Postparturition Changes in the Triacylglycerols of Cow Colostrum

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ABSTRACT: The changes in the triacylglycerol (TAG) composition of colostrum fat of three cows were studied. In addition to the determination of fatty acid composition by gas chromatography, the distribution of TAG according to the acyl carbon number (ACN) and molecular weight was analyzed utilizing both supercritical fluid chromatography (SFC) and ammonia negative-ion chemical ionization mass spectrometry (MS). Colostrum TAG contained substantially less stearic and oleic acids and more myristic and palmitic acids than the normal Finnish milk fat. The major trends in the changes of fatty acids and TAG were similar for each cow, although clear differences between individuals were found. During the first week of parturition, the proportions of short-chain fatty acids (C_4–C_{10}) typically increased as well as those of stearic and oleic acids, whereas the relative amounts of C_{12}–C_{16} acids decreased, especially those of myristic and palmitic acids. Distinct changes occurred also in TAG distributions: the proportions of molecules with ACN 38–40 increased and those with ACN 44–48 decreased. Although there were distinct differences between individuals shortly after delivery, both the fatty acid compositions and TAG distributions of the milk samples of the cows started to resemble each other after one week. The theoretical profiles of colostrum TAG calculated based on the fatty acid compositions differed clearly from the ACN distributions analyzed by SFC and MS. Thus, the analysis of TAG is essential, because the changes in molecular species composition of colostrum TAG cannot be estimated according to the fatty acid analysis alone. *Lipids 31*, 937–943 (1996).

The composition of milk fat triacylglycerols (TAG) has been extensively studied. However, most often information on the fatty acid composition, produced by gas chromatography (GC), has been found to be adequate. More specific information about milk fat has been achieved by analyzing the intact TAG by chromatographic methods, such as GC on both nonpolar (1,2) and polar stationary phases (3–5), and by high-performance liquid chromatography (HPLC) on reversed-phase columns (2,6–9) and ion-exchange columns loaded with silver ions (7,8,10–12). Furthermore, supercritical fluid chromatography (SFC) has been proved to be a useful technique for the analysis of TAG of various samples, including milk fat (13). In addition, milk fat TAG have been analyzed utilizing mass spectrometry (MS) in different modes usually in combination with chromatographic separation techniques (4,6,8,9,14–16). Chromatography prior to MS improves the interpretation of the results of complex mixtures. For some purposes, ammonia negative-ion chemical ionization MS, without chromatographic separation, has been found to be a rapid and sensitive technique for the analysis of TAG (17,18).

The colostrum of various species differs clearly from the mature milk. Some studies have been focused on the determination of fatty acid composition in cow colostrum and changes in the proportions of fatty acids after parturition (19–23). However, the TAG composition of colostrum has not been studied extensively. The present study was designed to provide information on the changes occurring in the composition of cow colostrum during the first week after parturition. The fatty acid compositions were determined by GC, and the TAG were separated according to the acyl carbon numbers (ACN) by SFC on a nonpolar column. In addition, molecular weight species of TAG were analyzed by ammonia negative-ion chemical ionization MS. The theoretical ACN distribution of TAG was calculated based on the fatty acid compositions and compared with that obtained on SFC and MS.

EXPERIMENTAL PROCEDURES

Materials. Milk samples were received from three cows, two of which were Ayrshires (cow 1, cow 2) and one Frisian (cow 3). Cow 2 was a descendant of cow 1. The cows were bred in Finland on the same farm and kept on the same diet consisting of oat meal, silage, concentrate, dry hay (*ad libitum*), minerals, and vitamins. The cows calved during a 10-d period in February 1993. Cow 1 had its fourth parturition, whereas cow 2 had its first and cow 3 its fifth calving. The milk samples were collected after every 12 h during the first week after parturition and stored at −20°C until the fat was separated by centrifugation and phase-exchange using sodium sulphate. TAG were purified from the total lipids by elution from short columns of Florisil™ with 10 mL of *n*-hexane/diethyl ether (4:1, vol/vol). Purified TAG fractions of 1 mg were dissolved in 1 mL of *n*-hexane for mass spec-

