Distribution of the Major Fatty Acids of Human Milk Between *sn*-2 and *sn*-1,3 Positions of Triacylglycerols

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Human milk triacylglycerols (TAG) were analyzed by tandem mass spectrometry. The SIMPLEX method and a simple linear model were used to interpret the distribution of fatty acids between the *sn*-2 and *sn*-1,3 positions in 24 major molecular weight groups of TAG. The number of regio-isomeric pairs of TAG varied between 3 and 18 in each of these groups. Hexadecanoic (16:0), tetradecanoic (14:0) and dodecanoic acids (12:0) typically occupied the *sn*-2 position in TAG containing less than 54 acyl carbons, whereas long-chain C18 and C20 acids were predominantly located at the primary positions. The positions of the three fatty acids within a TAG molecule were shown to depend on the fatty acid combination. The maximum of 12:0 in the *sn*-2 position appeared at acyl carbon number (ACN) 48, the maxima of 14:0 were at ACN 44 and ACN 50, and for 16:0 at ACN 46 and 52.

KEY WORDS: Human milk, regio-isomers, tandem mass spectrometry, triacylglycerols.

The proportions of fatty acids do not explain all the details concerning infants' digestion of fats and oils. The detailed structural information of the individual triacylglycerols (TAG) is needed to explain the mechanism of hydrolysis, absorption and transport, as well as the formation of structural and functional lipids. The specificities of human pancreatic and lingual lipases affect the proportions and rate of intake of the fatty acid (FA) moieties. The enzymes hydrolyze the FAs at *sn*-1 and *sn*-3 positions of TAG, producing FAs and 2-monoacyl-*sn*-glycerols.

The amount of TAGs in human milk is known to double, and the proportions of the FAs to change during the first week of lactation (1,2). The proportions of decanoic, dodecanoic and tetradecanoic acids all increase during lactation, whereas those of hexadecanoic and oleic acids decrease (2–5). At the same time, the average size of the TAGs becomes smaller (5). The same trend is observed when the prepartum mammary secretion is developed to colostrum (6). Thus, the proportions of the various regio-isomers may also vary during maturation of human milk.

The aim of this study was to identify TAG molecules of human milk and to describe the effect of FA combinations to the positioning of FAs between the primary (*sn*-1,3) and secondary (*sn*-2) positions within TAG molecules. The results are based on tandem mass-spectrometric analysis and computerized calculations of the proportions of regio-isomeric TAGs.

MATERIALS AND METHODS

Human milk TAGs. The origin of human milk and the isolation of TAGs have been previously described (7).

Tandem mass-spectrometric analysis. TAGs were analyzed with a TSQ-70 triple quadrupole mass spectrometer (Finnigan MAT, San Jose, CA) by using ammonia negative ion chemical ionization (NICI). The RCO_2^- and $[M - H - RCO_2H - 100]^-$ ions produced by collisional activation of $[M - H]^-$ parent ions were used to deduce the structures of the TAGs. Identification of the TAG regio-isomers was based on the discrimination of the production of $[M - H - RCO_2H - 100]^-$ ions according to the primary vs. secondary locations of FAs in TAGs (8).

Calculations of the proportions of regio-isomeric TAG. Possible TAGs are determined by the constituent FAs and the constraints on the total number of carbon atoms and double bonds. The calculations are divided into two steps. First, we compute the proportions of TAG when the FA chains' position is not considered, and from those results we compute the proportions of regio-isomer pairs of TAG.

In step 1, the positions of the FAs in the TAGs are not relevant. To compute the TAG relative abundances, R_{tag}, from the corrected experimental FA relative abundances, $R_{fa,exp}$, we have to solve a linear algebraic system, S_0, for each FA:

$$\sum_{\text{all tags}} (n_{tag,fa}/3)\, R_{tag} = R_{fa,exp} \qquad [1]$$

where $n_{tag,fa}$ is the number of different FA molecules in the TAG (0, 1, 2 or 3). In general, the number of FAs is greater than the number of possible TAGs. There is no exact solution as there are more equations than unknowns, and measurement errors. Error terms can be introduced, and an approximate solution is a set of R_{tag} that minimizes the errors. This is a typical linear programming problem. We use the SIMPLEX method as described by Dantzig (9) to solve the following system, S_1, Equations 2, 4 and 5 are for each FA:

$$\sum_{\text{all tags}} (n_{tag,fa}/3)\, R_{tag} + w_{fa}\, e_{fa} = R_{fa,exp} \qquad [2]$$

$$\sum_{\text{all tags}} R_{tag} = 100 \qquad [3]$$

$$|e_{fa}| \leqslant a_{fa} \qquad [4]$$

$$a_{fa} \leqslant A \qquad [5]$$

$$\sum_{\text{all tags}} a_{fa} + 100\, n_{fa}\, A = z \qquad [6]$$

where Equation 2 is the modified original system with error terms: e_{fa} are normalized errors, w_{fa} are weight factors, which allow working with different error criteria (see below). Equation 3 must be introduced because it is no longer a consequence of Equation 2. This is equivalent to saying that the sum of the error terms is zero.

