Apparent convergence (at 2-monoacylglycerol level) of phosphatidic acid and 2-monoacylglycerol pathways of synthesis of chylomicron triacylglycerols

Lu-Ying Yang and Arnis Kuksis[1]

Department of Biochemistry and Banting and Best Department of Medical Research, University of Toronto, Toronto, Ontario, M5G 1L6 Canada

Abstract Dietary fats are converted into chylomicron triacylglycerols via the 2-monoacylglycerol and phosphatidic acid pathways of acylglycerol formation. In view of the known positional and fatty acid specificity of the acyltransferases, the triacylglycerol structures resulting from the two pathways would be expected to differ, but this has not been demonstrated. We have performed stereospecific analyses on the chylomicron triacylglycerols from rats fed menhaden oil and the corresponding fatty acid alkyl esters, which would be expected to be assimilated via the monoacylglycerol and the phosphatidic acid pathways, respectively. The results show a remarkable similarity between the two triacylglycerol types in the fatty acid composition of the *sn*-1 and *sn*-3 positions, along with marked differences in the composition of the *sn*-2 positions. The triacylglycerols from rats fed oil retained about 85% of the original fatty acids in the *sn*-2 position, including a high proportion of the long chain polyunsaturates (e.g., 5–7% 20:5 and 4–5% 22:6). The triacylglycerols from rats fed the alkyl ester contained large amounts of endogenous fatty acids in the *sn*-2 position (e.g., 18% 16:1, 14% 18:1, 14% 18:2, and 2.5% 20:4), which approximated the composition of the *sn*-2 position of the presumed phosphatidic acid intermediates. The *sn*-1 position contained a much higher proportion of polyunsatured fatty acids (e.g., 12–13% 20:5, 5–6% 22:6) than the *sn*-2 position (e.g. 2–3% 20:5, 0–0.6% 22:6) of triacylglycerols from rats fed the ester. We conclude that the chylomicron triacylglycerols arising via the 2-monoacylglycerol and the phosphatidic acid pathways differ mainly in the composition of the fatty acids in the *sn*-2 position. The similarity in the acids of the *sn*-1 and *sn*-3 positions of the chylomicron triacylglycerols from rats fed oil or ester is consistent with a hydrolysis of the acylglycerol products of the phosphatidic acid pathway to 2-monoacylglycerols prior to reconversion to triacylglycerols via the monoacylglycerol pathway and secretion as chylomicrons.—**Yang, L. Y., and A. Kuksis.** Apparent convergence (at 2-monoacylglycerol level) of phosphatidic acid and 2-monoacylglycerol pathways of synthesis of chylomicron triacylglycerols. *J. Lipid Res.* 1991. **32:** 1173–1186.

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It is well known that intestine is capable of assimilating dietary fat via phosphatidic acid (1) and monoacylglycerol (2) pathways of acylglycerol synthesis, which under normal conditions contribute about 20% and 80%, respectively, to the total chylomicron triacylglycerol formation (3, 4). It is also known that the enzymes involved in the two pathways are associated with different subcellular structures (5, 6) and that the diacylglycerol intermediates from the two pathways remain segregated (7, 8). There is no evidence, however, that the triacylglycerols produced by the two pathways would appear in the lymph as different lipid particles. It is therefore apparent that the products of the two pathways of acylglycerol formation converge prior to chylomicron secretion, but the mechanism of such a convergence has not been determined.

Independent experiments in the adult liver, which presumably possesses only the phosphatidic acid pathway for triacylglycerol biosynthesis, have shown (9) the existence of microsomal and cytosolic pools of triacylglycerols. The microsomal pool is small in size and metabolically active (10), while the cytosolic pool is large, metabolically inactive, and may serve a storage function (11). The triacylglycerols stored in the cytosol become hydrolyzed and reesterified in microsomes before secretion (12). It is not known whether the reesterification takes place via the phosphatidic acid pathway. It has been estimated that a maximum of 15% of the hepatic triacylglycerols are

Abbreviations: VLDL, very low density lipoproteins; TLC, thin-layer chromatography; GLC, gas-liquid chromatography; HPLC, high performance liquid chromatography.

[1]To whom correspondence should be addressed at: Banting and Best Department of Medical Research, University of Toronto, 112 College Street, Toronto, Ontario, Canada M5G 1L6.

secreted without prior hydrolysis (13). If the lysosomal acid lipase responsible for the hydrolysis is inhibited by chloroquine, secretion of VLDL by hepatocytes is inhibited (14). It remains to be determined whether or not the triacylglycerol products of the phosphatidic acid pathway in the intestine are subject to a similar breakdown before secretion.

The possibility of triacylglycerol hydrolysis in intestine prior to transport out has been suggested by Mansbach, Arnold, and Garret (15). These authors thought that the triacylglycerol particles accumulated in the intestine during fat absorption were too large for secretion as chylomicrons (16). Since triacylglycerol also accumulated in the fasting intestine after chloroquine administration, Mansbach et al. (15) speculated that the endogenous triacylglycerols were hydrolyzed to free fatty acids and transported via the portal vein. This possibility, however, has not been experimentally confirmed.

