Biosynthesis of chylomicron triacylglycerols by rats fed glyceryl or alkyl esters of menhaden oil fatty acids

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Abstract We have previously shown great similarity in the distribution of fatty acids in the *sn*-1 and *sn*-3 positions of the chylomicron triacylglycerols (TG) from rats fed menhaden oil or its ethyl esters, and have proposed that the acylglycerol products of the phosphatidic acid (PA) pathway (ester feeding) are hydrolyzed to 2-monoacylglycerols (2-MG) prior to reconversion to TG via the 2-MG pathway (oil feeding) and secretion as chylomicrons. As the composition of the *sn*-2-position would also be retained if the TG were hydrolyzed only to the X-1,2-diacylglycerol (DG) stage before resynthesis, we have now retested the hypothesis by determining the molecular association and reverse isomer content of the *sn*-1,2- and *sn*-2,3-DG derived from the chylomicron TG and the PA resulting from the two feedings. The new data demonstrate a better than 90% homology among the molecular species of the PA from the oil and ester feeding, along with the characteristic association of the saturated acids with *sn*-1- and the unsaturated acids with *sn*-2-position. Due to increased proportion of unsaturated acids in the *sn*-1-position of the TG, there was only a 15–20% homology between the PA and the *sn*-1,2-DG moieties of the chylomicron TG from the oil and ester feeding. A lack of homology was also observed between the PA and free *sn*-1,2-DG, as well as between the free *sn*-1,2-DG and the *sn*-1,2-DG moieties of the chylomicron TG. On the basis of molecular association and the *sn*-1-/*sn*-3- reverse isomer content of the chylomicron TG a better than 90% homology was recognized between the chylomicron TG resulting from the oil and ester feeding. It is therefore concluded that hydrolysis to 2-MG followed by reesterification via the 2-MG pathway constitutes the most plausible mechanism for the transfer to chylomicrons of the TG arising from alkyl ester feeding.—**Yang, L-Y., A. Kuksis, and J. J. Myher.** Biosynthesis of chylomicron triacylglycerols by rats fed glyceryl or alkyl esters of menhaden oil fatty acids. *J. Lipid Res.* 1995. **36:** 1046–1057.

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It is generally accepted that dietary fats are converted into chylomicron triacylglycerols (TG) via the 2-monoacylglycerol (2-MG) and phosphatidic acid (PA) pathways (1). During oil absorption about 80% of the fatty acids originally in the *sn*-2-position are retained in the *sn*-2-position of the lymph TG, suggesting a minimum of 80% contribution from the 2-MG pathway (2, 3) and a direct transfer of these TG to lymph chylomicrons. As the acylglycerol tranferases involved are located on the cytoplasmic side (4, 5), the TG formed there must cross the lipid bilayer to the microsomal lumen, which may be facilitated by a TG transfer protein (6, 7). There is no evidence for a direct incorporation into chylomicrons of the TG synthesized via the PA pathway. We have recently observed that the lymph TG formed via the PA and 2-MG pathways possess close similarity in the fatty acid distribution of the corresponding *sn*-1- and *sn*-3-positions, while differing in the composition of the *sn*-2-position (8). We have pointed out that such similarity in the composition of the *sn*-1 and *sn*-3 positions could arise from a hydrolysis of the acylglycerol products of the PA pathway to 2-MG prior to reconversion to TG via the 2-MG pathway (e.g., PA pathway was only for TG storage). This suggestion is consistent with a cytoplasmic storage, lipolysis, and resynthesis of the liver TG before transfer to VLDL (9, 10). The rat liver, however, is believed to possess only the PA pathway for TG formation (11). Structural analyses of the very low density lipoprotein (VLDL) and liver TG have suggested that the lipolysis may proceed only to the diacylglycerol (DG) stage before resynthesis (12). As lipolysis of TG to X-1,2-DG in the enterocytes would also retain the fatty acid composition of the *sn*-2-position, we have retested our original hypothesis by performing

Abbreviations: TG, triacylglycerols; DG, diacylglycerols; PA, phosphatidic acid; MG, monoacylglycerol; VLDL, very low density lipoprotein; GLC, gas–liquid chromatography; TLC, thin-layer chromatography; DNPU, dinitrophenylurethane; HPLC, high performance liquid chromatography; FA, fatty acid; 16:0, 18:1, etc., number of acyl carbons:number of double bonds in a fatty acid; 16:0–18:1 and 16:0–18:1–18:2, etc., molecular species of diacylglycerols and triacylglycerols, respectively.

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detailed analyses of the molecular association and the reverse isomer content of the acylglycerol intermediates and end products of the TG synthesis during chylomicron secretion in rats fed menhaden oil or its ethyl esters. The results show that an intermediate lipolysis to *sn*-2-MG and resynthesis provides the most likely pathway to secretion of chylomicron TG during alkyl ester feeding.

MATERIALS AND METHODS

Experimental meals and animals

Menhaden oil was purchased from Zapata Haynie Corporation (Reedville, VA). Ethyl esters were prepared by treating the oil with 1 M sodium ethoxide in ethanol–toluene 60:40 as previously described (13). The fatty acid composition of the experimental meals was as described (8). The meals were fed by stomach tube to retired male breeder rats of Wistar strain (Charles River Canada, La Salle, Quebec), that had been equipped with thoracic duct cannulae as described (14), and the lymph was collected (15).

Isolation of TG

The TG from chylomicrons and villus cells was recovered by extraction with chloroform–methanol 2:1 (v/v) and were isolated by thin-layer chromatography (TLC) using heptane–isopropyl ether–acetic acid 60:40:4 (by vol) as the developing solvent (13). An aliquot of the purified TG was hydrogenated (13) to covert the unsaturated to fully saturated species, which were resolved (13) by high temperature gas–liquid chromatography (GLC) according to the number of total acyl carbons per acylglycerol molecule (carbon number).

Isolation of PA

The PA from chylomicrons and villus cells was isolated along with other glycerophospholipids by extraction with chloroform–methanol 2:1 (v/v) and was resolved by TLC as previously described (16). The fatty acid composition of the *sn*-1- and *sn*-2-positions of the PA was determined using phospholipase A_2, as described (8).

Structural analyses of TG

For this purpose the TG were subjected to random degradation to the *sn*-1,2-, *sn*-2,3-, and X-1,3-DG by the Grignard reaction (17). The DG were purified and resolved by boric acid TLC (18) into the *sn*-1,2(2,3)- and X-1,3-DG using chloroform–acetone 97:3 (v/v). The DG recovered by extraction of the gel with chloroform–acetone were converted into the 3,5-dinitrophenylurethane (DNPU) derivatives by reaction with 3,5-dinitrophenylisocyanate as previously described (19). The DNPU derivatives were resolved into the pure *sn*-1,2- and *sn*-2,3-DG by chiral phase high performance liquid chromatography (HPLC) on a column containing (R)-(+)-1-(1-naphthyl)ethylamine polymer using hexane–dichloromethane–ethanol 40:10:1 as the mobile phase (19). The *sn*-1,2- and *sn*-2,3-enantiomer peaks were separately collected and the DNPU groups removed by silolysis (20). The resulting trimethylsilyl (TMS) ethers were resolved by GLC on the basis of acyl carbon number on nonpolar capillary columns and into individual molecular species on polar capillary columns as previously described (21). Fatty acids of the original oil, ethyl esters and the *sn*-1,2-, *sn*-2,3-, and X-1,3-DG, as well as of any 2-MG resulting from Grignard degradation and of the fatty acids and lysophosphatidic acids released by phospholipase A_2 were determined by GLC of the methyl esters after acidic or alkaline transmethylation (13). Prior to GLC the fatty acid methyl esters were purified by TLC using a neutral lipid solvent (13).