Equation 4 represents constraints on the normalized errors, where a_{fa} are arbitrary positive variables. Equation 5 represents auxiliary constraints on the a_{fa}, where A is the maximum error. Equation 6 is the objective to be minimized; the emphasis is on minimizing A (with an arbitrary coefficient 100 × number of FA), which means getting the smallest possible maximal error and then

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minimizing the sum of the absolute values of the individual errors.

The use of weight factors is more easily understood when looking at the error:

$$e_{fa} = (R_{fa,exp} - R_{fa,calc})/w_{fa} \quad [7]$$

where the calculated $R_{fa,calc}$ equals:

$$\sum_{\text{all tags}} (n_{tag,fa}/3)\ R_{tag} \quad [8]$$

If w_{fa} is constant, the solution minimizes the error:

$$|R_{fa,exp} - R_{fa,calc}| \quad [9]$$

If $w_{fa} = R_{fa,exp}$, the solution minimizes the relative error:

$$|R_{fa,exp} - R_{fa,calc}\ |/R_{fa,exp} \quad [10]$$

In this paper we minimize the relative error.

We compute the relative abundancies of TAGs R'_{tag} and this time the FA in position *sn*-2 is relevant. One R_{tag} corresponds to one, two or three R'_{tag} (if the given TAG has three identical FA, two identical FA or three different FA). We use the following linear model for the signal:

$$S = S_c + S_e \quad [11]$$

with

$$S_c = \gamma_c R_c \quad [12]$$

and

$$S_e = \gamma_e R_e \quad [13]$$

where the c subscript denotes the secondary (*sn*-2) position, and the e subscript denotes the primary (*sn*-1 + *sn*-3) positions, R are relative abundances in the sample, and γ_c and γ_e are proportionality factors ($\gamma_c \neq \gamma_e$). With this model, the measured relative signal for a given $[M - H - RCO_2H - 100]^-$ ion can be written as:

$$S_{fa} = (\gamma_c + 2\gamma_e)^{-1} \sum_{\text{all tags}} (\gamma_c n_{tag,fa,c} + \gamma_e n_{tag,fa,e}) R'_{tag} \quad [14]$$

The coefficient $(\gamma_c + 2\gamma_e)^{-1}$ is introduced so that $\sum_{\text{all tags}} S_{fa} = 100$.

For a sample that has only one TAG (A-B-A), where fatty acid B is always in the *sn*-2 (*sn*-A-B-A):

$$S_B/S_A = \gamma_c/2\gamma_e \quad [15]$$

Measurements have shown this ratio to be constant, especially with the C16 and C18 fatty acids (8):

$$K_0 = \gamma_c/2\gamma_e \approx 0.148 \quad [16]$$

and Equation 14 can be written as:

$$S_{fa} = [2(1 + K_0)]^{-1} \sum_{\text{all tags}} (2K_0 n_{tag,fa,c} + n_{tag,fa,e})\ R'_{tag} \quad [17]$$

The same considerations apply as for system S_0, and we use the SIMPLEX method (9) to solve system S_2. Equations 18, 20 and 21 are for each FA; Equation 19 is for each nonregio-isomeric TAG:

$$[2(1 + K_0)]^{-1} \sum_{\text{all tags}} (2K_0 n_{tag,fa,c} + n_{tag,fa,e}) R'_{tag} + W_{fa} e_{fa} = S_{fa,exp} \quad [18]$$

$$\sum_{\text{all relevant regio-isomeric tags}} {}_{tag}R'_{tag} = R_{tag} \quad [19]$$

$$|e_{fa}| \leq a_{fa} \quad [20]$$

$$a_{fa} \leq A \quad [21]$$

$$\sum_{\text{all tags}} a_{fa} + 100\ n_{fa} A = z \quad [22]$$

Here, Equation 19 represents the conservation of the TAG abundancies computed, e.g., $R'_{A\text{-}B\text{-}C} + R'_{B\text{-}A\text{-}C} + R'_{A\text{-}C\text{-}B} = R_{A/B/C}$, where A-B-C = *sn*-A-B-C + *sn*-C-B-A, B-A-C = *sn*-B-A-C + *sn*-C-A-B, A-C-B = *sn*-A-C-B + *sn*-B-C-A and A/B/C is the combination of fatty acids A, B and C, each being located in any of the stereospecific positions. Although we use the same notation, the values of w_{fa}, e_{fa}, a_{fa}, A and z in systems S_1 and S_2 are not related.

RESULTS AND DISCUSSION

Ammonia NICI tandem mass spectrometry was used to investigate 24 molecular weight groups of human milk TAGs of varying numbers of acyl carbons (ACN) (from 38 to 54) and double bonds (DB) (from 0 to 4). Each of the $[M - H]^-$ parent ion groups of TAGs was separately analyzed by collisional activation: 38 (ACN):0 (DB) (2.3% of the total ion current of $[M - H]^-$ ions), 40:0 (3.2%), 42:0 (2.0%), 42:1 (3.8%), 42:2 (0.8%), 44:0 (2.0%), 44:1 (6.9%), 44:2 (1.7%), 46:0 (2.7%), 46:1 (9.6%), 46:2 (3.0%), 48:0 (1.3%), 48:1 (7.2%), 48:2 (5.3%), 48:3 (1.8%), 50:1 (6.2%), 50:2 (6.3%), 50:3 (2.7%), 52:1 (2.1%), 52:2 (12.1%), 52:3 (6.6%), 52:4 (1.8%), 54:2 (0.5%) and 54:3 (2.0%). The molecular weight species examined covered about 95% of the summed intensities of the $[M - H]^-$ ions.