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Abbreviations: ACN, acyl carbon number; FID, flame-ionization detector; GC, gas chromatography; HPLC, high-performance liquid chromatography; MS, mass spectrometry; SFC, supercritical fluid chromatography; TAG, triacylglycerols.

trometric analyses and for preparation of fatty acid methyl esters. An aliquot of the total lipid fraction was diluted in dichloromethane for supercritical fluid chromatographic analyses. All solvents were of HPLC-grade and were supplied by Merck (Darmstadt, Germany) or Rathburn (Walkerburn, Scotland).

GC. Fatty acid methyl esters of the TAG were prepared by sodium methoxide-catalyzed transesterification as described elsewhere (7). The analyses were obtained on a Hewlett Packard 5890 gas chromatograph (Palo Alto, CA), equipped with a split injector (split ratio 35:1) and a flame-ionization detector (FID). An OV-351 column (25 m × 0.20 mm i.d. with 0.20 μm film; HNU-Nordion Instruments Ltd., Helsinki, Finland) was used for GC with a three-step temperature programming initialized at 35°C with a 3-min isothermal period followed by a linear temperature increase at 10°C min^{-1} from 35 to 160°C, 2°C min^{-1} from 160 to 190°C, and 5°C min^{-1} from 190 to 230°C. The injector and detector temperatures were 225 and 240°C, respectively. Helium was used as a carrier gas with a column flow rate of 30 cm s^{-1} measured with propane at the initial conditions of the chromatographic program. Fatty acids were identified by reference to three standard mixtures of fatty acid methyl esters (ME61, ME68, and BR1; Larodan Fine Chemicals AB, Malmö, Sweden). The response correction factor for each fatty acid methyl ester, used for the conversion of percentage peak area to mol%, was determined by analyzing the reference mixture ME61.

SFC. The SFC analyses were conducted with a Lee Scientific Series 600 supercritical fluid chromatograph (Dionex, Salt Lake City, UT) equipped with an FID. The temperature of the FID was held at 340°C, and nitrogen was used as a make-up gas. An electrically and pneumatically controlled Valco switching valve (Valco Instruments Co. Inc., Houston, TX) with an internal loop volume of 1.0 μL was used for timed split/dynamic split injections with a loop-open time of 0.6 s. Frit restrictors (30 cm × 50 μm i.d.; Dionex) were installed both at the dynamic split outlet and at the detector end of the analytical column. SFC-grade CO_2 (Scott Specialty Gases, Plumsteadville, PA) was used as a carrier fluid with a column flow rate of 0.37 mL min^{-1} measured with propane at the initial conditions of the chromatographic program. An SB-Octyl-50 column (10 m × 50 μm i.d. with 0.25 μm film; Dionex) was used with linear density programming of CO_2 from 0.140 g mL^{-1} with a rate of 0.010 g mL^{-1} min^{-1} at a constant temperature of 140°C for the elution of TAG. Samples of 40 mg were dissolved in 1 mL of dichloromethane. The ACN distributions of TAG in the figures were means of three determinations without applying response factors.

MS. Ammonia negative-ion chemical ionization of TAG was obtained on a Finnigan MAT (San Jose, CA) TSQ-700 triple quadrupole instrument with a direct exposure probe for sample introduction (17,18). An aliquot of 1.0 μL of TAG dissolved in *n*-hexane was applied to the rhenium wire of the direct exposure probe. The probe was introduced into the ion source after the sample solvent was evaporated at ambient temperature. The sample was vaporized in the ion source by heating the rhenium wire with the current rate of 40 mA s^{-1}. The pressure of ammonia (≥ 99.998%; Prax Air, Oevel, Belgium) was 8500 mtorr, and the ion source temperature was 200°C. The electron energy applied was 70 eV and the filament current 400 μA. The *m/z* values were scanned from 450 to 950 with a scanning time of 0.5 s. Ammonia negative-ion chemical ionization MS of TAG yielded simple spectra containing abundant $[M - H]^-$ ions only in the scanned molecular weight region. Scans containing ions representing deprotonated TAG were averaged in each analysis. The abundance of the $[M - H]^-$ ions in the averaged spectrum was corrected according to the ^{13}C and ^{18}O isotopes, before expressing the results as the mean of four analyses. No corrections were made according to the mass spectrometric response factors of TAG.

