Positional Distribution of Fatty Acids in the Triglycerides of Bovine Milk Fat with Elevated Levels of Linoleic Acid

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ABSTRACT

A stereospecific distribution of fatty acids in bovine milk fat containing 15.5% linoleic acid has been compared with the distribution in bovine milk fat containing a normal level (1.8%) of linoleic acid. The positional distribution was obtained by the separate analysis of milk fat triglycerides of high, medium, and low molecular weight. The order of preference for linoleic acid in the high molecular weight triglycerides was position 3 > position 2 > position 1. There was an accompanying altered distribution of myristic acid and palmitic acid in favor of position 1 at the expense of position 3. However, the proportions of myristic acid and palmitic acid in position 2, relative to positions 1 and 3 were identical in the high molecular weight fractions of the two milk fats. The distribution of linoleic acid in the medium molecular weight triglycrides of linoleic-rich milk fat was position 1 = position 2 > position 3. This resulted in a change in the distribution of 18 carbon monounsaturated fatty acids in favor of position 2 at the expense of position 1, but the distribution of myristic acid and palmitic acid was virtually unaltered. The distribution of linoleic acid in the low molecular weight triglycerides was position 2 > position 1 > position 3. The amounts of myristic acid and palmitic acid in position 2 and of palmitic acid in position 1 decreased in the low molecular weight triglycerides of the milk fat containing elevated levels of linoleic acid. Changes in the distribution of fatty acids which were observed in the separate analysis of the high, medium, and low molecular weight triglycerides were not apparent when comparing the distribution in the total milk fats. For example, the distribution of myristic acid and palmitic acid appeared to be unchanged, while the distribution of 18 carbon monounsaturated fatty acids was slightly altered in favor of positions 2 and 3. Moreover, linoleic acid showed an almost equal preference for the three positions of the glycerol moiety in milk fat containing elevated levels of this fatty acid with some concentration at position 2.

INTRODUCTION

Linoleic acid shows a preference for position 2 in the great majority of plant and animal depot triglycerides (1). This structural preference was maintained when rats were fed corn oil (2) and when sheep were fed protected safflower oil (3) although appreciable 18:2 also entered position 3 (4).

The positional distribution of 18:2 in the milk fat of ruminants has been determined only in milk fats containing low levels of this fatty acid (FA) (5-8) although recently Smith et al. (9) have used the pancreatic lipase technique to examine polyunsaturated milk fat. These studies indicated that 18:2 in bovine milk fat, as in other animal fats excepting that of the pig (10), preferred position 2 of these triglycerides although the inaccuracies in determining the distribution of minor components should be recognized.

This investigation examines the stereospecific distribution of 18:2 in milk fat with elevated levels of this fatty acid and its effect on the normal stereospecific distribution of the other constituent fatty acids. In view of the presence of short chain fatty acids in the 3-position of almost 50% of the triglycerides, bovine milk fat is an interesting natural fat in which to follow these effects. The possibility has been examined that the distribution of fatty acids in the high and low molecular weight triglycerides was affected differently by the incorporation of 18:2.

EXPERIMENTAL PROCEDURES

The milk used for stereospecific analysis was obtained from a pair of monozygous twin cows fed the same basic diet. The diet of one cow was supplemented with formaldehyde-treated sunflower seed (11). The fats with normal and elevated levels of 18:2 were each separated into triglyceride fractions of high, medium, and low molecular weight (11). Stereospecific analysis of each fraction was carried out essentially by

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TABLE I

Fatty Acid Composition of the 1,2(2,3)-Diglyceride Intermediates in the Stereospecific Analysis of the Triglyceride of Milk Fat from Monozygous Twin Cows Fed Control and 18:2-rich Diets