The ammonia NICI mass spectrum of a TAG mixture overestimates the low-mass TAGs due to the high specific abundances of their $[M - H]^-$ ions. Thus, the proportions displayed in parentheses do not represent the correct ratios of the TAG groups. Our research was focused on the analysis of the FA distribution between the primary and secondary positions of TAG, not on the molecular weight distribution of TAG. The goal was to distinguish between the molecular species within each TAG molecular weight group of human milk.

Proportions of the various pairs of TAG regio-isomers within each molecular weight group were calculated according to the intensities of RCO_2^- and $[M - H - RCO_2H\ 100]^-$ ions of the CA-daughter spectra (7,8). The calculations of the proportions of regio-isomeric TAG are implemented as a computer program (this program runs under DOS). The SIMPLEX method guarantees an optimal solution for the objective we choose to optimize. However, this method does not indicate if there is more than one optimal solution. Some more precise mathematical investigations would be needed to determine if this

situation can occur or if this class of problem always has a unique solution.

The formula we used for Equations 6 or 22 contains the arbitrary coefficient $100 \times n_{fa}$. This coefficient has been chosen because it is sufficient to achieve our goal of minimizing the maximum error A first. When using a coefficient of $1000 \times n_{fa}$, one gets identical results with a relative precision better than 1% for each TAG.

The calculations were organized into two steps: Proportions of TAG when the FA chain position is not considered (R_{tag}) and, from those results, the proportions of regioisomeric TAG (R'_{tag}). Actually, the latter could be computed directly from the $R_{fa,exp}$ and $S_{fa,exp}$ by eliminating R_{tag} between systems S_1 and S_2. Once the R'_{tag} are known, it is easy to compute R_{tag} with Equation 19. We decided to keep this two-step method so that the R_{tag} are computed without using the model introduced in step 2 (Equations 11–16). The validity of the model will be further tested by analyzing known mixtures of TAG and comparing the computed proportions with the known proportions. This is of primary importance because we need more detailed information on the effect of complex matrices on the mass spectrometric and the tandem mass spectrometric fragmentation. The program allows us to specify the weight factors for the errors:

$$w_{fa} = \alpha R_{fa,exp} + \beta \quad [23]$$

We have been using $\alpha = 1$ and $\beta = 0$, i.e., the relative error (Equation 7). The relative error can be seen as giving too much importance to the small values in the computations; an error of 0.1% for a 1.0% measurement is as important as an error of 1.0 for a 10.0% measurement. It is possible to use different coefficients in order to improve this situation. The coefficient α can also be viewed as the relative error on big measurements, and β the best absolute error on small measurements.

The spectrum of the TAG 40:1, molecular weight 692.6, could not be resolved because the ion clusters RCO_2^- and $[M - H - RCO_2H - 100]^-$ partially overlapped. All 24 molecular weight groups of TAG analyzed were mixtures of several molecular species. With the sensitivity of this tandem mass-spectrometric method, 2–12 FA combinations and 3–18 pairs of regio-isomers were identified.

The tandem mass-spectrometric analyses of human milk TAGs 40:0, 46:1, 50:3 and 52:4, which represent TAG of various sizes and degrees of unsaturation, are shown as examples in Table 1. All the regio-isomer pairs of TAG exceeding the calculated 1% level in the corresponding molecular weight species are listed. The errors between the measured and the calculated intensities of the RCO_2^- and $[M - H - RCO_2H - 100]^-$ ions varied typically from 0 to 10% for the major fatty acids.

Table 1 shows that the proportions of the $[M - H - RCO_2H - 100]^-$ ions for the saturated FAs (RCO_2H) were low among all the $[M - H - RCO_2H - 100]^-$ ions, when compared with the proportions of the corresponding saturated $[RCO_2]^-$ ions among all the deprotonated FA ions. Stearic acid (18:0) was an exception. Oleic (18:1) and linoleic (18:2) acids were the two dominating unsaturated FAs and seemed to be almost entirely located at the secondary *sn*-1,3 positions.

All pairs of the regio-isomeric TAGs, the proportions of which exceeded 10% of the corresponding molecular weight group, are listed in Table 2 according to the ACNs and DBs. Of the 66 regio-isomer TAG pairs, only five contain an unsaturated FA at the *sn*-2 position, i.e., 16:1 once, 18:1 three times and 18:2 once. The typical FAs occupying the secondary position are 16:0, 14:0 and 12:0. It has to be stated that positions *sn*-1 and *sn*-3 cannot be distinguished from each other with the method applied.

It is clear from Table 2 that the distribution of any FA between the primary and secondary positions within a TAG depends on the two other acids of the combination, e.g., if the content of 16:0 in a certain molecular weight group TAG was not abundant, 14:0 and 12:0 typically occupied the *sn*-2 position.