We have demonstrated that the free fatty acids arising from hydrolysis of fatty acid methyl or ethyl esters of menhaden (17), mustardseed (18), rapeseed (17), and corn (19) oils are effectively incorporated into lymph chylomicron triacylglycerols in the absence of lumenal 2-monoacylglycerols. We were surprised to discover that the fatty acids in the *sn*-1 and *sn*-3 positions of the chylomicron triacylglycerols from rats fed mustardseed oil acids as methyl esters were similar to those in the *sn*-1 and *sn*-3 positions of the triacylglycerols obtained by feeding mustardseed oil (18). We suggested among other possibilities that the identity in the composition of the outer positions of the triacylglycerol molecules arising from the oil and ester feeding could have resulted from a hydrolysis of the triacylglycerols arising from the phosphatidic acid pathway into 2-monoacylglycerols followed by reacylation via the monoacylglycerol pathway. In the present study we have used menhaden oil and its ethyl or methyl esters as much more complex markers for the exogenous fatty acids and 2-monoacylglycerols, and have obtained evidence which favors the hydrolysis hypothesis over other hypotheses.

MATERIALS AND METHODS

Experimental meals

Menhaden oil was purchased from Zapata Haynie Corporation (Reedville, VA). The methyl and ethyl esters were prepared by treating the oil with 1 M sodium methoxide or ethoxide in the corresponding alcohol-toluene 60:40 as previously described (20). **Table 1** gives the fatty acid composition of the experimental meals.

Animals and surgical procedures

Retired male breeder rats of Wistar strain (Charles River Canada, La Salle, Quebec) weighing 380–450 g at surgery were fed formula stock diet ad libitum until used for experimentation. One hour prior to surgery the animals were given 1 ml of menhaden oil or its fatty acid alkyl esters. For surgery the animals were anesthetized by intraperitoneal injection of sodium pentobarbital (Abbott Laboratories, Montreal, Quebec) at the dose level of 7.3 mg/100 g body weight. The thoracic duct was cannulated as described by Bollman, Chain, and Grindlay (21) and the lymph was collected (17, 19). During this time the animals were partially immobilized but had free access to drinking water.

TABLE 1. Fatty acid composition of original menhaden oil and its corresponding fatty acid alkyl esters

Fatty Acids	Experiment 1		Experiment 2	
	Oil	EE	Oil	ME
	mole %			
14:0	9.1	9.2	9.3	9.7
15:0	0.6	0.6	0.5	0.6
16:0	16.7	16.9	16.8	18.3
16:1n-7	12.0	11.9	12.7	12.4
17:0	0.7	1.0		0.8
16:2n-4	2.1	2.1		
18:0	2.7	4.8	2.6	3.0
16:3n-4	2.1		2.1	2.1
18:1n-9	8.8	8.8	9.7	9.3
18:1n-7	3.2	3.3	3.7	3.6
16:4n-1	2.2	2.2	2.4	2.3
18:2n-6	1.5	1.5	1.4	1.5
18:3n-6	0.3	0.4		
20:0	0.2	0.5	0.1	
18:3n-3	1.1	1.1	1.1	1.1
20:1n-9	1.5	1.5	1.3	1.5
20:1n-7	0.3	0.2		
18:4n-3	3.6	3.7	3.6	3.6
20:3n-6	0.2	0.2	0.1	0.2
20:4n-6	0.8	0.9	1.3	1.3
22:1n-11	0.5	0.6		
20:4n-3	1.5	1.5	1.5	1.5
20:5n-3	15.3	15.2	16.9	14.7
22:4n-6 + 21:5n-3	1.2	0.9		
22:4n-3	0.4	0.3		
22:5n-3	2.2	2.2	2.3	2.3
22:6n-3	9.3	8.6	9.1	8.1

Average of two analyses; ME, methyl esters; EE, ethyl esters; Oil, original glyceryl esters.

Experimental design

Lymph was collected from a total of four animals. One rat of each pair received menhaden oil to ensure triacylglycerol formation via the 2-monoacylglycerol pathway; the other rat received methyl or ethyl esters of menhaden oil fatty acids to ensure triacylglycerol formation via the phosphatidic acid pathway. The lymph was collected continuously in larger or smaller volumes as required, while the animals received 1 ml of the oil or ester 1 h before surgery and 6 and 12 h after surgery. In experi-

ment 1, one animal from oil feeding and one animal from ethyl ester feeding provided complete lymph collections from two consecutive meals each. These lymph samples supplied the triacylglycerols for positional and stereospecific analyses. In experiment 2, the other pair of rats provided triacylglycerols from menhaden oil and methyl ester feeding which were analyzed by pancreatic lipase hydrolysis. The chylomicrons were isolated by ultracentrifugation and were characterized as previously described (19). In addition, four more animals were fed oil and four more the ethyl esters, all being killed 2 h later to provide the fatty acid composition of the phosphatidic acids. Four other animals that received ethyl esters were killed 2 h later to provide the phosphatidic acid for positional analy- fatty acids. Finally, two additional animals received ethyl esters and were killed 2 h later to provide the fatty acid composition of endogeneous 2-monoacylglycerols.

Isolation of chylomicron triacylglycerols

Total lipid extracts of chylomicrons were obtained with chloroform–methanol 2:1 and the triacylglycerols were isolated by TLC using heptane–isopropyl ether–acetic acid 60:40:4 as the developing solvent (20). The triacylglycerols were recovered by suspending the gel scrapings in chloroform–methanol 2:1, washing with water, and drying with anhydrous sodium sulfate.