Milk fat	Mol wt fraction		Fatty acid composition (mole %)															
			4:0	6:0	8:0	10:0	10:1	12:0	14:0	14:1	15:0	16:0	16:1	17:0	18:0	18:1	18:2	18:3	20:1
Control	High	Exp.[a]	---	0.2	1.4	4.3	---	4.3	16.1	1.3	1.6	28.0	2.0	1.3	14.2	23.3	1.3	0.7	---
		Calc.[b]	---	0.3	1.0	3.6	---	4.4	16.1	1.0	1.3	29.7	2.0	1.8	14.1	23.5	1.6	0.7	---
18:2-rich	High	Exp.[a]	---	0.9	1.8	3.8	0.1	3.6	10.4	1.1	1.0	17.7	1.7	0.8	16.0	23.8	16.0	1.4	---
		Calc.[b]	---	0.8	1.5	3.8	---	4.0	10.7	0.5	0.7	18.0	1.5	0.6	16.4	24.5	16.1	1.0	---
Control	Medium	Exp.[a]	4.6	10.0	3.2	5.1	0.4	4.9	13.5	0.8	1.1	26.2	2.7	0.8	10.6	14.3	1.0	0.7	0.2
		Calc.[b]	4.0	8.3	4.0	6.0	0.4	5.2	14.5	1.3	1.1	25.7	2.0	0.7	10.1	14.7	1.1	0.8	0.2
18:2-rich	Medium	Exp.[a]	7.0	11.9	3.3	4.7	0.2	3.6	8.8	0.8	0.7	15.3	1.5	0.5	13.8	16.5	10.5	0.5	0.5
		Calc.[b]	6.1	9.6	4.0	5.0	0.3	3.3	9.0	0.3	0.7	15.6	1.0	0.6	14.4	16.3	12.0	0.7	0.8
Control	Low	Exp.[a]	22.5	5.9	2.4	4.5	0.7	5.2	16.1	1.0	1.0	21.0	1.3	0.1	5.6	10.3	1.0	0.7	0.3
		Calc.[b]	18.8	5.5	3.1	4.9	0.6	5.1	14.2	1.0	1.2	21.5	1.5	0.4	6.9	12.4	1.2	1.1	0.7
18:2-rich	Low	Exp.[a]	22.0	4.6	2.3	3.9	0.2	4.2	9.5	0.4	0.7	13.6	1.5	0.5	8.3	13.4	14.4	0.6	---
		Calc.[b]	18.2	5.8	3.3	5.1	0.4	4.4	10.5	0.7	0.7	13.3	1.1	0.2	7.5	13.3	14.8	0.9	---

[a]Obtained experimentally.
[b][3 x (TG) + (2-MG)] / 4.

the scheme proposed by Brockerhoff (12) and modified by Christie and Moore (13). The 1,2(2,3)-diglyceride intermediates from the fraction of high molecular weight were prepared by partial deacylation with a Grignard reagent (13) whereas those from the fractions of low and medium molecular weight were prepared by digestion with pancreatic lipase (14) as described by Taylor and Hawke (8). The 1,2-diacyl-*sn*-glycerol-1-phosphoryl phenols (2,3-PL) were prepared from the 1,2(2,3)-diglycerides (8) and purified by TLC on Silica Gel G with a solvent system of chloroform: methanol: 14M-ammonia (80:20:2, by vol). The phosphatidyl phenols were digested with phospholipiase A_2 *(Ophiophagus hannah* snake venom, Sigma Chemical Co. St. Louis, MO), then isolated and separated by TLC as previously described (8). The phosphatidyl phenols were extracted from the adsorbent with two 30 ml portions of chloroform:methanol:water (60:30:3, by vol) and two 30 ml portions of chloroform:methanol:water (30:60:3, by vol). Solvents were either evaporated under N_2, or, alternatively, the bulk of the solvent was removed on a rotary evaporator at temperatures below 35 C and the remaining solvent removed under N_2. Butylated hydroxyanisole was added to all TLC solvents to minimize oxidation.

Gas liquid chromatographic (GLC) analyses of the triglycerides as their fatty acid methyl esters were carried out as previously described (11). Methyl esters of fatty acids from the triglycerides, diglycerides, and monoglycerides were prepared by transesterification using the method of Shehata et al. (15). Methyl esters were prepared from the phosphatidyl phenols by transesterification with sodium methoxide using a method adapted from that of Christopherson and Glass (16). A 1-4 mg sample of lipid dissolved in 60 μl light petroleum (bp 50-60 C) was placed in a Kontes reaction vial and 4 μl 2M-sodium methoxide added. The vial was capped securely and gently rotated for 5 min to facilitate mixing. After standing for a further 5 min, 1-8 μl of the light petroleum layer was injected into the GLC.