If the TAG structures presented in Table 2 are compared with the previous results of Currie and Kallio (7), there are some differences, which may be due to more accurate methods of calculation. However, by introducing a new dimension, the ratios of the pairs of TAG regio-isomers, the overall picture of human milk TAG remains unaltered. Two significant digits have not been shown to be the correct level of accuracy, but have to be verified in further model investigation.

All 24 TAG groups were investigated analogously to the examples shown in Table 1. The specific distribution of FAs between secondary (*sn*-2) and primary (*sn*-1 and *sn*-3) positions of the TAGs within each molecular weight group was calculated, and the results are outlined in Figure 1. Each FA exceeding the proportion of 5% in any of the molecular weight groups of TAG was taken into account, whereas trace acids are not shown on Figure 1. In all, ten FAs fulfilled these conditions in at least one group of TAG.

The bars in Figure 1a show the proportions of FAs located at the secondary position of the corresponding molecular weight TAG. Accordingly, Figure 1b shows the distribution of FAs in the primary positions. The long-chain FAs, C18 and C20, are almost absent from the *sn*-2 position in TAGs of ACN 38–52. The low content of TAGs having 54 or more acyl carbon atoms is characteristic of human milk, even though more than half of the FAs contain 18 carbon atoms. This can be explained by the fact that the formation of triacyl-*sn*-glycerols with stearoyl, oleoyl, linoleoyl and linolenoyl groups at the *sn*-2 position is highly unfavorable in human mammary glands.

More than half of the hexadecanoic and tetradecanoic acids were almost always (when in existence) at the secondary position among a unimolecular weight fraction of TAG. An interesting cyclic appearance in the positioning of the FAs 12:0, 14:0 and 16:0 can be observed (Fig. 1). This trend shows that the enzymatic system of the human mammary glands is able to distinguish between the primary and secondary positions, depending on the combination of the three FAs. Clear maxima of 12:0 in position *sn*-2 existed at ACN 48, of 14:0 at ACN 44 and ACN 50 and of 16:0 at ACN 46 and of ACN 52. The maximum of FA 12:0 at ACN 42 was less abundant, although still evident.

The structure of human milk TAGs has been reviewed extensively (10–12). It is well known that the distribution of FAs in human milk TAG is not random. Freeman *et al.* (13) were the first to show that the majority of 16:0 is esterified at the *sn*-2 position and 18:0, 18:1 and 18:2 are esterified at the *sn*-1,3 positions. Several studies on FA combinations (14,15) and on the distribution of the most abundant FAs in human milk between the *sn*-2 and *sn*-1,3

TABLE 1

Examples of the Analysis of Regio-Isomeric Triacylglycerols (TAG) of Human Milk by Ammonia Negative Ion Chemical Ionization Tandem Mass Spectrometry

ACN 42, 0 DB[a]

	Measured proportions (%) of:		Calculated proportions[d] (%) of:			
FA[b]	FA[c]	$[M - H - FA - 100]^-$	FA	± error %	$[M - H - FA - 100]^-$	± error %
12:0	31.2	29.7	30.9	−1.0	32.8	+10.5
14:0	27.7	24.4	27.7	+0.1	24.4	−0.0
16:0	25.9	22.9	26.2	+1.0	22.9	−0.0
18:0	8.7	14.4	8.8	+1.0	11.5	−20.2
10:0	6.5	8.6	6.4	−1.0	8.4	−2.3

TAG FA combinations[e]	Proportion (%)
16:0/14:0/12:0	68.7
18:0/14:0/10:0	14.4
18:0/12:0/12:0	12.0
16:0/16:0/10:0	4.9
14:0/14:0/14:0	0.0

Regio-isomers of TAG	Proportion (%)
sn-14:0-16:0-12:0 + *sn*-12:0-16:0-14:0	32
sn-16:0-14:0-12:0 + *sn*-12:0-14:0-16:0	24
sn-18:0-14:0-10:0 + *sn*-10:0-14:0-18:0	14
sn-16:0-12:0-14:0 + *sn*-14:0-12:0-16:0	13
sn-18:0-12:0-12:0 + *sn*-12:0-12:0-18:0	12
sn-16:0-16:0-10:0 + *sn*-10:0-16:0-16:0	5

ACN 46, 1 DB[a]

	Measured proportions (%) of:		Calculated proportions[d] (%) of:			
FA[b]	FA[c]	$[M - H - FA - 100]^-$	FA	± error %	$[M - H - FA - 100]^-$	± error %
18:1	33.1	41.8	31.3	−5.4	40.9	−2.1
16:0	25.7	14.3	25.1	−2.2	14.3	−0.0
12:0	24.7	26.2	26.3	+6.5	29.1	+11.0
14:0	12.8	10.1	13.6	+6.5	11.3	+12.3
16:1	1.9	2.3	2.0	+6.5	2.3	−0.0
18:0	1.7	2.2	1.6	−6.5	2.1	−5.6

TAG FA combinations[e]	Proportion (%)
18:1/16:0/12:0	74.1
18:1/14:0/14:0	19.8
18:0/16:1/12:0	4.9
16:0/16:1/14:0	1.3