Calculations

The stereospecific positional distribution of the fatty acids was determined by calculation from the fatty acid composition of total TG and the *sn*-1,2- and *sn*-2,3-DG recovered from the chiral column (8). The molecular association of the fatty acids and reverse isomer content of the TG and the derived *sn*-1,2- and *sn*-2,3-DG were determined by calculation on the basis of the knowledge of the fatty acid composition of the *sn*-1-, *sn*-2-, and *sn*-3-positions of the acylglycerols, assuming 1-random, 2-random, 3-random distribution. This calculation provides both molecular association and reverse isomer composition for the DG and TG enantiomers. The molecular association gives the exact pairs of fatty acids in individual DG and the exact triplets of fatty acids in individual TG. The reverse isomers of *sn*-1,2-DG describe species, where the fatty acids have been interchanged between the *sn*-1- and *sn*-2-positions (e.g., 16:0–18:1 vs. 18:1–16:0). The reversal of the fatty acids between the *sn*-2- and *sn*-3-positions (e.g., 18:1–18:2 vs. 18:2–18:1) gives the reverse isomers for the *sn*-2,3-DG. In the *sn*-1- and *sn*-3-reverse isomers (enantiomers) of the TG the fatty acids are interchanged in the *sn*-1- and *sn*-3-positions (e.g., 16:0–18:1–18:2 vs. 18:2–18:1–16:0). Likewise, the molecular association and reverse isomer content of the PA were calculated by the 1-random 2-random method and the knowledge of the fatty acids in the *sn*-1- and *sn*-2-position (8). The homology between lipid classes and enantiomers was calculated by subtraction of the compositions of the corresponding molecular species and expression of the proportion of common species as per cent of total. Thus, a 100% homology would refer to complete identity of species in the compared lipid classes, while a 50% homology would indicate that only one half of the species was common to both lipid classes.

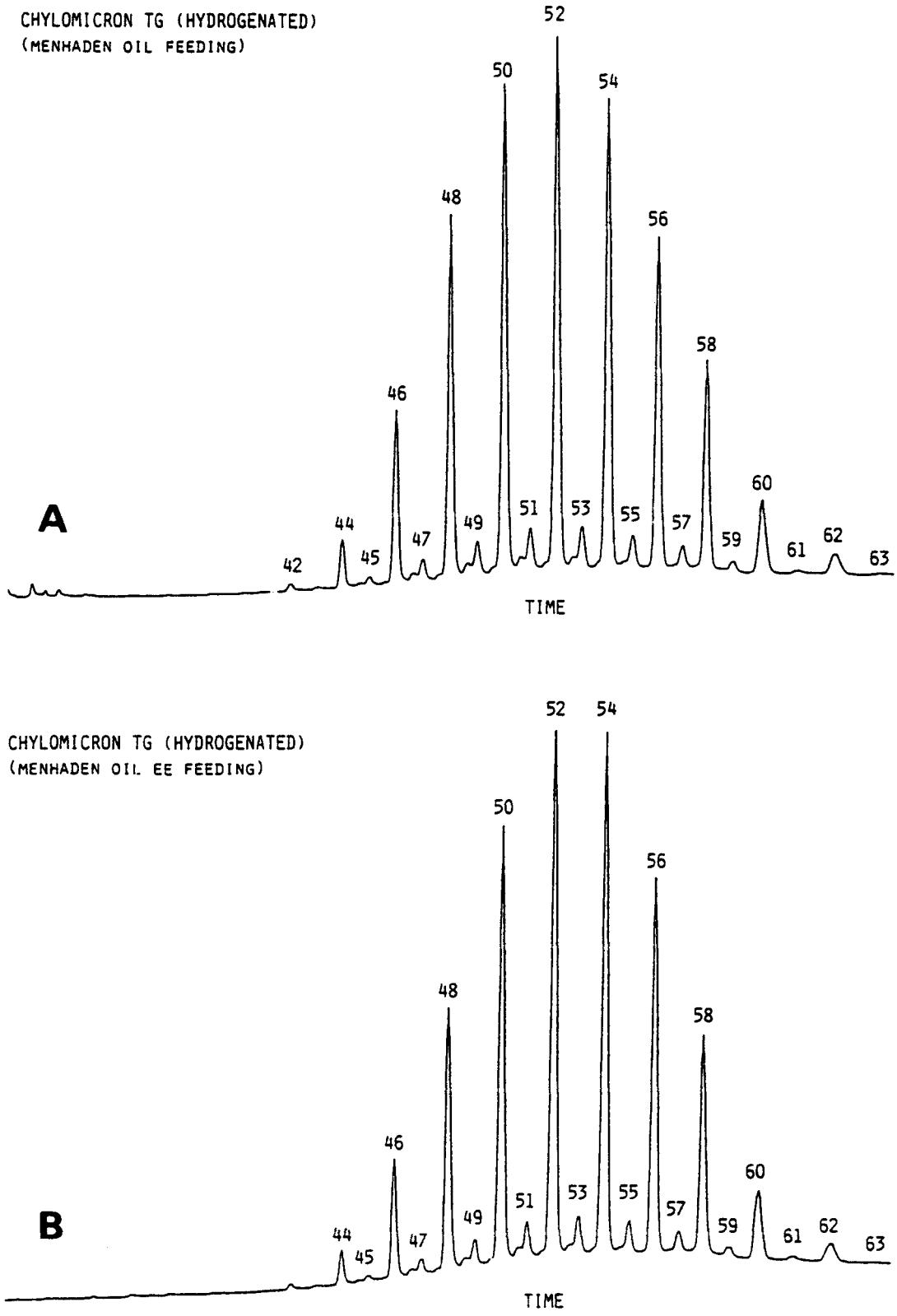

Fig. 1. GLC profiles of hydrogenated TG from lymph chylomicrons of rats fed either menhaden oil (A) or ethyl esters of menhaden oil fatty acids (B). Peaks are identified by number of acyl carbons (e.g., peak 54, tristearin or any other combination of three fatty acids to give a total of 54 acyl carbons). GLC conditions: instrument, Hewlett-Packard (Palo Alto, CA) Model 5880 equipped with a nonpolar column (8 m × 0.32 mm ID fused silica open tubular capillary) coated with chemically bonded SE-54. Temperature program: 40°C (isothermal for 0.5 min), 30°C/min to 150°C, 20°C/min to 230°C, 10°C/min to 280°C, 5°C/min to 350°C, and then holding to the end of the run. Detector, 350°C; injector, unheated on column. Carrier gas: H_2 at 8 psi head pressure. Elution time: C_{48} at 18.1 min; C_{66} at 29.2 min. Sample: 1 μl of a 0.1% solution of hydrogenated TG in hexane.