Stereospecific analysis

The stereospecific distribution of fatty acids in the triacylglycerols was determined by calculation after a chiral phase HPLC resolution (22) of the *sn*-1,2(2,3)-diacylglycerols prepared by random degradation. The *sn*-1,2(2,3)-diacylglycerols were generated from the chylomicron triacylglycerols by Grignard reaction and were purified and resolved from the X-1,3-diacylglycerols by boric acid TLC (23). The R_f values for *sn*-1,2(2,3)- and X-1,3-diacylglycerols in the chloroform–acetone 97:3 solvent system were 0.43 and 0.6, respectively. The enantiomers were resolved as the 3,5-dinitrophenylurethane derivatives on an HPLC column containing (R)-(+)-1-(1-naphthyl)ethylamine polymer using hexane–dichloromethane–ethanol 40:10:1 as the mobile phase (22). The fatty acid composition of the *sn*-2 position was calculated by subtracting the fatty acid composition of chylomicron triacylglycerols from the fatty acid composition of the *sn*-1,2(2,3)-diacylglycerols, while the fatty acid compositions of the *sn*-1 and *sn*-3 positions were obtained by subtracting the composition of the *sn*-2 position from the fatty acid composition of the appropriate diacylglycerol enantiomer (24). The latter values were cross-checked by subtracting the fatty acids of the appropriate enantiomer from those of the original triacylglycerol. The overall correctness was assessed by comparing the reconstituted total to the total of the original triacylglycerols.

In addition, the fatty acid composition of the *sn*-2 position of the triacylglycerols was determined directly by pancreatic lipase hydrolysis (23). The *sn*-2-monoacylglycerols were isolated by borate TLC (23). The fatty acid composition of the combined outer positions was obtained by subtraction of the fatty acid composition of the *sn*-2 position from that of the total chylomicron triacyglycerols.

Isolation of phosphatidic acid intermediates

For this purpose mucosal villus cells were isolated and total lipid extracts were obtained as described (17). Phosphatidic acid standard was prepared from egg yolk phosphatidylcholine by hydrolysis with cabbage phospholipase D (25). The total cellular lipids were resolved by TLC on silica gel H containing 7% magnesium acetate with chloroform–methanol–ammonia–water 65:35:1:3 as the mobile phase. The phosphatidic acid, which migrated just above the origin, was extracted with chloroform–methanol–water–acetic acid 50:39:10:1 and purified in an acidic TLC system using chloroform–methanol–acetone–acetic acid–water 30:10:40:10:5 as mobile phase (26).

Hydrolysis with phospholipase A_2

The positional distribution of fatty acids in phosphatidic acid was determined by hydrolysis with phospholipase A_2 (*Crotalus adamanteus*, Sigma Chemical Co., St. Louis, MO) (27). To prevent peroxidation, butylated hydroxytoluene was incorporated into the digestion buffer (25 μg/ml) and the digestion was conducted under nitrogen. The purified phosphatidic acid was suspended in 2 ml of 0.2% Tris (0.01% $CaCl_2$) buffer (pH 7.3) containing 10 units enzyme and incubated for 2 h at 37°C. Total lipids were extracted with chloroform–methanol 2:1 and the recovered free fatty acids, lysophosphatidic acid, and residual phosphatidic acid were separated by TLC using a double development (26) with chloroform–methanol–acetone–acetic acid–water 30:10:40:10:5 as the mobile phase in the first development (up to 2 cm from the top of the plate) and heptane–isopropyl ether–acetic acid 60:40:4 as the mobile phase in the second development (to the top).

Analysis of fatty acids

Fatty acids were determined by GLC of the methyl esters after acidic or alkaline transmethylation (20). The fatty acid methyl esters were purified by TLC using heptane–isopropyl ether–acetic acid 60:40:4 as developing solvent. Fatty acid methyl and ethyl esters were resolved by GLC using a polar capillary column (RTx 2330, Restek Corp., Port Matilda, PA) as previously described (28).

RESULTS

Chromatographic analysis

Fig. 1 shows the separation of the *sn*-1,2- and *sn*-2,3-diacylglycerol moieties of the chylomicron triacylglycerols from the oil and ethyl ester feeding. An essentially complete resolution was obtained for both samples with *sn*-1,2-enantiomer emerging cleanly ahead of the *sn*-2,3-enantiomer. An identical separation was obtained for the *sn*-1,2- and *sn*-2,3-diacylglycerols derived from the chylomicron triacylglycerols isolated from a second oil and ester feeding (chromatograms not shown). The overlap between the longest retained *sn*-1,2- and the earliest eluted *sn*-2,3-diacylglycerol species was minimal. The purity of the collected fractions was verified by rechromatography. These separations are similar to those obtained previously