Regio-isomers of TAG	Proportion (%)
sn-18:1-16:0-12:0 + *sn*-12:0-16:0-18:1	61
sn-18:1-14:0-14:0 + *sn*-14:0-14:0-18:1	20
sn-18:1-12:0-16:0 + *sn*-16:0-12:0-18:1	14
sn-18:0-12:0-16:1 + *sn*-16:1-12:0-18:0	4
sn-16:0-14:0-16:1 + *sn*-16:1-14:0-16:0	1
sn-18:0-16:1-12:0 + *sn*-12:0-16:1-18:0	1

ACN 50, 3 DB

	Measured proportions (%) of:		Calculated proportions[d] (%) of:			
FA[b]	FA[c]	$[M - H - FA - 100]^-$	FA	± error %	$[M - H - FA - 100]^-$	± error %
18:2	30.3	34.8	28.4	−6.3	37.1	+6.6
18:1	25.1	29.7	24.9	−0.8	30.2	+1.7
14:0	22.9	12.0	24.3	+6.3	12.0	−0.0
16:0	7.7	4.8	8.2	+6.3	4.8	−0.0
16:1	6.6	7.2	6.9	+3.9	7.9	+10.1
18:3	2.7	4.1	2.5	−6.3	3.3	−19.3
18:0	1.7	1.6	1.8	+6.3	1.6	+0.0
12:0	1.4	1.1	1.5	+6.3	1.1	+0.0
20:3	0.9	1.4	0.8	−6.3	1.1	−21.3
20:2	0.7	1.3	0.7	−6.3	0.9	−34.0

(continued)

TABLE 1 (continued)

TAG FA combinations[e]	Proportion (%)
18:1/18:2/14:0	70.1
18:2/16:0/16:1	15.1
18:3/16:0/16:0	4.7
18:0/18:3/14:0	2.9
18:1/16:1/16:1	2.7
20:3/18:0/12:0	2.5
20:2/18:1/12:0	1.9
20:3/16:0/14:0	0.0
20:2/16:1/14:0	0.0

Regio-isomers of TAG	Proportion (%)
sn-18:1-14:0-18:2 + *sn*-18:2-14:0-18:1	64
sn-18:2-16:0-16:1 + *sn*-16:1-16:0-18:2	15
sn-18:2-18:1-14:0 + *sn*-14:0-18:1-18:2	6
sn-18:3-16:0-16:0 + *sn*-16:0-16:0-18:3	5
sn-18:1-16:1-16:1 + *sn*-16:1-16:1-18:1	3
sn-18:3-18:0-14:0 + *sn*-14:0-18:0-18:3	3
sn-20:3-12:0-18:0 + *sn*-18:0-12:0-20:3	3
sn-20:2-18:1-12:0 + *sn*-12:0-18:1-20:2	2

ACN 52, 4 DB[a]

	Measured proportions (%) of:		Calculated proportions[d] (%) of:			
FA[b]	FA[c]	$[M - H - FA - 100]^-$	FA ± error %		$[M - H - FA - 100]^-$	± error %
18:2	37.4	42.4	35.8	−4.3	44.5	+4.9
16:0	22.2	11.0	24.2	+8.9	11.0	−0.0
18:1	19.0	22.4	17.3	−8.9	22.4	+0.0
18:3	9.6	14.2	10.1	+4.8	13.1	−7.4
16:1	6.0	3.7	6.5	+8.9	3.7	+0.0
14:0	2.5	0.9	2.6	+4.6	1.3	+49.2
20:3	1.3	2.7	1.4	+8.9	1.9	−31.5
18:0	0.9	0.6	0.9	+0.0	0.6	−0.0
20:2	0.6	0.6	0.7	+8.9	0.8	+28.6
20:4	0.5	1.4	0.5	+8.9	0.7	−49.2

TAG FA combinations[e]	Proportion (%)
18:2/18:2/16:0	43.4
18:1/18:3/16:0	29.1
18:1/18:2/16:1	18.5
20:3/18:1/14:0	4.2
20:2/18:2/14:0	2.0
20:4/18:0/14:0	1.6
18:0/18:3/16:1	1.1
20:4/16:0/16:0	0.0
20:3/16:0/16:1	0.0
20:2/16:1/16:1	0.0

Regio-isomers of TAG	Proportion (%)
sn-18:2-16:0-18:2	39
sn-18:1-16:0-18:3 + *sn*-18:3-16:0-18:1	28
sn-18:1-16:1-18:2 + *sn*-18:2-16:1-18:1	16
sn-18:2-18:2-16:0 + *sn*-16:0-18:2-18:2	5
sn-20:3-14:0-18:1 + *sn*-18:1-14:0-20:3	4
sn-18:1-18:2-16:1 + *sn*-16:1-18:2-18:1	3
sn-20:2-14:0-18:2 + *sn*-18:2-14:0-20:2	2
sn-18:3-18:0-16:1 + *sn*-16:1-18:0-18:3	1

[a]ACN, acyl carbon number; DB, double bond.
[b]Only the molecular weight of the fatty acid (FA) known.
[c]Calculated from the intensities of the RCO_2^--ions with empirically determined molar correction factors for FA: 10:0 (1.6), 10:1 (2.0), 12:0 (1.5), 12:1 (1.9), 14:0 (1.3), 14:1 (1.7), 16:0 (1.1), 16:1 (1.5), 16:2 (1.7), 18:0 (1.0), 18:1 (1.3), 18:2 (1.6), 18:3 (1.9), 20:0 (1.0), 20:1 (1.1), 20:2 (1.1), 20:3 (1.1), 20:4 (1.1), 22:1 (1.0).
[d]The definitions as described in the Materials and Methods section.
[e]Each combination of three FAs contains all the regio-isomeric TAG.