RESULTS

Figure 1 gives the carbon number profiles of the lymph chylomicron TG recovered from rats receiving menhaden oil or its ethyl esters. Prior to GLC the sample was hydrogenated to avoid degradation of the polyunsaturated long chain TG on the GLC column. The peaks are identified by the number of acyl carbon atoms in the fatty acids making up the TG molecule (e.g., peak 54 could be due to tristearoylglycerol or to any other combination of fatty acids with a total of 54 acyl carbons). The minor odd carbon number peaks are due to the presence of traces of odd carbon number fatty acids in these TG. The elution profiles are closely similar. **Table 1** compares the experimental values to those calculated from the knowledge of the positional distribution of the fatty acids by the 1-random, 2-random, 3-random multiplication. There is good agreement between the experimental and calculated values in each instance, indicating correct identification of the fatty acids and complete recovery of the TG from the GLC column. Interestingly, the chylomicron triacylglycerol values differ little from those of the original oil TG, which attests to a comparable absorption of the two fat meals and an apparently similar association of the fatty acids during the TG biosynthesis.

Table 2 gives the acyl carbon number distribution of the enantiomeric *sn*-1,2- and *sn*-2,3-DG moieties derived from the chylomicron TG. The determined values are compared to the values calculated from a 1-random, 2-random, 3-random distribution based on knowledge of the positional distribution of the fatty acids. The close agreement between the calculated and determined values shows that the chiral HPLC column gave complete or representative recoveries of both short and long chain spe-

TABLE 1. Carbon number profiles of hydrogenated triacylglycerols of menhaden oil and of chylomicrons from rats fed menhaden oil or its fatty acid ethyl esters

Carbon Number[a]	Chylomicrons				Menhaden Oil	
	Oil-Fed		Ester-Fed			
	Exp.[b]	Calc.[b]	Exp.	Calc.	Exp.	Calc.
	mole %					
42	0.03	0.08	0.01	0.03	0.11	0.11
43	0.02	0.02	N.D.[c]	0.08	0.04	0.02
44	1.51	1.00	1.37	0.55	1.12	1.24
45	0.34	0.17	0.26	0.10	0.28	0.21
46	5.58	4.69	5.12	3.11	4.67	5.38
47	0.95	0.58	1.03	0.42	0.90	0.64
48	12.01	11.57	11.41	8.79	10.33	11.84
49	1.56	1.05	1.75	0.87	1.61	0.98
50	17.04	17.50	16.76	15.59	15.47	16.21
51	1.85	1.27	2.19	1.20	2.07	1.11
52	18.29	19.27	19.21	20.10	18.54	17.91
53	1.80	1.34	2.14	1.38	2.28	1.19
54	15.74	16.83	16.61	19.30	16.04	16.26
55	1.35	1.13	1.61	1.10	1.80	0.93
56	10.79	11.56	11.01	14.20	11.68	11.89
57	0.81	0.73	0.89	0.74	1.03	0.62
58	5.99	6.39	5.48	7.71	6.81	7.42
59	0.38	0.42	0.39	0.37	0.53	0.37
60	2.61	2.89	1.90	3.00	3.15	3.71
61	0.12	0.18	0.09	0.11	0.18	0.16
62	0.85	0.99	0.53	0.71	1.00	1.34
63	0.04	0.05	N.D.	0.01	0.04	0.05
64	0.25	0.26	0.23	0.08	0.32	0.35
65	0.02	0.02	N.D.	0.001	N.D.	0.00
66	0.09	0.05	N.D.	0.005	N.D.	0.06

[a]Carbon number, total number of acyl carbons per TG molecule resolved by GLC. GLC and column conditions are as described in the legend of Fig. 1.

[b]Exp., experimental; Calc., calculated. Calculated values were obtained by the 1-random, 2-random, 3-random method and the knowledge of the fatty acids in the *sn*-1-, *sn*-2-, and *sn*-3-positions.

[c]N.D., not detected.

TABLE 2. Carbon number profiles of the enantiomeric diacylglycerol moieties of the triacylglycerols of lymph chylomicrons from rats receiving menhaden oil or its fatty acid ethyl esters

Carbon Number[a]	Oil-Fed				Ester-Fed			
	sn-1,2-		*sn*-2,3-		*sn*-1,2-		*sn*-2,3-	
	Exp.[b]	Calc.[b]	Exp.	Calc.	Exp.	Calc.	Exp.	Calc.
	mole %							
28	1.77	1.13	1.70	0.98	1.49	0.69	1.45	0.75
30	9.06	8.43	9.83	7.59	9.41	6.82	9.16	6.37
32	24.16	21.93	22.01	20.00	24.16	21.88	20.43	18.26
34	26.79	26.39	25.86	24.17	28.41	29.18	24.23	24.31
36	20.06	20.64	21.78	21.49	21.91	21.39	23.88	24.97
38	11.79	13.39	11.12	15.32	11.23	13.18	15.00	18.09
40	4.85	5.89	6.43	6.98	3.18	4.22	5.20	6.29
42	0.86	1.87	1.23	2.64	0.17	0.53	0.50	0.81
44	0.13	0.50	0.03	0.73	0.04	0.004	0.16	0.06

[a]Carbon number, total number of acyl carbons per DG molecule resolved by GLC. GLC and column conditions are as described in the legend of Fig. 1.

[b]Exp., experimental, Calc., calculated. Calculated values were obtained by the 1-random, 2-random, 3-random method and the knowledge of the fatty acids in the *sn*-1-, *sn*-2-, and *sn*-3-positions.

cies for both DG enantiomers. There is a slightly closer similarity in the distributions of the carbon numbers between the *sn*-1,2- and *sn*-2,3-DG after oil feeding than after ester feeding (e.g., C_{38}, 11.79 vs. 11.12 and 11.23 vs. 15.00, as an extreme difference).

Figure 2 shows the GLC profiles of the molecular species of the *sn*-1,2-DG moieties derived from the chylomicron TG of oil- and ester-fed rats, respectively. Some 70 distinct molecular species are recognized in the elution sequence. Despite the complexity of the pattern, it is possible to recognize differences in peak proportions. Thus, the *sn*-1,2-DG moieties of the TG resulting from the ester feeding contain markedly higher proportions of peaks 28, 38, 44, and 62, while the *sn*-1,2-DG moieties of the TG resulting from oil feeding contain increased proportions of peaks 7, 15, 24, 33, and 47. Differences of considerably lower magnitude between the oil and ester feeding are seen for the *sn*-2,3-DG moieties (**Fig. 3**). The quantitative differences between the various DG enantiomers are best seen from the tabulated data presented in **Table 3.** There is great similarity in the quantitative proportions between the corresponding species of the *sn*-1,2-DG moieties from the oil and ester feeding. Clearly absent are the large proportions of species containing saturated acids in *sn*-1- and unsaturated acids in *sn*-2-positions of the *sn*-1,2-DG moieties of TG from ester feeding, which were anticipated on the basis of the composition of the PA recovered from the cells and the known fatty acid specificity of the in vitro acylation of glycerophosphate by rat intestinal microsomes (22). Instead, the *sn*-1,2-DG of the TG from ester feeding contained high proportions of unsaturated and polyunsaturated species in the *sn*-1-position, very much like those arising from the 2-MG pathway. Table 3 also compares the molecular species of the *sn*-2,3-DG moieties of the chylomicron TG from the oil and ester feeding, which show only marginal differences at this level of comparison. For both oil and ester feeding, however, the composition of the *sn*-1,2- and *sn*-2,3-DG moieties differed significantly, demonstrating that considerable acyl chain specificity was exerted by the acyl-transferases involved in the reesterification processes. It was estimated by molecular species subtraction that the PA possess only a 15–20% homology to the *sn*-1,2-DG of the chylomicron TG of the ester- or oil-fed rats. We have previously demonstrated (8) identical fatty acid composition for the PA of the villus cells and chylomicrons from oil- and ester-fed rats, yielding identical profiles of the molecular species. In addition, Table 3 includes the values for the *sn*-1,2- and *sn*-2,3-DG species calculated from the known positional distribution of the fatty acids (8) by the 1-random-2-random, and the 2-random-3-random method. There is good agreement between the structures, although the total mass measured experimentally was attributed to only 150 species, while the calculation distributed it among a total of 8,000 species, most of which contained little mass.

