (HPLC: 14 fractions) were named "saturates" (comparable with our Ag-HPLC SSS fraction in Table I), "monoenes" (comparable with pooled data of our SSE and SSM fractions), "dienes 1" and "dienes 2" (SEM + SMC/CHOL + SMM + SSD/EMM), "trienes" (MMM/SED + SMD) and "tetraenes/polyenes" (MED + MMD + SDD/SSTri + MDD/SMTri + DDD/other). The (pooled) abundances of the saturates, monoenes and trienes of the present study ("mol% of Ag-HPLC fractions" in Table I) are in reasonable agreement with those of their sample 3. For the dienes we found a 9.4% (absolute value) higher abundance, whereas our polyene fraction proved to be 8.3% lower. Underestimation of PUFAs with three or more double bonds in the present study may explain the latter difference.

Generally the abundances of (pooled) RP-HPLC subfractions ("mol% of total HM" in Table I) proved to be in good agreement with results for sample 3 of Breckenridge *et al.* [6], who used packed-column GC analyses of intact TGs residing in the silver ion thin-layer chromatographic fractions. Except for the saturates, the quantitatively major RP-HPLC subfractions were found to be the same and their relative quantities compared well. However, the minor subfractions showed greater differences. For the saturates they found a carbon number range from C_{40} to C_{54}, with C_{46} and C_{48} being the most important. These data contrast with our results, which indicate a range from C_{32} to C_{52}, with C_{42} and C_{44} as the major constituents (see also Fig. 1C and D). The difference may be caused by an apparent discrepancy in their results for the saturates: calculation of the mean carbon number of this fraction yields 46.7, whereas calculation by taking three times the mean chain length of its FAs gives a value of 44.7. In our study these data amount to 43.1 and 43.8, respectively.

The FA compositions of total human milk and (pooled) Ag-HPLC fractions (Table II) compare favourably with the results of Breckenridge *et al.* [6] for sample 3. In all fractions our 16:1 data were lower, whereas also the minor FAs showed differences.

Breckenridge *et al.* [6] reported the presence of 13 major TGs in human milk, of which 8 are consistent with the 8 quantitatively most important found in the present study (see Table III). The positions of the remaining 5 in Table III are: 18:1/18:1/18:1 (position 13), 18:0/18:1/18:1 (30), 14:0/16:0/18:0 (42), 16:0/16:0/18:0 (79) and 16:0/18:0/18:0 (156). The last three belong to the SSS class, the fraction that seems to give rise to some discrepancies between the two studies.

Consistent with our results in Table III, the quantitative data given for sample 1 in Breckenridge *et al.* [6] (second day of lactation) indicate 16:0/18:1/18:1 (their result: 16.6%) and 16:0/18:1/18:2 (9.3%) as the two major TGs. Calculation from their total FA composition revealed that the abundances of these TGs were, respectively, 1.48 (our value 1.86, Fig. 4C) and 1.56 (1.71) times higher than would have been expected from random distribution. A 2.25 times higher than random distribution was found for 16:0/18:2/18:2 (our value 2.38). Lower than random were 18:0/18:1/18:1 (their value 0.82; our value 0.40), 18:0/18:1/18:2 (0.91; 0.65), 18:1/18:1/18:1 (0.56; 0.72) and 18:1/18:1/18:2 (0.90; 0.85). In contrast to our results 18:0/18:2/18:2 was found to be 3.48 times higher (our value 0.63).

Systematics in the fatty acid composition of human milk triglycerides

Deviation from random distribution of FAs among the three positions at the glycerol moiety (Figs. 2 and 4) seems to follow a systematic pattern, which suggests maintenance of a certain degree of TG polarity. Modulators of the highly apolar nature of LCSAFAs (16:0 and 18:0) are unsaturated FAs (18:1 and 18:2) and MCSAFAs (notably 12:0). Myristic acid (14:0) seems to hold an intermediate position between LCSAFAs and MCSAFAs. Arguments in support of this thesis are:

(1) Lower abundance of the SSS fraction; avoidance of combinations of three LCSAFAs or two LCSAFAs with one MCSAFA (12:0 more preferred than 14:0); and preference for one LCSAFA (notably 18:0) combined with two MCSAFAs (12:0 more preferred than 14:0), or combination of three MCSAFAs (14:0/14:0/14:0 more preferred than 12:0/12:0/12:0).

(2) Moderate preference to combine two LCSAFAs in the SSX group with 18:1 or 18:2 and an apparent decrease in this preference when LCSAFAs are replaced by MCSAFAs (notably 12:0). Modulation with the more polar 18:2 seems especially necessary in case of two LCSAFAs, whereas combination with the less polar 18:1 becomes more preferred when LCSAFAs are replaced by MCSAFAs.

(3) Higher abundance of the SXX group, in which (except for 18:0) decreasing preference was noted with decreasing SAFA chain length.

(4) About as expected abundances in the XXX group, with an increased preference for 18:2 to combine with 18:2.