RESULTS AND DISCUSSION

Application of Stereospecific Analysis to Bovine Milk Fat

Pancreatic lipase hydrolyses triglycerides containing 4:0 in preference to those containing longer chain fatty acids (17) and consequently acceptable data for the positional distribution of fatty acids in bovine milk fat are more difficult to obtain (18). The standard pro-

cedures for stereospecific analysis could, however, be applied to the high molecular weight fraction obtained by silicic acid column chromatography as this fraction contained no detectable butyric acid (4:0). Furthermore, partial deacylation with a Grignard reagent, used in standard procedures, is not suitable to provide diglycerides from low and medium molecular weight triglycerides containing 4:0 in the 3-position, due to the difficulty of separating the 1,2(2,3)-diglyceride and the 1,3-diglyceride products (8). However, with the narrower range of triglyceride species present in the low and medium molecular weight triglyceride fractions, pancreatic lipase gives relatively representative 1,2(2,3)-diglycerides over short reaction times (8). A check on the reliability of the procedures adopted was obtained by comparing the fatty acid compositions of the experimentally produced diglycerides with those obtained by calculation (19) (Table I). With the exception of 4:0 in the low molecular weight fractions and 6:0 in the medium molecular weight fractions, proportions of major components (> 10% of the total) obtained by analysis and by calculation showed deviations of less than 2%. Values of 4:0 obtained by analysis were 16-17% higher in the fractions of low molecular weight and values for 6:0 were 17-19% higher in the fraction of medium molecular weight than obtained by calculation (Table I). In view of possible errors arising from the above-mentioned shortcomings of the procedures, the fatty acid composition at position 3 has been determined by two alternative methods of calculation (Table II). Notwithstanding the different values obtained, either method gave a clear indication of the specific nature of the placement of the major fatty acids between the three positions.

Gel filtration seems to be a less satisfactory alternative to silicic acid column chromatography for the fractionation of bovine milk triglycerides because the proportion of 4:0 in the fraction of highest molecular weight contained 7.2% 4:0, which was very little lower than the 8.1% 4:0 present in the total triglycerides (20). Moreover, those workers found that the nature of the fractionation did not permit the stereospecific analysis of two further fractions of lower molecular weight.

Effects of Elevated 18:2 on Stereospecific Distribution

A comparison of the stereospecific distribution of the fatty acids in the high molecular weight fractions of the two milk fats showed that increases in the levels of 18:2 in each of the three positions were paralleled by decreases in the levels of 14:0 and 16:0 (Table II). The presence of 18:2 in the triglycerides altered the proportional distribution of 14:0 and 16:0 in favor of position 1 at the expense of position 3 (Table III). It would seem that the preference of 18:2 for position 3 over position 1 has the effect of diverting the available 14:0 and 16:0 into position 1. On the other hand, there were identical proportions of 14:0 and 16:0 in position 2 in the triglycerides of both 18:2-rich and normal milk fat. The amounts of 18:0 and 18:1 were hardly altered in any of the three stereospecific positions (Table II) and, consequently, the distribution of these two fatty acids remained unaltered (Table III). Although the trends of distribution of 18:0 and 18:1 were similar to those found by Breckenridge and Kuksis (6) for a high molecular weight distillate, a higher degree of symmetry was found in the distribution of 18:0 in the high molecular weight fraction isolated by silicic acid column chromatography. This may be attributed to a more distinct fractionation of milk fat on silicic acid judged by the absence of 4:0 and the lower 6:0 level. A fraction of high molecular weight obtained by gel filtration exhibited an approximately random distribution of 18:0 and 18:1 (20), but this fraction contained 7.2% 4:0 and is clearly not comparable to the high molecular weight fraction obtained by silicic acid column chromatography. Moreover, the use of the standard procedure of stereospecific analysis (13) with this fraction would have been subject to the same errors as with total milk triglycerides. However, there was agreement in other characteristic features, e.g., the highest proportion of 18:2 was incorporated at position 3, in the high molecular weight fraction in milk triglycerides from cows fed protected 18:2.