Figure 4 compares the calculated reverse isomer ratios for the *sn*-1,2- and *sn*-2,3-DG moieties of the chylomicron TG from oil and ester feeding. As the reverse isomers cannot be resolved experimentally, their ratios were obtained by the 1-random, 2-random, 3-random multiplication of the fatty acids in the *sn*-1-, *sn*-2- and *sn*-3-positions. A ratio of 1 indicates a racemate, while the ratios above and below 1 indicate the preponderance of one or the other enantiomer. Among the *sn*-1,2-DG moieties of TG from the ester feeding, the species with the saturated and monounsaturated fatty acids in the *sn*-1-position and the dienoic, trienoic, and tetraenoic fatty acids in the *sn*-2-position appear to predominate, except in combinations with tetraenoic and pentaenoic eicosanoates, which yield more of the reverse isomers. In the case of the *sn*-1,2-DG moieties of TG from the oil feeding, the major species appear in nearly 1:1 ratio. Special exceptions are the 18:0–20:5, 18:1–18:4, and 20:1–20:5 species, where the normal isomer (as written) exceeds the reverse isomer 3- to 4-fold. In the case of the *sn*-2,3-DG moieties of the TG from ester feeding, the isomer ratios were also close to 1 in most instances. Exceptions are provided by the 14:0–20:5, 16:0–20:5, 16:1–20:5, and 18:1–20:5 combinations, which are present in 15- to 20 times greater proportion in the normal (as written) isomer form than in the reverse isomer form. This is not so during oil feeding, where the polyunsaturated fatty acids have been preserved in the *sn*-2-position by the 2-MG pathway, and the isomer ratio approximates 1. The slight excess of the normal over the reverse isomer for the species containing saturated and di- and tri-unsaturated octadecanoates obtained during the ester and oil feeding could reflect a direct contribution of the PA pathway, estimated at 10–20% of the total. It should be noted that the reverse isomer ratios calculated for the DG moieties reflect different TG species, and cannot be related to any specific molecular associations in the TG molecules.

Figure 5 gives the isomer ratios for the major species of chylomicron TG from rats receiving menhaden oil or its alkyl esters. Both feedings produce largely enantiomers (isomer ratios 2–9) especially for those containing the polyunsaturated fatty acids. Thus, both oil and ester feeding yielded 2- to 3-times more of the 16:0–16:0–18:2, 16:0–16:1–18:2, 16:0–14:0–20:4, 16:0–16:0–20:4, 16:0–16:0–22:5, 16:0–18:1–20:5, 18:1–16:3–20:5, 18:1–18:2–22:6, and 18:1–18:3–20:5 species than of the reverse isomers. The oil feeding yielded nearly 4-times as much 14:0–16:0–18:4 and 16:1–16:1–18:4 species than of their reverse isomers, while ester feeding yielded these species in nearly racemic amounts. In contrast, the ester feeding yielded nearly 4-times as much of the 18:2–16:1–20:5, and nearly 9-times as much of the 18:3–16:0–20:5 and 16:2–16:0–20:5 isomer

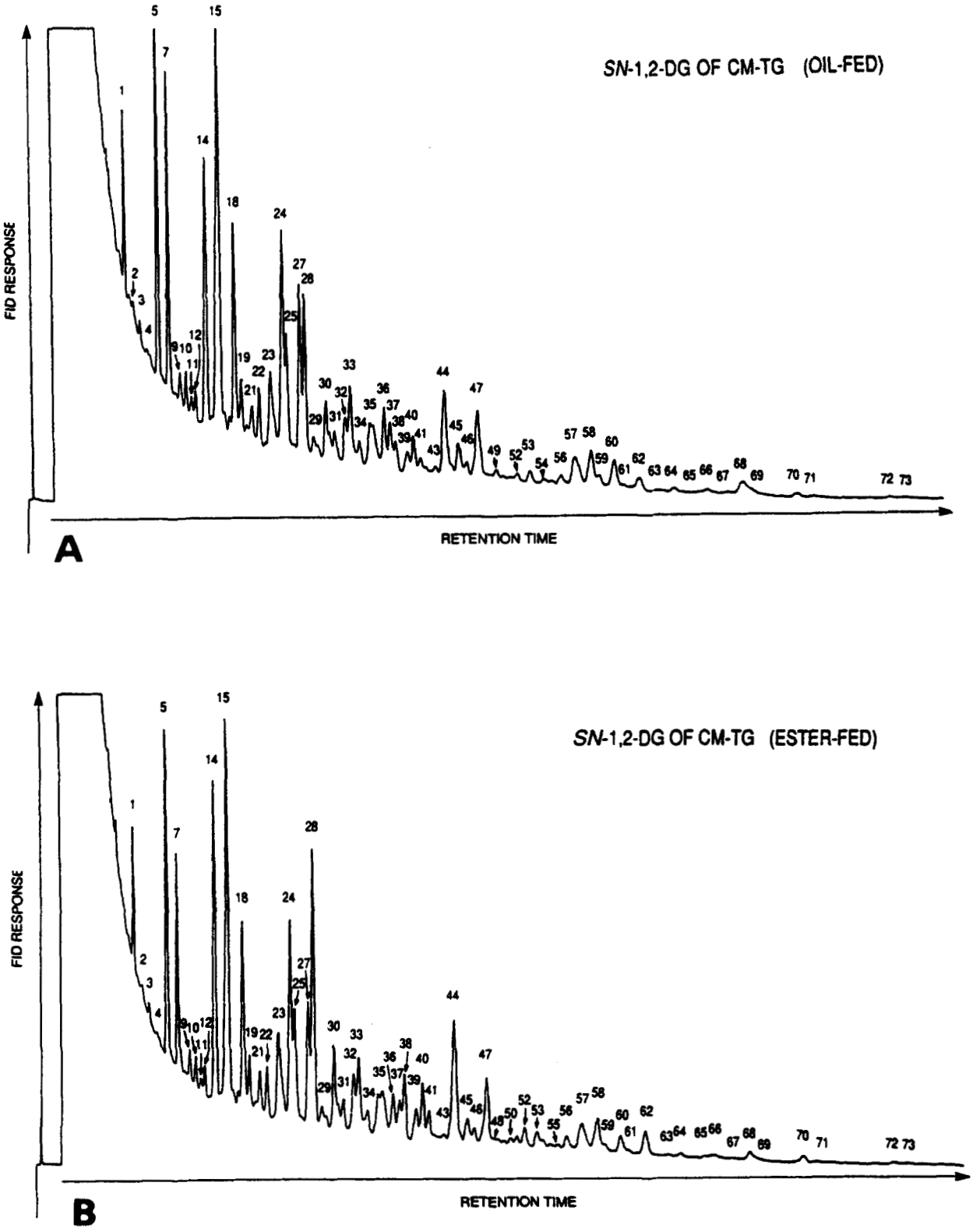

Fig. 2. Comparison of polar capillary GLC profiles of molecular species of (A) the *sn*-1,2-DG derived from the chylomicron TG of rats receiving menhaden oil and (B) the *sn*-1,2-DG derived from the chylomicrons of rats receiving the ethyl esters of menhaden oil fatty acids. Peak identification is as given in figure and in Table 3. GLC conditions: instrument, Hewlett-Packard Model 5880 equipped with a polar capillary column (15 m × 0.32 mm ID) wall-coated with cross-bonded film of RTx-2330 (Restek Corp., Port Matilda, PA). Carrier gas, H_2 at 3 psi head pressure. Temperature program: 240–260°C at 1°C/min, then isothermal at 260°C. Sample: 1 μl of approx. 0.1% solution of TMS-treated lipid mixture in hexane.