From the view of polarity the most remarkable observation is that the apolarity of a single 16:0 or 16:0/16:0 is notably modulated by unsaturated FAs, whereas a single 18:0 (18:0/18:0 not observed) seems to be modulated more than expected by MCSAFAs. In the 16:0/18:0 combination the third FA is preferentially an unsaturated FA. This is at least partially consistent with different *sn* positions of the majority of 16:0 and 18:0 at the glycerol moiety. Breckenridge *et al.* [6] found 10:0 and 12:0 to be mainly at *sn*-3, 14:0 and 16:0 mainly at *sn*-2, 18:0 mainly at *sn*-1 and the majority of 18:1 and 18:2 about equally divided amongst *sn*-1 and *sn*-3. Consistent with this observation it may be expected that 16:0 will notably reside in the SXX group, with 16:0 at *sn*-2. Positioning of 18:0 at *sn*-1 leaves the possibility of combining it with the following main *sn*-2/*sn*-3 combinations: 16:0/unsaturated FA, 16:0/12:0, 14:0/unsaturated FA, and 14:0/12:0. TGs harbouring these FA compositions were found to have abundances that are close to or higher than expected from random distribution.

Possible consequences for optimal structures of infant formula triglycerides

The human milk TGs, as presented in Table III, are supposed to be easily hydrolysed by the concerted actions of BSSL and lingual, gastric and pancreatic lipases [1,3]. Their structures are not necessarily optimal for use in infant formulas. Deprivation of BSSL may create a special need for TGs that are susceptible to the actions of lingual and gastric lipases in both stomach and duodenum. The resulting hydrolysis products are considered to accelerate subsequent fat digestion in the duodenum, because of their stimulating action on cholecystokinin release (which stimulates secretion of gastric [23,24] and pancreatic [5,23] lipases) and their assistance in binding of pancreatic lipase–colipase to fat droplets [3,5,23]. Rapid lipolysis seems important, since inadequate attuning between small intestinal lipolysis rate and passage time may contribute to the relatively low fat absorption coefficient of preterm neonates in the first post-partum weeks [8].

TG structures that fulfil the stereospecific requirements for intragastric lipolysis will have an MCFA (notably 12:0) at *sn*-3. Together with a long-chain FA (LCFA, C > 12) at *sn*-1 these structures are not only easily hydrolysed by lingual and gastric lipases, but the resulting diglycerides, monoglycerides and FFAs are eligible to reach the duodenum. Liberated MCFAs will, dependent on chain length, be absorbed as FFAs via the portal system [5,23,25], which prevents their assistance in duodenal lipolysis.

The sum of human milk TGs containing one or more MCFAs amounts to 19.0 mol/100 mol (Table III). In view of the preferential *sn* positions of the FAs, the most likely and abundant lingual and gastric lipase substrates will have an MCFA at *sn*-3, 14:0 or 16:0 at *sn*-2, and 18:0, 18:1 or 18:2 at *sn*-1 [6,7]. A highly apolar LCSAFA species at *sn*-1 may, when liberated, form insoluble calcium soaps, which results in diminished absorption. Therefore, MCFAs at *sn*-3 and unsaturated LCFAs at *sn*-1 may be the optimal structures for lingual and gastric lipases to produce hydrolysis products that stimulate cholecystokinin release, assist in further duodenal TG hydrolysis by virtue of their emulsifying properties and are themselves easily taken up in the duodenum. Table III contains 10 TGs that are potentially consistent with these structures (numbers 7, 19, 21, 31, 45, 62, 76, 103, 110 and 130). Together they amount to 7.2 mol/100 mol TGs. The quantitatively most important are 12:0/16:0/18:1 (3.1%), 12:0/16:0/18:2 (1.3%) and 12:0/14:0/18:1 (1.1%). Such structures are not expected to be abundant in corn oil, soybean oil or semisynthetic medium-chain TGs (MCT; containing 6:0–10:0), which form the usual basis for (premature) infant formula fat.

Modern formulas for premature babies contain coconut oil TGs, which harbour about 72.9% MCFAs (51.4% 12:0; ref. 26). However, compared with an MCT/corn oil/low-coconut oil/other fat (39:39:18:4)-containing formula, a blend with high-coconut oil/soybean oil/lecithin (54:44:2) did not improve lipid absorption (about 84%) in premature babies [23]. The underlying reason may be found in coconut TG structure. The *sn*-3 position of coconut oil is for 85.9% occupied by MCFAs (37.8% 12:0; ref. 26), which makes its TGs excellent substrates for lingual and gastric lipases. The percentage LCFAs at *sn*-1 equals 51.9%; the percentage unsaturated LCFAs at *sn*-1 is, however, only 3.7%. This implies that the high-coconut oil containing blend mentioned above contains a maximum of 2 mol/100 mol TGs that are both optimal structures for lingual and gastric lipases and produce easily absorbable hydrolysis products that potentiate duodenal TG hydrolysis.

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