Because of the predominance of short chain fatty acids in position 3 of the triglycerides of the low molecular weight fraction, it follows that the effect of increased 18:2 content was restricted to the fatty acids in positions 1 and 2. Linoleic acid was a major component of the fatty acids at both positions 1 and 2 (Table II) in this fraction of the 18:2-rich milk fat, with 18:2 showing a slight preference for position 2 (Table III). Oleic acid was preferentially esterified at position 1 of both milk fats, but the 18:2-rich milk fat tended to have a higher proportion of the 18:1 in position 3 and a lower proportion in position 1 than the control milk fat. The elevated 18:2-content led to large decreases in the amounts of 14:0 and 16:0 in position 2 and of 16:0 in position 1 (Table II). However, as observed in rabbit adipose tissue (21), changes in the proportional distribution

TABLE II

Positional Distribution of Fatty Acids in the Milk Triglycerides and Milk Triglyceride Fractions from Monozygous Twin Cows Fed a Control Diet or a Similar Diet Supplemented with Protected 18:2

Milk fat	Mol wt fraction	Position[a]	Fatty acid composition (mole %)															
			4:0	6:0	8:0	10:0	10:1	12:0	14:0	14:1	15:0	16:0	16:1	17:0	18:0	18:1	18:2	18:3	20:1
		Total	---	0.4	1.1	3.8	---	4.0	13.3	0.7	1.3	27.3	1.9	0.9	16.4	26.5	1.7	0.7	---
		1	---	---	1.7	3.6	---	4.0	8.2	0.4	1.9	29.1	1.7	0.6	18.9	27.2	1.5	1.1	---
Control	High	2	---	---	0.6	3.0	---	5.9	24.4	1.0	1.9	36.9	2.3	0.6	7.2	14.4	1.2	0.6	---
		3[b]	---	2.2	2.6	7.6	---	4.7	6.2	3.0	1.7	15.5	0.9	0.6	18.6	34.2	0.8	1.4	---
		3[c]	---	1.2	1.0	4.8	---	2.1	7.3	0.7	0.1	15.9	1.7	1.5	23.1	37.9	2.4	0.4	---
		Total	---	1.1	1.5	3.5	---	3.2	8.8	0.6	0.7	16.2	1.4	0.7	18.6	26.5	16.1	1.1	---
		1	---	—	0.3	1.3	0.1	2.5	8.0	0.4	1.3	21.8	2.4	1.5	22.0	25.7	11.7	1.0	---
18:2-rich	High	2	---	---	1.6	4.7	---	6.2	16.4	0.2	0.6	23.3	1.7	0.3	9.8	18.4	16.0	0.8	---
		3[b]	---	2.8	1.6	1.7	0.2	1.6	2.8	0.4	2.0	7.1	1.5	1.3	21.0	33.6	18.6	3.8	---
		3[c]	---	3.3	2.6	4.5	0.1	0.9	2.0	1.2	0.2	3.5	0.1	0.3	24.0	35.4	20.6	1.4	---
		Total	5.3	10.4	4.3	5.9	0.5	4.5	11.8	1.4	1.0	23.6	2.1	0.8	11.1	15.3	5.0	0.8	0.2
		1	---	---	0.7	0.9	---	2.9	9.8	---	0.4	32.3	1.5	0.3	19.8	25.7	2.1	1.9	1.6