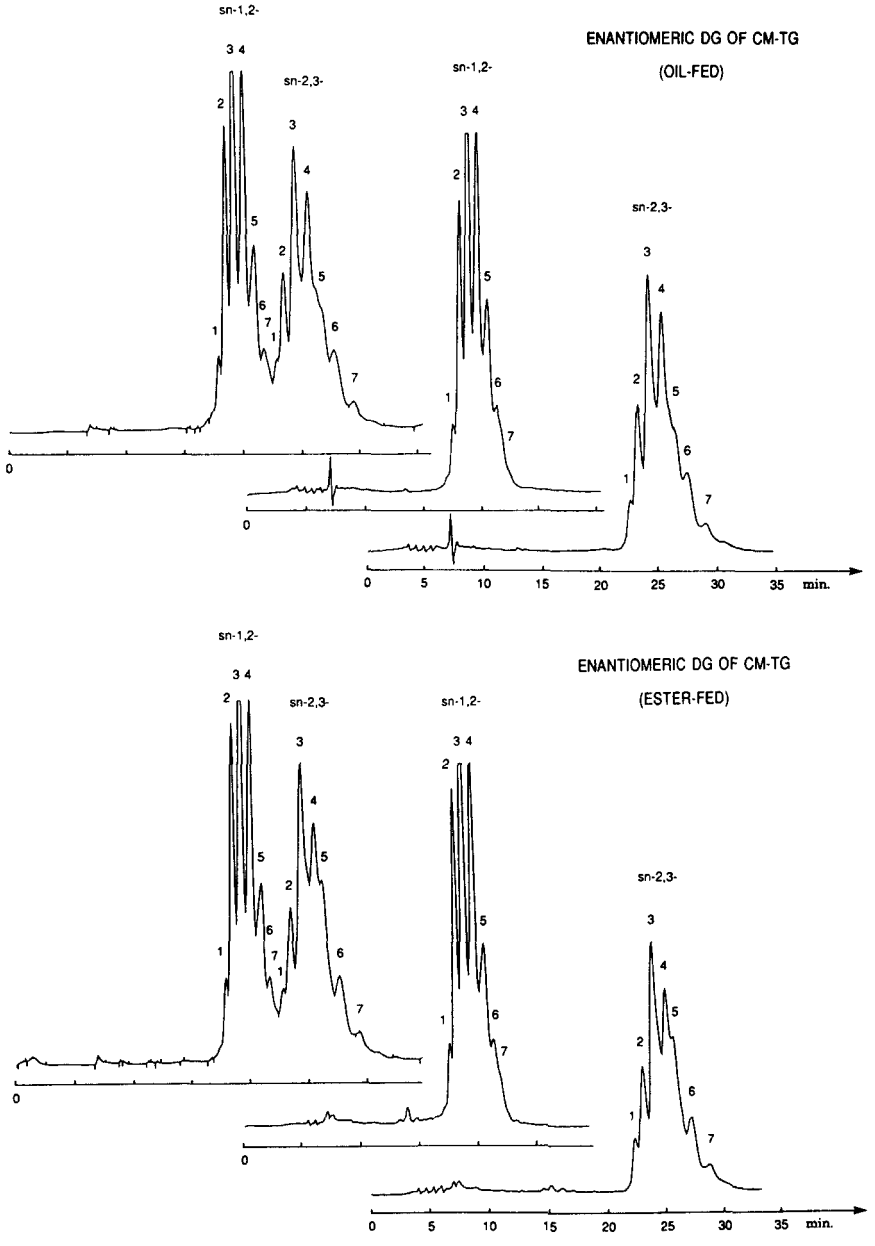

Fig. 1. Initial resolution and rechromatography by chiral phase HPLC of *sn*-1,2- and *sn*-2,3-diacylglycerol moieties of chylomicron triacylglycerols as their 3,5-dinitrophenylurethane derivatives. Top panel: menhaden oil feeding; bottom panel: ethyl ester feeding. Instrument: Hewlett-Packard Model 1084 liquid chromatograph equipped with conventional column (25 cm × 4.6 mm i.d.) containing (R)-(+)-1-(1-naphthyl)ethylamine polymeric chiral phase covalently bonded to 300 Å wide-pore spherical silica particles (5 μm) (YMC-Pack, A-KO3, YMC Inc. Kyoto, Japan); solvent, n-heptane-1,2-dichloromethane–ethanol 40:10:1 (by vol) at 0.5 ml/min; temperature, 20°C; peak detection, 254 nm (λ_{max}).

for the *sn*-1,2- and *sn*-2,3-diacylglycerol moieties derived from the dietary menhaden oil triacylglycerols (22).

Table 2 gives the fatty acid composition of the *sn*-1,2- and *sn*-2,3-diacylglycerol moieties of the chylomicron triacylglycerols from the two oil feedings as obtained by transmethylation of the fractions collected by chiral phase HPLC. In each instance the combined values for the fatty acids in the *sn*-1,2- and *sn*-2,3-diacylglycerols agreed closely with the values for the unresolved *sn*-1,2(2,3)-diacylglycerols (reconstitution values not shown). Although the same oil was fed both times, there were minor differences in the fatty acid composition of the corresponding diacylglycerol enantiomers, reflecting minor differences in the fatty acid composition of the recovered chylomicrons. In both instances, however, the differences between the *sn*-1,2- and *sn*-2,3-enantiomers from the same meal were much greater than those seen for corresponding enantiomers between meals.

Table 3 gives the fatty acid composition of the *sn*-1,2- and *sn*-2,3-diacylglycerol moieties similarly recovered from the chylomicron triacylglycerols after the two ethyl ester feedings. Again, there is an excellent agreement between the composition of the original *sn*-1,2(2,3)-diacylglycerols derived by Grignard degradation and the composition obtained by summation of the acids in the resolved *sn*-1,2- and *sn*-2,3-diacylglycerols. A comparison of the corresponding *sn*-1,2- and *sn*-2,3-diacylglycerols from the oil and ester feeding shows great similarity in the fatty acid composition, although the differences between the *sn*-1,2- and *sn*-2,3-diacylglycerols from the ester feeding were somewhat greater than those seen following the oil feeding.