RESULTS AND DISCUSSION

Fatty acid compositions. The changes in the fatty acid composition of colostrum TAG during the first week after parturition were determined by GC as fatty acid methyl esters. Altogether 26 fatty acids were separated and identified from the colostrum fat samples. The most abundant fatty acids were butyric acid, myristic acid, palmitic acid, stearic acid, and oleic acid, together representing more than 75 mol% of the total fatty acids in each sample. Compared with the composition of normal Finnish milk fat (8), the colostrum TAG were substantially lower in stearic and oleic acids, and richer in myristic and palmitic acids.

The major trends in the changes of the proportions of fatty acids were similar for each cow, although variation between individuals was found (Fig. 1). The proportions of the short-chain fatty acids, from C_4 to C_{10}, increased after parturition with the exception of cow 1. The relative amounts of fatty acids with 12–16 acyl carbon atoms typically decreased, especially those of myristic acid and palmitic acid. Furthermore, the proportions of stearic acid, oleic acid, and *cis*-vaccenic acid (data not shown) increased after parturition with the exception of cow 2. The relative amounts of linoleic acid and α-linolenic acid (data not shown) were less than 2 and 0.6 mol%, respectively, in each sample, and only minor changes in their proportions were observed. In general, the proportions of different fatty acids started to resemble each other more and approached the composition of normal Finnish milk fat after one week, even though there were great differences between individuals shortly after delivery. For example, the proportion of $C_{18:1}$ fatty acids in the milk samples of the three cows approached the value of 20 mol% which is typical for Finnish winter milk.

Our results are in good accordance with those reported by Senft and Klobasa (20) and Baše *et al.* (22). There are also contradictory results as reviewed by Christie (24) and Hawke and Taylor (25): the proportion of palmitic acid has been reported to remain relatively constant or increase slightly, and the relative amounts of stearic acid and oleic acid have been found to diminish after calving. During early lactation, the fatty acids are mobilized extensively from the adipose tissue