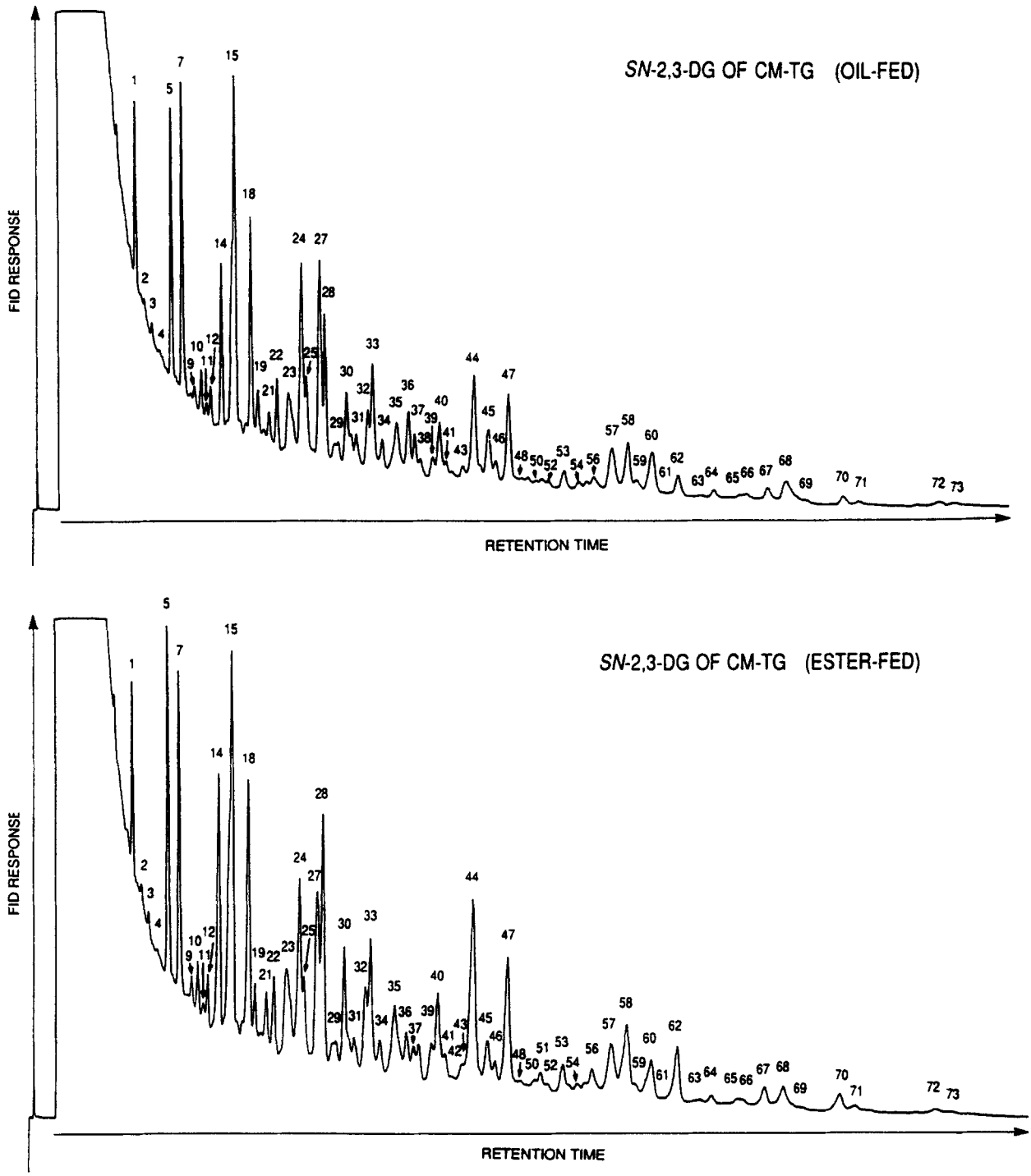

Fig. 3. Comparison of polar capillary GLC profiles of molecular species of (A) the *sn*-2,3-DG derived from the chylomicron TG of rats receiving menhaden oil and (B) the *sn*-2,3-DG derived from the chylomicron TG of rats receiving the ethyl esters of menhaden oil fatty acids. Peak identification is as given in figure and in Table 3. GLC conditions as given in Fig. 2.

than the corresponding reverse isomer, while oil feeding yielded these species in nearly racemic proportions. A comparison of the calculated distributions of the major DG species (over 40% of total) arising from the oil and ester feeding, including the reverse isomers, revealed better than 90% homology.

DISCUSSION

The structural analyses of the molecular species of the TG recovered from the lymph chylomicrons of rats fed either menhaden oil or the corresponding fatty acid ethyl esters indicates a great similarity in the overall molecular

TABLE 3. Experimental and calculated molecular species of the enantiomeric diacylglycerol moieties of the triacylglycerols of lymph chylomicrons and phosphatidic acid from the villus cells of rats fed menhaden oil or its fatty acid ethyl esters