Fig. 2A compares the fatty acid composition of the phosphatidic acids recovered from the villus cells during the oil and ester assimilation. Since the phosphatidic acids occur at low concentrations and are difficult to isolate and characterize, the results are presented as means ± SD for four rats receiving menhaden oil or the corresponding fatty acid ethyl esters. There is great similarity in the fatty acid compositions, which are characterized by a high proportion of endogenous fatty acids. As a result the anticipated close similarity between the fatty acid compositions of the phosphatidic acids and the *sn*-1,2-diacylglycerols of chylomicron triacylglycerols arising from the ester feeding (Table 3) is not realized. Especially noticeable is the much higher content of 16:1n-7 in the *sn*-

TABLE 2. Fatty acid composition of diacylglycerol moieties of lymph chylomicron triacylglycerols from a rat fed menhaden oil

Fatty Acids	Meal 1[a]			Meal 2[a]		
	sn-1,2-	*sn*-2,3-	*sn*-1,2(2,3)-	*sn*-1,2-	*sn*-2,3-	*sn*-1,2(2,3)-
	mole %					
14:0	10.9	11.3	11.1	9.9	9.3	9.7
15:0	0.9	0.7	0.8	0.8	0.7	0.7
16:0	23.3	18.0	19.9	19.5	14.8	16.5
16:1n-7	14.5	15.0	14.7	15.1	14.8	14.8
17:0	0.8	0.6	0.7	0.9	0.8	0.6
16:2n-4	2.2	2.7	2.5	2.3	2.3	2.6
18:0	3.7	1.4	2.4	3.0	1.1	1.9
16:3n-4	1.8	2.4	2.2	0.9	2.9	1.9
18:1n-9	12.9	11.3	11.6	13.4	12.3	12.5
18:1n-7	4.3	2.8	3.4	4.4	3.0	3.6
16:4n-1	1.0	2.4	1.7	0.7	2.2	1.6
18:2n-6	4.7	4.9	4.4	5.4	5.6	5.0
18:3n-6	0.3	0.3	0.3	0.3	0.4	0.3
20:0	0.2	0.1	0.1	0.2	0.1	0.1
18:3n-3	1.0	1.4	1.3	1.2	1.6	1.4
20:1n-9	1.5	1.1	1.2	1.6	1.0	1.3
20:1n-7	0.3	0.3	0.3	0.3	0.2	0.3
18:4n-3	1.8	3.2	2.7	2.1	3.7	3.0
20:3n-6	0.2	0.2	0.2	0.2	0.2	0.2
20:4n-6	1.4	1.6	1.5	1.5	1.7	1.7
22:1n-11	0.4	0.3	0.3	0.3	0.3	0.4
20:4n-3	0.9	1.3	1.2	1.1	1.4	1.3
20:5n-3	5.5	8.8	7.8	7.3	10.2	9.3
22:4n-6 + 21:5n-3	0.6	0.7	0.7	0.7	0.8	0.8
22:4n-3	0.2	0.3	0.3	0.3	0.3	0.3
22:5n-3	1.2	1.8	1.6	1.8	2.0	1.9
22:6n-3	3.7	5.0	5.2	4.7	6.0	6.3

sn-1,2(2,3)-Diacylglycerols were from Grignard degradation; *sn*-1,2 and -2,3-diacylglycerols were as resolved by chiral phase HPLC.

[a]Values for meals 1 and 2 from the same animal receiving 1 ml oil 6 and 12 h after surgery.

TABLE 3. Fatty acid composition of diacylglycerol moieties of lymph chylomicron triacylglycerols from a rat fed menhaden oil fatty acids as ethyl esters

Fatty Acids	Meal 1[a]			Meal 2[a]		
	sn-1,2-	*sn*-2,3-	*sn*-1,2(2,3)-	*sn*-1,2-	*sn*-2,3-	*sn*-1,2(2,3)-
	mole %					
14:0	8.7	8.8	8.5	8.1	8.4	8.4
15:0	0.8	0.9	0.7	0.8	0.7	0.7
16:0	21.5	14.8	17.6	22.4	16.5	18.9
16:1n-7	14.4	14.3	14.1	13.2	13.1	13.4
17:0	0.6	0.4	0.6	0.9	0.7	0.6
16:2n-4	2.4	2.8	2.6	2.2	2.6	2.5
18:0	4.1	1.0	2.6	4.4	1.3	2.6
16:3n-4	1.7	3.3	2.6	1.1	2.2	2.2
18:1n-9	11.2	9.5	10.1	11.3	9.8	10.1
18:1n-7	3.8	2.3	3.1	3.6	2.3	2.9
16:4n-1	1.0	2.8	2.0	1.0	2.6	1.8
18:2n-6	8.9	8.6	8.4	9.7	9.7	9.4
18:3n-6	0.4	0.4	0.4	0.3	0.4	0.4
20:0	0.2	0.1	0.1	0.2	0.1	0.1
18:3n-3	1.1	1.4	1.3	1.1	1.4	1.2
20:1n-9	0.7	0.6	0.6	0.8	0.5	0.7
20:1n-7	1.3	1.0	1.1	1.1	0.7	0.9
18:4n-3	2.6	4.4	3.5	2.4	4.1	3.3
20:3n-6	0.2	0.2	0.2	0.2	0.2	0.2
20:4n-6	1.8	2.0	2.0	2.0	2.3	2.1
22:1n-11	0.2	0.1	0.2	0.2	0.1	0.2
20:4n-3	0.9	1.1	1.0	0.8	1.0	1.0
20:5n-3	7.5	12.3	10.5	7.6	12.2	10.1
22:4n-6 + 21:5n-3	0.5	0.7	0.8	0.4	0.6	0.6
22:4n-3	0.2	0.2	0.3	0.2	0.2	0.2
22:5n-3	0.8	1.5	1.3	0.9	1.5	1.3
22:6n-3	2.7	4.7	4.0	3.1	5.0	4.2

sn-1,2(2,3)-Diacylglycerols were from Grignard degradation; *sn*-1,2- and -2,3-diacylglycerols were as resolved by chiral phase HPLC.