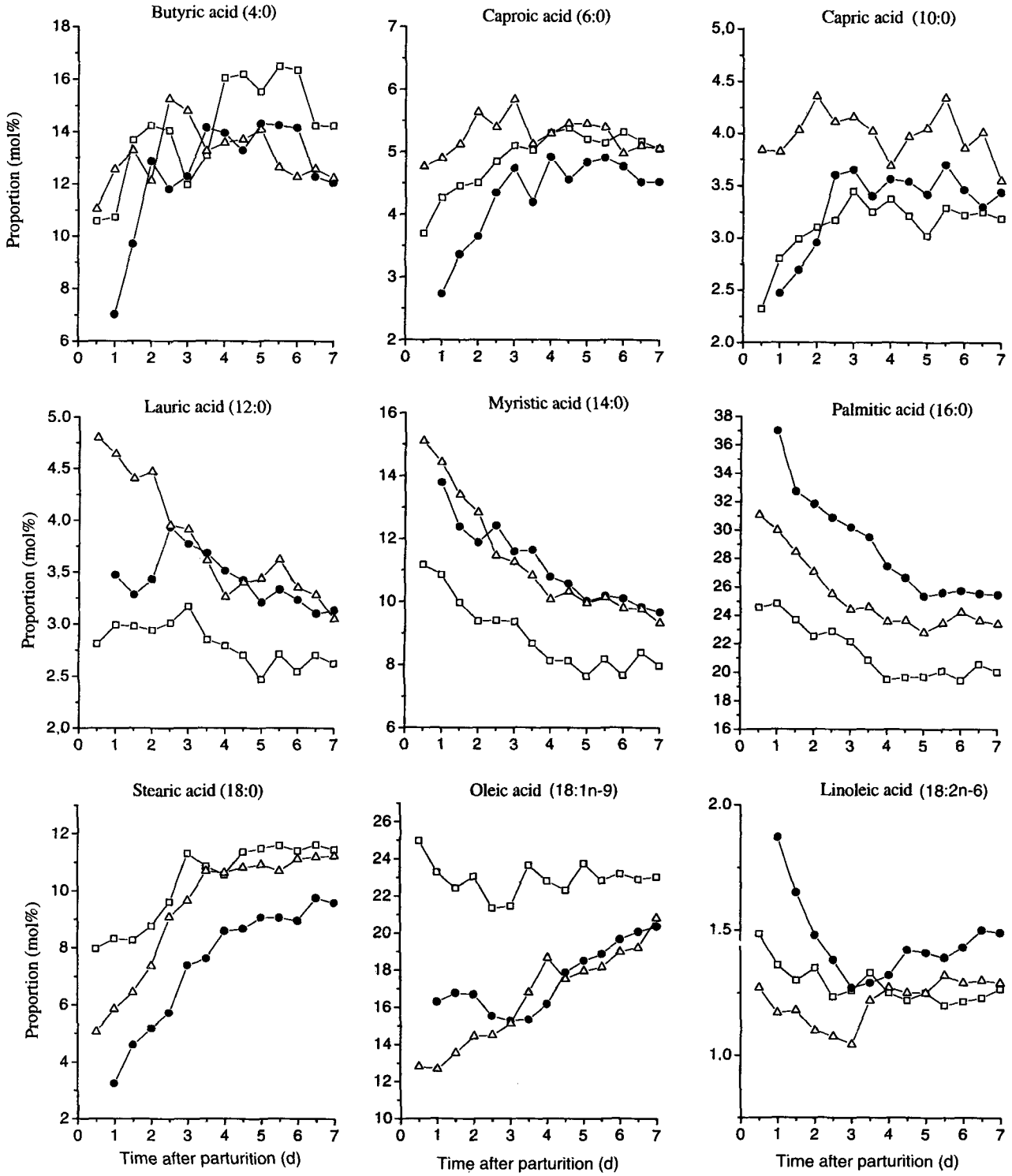

FIG. 1. Changes in the proportions (mol%) of fatty acids in the milk fat triacylglycerols of three Finnish cows (△ = cow 1, □ = cow 2, ● = cow 3) during the first week after parturition. The most abundant fatty acids of groups representing C_4–C_{10} acids, C_{12}–C_{16} acids, and C_{17}–C_{18} fatty acids are presented. The method for the analysis of fatty acids is described in the Experimental Procedures section.

for milk fat synthesis. A decline in the proportions of C_{18} fatty acids has been assumed to reflect the drop in the extent of lipid mobilization from the adipose tissue after parturition (24). The most abundant fatty acids in the tallow of Finnish cows are palmitic, stearic, and oleic acids. Thus, the diminished mobilization of storage lipids would partially explain the decrease in the proportion of palmitic acid, but not the observed increase in the proportions of stearic acid and oleic acid in the present study. The high content of myristic acid in the milk TAG shortly after delivery and the increase in the proportions

of short-chain fatty acids indicate distinct changes in the *de novo* synthesis of fatty acids after parturition.

Although significant changes in the proportions of individual fatty acids occurred during the first week after parturition, the proportions of total saturates (approximately 70 mol%) and unsaturates (approximately 30 mol%) were nearly constant. In general, the most distinct changes in fatty acids occurred during the first four days, after which the composition slowly approached that of normal milk fat. The fatty acid compositions, however, did not provide information concerning changes at the molecular level, thus, other techniques were required.