TABLE 2

Proportions of the Major Triacylglycerol (TAG) of Human Milk Analyzed by Ammonia Negative Ion Tandem Mass Spectrometry

MW[a]	ACN:DB[a]	Regio-isomers of TAG	Proportion (%)[b]
666.6	38:0	*sn*-14:0-12:0-12:0 + *sn*-12:0-12:0-14:0	34
		sn-12:0-14:0-12:0	26
		sn-16:0-12:0-10:0 + *sn*-10:0-12:0-16:0	22
694.6	40:0	*sn*-14:0-14:0-12:0 + *sn*-12:0-14:0-14:0	31
		sn-16:0-12:0-12:0 + *sn*-12:0-12:0-16:0	30
		sn-14:0-16:0-10:0 + *sn*-10:0-16:0-14:0	16
		sn-12:0-16:0-12:0	10
722.6	42:0	*sn*-14:0-16:0-12:0 + *sn*-12:0-16:0-14:0	32
		sn-16:0-14:0-12:0 + *sn*-12:0-14:0-16:0	24
		sn-18:0-14:0-10:0 + *sn*-10:0-14:0-18:0	14
		sn-16:0-12:0-14:0 + *sn*-14:0-12:0-16:0	13
		sn-18:0-12:0-12:0 + *sn*-12:0-12:0-18:0	12
720.6	42:1	*sn*-18:1-12:0-12:0 + *sn*-12:0-12:0-18:1	62
		sn-18:1-14:0-10:0 + *sn*-10:0-14:0-18:1	17
718.6	42:2	*sn*-18:2-12:0-12:0 + *sn*-12:0-12:0-18:2	61
		sn-18:2-10:0-14:0 + *sn*-14:0-10:0-18:2	25
750.7	44:0	*sn*-16:0-16:0-12:0 + *sn*-12:0-12:0-16:0	37
		sn-16:0-14:0-14:0 + *sn*-14:0-14:0-16:0	18
		sn-18:0-16:0-10:0 + *sn*-10:0-16:0-18:0	17
		sn-18:0-14:0-12:0 + *sn*-12:0-14:0-18:0	16
		sn-18:0-12:0-14:0 + *sn*-14:0-12:0-18:0	11
748.7	44:1	*sn*-18:1-14:0-12:0 + *sn*-12:0-14:0-18:1	50
		sn-18:1-16:0-10:0 + *sn*-10:0-16:0-18:1	24
		sn-18:1-12:0-14:0 + *sn*-14:0-12:0-18:1	12
746.7	44:2	*sn*-18:2-14:0-12:0 + *sn*-12:0-14:0-18:2	50
		sn-18:2-16:0-10:0 + *sn*-10:0-16:0-18:2	20
		sn-18:2-12:0-14:0 + *sn*-14:0-12:0-18:2	14
778.7	46:0	*sn*-18:0-16:0-12:0 + *sn*-12:0-16:0-18:0	48
		sn-16:0-16:0-14:0 + *sn*-14:0-16:0-16:0	21
		sn-18:0-14:0-14:0 + *sn*-14:0-14:0-18:0	14
		sn-16:0-14:0-16:0	12
776.7	46:1	*sn*-18:1-16:0-12:0 + *sn*-12:0-16:0-18:1	61
		sn-18:1-14:0-14:0 + *sn*-14:0-14:0-18:1	20
		sn-18:1-12:0-16:0 + *sn*-16:0-12:0-18:1	14
774.7	46:2	*sn*-18:2-16:0-12:0 + *sn*-12:0-16:0-18:2	49
		sn-18:2-14:0-14:0 + *sn*-14:0-14:0-18:2	13
		sn-18:2-12:0-16:0 + *sn*-16:0-12:0-18:2	11
806.7	48:0	*sn*-18:0-14:0-16:0 + *sn*-16:0-14:0-18:0	47
		sn-18:0-16:0-14:0 + *sn*-14:0-16:0-18:0	38
804.7	48:1	*sn*-18:1-16:0-14:0 + *sn*-14:0-16:0-18:1	50
		sn-18:0-12:0-18:1 + *sn*-18:1-12:0-18:1	18
		sn-18:1-14:0-16:0 + *sn*-16:0-14:0-18:1	16
802.7	48:2	*sn*-18:1-12:0-18:1	43
		sn-18:2-16:0-14:0 + *sn*-14:0-16:0-18:2	16
		sn-18:2-14:0-16:0 + *sn*-16:0-14:0-18:2	11
800.7	48:3	*sn*-18:1-12:0-18:2 + *sn*-18:2-12:0-18:1	69
832.8	50:1	*sn*-18:1-16:0-16:0 + *sn*-16:0-16:0-18:1	62
		sn-18:0-14:0-18:1 + *sn*-18:1-14:0-18:0	25
830.8	50:2	*sn*-18:1-14:0-18:1	56
		sn-18:2-16:0-16:0 + *sn*-16:0-16:0-18:2	27
828.7	50:3	*sn*-18:1-14:0-18:2 + *sn*-18:2-14:0-18:1	64
		sn-18:2-16:0-16:1 + *sn*-16:1-16:0-18:2	15
860.8	52:1	*sn*-18:0-16:0-18:1 + *sn*-18:1-16:0-18:0	81
		sn-18:0-18:1-16:0 + *sn*-16:0-18:1-18:0	18
858.8	52:2	*sn*-18:1-16:0-18:1	79
		sn-18:0-16:0-18:2 + *sn*-18:2-16:0-18:0	12
856.8	52:3	*sn*-18:1-16:0-18:2 + *sn*-18:2-16:0-18:1	78
854.7	52:4	*sn*-18:2-16:0-18:2	39
		sn-18:1-16:0-18:3 + *sn*-18:3-16:0-18:1	28
		sn-18:1-16:1-18:2 + *sn*-18:2-16:1-18:1	16
886.8	54:2	*sn*-18:1-18:1-18:0 + *sn*-18:0-18:1-18:1	44
		sn-18:1-18:0-18:1	23
		sn-20:1-16:0-18:1 + *sn*-18:1-16:0-20:1	13
884.8	54:3	*sn*-18:0-18:2-18:1 + *sn*-18:1-18:2-18:0	43
		sn-18:1-18:1-18:1	22
		sn-18:1-18:0-18:2 + *sn*-18:2-18:0-18:1	18