Peak No.[a]	Molecular Species	Oil-Fed				Ester-Fed				Oil-Fed
		sn-1,2-		*sn*-2,3-		*sn*-1,2-		*sn*-2,3-		PA
		Exp.[b]	Calc.[b]	Exp.	Calc.	Exp.	Calc.	Exp.	Calc.	Calc.
		mole %								
1	14:0-14:0	1.42	0.99	1.70	0.86	1.23	0.59	1.22	0.66	0.07
3	14:0-15:0	0.15	0.15	0.17	0.13	0.14	0.10	0.10	0.10	0.02
5	14:0-16:0	4.40	3.89	3.71	2.68	4.70	3.65	3.86	2.55	1.24
7	14:0-16:1(n-7)	3.66	3.02	4.24	2.79	3.22	1.92	3.68	2.01	0.04
10	14:0-16:2(n-4)	0.42	0.48	0.56	0.34	0.41	0.51	0.52	0.32	0.01
11	14:0-17:0 + 15:0-16:0	0.23	0.45	0.22	0.51	0.19	0.29	0.18	0.39	0.21
12	15:0-16:1 + 14:0-16:3(n-4)	0.35	0.40	0.52	0.76	0.43	0.31	0.55	0.53	0.01
14	16:0-16:0 + 14:0-16:4(n-1)	4.99	3.95	3.18	2.41	6.36	5.13	3.85	2.98	4.81
15	14:0-18:1 + 16:0-16:1(n-7) + 18:0-14:0	10.97	9.45	11.07	7.27	9.60	8.39	8.58	5.76	2.77
18	16:1(n-7)-16:1(n-7) + 14:0-18:2(n-6)	4.13	3.34	4.88	3.24	4.11	2.89	4.39	2.94	0.39
19	16:0-16:2(n-4)	1.30	0.89	1.20	0.81	1.18	1.01	0.96	0.76	0.12
21	16:0-16:3(n-4) + 14:0-18:3(n-3)	0.89	0.60	0.87	1.07	1.28	0.66	0.99	0.95	0.02
22	16:1(n-7)-16:2(n-4)	1.06	0.70	1.41	0.81	0.98	0.47	1.12	0.60	0.01
23	16:1(n-7)-16:3(n-4) + 14:0-18:4(n-3) + 16:0-18:0 + 16:0-16:4(n-1)	2.97	2.15	2.79	2.57	3.41	2.95	3.04	2.74	12.14
24	16:0-18:1(n-9) + 14:0-20:1(n-9)	6.09	5.55	5.37	3.70	5.39	5.28	2.81	2.93	5.86
25	16:0-18:1(n-7) + 16:1(n-7)-18:0	2.45	2.56	1.77	1.22	2.44	3.06	1.18	1.20	2.77
26	16:1(n-7)-16:4(n-1)	0.14	0.24	0.00	0.65	0.09	0.17	0.09	0.74	
27	16:1(n-7)-18:1(n-9)	4.20	4.06	5.41	3.64	2.95	2.80	3.89	2.22	0.36
28	16:1(n-7)-18:1(n-7) + 16:0-18:2(n-6)	3.38	3.40	3.21	2.44	6.10	5.56	4.14	3.35	5.38
29	18:0-16:2(n-4)	0.76	0.15	0.91	0.06	0.54	0.28	0.77	0.07	0.26
30	16:1(n-7)-18:2(n-6) + 18:1(n-9)-16:2(n-4)	2.04	2.26	2.55	2.36	2.54	2.58	2.72	2.54	0.36
31	18:0-16:3(n-4)	0.77	0.12	0.96	0.06	0.74	0.16	0.61	0.05	0.05
32	16:0-18:3(n-3)	1.19	0.47	1.49	0.47	1.48	0.50	1.71	0.43	0.10
33	18:1(n-9)-16:3(n-4) + 14:0-20:5(n-3) + 16:0-18:4(n-3)	2.22	2.52	2.66	3.82	2.11	2.57	2.59	3.95	0.09
										0.26
34	18:0-16:4(n-1) + 16:1(n-7)-18:3(n-3) + 16:3(n-4)-18:1(n-9) + 18:0-18:0	0.57	0.60	0.78	0.69	0.62	0.51	0.69	0.50	4.77
35	16:1(n-7)-18:4(n-3) + 18:1(n-9)-16:4(n-1) + 18:0-18:1(n-9) + 18:0-18:1(n-7)	2.86	2.45	2.19	2.30	2.28	2.46	2.53	2.26	9.89
36	18:1(n-9)-18:1(n-9)	2.08	1.73	1.76	1.86	1.38	0.83	1.10	1.82	1.41
37	18:1(n-9)-18:1(n-7) + 14:0-21:5(n-3) + 15:0-20:5(n-3)	1.44	1.30	1.04	0.89	1.03	1.05	0.66	0.74	0.21
38	18:0-18:2(n-6)	0.83	0.37	0.47	0.12	1.93	1.22	0.75	0.30	9.88
40	18:1(n-9)-18:2(n-6)	1.16	1.43	1.59	1.35	1.68	1.98	2.16	1.51	1.42
43	16:0-20:3(n-3) + 18:0-18:3(n-3)	0.23	0.08	0.44	0.03	0.25	0.14	0.53	0.04	0.94
44	16:0-20:5(n-3) + 16:0-20:4(n-6)	3.67	2.84	3.90	3.25	4.86	3.32	6.20	5.05	4.81
45	14:0-22:5(n-3) + 14:0-22:6(n-3)	1.31	1.29	1.86	1.14	0.89	0.83	1.26	1.32	0.32
46	16:1(n-7)-20:3(n-3) + 18:1(n-9)-18:3(n-3)	0.51	0.32	0.63	0.39	0.50	0.22	0.58	0.25	0.09
47	16:1(n-7)-20:5(n-3)	2.96	2.84	2.97	3.25	2.57	3.32	3.61	5.05	0.08
53	16:2(n-4)-20:5(n-3) + 18:0-20:4(n-6)	0.45	0.33	0.66	0.55	0.58	0.47	0.77	0.80	8.81
56	16:3(n-4)-20:5(n-3) + 18:0-20:5(n-3) + 18:1(n-9) + 20:4(n-6)	0.73	0.56	0.71	0.79	0.93	0.54	1.25	0.69	3.54
57	16:0-22:6(n-3) + 16:0-22:5(n-3)	3.39	1.83	2.75	1.87	2.94	1.36	3.36	2.11	1.30
58	18:1(n-9)-20:5(n-3)	2.42	1.95	2.68	2.57	2.93	1.95	4.38	3.12	0.33
59	18:1(n-7)-20:5(n-3)	0.76	0.64	0.64	0.57	0.61	0.34	0.32	0.43	
60	16:1(n-7)-22:6(n-3)	2.66	1.42	2.50	1.77	1.34	1.10	2.37	1.71	0.08
62	18:4(n-3)-20:5(n-3)	1.21	1.20	0.85	0.72	1.69	1.53	3.10	2.90	
65	16:4(n-1)-22:6(n-3) + 16:4(n-1)-22:5(n-3)	0.29	0.08	0.32	0.34	1.09	0.15	0.47	0.26	
66	18:0-22:6(n-6) + 18:0-22:5(n-3)		0.40		0.17		0.10		0.11	3.10
67	18:1(n-9)-22:6(n-3) + 18:1(n-9)-22:5(n-3)	0.39	1.74	0.76	1.99	0.03	1.06	0.91	1.70	0.34
68	18:1(n-7)-22:6(n-3) + 18:1(n-7)-22:5(n-3)	2.62	0.59	1.90	0.43	0.64	0.16	1.66	0.22	
72	20:5(n-3)-20:5(n-3)	0.14	0.53	0.26	0.94	0.21	0.29	0.29	0.49	0.03
73	18:4(n-3)-22:6(n-3) + 18:4(n-3)-22:5(n-3)	0.15	0.27	0.38	0.57	0.42	0.24	0.39	0.39	
C42		0.77	0.56	1.23	0.29	0.17	0.56	0.50	0.29	
C44		0.12	0.25	0.03	0.56	0.04	0.25	0.16	0.56	
Others		6.11	20.60	5.85	25.93	6.77	22.19	6.45	24.59	15.98

The phosphatidic acids were from villus cells of rats receiving menhaden oil.

[a]Peak number is as given in Fig. 2 and Fig. 3.

[b]Exp., experimental, Calc., calculated. Calculated values were obtained by the 1-random, 2-random, 3-random method and the knowledge of the fatty acids in the *sn*-1-, *sn*-2-, and *sn*-3-positions.