TAG distributions. The TAG compositions of the colostrum samples were measured with both SFC and MS. SFC separated TAG mainly according to the combined number of acyl carbons on a nonpolar stationary phase (Fig. 2). Colostrum TAG were separated into groups representing molecules with 26–54 acyl carbons. The presence of isobaric TAG, differing substantially in the chainlength of the fatty acyl residues, resulted in a partial separation within most of the ACN-groups. For example, 1,3-dipalmitoyl-2-butanoyl-*sn*-glycerol eluted well-separated after trilauroylglycerol as tested with reference compounds. In addition to the molecular association of fatty acids, the steric interactions between the fatty acid moieties and the stationary phase have an effect on the retention. The differences in unsaturation of the analytes were less important on the separation, causing only slight broadening of the chromatographic peaks. Figure 2 shows that the distribution of TAG changed clearly after parturition. For example, a decline in the proportions of molecules having from 42 to 48 acyl carbons was found. The repeatability of the analysis was acceptable, the coefficient of variation typically being less than 3% for the components corresponding to more than 1% of the total.

The advantage of MS was the unit resolution that made it possible to achieve information on the molecular species composition of TAG. The *m/z* values of the $[M - H]^-$ ions characterized the combined number of acyl carbons and double bonds in the acyl chains of TAG, and the abundances of the $[M - H]^-$ ions defined the proportions of different molecular weight species of TAG. In addition to the ACN distribution, the analysis of colostrum TAG by ammonia negative-ion chemical ionization MS produced information on the saturated, monoenoic, dienoic, and polyenoic TAG consisting of fatty acids with even or odd numbers of carbon atoms without chromatographic separation. TAG containing *cis*- or *trans*-fatty acids could not be separated with the method used.

Information on the ACN distribution of TAG was achieved from both SFC and MS determinations. Figure 3 shows as an example the measured ACN distributions of the TAG of the milk samples of cow 1. The proportions of different ACN-groups, analyzed either by SFC or MS, differed to some extent, which was due to the differences in molecular discrimination of the analytical techniques. Nevertheless, both methods provided similar trends for the changes of colostrum TAG, and therefore, are useful for biological screening purposes.