[a]MW, molecular weight; ACN, acyl carbon number; DB, double bond.
[b]Only regio-isomer pairs exceeding 10% proportion included.

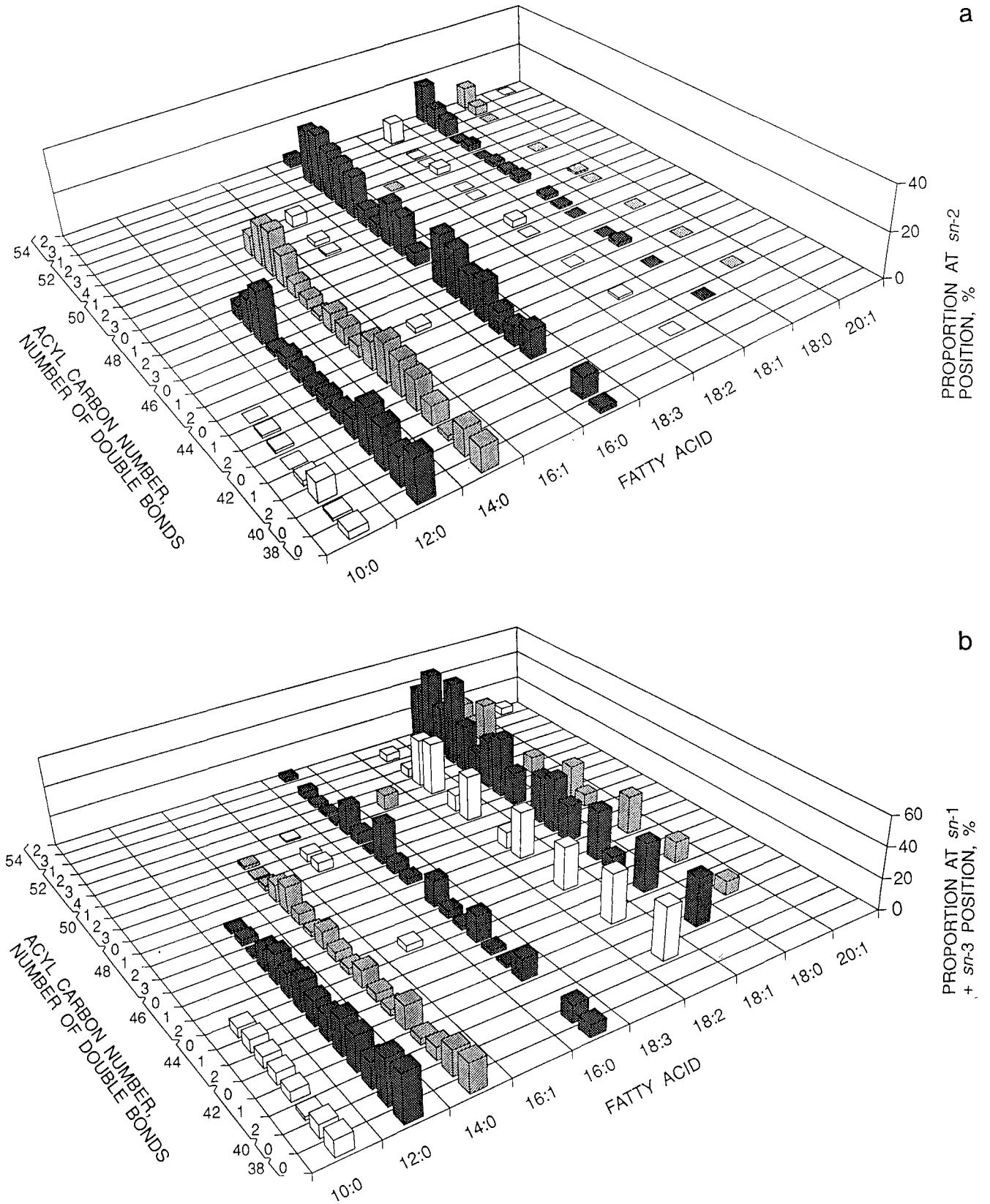

FIG. 1. Distribution of fatty acids (FAs) in positions *sn*-2 (a) and *sn*-1,3 (b) in triacylglycerols (TAG) of various molecular weight groups of human milk. FAs exceeding a 5% proportion of a TAG group were taken into account. The sum of the bars in each acyl carbon number/double bond group in a and b is 100%.

positions have been published. The greatest polarizations were observed for 18:1 (81–92% at *sn*-1,3), 18:0 (80–83% at *sn*-1,3), 18:2 (67–84% at *sn*-1,3), 18:3 (77% at *sn*-1,3), 16:0 (70–86% at *sn*-2) and 14:0 (41–81% at *sn*-2) (13,16–19).

According to Breckenridge *et al.* (17), the saturated TAG mainly consist of 1-stearoyl-2-palmitoyl-*sn*-glycerol with saturated FAs from 14:0 to 18:0 at the position *sn*-3. This is in accordance with our results. In addition, we noted that in TAG of low molecular weight species (ACN 44 and less) 14:0, 12:0 and even 10:0 also occupy the secondary position. The monoenoic TAGs were typically comprised of 1-oleoyl-2-palmitoyl-*sn*-glycerols with 12:0–18:0 at the position *sn*-3 (17). According to Table 2, this primary–secondary distribution is unexceptional in the TAG species 46:1, 48:1, 50:1 and 52:1. The results of Christie and Clapperton (18) were analogous to those reported by Breckenridge *et al.* (17). Breckenridge *et al.* (17) summarized the information of the major stereo-isomers of human milk TAG. The results are in accordance with the regio-isomeric structures listed in Table 2, with two exceptions. According to our tandem mass-spectrometric analysis, the major trienoic TAG of ACN 54 was the combination *sn*-18:0-18:2-18:1 + *sn*-18:1-18:2-18:0 (43%), and not *sn*-18:1-18:1-18:1. Breckenridge *et al.