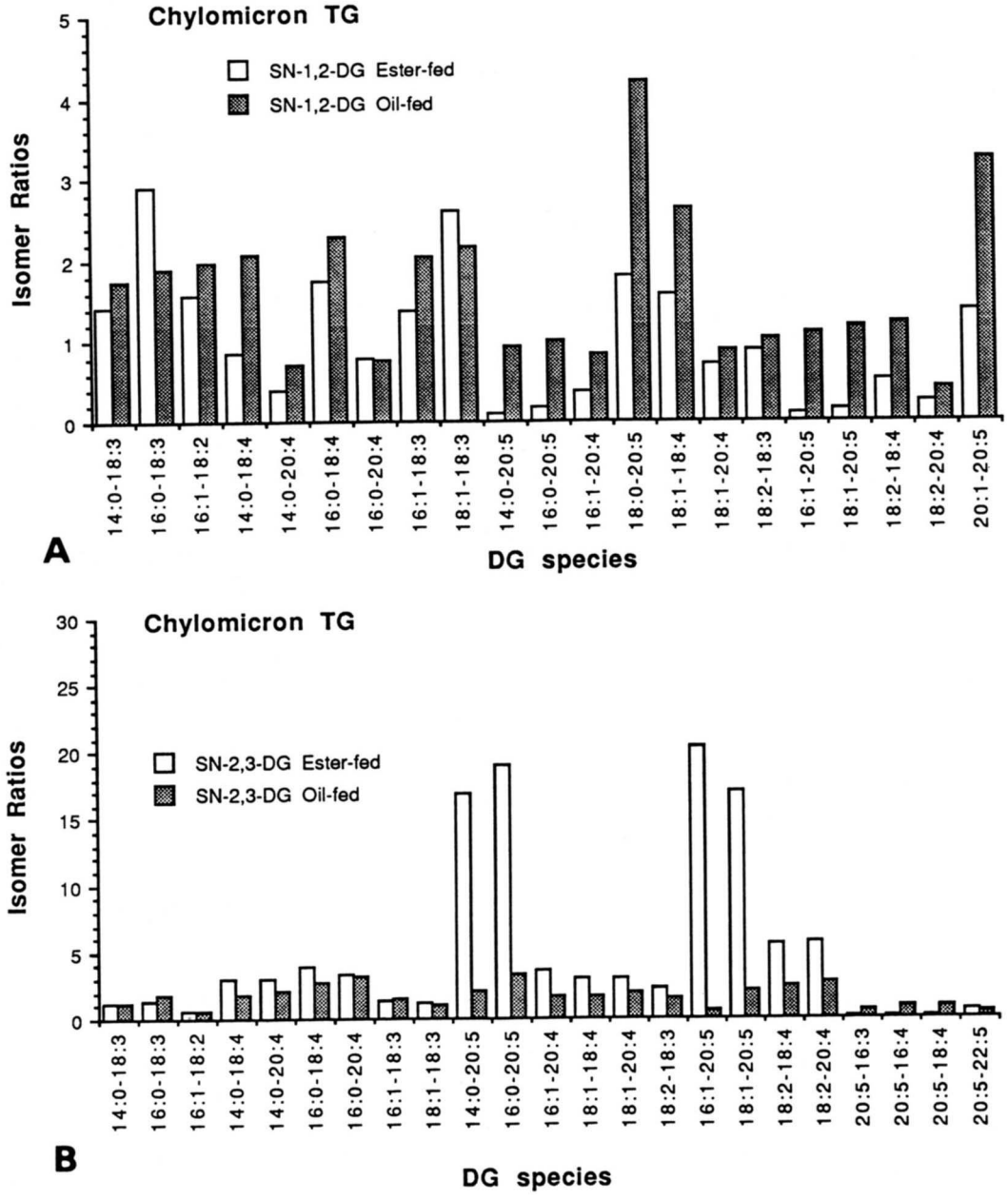

Fig. 4. Reverse isomer ratios of molecular species for (A) the *sn*-1,2- and (B) the *sn*-2,3-DG moieties of the chylomicron TG derived from the ester (open bars) and oil (closed bars) feeding. The molecular species are identified using abbreviated notation for the component fatty acids with the *sn*-1-fatty acid on the left in A and the *sn*-2-fatty acid on the left in B. The reverse isomer content was calculated as explained in Methods. The ratios were obtained by dividing the values for the normal isomer (as written) by the values for the reverse isomer: e.g., 14:0–18:3/18:3–14:0 for first column in A.

association of the fatty acids. The results also show a closely similar molecular association of the fatty acids in the corresponding *sn*-1,2- and *sn*-2,3-DG moieties of chylomicron TG. This would be anticipated during the oil feeding if fatty acid pools of similar composition were equally accessible for the acylation of both *sn*-1- and *sn*-3-positions of the 2-MG, as claimed by Breckenridge and Kuksis (23) on the basis of the isolation of significant amounts of free *sn*-1,2- and *sn*-2,3-DG from the mucosal cells during fat absorption. If the acylation of the *sn*-2-MG had proceeded exclusively via the *sn*-1,2-DG intermediates as suggested from the in vitro work of Johnston et al. (24) and Coleman et al. (25), then fatty acid pools of similar composition must have been available for the acylation of the