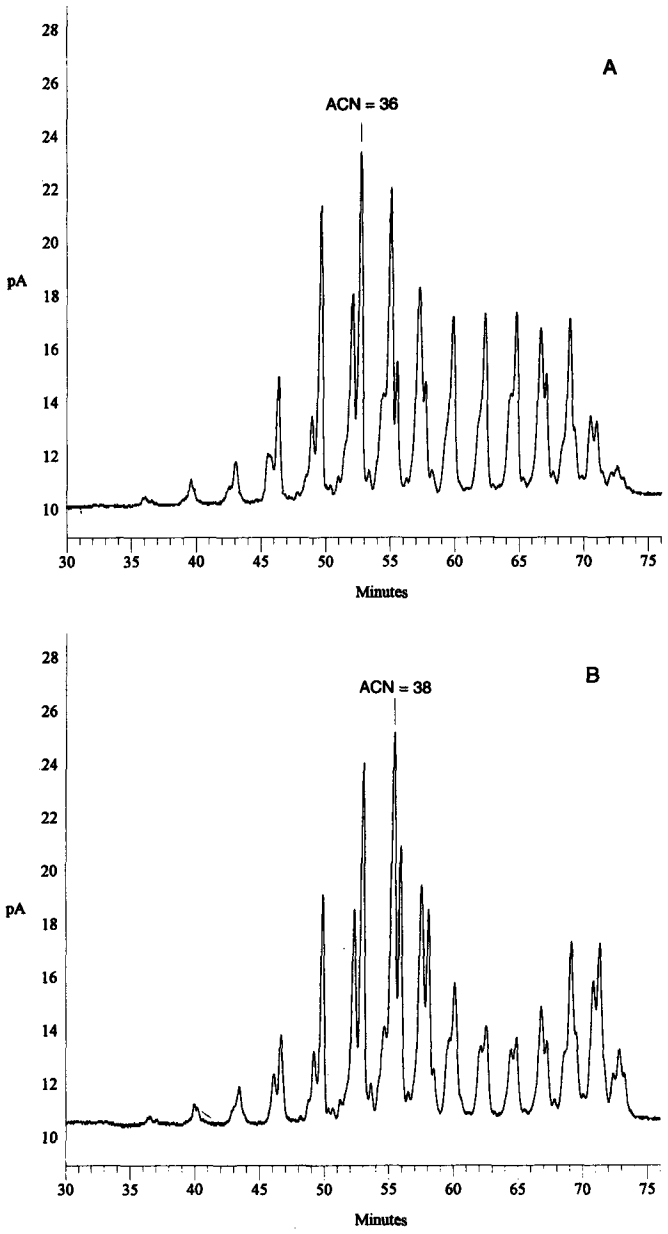

FIG. 2. Supercritical fluid chromatographic separation of the triacylglycerols of the colostrum samples: A, 1 d after parturition; and B, 7 d after parturition of the cow 1 on an SB-Octyl-50 column (10 m × 50 μm i.d. with 0.25 μm film; Dionex, Salt Lake City, UT). ACN, acyl carbon number.

The differences in the TAG profiles between the cows studied were marked; however, similarities were also found. The general distribution profile of TAG of each sample was bimodal. TAG with ACN 36–40 were the most abundant components among the molecules, which may contain butyric acid. The maximum of the TAG, which do not contain butyric acid, was in the region of ACN 46–52. The composition changed substantially during the first week after parturition: the proportions of TAG with ACN 38–40 increased and those with ACN 44–48 decreased. The alterations in the proportions of molecules with 50–54 acyl carbons varied from cow

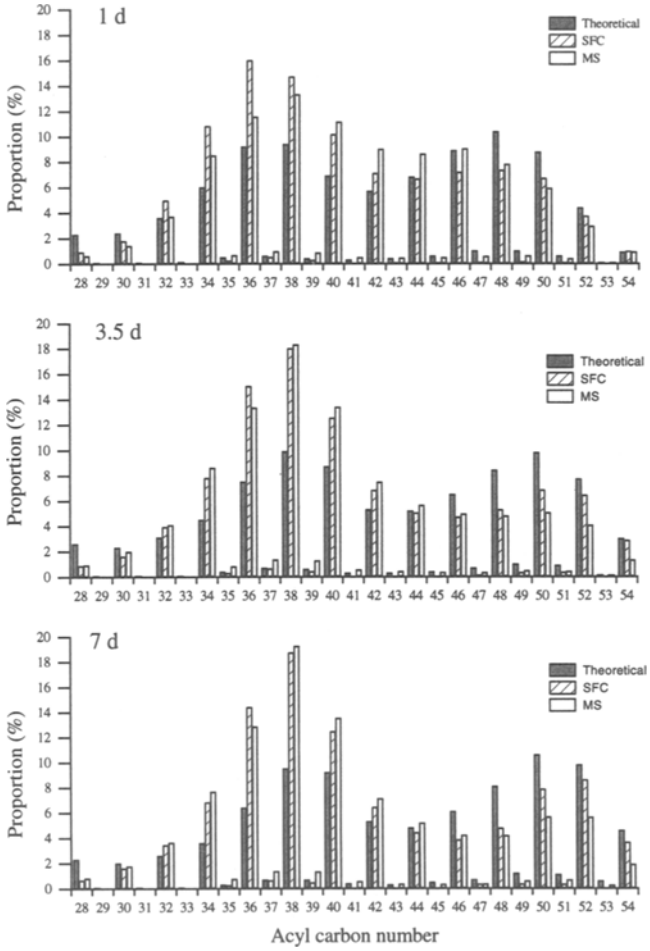

FIG. 3. Acyl carbon number (ACN) distribution of milk fat triacylglycerols 1, 3.5, and 7 d after parturition of the cow 1 determined by supercritical fluid chromatography (SFC) and mass spectrometry (MS). The theoretical ACN distribution of triacylglycerols is calculated according to the fatty acid compositions, assuming a 1,2,3-random distribution of fatty acids.