[a]Meals 1 and 2 as described in Table 2.

1,2-diacylglycerols (13%) and its relative absence from the phosphatidic acids (2–3%). In contrast, 18:0 and 20:4n-6 are present in much larger amounts in the phosphatidic acid (23% and 8%, respectively), but only in small amounts in the corresponding diacylglycerol moieties (4% and 2%, respectively). Fig. 2B shows that the saturated acids are associated primarily with *sn*-1 position and the unsaturated acids with the *sn*-2 position.

Calculation of positional distribution of fatty acids

Table 4 gives the fatty acid composition of the *sn*-1, *sn*-2, and *sn*-3 positions of the chylomicron triacylglycerols derived from the two oil feedings, as calculated by subtraction of the appropriate fatty acid compositions in Table 2. These values are accurate as seen from the excellent agreement between the reconstituted and the original fatty acid composition of the chylomicron triacylglycerols (reconstitution data not shown). Furthermore, there is an excellent agreement between the results from the two oil feedings. Assuming that the acylation of 2-monoacylglycerols to triacylglycerols is solely responsible for the final structure, it must be concluded that the synthetic process is non-random with the saturated fatty acids (16:0 and 18:0) being preferentially located in the *sn*-1 position and the polyunsaturated fatty acids (16:3, 18:4, 20:5, and 22:6) primarily in the *sn*-3 position. The fatty acids in the *sn*-2 position are closely similar to those found in the 2-monoacylglycerols isolated from the villus cells during the oil absorption (**Fig. 3A**). They are also similar to those in the *sn*-2 position of the original menhaden oil (29). They differ greatly from those of the *sn*-2-monoacylglycerols isolated from the villus cells during the ester feeding (Fig. 3B).

Table 5 gives the fatty acid composition of the *sn*-1, *sn*-2, and *sn*-3 positions of the chylomicron triacylglycerols derived from the two ester feedings. Again there is an excellent agreement between the reconstituted fatty acid composition and that determined directly for the chylomicron triacylglycerols (reconstitution data not shown). Assuming that the acylation of the *sn*-1,2-diacylglycerols derived from the phosphatidic acids was solely responsible for the chylomicron triacylglycerol structure, it would have been anticipated that a close similarity would exist between the triacylglycerols and the phosphatidic acids

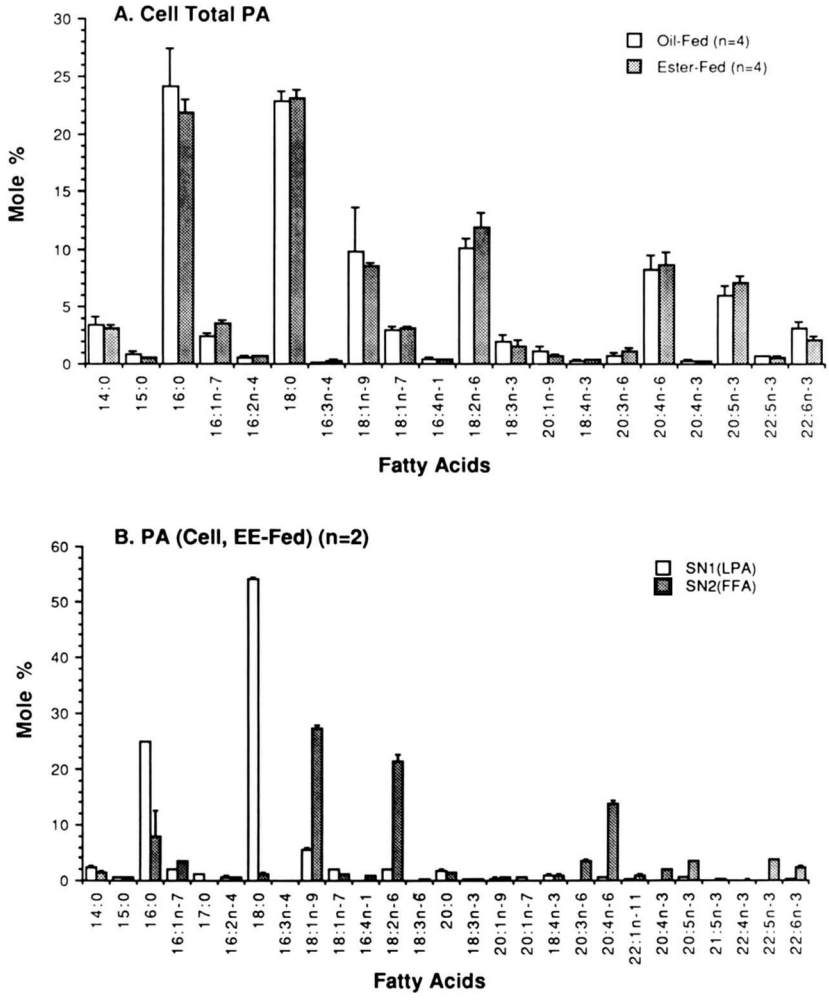

Fig. 2. Fatty acid composition of phosphatidic acid. A: Total fatty acid of phosphatidic acid isolated from intestinal mucosal cells during absorption of menhaden oil or its ethyl esters. B: Positional distribution of fatty acids in phosphatidic acids isolated from mucosal cells during absorption of menhaden oil fatty acid ethyl esters; n, number of animals.