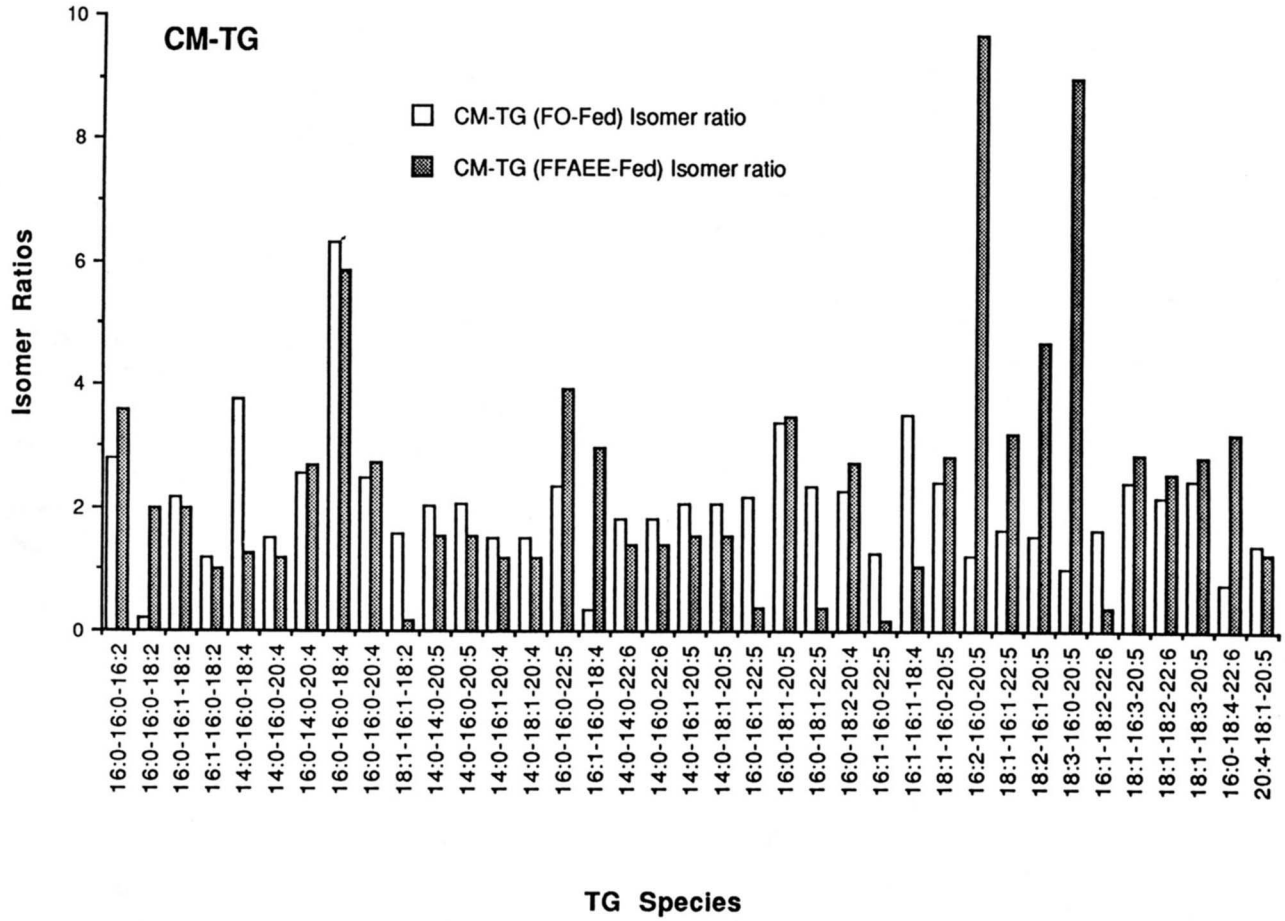

Fig. 5. Reverse isomer ratios for selected major TG species recovered from the chylomicrons of rats fed menhaden oil (open bars) and ethyl esters of menhaden oil fatty acids (closed bars). The molecular species are identified using abbreviated notation for the component fatty acids with the *sn*-1-fatty acid on the left and the *sn*-3-fatty acid on the right. The reverse isomer content was calculated as explained in Methods. The ratios were obtained by dividing the values for the normal isomer (as written) by the values for the reverse isomer: e.g., 16:0–16:0–18:2/18:2–16:0–16:0 for the second column in the figure.

sn-1-position of the 2-MG and the *sn*-3-position of the resulting *sn*-1,2-DG. In the present study the recovery of the free *sn*-2,3-DG averaged 10–20% of the total *sn*-1,2/2,3-DG (L-Y. Yang and A. Kuksis, unpublished results). As the *sn*-1,2- and *sn*-2,3-DG moieties contain the same fatty acid in the *sn*-2-position, the relative fatty acyl group preference for the *sn*-1- and the *sn*-3-acyltransferases was assessed from the reverse isomer ratios determined for the major DG species. While a discrimination between the medium chain length saturated and oligounsaturated acids was not obvious, that for the polyunsaturated acids was conspicuous. Thus, the 18:4 and especially the 20:5 acids were largely excluded from the *sn*-1-position, while the *sn*-1,2-DG, with the polyunsaturated fatty acids in the *sn*-2-position, were present in an overwhelming proportion as a result of the retention of the composition of the *sn*-2-position of the original oil.

In the *sn*-2,3-DG moieties, significantly more of the polyunsaturated fatty acid was placed in the *sn*-3-position when compared to the *sn*-1-position of the *sn*-1,2-DG moieties. It must, therefore, be concluded that the 2-MG acylation does not proceed indiscriminately, but shows preference for the placement of the more saturated and oligounsaturated fatty acids in the *sn*-1-position. A calculation of the reverse isomer ratio in the lymph chylomicron TG from the oil-fed animals indicates that the preference for the placement of the long chain polyunsaturated fatty acids for the *sn*-3-position of the TG molecule is genuine, as the comparison is now being made to the *sn*-1-position of the same TG molecule.

Such a fatty acid positional specificity is not known to be characteristic of the 2-MG or the X-1,2-DG acyltransferase activity measured in vitro (22). It is possible that part of this non-randomness in the fatty acid distribution in the TG arising from oil feeding was due to a significant contribution from the PA pathway, which is known to place the saturated and the oligounsaturated fatty acids in the *sn*-1-position and the polyunsaturated and other unusual fatty acids in the *sn*-3-position of the TG molecule during TG synthesis by rat intestinal villus cells (22).

However, detailed comparisons of the molecular species of the PA and the *sn*-1,2-DG moieties of the chylomicron TG from the oil feeding revealed only a limited homology, corresponding to a maximum contribution of 15–20% in keeping with the 80% recovery of the dietary 2-MG in the chylomicron TG.

Polheim et al. (26) had suggested that the 2-MG might inhibit the PA pathway, while Brindley (27) has attributed this in vitro phenomenon to the detergent effect brought on by unnaturally high 2-MG concentrations in the incubation medium. A limited contribution from the PA pathway is also likely because of the excess of free fatty acids entering the villus cell when compared to the proportion of the 2-MG (13).

As the PA synthesis proceeds via a preferential incorporation of the saturated fatty acids in the *sn*-1-position and the oligounsaturated acids in the *sn*-2-position (8, 22), it was anticipated that the *sn*-1,2-DG moieties of these TG would show higher homology to the *sn*-1,2-DG moieties of the PA than the 10–20% observed experimentally. A comparison of the fatty acid association in the *sn*-1,2- and *sn*-2,3-DG moieties of the chylomicron TG from the ethyl ester-fed rats unexpectedly revealed close similarity, although the *sn*-2,3-enantiomers did contain a slightly higher proportion of the polyunsaturated species than the *sn*-1,2-DG. Furthermore, the reverse isomer ratios in the *sn*-1,2-DG revealed that the *sn*-1-position contained high proportions of the polyunsaturated fatty acids, which normally should have been absent from this position. The proportion of the isomers containing the polyunsaturated fatty acids in the *sn*-3-position of the *sn*-2,3-DG is greatly favored. We have since performed similar calculations on the data obtained in similar studies on the glyceryl and alkyl ester absorption derived from corn (15) and mustard seed (17) oil and have demonstrated comparable similarities in the structure of the corresponding chylomicron TG.

Mechanism of chylomicron TG synthesis and secretion

We have previously suggested that the TG arising via the PA pathway undergo lipolysis to 2-MG and reesterification prior to incorporation into the chylomicron TG (8). Such a mechanism was consistent with the findings in rat liver, where the TG from the PA pathway are transferred to the VLDL via a transient storage and lipolysis in the cytoplasm (9, 10, 12). In view of the recent isolation of the TG transfer protein (28, 29) and the demonstration that a lack of this protein may be responsible for the abetalipoproteinemia and the inability to transfer to plasma of apolipoprotein B from either liver or intestine (28), the earlier hypothesis (8) of the convergence of the PA and the 2-MG pathways of TG synthesis may be rationalized along the lines suggested for the VLDL formation in rat liver (7, 8). The TG generated by the PA pathway would initially accumulate at the site of the synthesis between the two halves of the lipid bilayer, where it would interact with apolipoprotein B to generate the small TG-poor particle (29). The lipid bilayer is capable of limited TG uptake (30, 31). The excess TG would enter cytoplasmic storage, where it would undergo lipolysis and resynthesis via the 2-MG pathway and a transfer to the TG-rich particle with the help of the TG transfer protein (28, 29). The larger TG-rich particle would eventually fuse with the smaller particle (29). The lipolysis and reesterification would result in the loss of distinction between the compositions of the fatty acids in the *sn*-1- and *sn*-3-positions, as observed experimentally. Because of the high retention of the original 2-MG composition in the *sn*-2-position of the chylomicron TG during oil feeding, it is possible that these TG are transferred directly to the chylomicrons without a transient storage in the cytoplasm. A confirmation of the proposed convergence of the 2-MG and the PA pathways requires the demonstration of an endogenous lipase, which attacks the stored TG yielding largely *sn*-2-MG, and the isolation of the TG transfer protein in association with the acyltransferases of the 2-MG pathway. In preliminary communications, Hulsmann, Stam, and Breeman (32) and Rao and Mansbach (33) have reported the presence of both neutral and alkaline endogenous lipases in the small intestine that preferentially attack the primary ester bonds of TG.

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