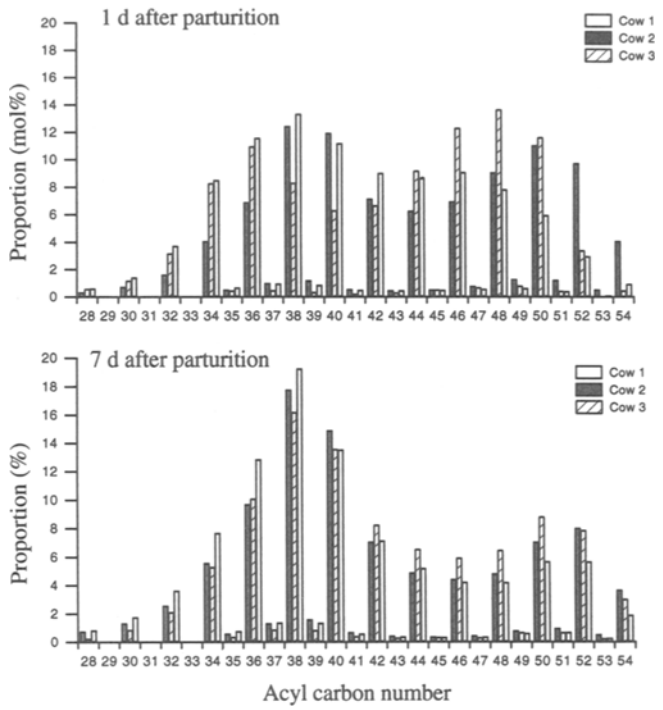

FIG. 4. Acyl carbon number distribution of milk fat triacylglycerols of three Finnish cows 1 and 7 d after parturition. The results are based on the mass spectrometric data.

to cow and may be explained by the great differences in the proportions of $C_{18:1}$ fatty acids between individuals (Fig. 1). Figure 4 presents the TAG distributions, based on MS-data, of the colostrum of each cow collected 1 d and 7 d after parturition. The TAG profiles showed distinct differences between individual cows shortly after delivery. After one week, the changes between the cows were clearly diminished and the compositions of milk TAG were much alike.

For comparison, the distribution of the TAG of each colostrum sample was calculated according to the fatty acid composition, assuming a 1,2,3-random distribution of fatty acids. The theoretical distributions of TAG clearly differed from those measured by SFC and MS (Fig. 3): the proportions of TAG with ACN 34–42 were underestimated and the proportions of TAG with ACN 28 and 46–52 overestimated. The nonrandom structure of TAG of mature milk is well known (26,27), and similar deviations from the random distribution have been reported elsewhere (9,28). The molecular association of fatty acids during the biosynthesis of milk fat TAG is not a random process; therefore, it is not possible to estimate the changes occurring in molecular species composition of TAG based on the fatty acid analysis alone.

In addition to ACN distributions obtained on SFC and MS, the mass spectrometric analysis provided information on differently unsaturated molecular species of TAG. The proportions of saturated, monoenoic, dienoic and trienoic TAG in the colostrum of the three cows studied are presented in Table 1. The differences in the TAG composition of the milk samples after 1 d of parturition of each cow were distinct. After one week of parturition, the TAG compositions were more alike; 32–44 mol% saturated TAG, 41–45 mol% monoenes, 12–19 mol% dienes, and 2–4 mol% trienes. This kind of composition of the TAG is typical for Finnish winter butter fat (8).

As an example, the changes in the distribution of saturated and monoenoic TAG in the milk samples of cow 1 are shown in Figure 5. The most abundant saturated components were 34:0, 36:0, and 38:0. The proportion of 38:0 increased after parturition in the colostrum of each cow, whereas the proportions of 44:0–48:0 declined. The changes in the relative amounts of 28:0–36:0 varied from cow to cow. The profiles of monoenoic TAG were bimodal, closely resembling the ACN profiles. The most abundant monoenoic TAG were 36:1, 38:1, 40:1, 46:1, 48:1, and 50:1. Typically, the proportions of TAG 28:1–40:1 increased and those of TAG 44:1–50:1 decreased after parturition.