(Fig. 2B) in the fatty acid composition of the *sn*-1 and *sn*-2 positions. There are significant differences. Thus, the *sn*-1 position of the triacylglycerols lacks much of the 18:0 but contains much more of polyunsaturated fatty acids than found in the *sn*-1 position of the phosphatidic acids. The *sn*-1 position of the triacylglycerols contains much more polyunsaturated fatty acids than the *sn*-2 position. Furthermore, much of the 20:4n–6 found in the *sn*-2 position of the phosphatidic acids is not seen in the *sn*-2 position of these triacylglycerols. There is also a large excess of 14:0 in the *sn*-2 position of these triacylglycerols. These results clearly show that the *sn*-1,2-diacylglycerol moieties of the phosphatidic acids cannot be the direct precursors of chylomicron triacylglycerols during ethyl ester absorption.

The data in Tables 4 and 5 allow a comparison between the positional distribution of fatty acids in the chylomicron triacylglycerols recovered from oil and ester feeding. The fatty acids in the *sn*-2 position of triacylglycerols from ester feeding differ from those in the triacylglycerols from oil feeding by a greatly reduced content of the polyunsaturated fatty acids. This is due to the retention of about 85% original fatty acids in the *sn*-2 position of the corresponding chylomicron triacylglycerols. However, there is remarkable similarity in the fatty acid composition between the corresponding *sn*-1 and *sn*-3 positions of the triacylglycerols from the oil and ester feeding. This similarity is best seen after correction for differences in the total fatty acid composition by expressing the results as a positive or negative difference between the composi-

TABLE 4. Positional distribution and overall composition of fatty acids in lymph chylomicron triacylglycerols from a rat fed menhaden oil

Fatty Acids	Meal 1[a]				Meal 2[a]			
	sn-1-	*sn*-2-	*sn*-3-	TG[b]	*sn*-1-	*sn*-2-	*sn*-3-	TG[b]
	mole %							
14:0	10.0	11.8	10.8	10.8	9.8	10.1	8.5	9.5
15:0	0.8	0.9	0.6	0.7	0.6	0.9	0.5	0.6
16:0	23.1	23.5	12.6	18.7	20.0	19.0	10.5	15.7
16:1n-7	13.5	15.4	14.6	14.5	16.1	14.2	15.4	15.1
17:0	0.9	0.6	0.6	0.7	1.2	0.6	1.0	0.6
16:2n-4	1.7	2.7	2.6	2.5	1.9	2.7	2.8	2.5
18:0	6.2	1.3	1.5	2.8	4.8	1.1	1.0	2.2
16:3n-4	−0.2	3.9	1.0	1.6	−0.8	2.6	3.2	1.7
18:1n-9	14.0	11.8	10.9	11.5	13.1	13.7	10.9	12.1
18:1n-7	6.6	2.0	3.6	3.9	6.4	2.4	3.5	4.0
16:4n-1	0.0	2.0	2.7	1.6	−0.3	1.7	2.7	1.6
18:2n-6	3.4	6.0	3.8	3.8	3.9	6.8	4.5	4.5
18:3n-6	0.2	0.3	0.3	0.3	0.2	0.4	0.3	0.3
20:0	0.4	0.1	0.1	0.2	0.2	0.1	0.1	0.1
18:3n-3	0.7	1.4	1.4	1.3	0.9	1.5	1.6	1.4
20:1n-9	2.4	0.5	1.8	1.4	2.4	0.8	1.3	1.5
20:1n-7	0.5	0.1	0.4	0.3	0.6	0.1	0.3	0.3
18:4n-3	0.7	2.9	3.6	2.7	1.4	2.9	4.5	3.0
20:3n-6	0.2	0.2	0.3	0.2	0.2	0.3	0.2	0.2
20:4n-6	1.5	1.3	1.8	1.6	1.6	1.5	2.1	1.8
22:1n-11	0.8	0.1	0.5	0.4	−0.2	0.8	−0.4	0.2
20:4n-3	1.4	0.5	2.2	1.4	1.5	0.8	2.0	1.5
20:5n-3	6.5	4.6	13.1	8.9	7.4	7.2	13.1	10.0
22:4n-6 + 21:5n-3	0.8	0.4	1.1	0.8	0.8	0.6	1.0	0.8
22:4n3	−0.1	0.4	0.2	0.2	0.3	0.3	0.4	0.3
22:5n-3	0.8	1.5	2.1	1.6	1.6	2.1	1.9	1.8
22:6n-3	3.4	4.0	6.1	5.7	4.5	4.8	7.2	6.7

[a]Meals 1 and 2 as described in Table 2. The fatty acid compositions of *sn*-1, *sn*-2, and *sn*-3 positions were determined by subtraction of the fatty acid values in Table 2 and as described in the text.
[b]Original composition of the chylomicron triacylglycerols.

tions of the *sn*-1 and *sn*-3 positions in the triacylglycerols from oil and ester feeding (**Fig. 4A**) and a ratio of *sn*-1/*sn*-1 + *sn*-3 fatty acids in the triacylglycerols (Fig. 4B). There is very little difference in the ratios; only the proportions of the minor fatty acids show noticeable discrepancies which may not be significant. Nearly identical fractions of each major fatty acid are present in both instances.