Control	Medium	2	---	1.8	3.0	6.1	0.3	7.3	22.6	1.2	1.6	32.1	1.6	0.5	7.2	12.7	1.3	0.7	---
		3[b]	19.0	30.2	7.0	5.3	0.3	2.3	1.8	0.8	0.4	10.1	2.4	0.5	9.6	8.3	1.1	0.7	0.0
		3[c]	15.9	29.4	9.2	10.7	0.2	3.3	3.0	3.0	1.0	6.4	3.2	1.6	6.3	7.5	0.4	0.2	1.0
		Total	8.2	12.3	3.8	4.5	0.3	2.6	7.3	0.4	0.6	14.0	0.9	0.6	15.3	15.9	11.6	0.7	1.0
		1	---	---	0.4	1.9	---	2.8	7.6	---	1.0	19.5	1.6	0.9	28.5	21.4	13.4	0.8	0.2
18:2-rich	Medium	2	---	1.4	4.8	6.7	0.3	5.3	14.8	0.7	0.9	20.4	1.3	0.5	11.6	17.5	13.1	0.9	0.3
		3[b]	24.2	36.0	3.6	2.9	0.1	1.5	1.5	0.1	0.5	8.5	0.5	0.3	8.2	8.1	3.9	0.1	0.1
		3[c]	24.6	35.5	6.2	4.9	0.6	0.3	0.0	0.5	0.1	2.1	0.2	0.4	5.8	8.8	8.3	0.4	2.5
		Total	25.0	6.2	2.3	3.6	0.5	3.8	11.5	0.9	0.9	20.2	1.4	0.4	7.6	12.6	1.2	1.2	0.8
		1	--	---	0.4	1.2	0.1	2.9	10.3	0.2	1.7	34.4	1.6	0.7	19.8	23.5	2.1	0.8	0.4
Control	Low	2	0.1	3.2	5.6	8.9	0.8	9.0	22.3	1.4	2.2	25.5	1.6	0.3	4.8	11.8	1.1	0.9	0.5
		3[b]	73.7	15.0	0.8	0.7	0.0	0.8	2.7	0.0	0.4	6.5	1.4	0.1	2.0	1.0	0.7	0.3	1.5
		3[c]	75.0	15.4	0.9	0.7	0.6	0.5	1.9	1.1	1.2	0.7	1.0	0.2	1.8	2.5	0.4	1.5	1.5
		Total	24.3	5.9	2.3	3.7	0.3	3.4	9.1	0.6	0.6	13.1	1.0	0.2	8.0	13.2	13.5	0.8	---
		1	---	---	1.5	2.6	0.1	3.4	8.7	0.3	2.1	23.3	2.1	0.6	18.9	19.6	15.5	1.3	---
18:2-rich	Low	2	---	5.6	6.3	9.2	0.6	7.5	14.5	0.8	1.0	13.7	1.5	0.3	5.8	13.7	18.5	1.0	---
		3[b]	71.0	9.0	2.1	1.6	0.2	0.9	3.3	0.0	0.6	7.5	1.3	0.5	3.6	3.9	2.5	0.2	---
		3[c]	72.9	12.1	0.9	0.7	0.1	0.7	4.1	0.7	1.3	2.3	0.6	0.3	0.7	6.3	6.5	0.1	---
		Total	11.9	5.3	2.3	4.1	0.3	3.5	11.9	1.0	1.0	22.1	2.0	0.9	11.9	18.6	1.8	0.8	0.5
		1	---	---	0.9	2.0	---	3.3	9.4	0.2	1.5	32.1	1.6	0.6	19.5	25.3	1.9	1.1	0.5
Control	Milk fat	2	---	1.8	3.3	6.2	0.4	7.5	23.1	1.2	2.0	30.9	1.9	0.4	6.1	12.9	1.2	0.9	0.2
		3	36.3	13.2	2.2	3.8	0.3	1.8	3.9	1.3	0.1	8.9	1.6	0.6	9.2	14.9	0.9	0.8	0.1
		Total	10.4	5.4	2.1	3.2	0.2	2.6	7.7	0.5	0.7	14.1	1.1	0.5	14.1	20.6	15.5	0.7	0.7
		1	---	---	0.8	1.9	0.1	2.9	8.2	0.3	1.5	21.9	2.1	1.0	22.1	22.6	13.5	1.1	---
18:2-rich	Milk fat	2	---	2.4	4.0	6.8	0.3	6.5	15.3	0.5	0.8	19.1	1.5	0.3	8.7	16.5	16.4	0.9	0.1
		3	31.8	12.2	1.3	1.7	0.1	0.7	2.6	0.5	0.4	5.2	0.5	0.4	11.6	18.4	11.3	1.2	0.2

[a]Relative to *sn*-glycerol-3-phosphate.
[b]2 x (2,3-PL)-(2-MG).
[c]3 x (TG)-(1-PL)-(2-MG).