* (17) also stated the isomer *sn*-16:0-18:2-18:2 to be one of the major tetraenoic TAG in human milk. According to our results, the isomer *sn*-18:2-16:0-18:2 dominated the species ACN 52 with 4 DB, and linoleic acid occupied almost entirely one of the primary positions. The comparison of these two reports is not a comment about the reliability of either. This small difference may be caused by biological variation.

The effect of the FA combinations on the intramolecular distribution in the saturated TAGs is summarized in Figure 2. With an increase in the number of acyl carbons, decanoic and dodecanoic acids are concentrated more in the primary positions. For dodecanoic acid, the correlation between the proportion at the *sn*-2 position and ACN is highly linear; $y = 171.2 - 3.5x$, $r = -0.989$ (Fig. 2). The graphs of tetradecanoic and hexadecanoic acids are less regular. However, an increasing trend with increasing molecular weight of TAG can be observed.

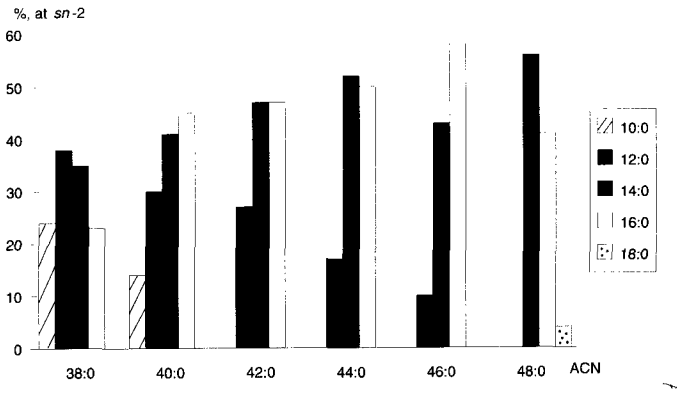

FIG. 2. Distribution of the major saturated FAs of human milk in positions *sn*-2 and *sn*-1,3 in saturated TAGs of human milk (ACN, acyl carbon number). See Figure 1 for abbreviations.

The above figures describe the FA preferences of human milk TAGs synthesized by complex multistage biochemical processes. The results do not, however, give an indication of the various pathways and positional preferences. We also have to bear in mind that the calculations within most of the molecular weight species are statistically averaged results. In addition, the errors in some examples may be high. All this suggests is that the results are to be read as profiles (shown in Figs. 1 and 2) which are typical for human milk and differ clearly from any other fats and oils preliminarily studied so far. Common calibration of the quantitative tandem mass spectrometric method is in progress.

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