The data presented in this study, i.e., changes in the fatty acid compositions and TAG profiles, improves our under-

TABLE 1
Changes in the Proportion (mol%) of Triacylglycerols, Differing in the Degree of Unsaturation, in the Milk Samples of Three Finnish Cows During the First Week After Parturition[a]

Triacylglycerols	Time after parturition (d)	Proportion (mol%) Cow 1	Cow 2	Cow 3
Saturated	1	51.6	27.4	41.6
	3.5	48.7	32.4	37.6
	7	44.0	32.4	36.5
Monoenes	1	37.2	45.2	43.8
	3.5	39.1	44.4	44.1
	7	41.1	44.7	43.2
Dienes	1	9.4	21.7	12.1
	3.5	10.3	18.7	15.0
	7	12.4	18.7	16.4
Trienes	1	1.7	4.8	2.3
	3.5	1.8	4.0	3.0
	7	2.3	3.8	3.3
Others	1	0.2	0.9	0.2
	3.5	0.1	0.5	0.3
	7	0.3	0.4	0.6

[a]Results calculated from mass spectrometric data.

standing of biological changes in colostrum TAG. Apparent differences between individuals were found, which may be due to genetic factors including the breed of a cow, the age, and the time of delivery. Changes in the fatty acid composition of the milk samples determined by capillary GC did not provide adequate information about the changes in TAG compositions. Comparisons of the ACN distributions of colostrum TAG, measured by SFC and MS, with the theoretical ones, based on the fatty acid compositions, clearly showed that the molecular association of fatty acids during the biosynthesis of milk fat is not a random process. Both SFC and MS are convenient techniques for the analysis of TAG. The ACN distribution of TAG is achieved directly from the SFC data sheet, whereas MS data providing more detailed information require extensive calculations. Therefore, sophisticated computer programs for the handling of MS data would be advantageous in routine analysis. The present study clearly showed that it was not possible to estimate the changes in the molecular species composition of colostrum TAG according to the fatty acid analysis alone. Instead, both SFC and MS analyses provided valuable information about TAG compositions in the colostrum.

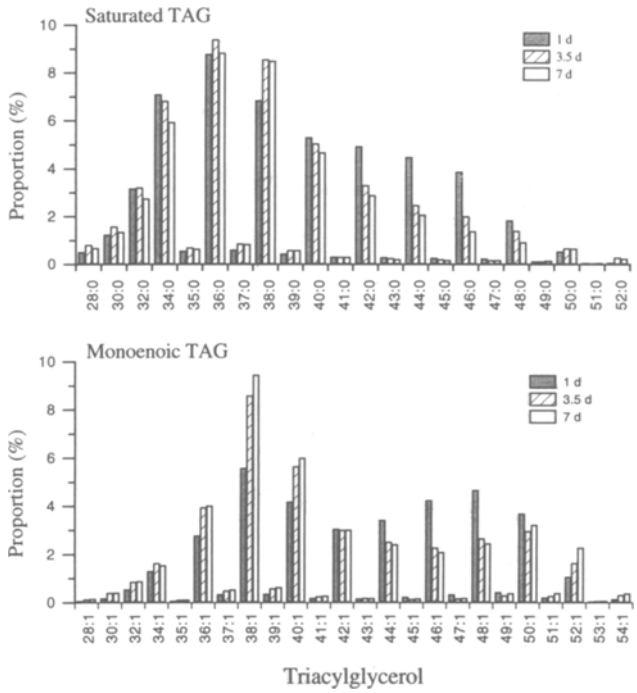

FIG. 5. Changes in the distribution of saturated and monoenoic triacylglycerols (TAG) in the milk fat of cow 1 during the first week after parturition (1, 3.5, and 7 d).

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