TABLE III

Proportional Distribution of Major Fatty Acids in the Milk Triglycerides and Milk Triglyceride Fractions of High, Medium, and Low Molecular Weight of Monozygous Twin Cows Fed a Control Diet or a Similar Diet Supplemented with Protected 18:2

Milk fat	Mol. wt fraction	Position[a]	4:0	6:0	8:0	10:0	12:0	14:0	16:0	18:0	18:1	18:2
		1	---	---	41	28	30	21	36	40	35	35
Control	High	2	---	---	15	23	44	62	45	15	19	28
		3[b]	---	100	44	49	26	17	19	45	46	37
		1	---	---	8	14	26	30	43	41	33	25
18:2-rich	High	2	---	---	40	52	62	61	46	18	23	34
		3[b]	---	100	52	34	13	9	11	41	44	41
		1	---	---	6	6	22	28	44	57	56	57
Control	Medium	2	---	6	25	41	56	65	44	21	27	35
		3[b]	100	94	69	53	22	7	11	23	17	8
		1	---	---	4	15	32	34	43	60	45	41
18:2-rich	Medium	2	---	4	48	54	61	63	45	25	37	40
		3[b]	100	96	48	31	7	4	12	15	18	19
		1	---	---	7	12	24	29	54	80	65	55
Control	Low	2	---	17	93	88	75	64	40	20	33	29
		3[b]	100	83	---	---	1	7	6	---	2	16
		1	---	---	19	22	31	32	56	73	51	40
18:2-rich	Low	2	---	35	81	78	68	54	33	22	36	48
		3[b]	100	65	---	---	1	14	11	5	13	12
		1	---	---	14	17	26	26	45	56	48	48
Control	Milk fat	2	---	12	52	51	60	63	43	18	24	30
		3[b]	100	88	34	32	14	11	12	26	28	22
		1	---	---	13	18	29	31	48	52	39	33
18:2-rich	Milk fat	2	---	16	66	66	64	59	41	21	29	40
		3[b]	100	84	21	16	7	10	11	27	32	27

[a]Relative to *sn*-glycerol-3-phosphate.

[b]Calculated using the average of the two calculations for position 3.

of the major fatty acids arising from the availability of high levels of 18:2 were minor, and the fatty acids remained distributed within the triglycerides in a characteristic manner.

Smith et al. (9) used the pancreatic lipase technique to show an additional introduction of oleic acid and linoleic acid at position 2 in 18:2-rich milk. Stereospecific analysis shows that this arises from a substantial reduction in the proportions of these two fatty acids in position 1 and also shows that the proportions increase in position 3 as well as in position 2. Moreover, it should be emphasized that the order of preference $2 > 1 > 3$ for the positional introduction of linoleic in total milk fat is an average order. The order of preference for 18:2 in the constituent triglycerides of high molecular weight is $3 > 2 > 1$ and that in the constituent triglycerides of medium and low molecular weight is $2 \geqslant 1 > 3$.

Relationship of Bovine Milk Fat to Other Animal Fats

Assessing the extent to which the triglycerides of bovine milk fat follow the trends in the stereospecific placement of fatty acids in animals (1) is complicated by the placement of short chain fatty acids in position 3 in almost 50% of the triglycerides. Consequently, a clear representation of the positional distribution of fatty acids in the remaining constituent triglycerides is obtained only by a separate analysis of the high and low molecular weight triglycerides as conducted in the present study. For example, palmitic acid was preferentially located in position 2 in the high molecular weight triglycerides, with the least present in position 3 (Table III), as has been found in human milk fat (22). However, 16:0 was preferentially located in position 1 in the low molecular weight triglycerides and this resulted in an almost even distribution between positions 1 and 2 in the total milk fat (Tables II and III). Oleic acid was preferentially located in positions 1 and 3 in the high molecular weight triglycerides with only about 20% in position 2 which is very similar to the distribution of 18:1 in human milk fat (22). However, 18:1 was virtually absent in position 3 of the low molecular weight triglycerides and, consequently, a higher proportion was located in positions 1 and 2. Nevertheless, the ratio in these two positions was about the same in the low molecular weight triglycerides as in the high molecular weight triglycerides. Therefore, although the overall distribution of oleic acid in milk fat favored position 1 with the proportion in position 3 exceeding only slightly the proportion in position 2 (Table III), this distribution did not reflect the distribution patterns in the constituent triglycerides. Stearic acid (18:0) had a similar pattern of distribution within the high and low molecular weight triglycerides to that of 18:1.

The distribution of 18:2 in high molecular

weight triglycerides of bovine milk and in human milk triglycerides, containing 16 and 11% 18:2, respectively (22), is reasonably similar in that slightly more than 40% is located in position 3 of the triglycerides. In contrast, 18:2 showed a slight preference for position 2 over position 1 in the low molecular weight triglycerides which leads to the similarities in the distribution of fatty acids of high molecular weight triglycerides of bovine milk and the triglycerides of human milk being overlooked when the distribution of fatty acids in the total triglycerides are compared (22). The triglycerides in the fraction of medium molecular weight which was 20% of the total milk triglycerides, like the milk fat itself, tended to have distributions of the major fatty acids which were averages of the high and low molecular weight fractions.

Changes in Individual Species of Triglycerides

The individual molecular species of triglycerides which undergo the greatest quantitative changes with the presence of elevated levels of 18:2 for esterification may be assessed from the triglyceride composition (11) and the positional distribution of the fatty acids reported here. Decreases in the level of saturated triglycerides such as *sn*-glycerol-1-palmitate-2-myristate-3-stearate and *sn*-glycerol-1,2-dipalmitate-3-stearate or 3-butyrate, monoene triglycerides such as *sn*-glycerol-1-palmitate-2-myristate-3-oleate and *sn*-glycerol-1-oleate-2-palmitate-3-stearate or 3-butyrate, and diene triglycerides such as *sn*-glycerol-1-oleate-2-palmitate-3-oleate appear likely. On the other hand, it may be deduced that there was an increase in the level of diene triglycerides such as *sn*-glycerol-1-stearate-2-palmitate-3-linoleate and *sn*-glycerol-1-stearate-2-linoleate-3-stearate or 3-butyrate, triene triglycerides such as *sn*-glycerol-1-stearate-2-linoleate-3-oleate and *sn*-glycerol-1-oleate-2-linoleate-3-butyrate, and tetraene triglycerides such as *sn*-glycerol-1-stearate-2,3-dilinoleate and *sn*-glycerol-1,2-dilinoleate-3-butyrate.

Biosynthesis of Milk Fat Triglycerides

Similarities in the fatty acid composition in positions 1 and 2 of bovine milk triglycerides of differing fatty acid composition in the position 3 (6) have been cited as evidence for a common pool of 1,2-diglycerides being acceptors for acyl groups in the final step of triglyceride biosynthesis via the glycerol-3-phosphate pathway (6,23). However, an interesting pattern of distribution with respect to 16:0 in positions 1 and 2 of triglycerides has emerged from the fractionations carried out in the present study and in the earlier work of Taylor and Hawke (8). Palmitic acid shows a preference for the 2-position relative to the 1-position in the high molecular weight triglycerides, is evenly distributed in the medium molecular weight triglycerides, and favors the 1-position relative to the 2-position in the low molecular weight triglycerides. Dimick et al. (24) and Barbano and Sherbon (25) also observed a greater relative abundance of 16:0 in position 2 of bovine milk triglycerides of higher molecular weight and higher melting point, respectively. The high proportions of 16:0 in position 2 of the low density lipoproteins of ruminant blood serum (26) and the above data are not inconsistent with the suggestion that a 2-monoglyceride pathway has some role in the biosynthesis of the high molecular weight triglycerides of ruminant milk (24), although metabolic and compositional studies suggest that the glycerol-3-phosphate pathway involving 1,2-diglycerides as intermediates is likely to be the dominant route in the synthesis of bovine milk triglycerides (27).

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