A comparable similarity is seen in the ratios of fatty acids in positions *sn*-1 + *sn*-3/*sn*-1 + *sn*-2 + *sn*-3 in the chylomicron triacylglycerols from the oil feeding (two meals each, **Fig. 5A**) and from ethyl and methyl ester feeding (Fig. 5B). These results show that the distribution of fatty acids in the outer positions does not differ from meal to meal or from animal to animal after oil or alkyl ester feeding.

From the data in Fig. 3B and Table 5, it can be calculated that at most 20% of the total phosphatidic acid pool could have served as a direct precursor of the *sn*-1,2-diacylglycerols eventually incorporated into the triacylglycerols. The bulk of the diacylglycerols or triacylglycerols originating from the phosphatidic acid pathway, therefore, may have been hydrolyzed to 2-monoacylglycerols by endogenous lipases. The resulting 2-monoacylglycerols would then become subject to reacylation via the 2-monoacylglycerol pathway and secretion as chylomicrons. This possibility is consistent with the isolation of *sn*-2-monoacylglycerols from the villus cells during fatty acid ethyl ester absorption. The fatty acid composition of the cellular *sn*-2-monoacylglycerols is similar to that of the *sn*-2 position of the chylomicron triacylglycerols (Fig. 3B).

DISCUSSION

The existence of two pathways of triacylglycerol biosynthesis in the intestinal mucosa was discovered about 30 years ago, but their relative contributions to the formation of chylomicron triacylglycerols have remained uncertain. Recent work in liver (14) and intestine (15) has suggested that the triacylglycerols arising via the phosphatidic acid

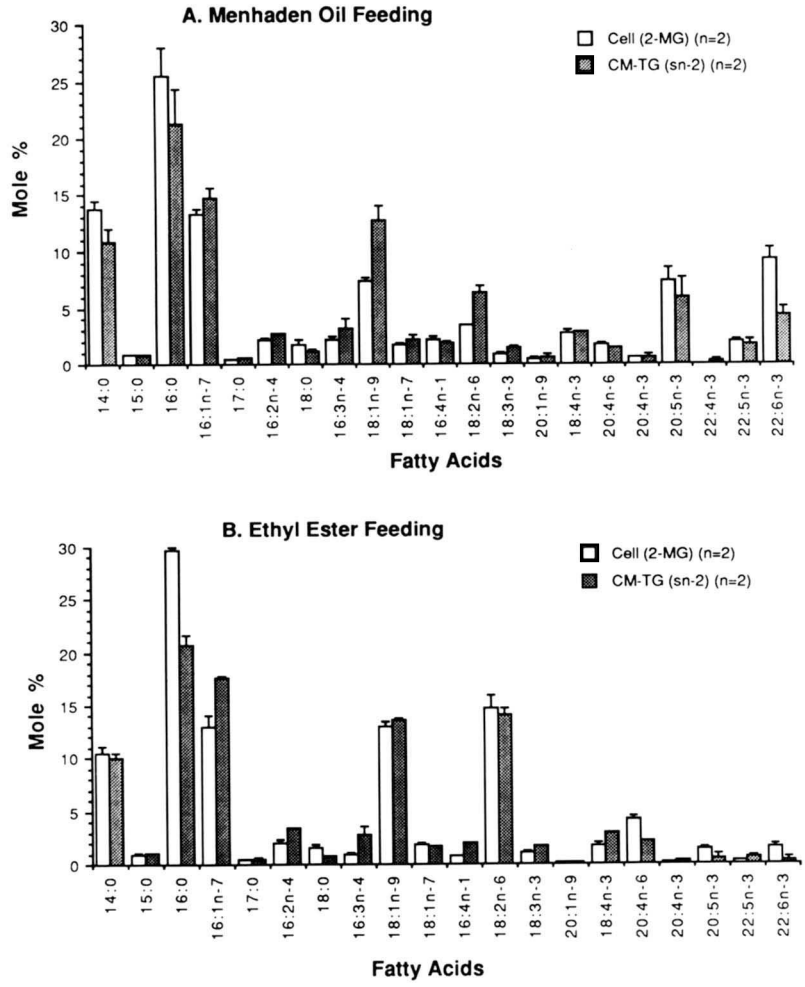

Fig. 3. Fatty acid composition of *sn*-2 position. A: Fatty acids of 2-monoacylglycerols of mucosal cells and of *sn*-2 position of chylomicron triacylglycerols after menhaden oil feeding. B: Fatty acids of 2-monoacylglycerols of mucosal cells and of *sn*-2 position of chylomicron triacylglycerols from menhaden oil fatty acid ethyl ester feeding; n, number of animals for Cell 2-MG; number of meals per animal for CM-TG.

pathway may not be available for direct secretion and may undergo lipolysis prior to reesterification and transfer out of the cell. The pathway responsible for the reesterification has not been investigated. These suggestions, however, are consistent with our recent observation (18) that feeding mustardseed oil or the methyl esters of its fatty acids yields chylomicron triacylglycerols with closely similar fatty acids in the outer positions. We have pointed out that this finding would be consistent with a lipolysis of the products of the phosphatidic acid pathway. In the present study we have used menhaden oil to provide more distinct markers for exogenous fatty acids and 2-monoacylglycerols, and have been able to obtain more direct evidence for the convergence of the phosphatidic acid and monoacylglycerol pathways of intestinal triacylglycerol synthesis at the *sn*-2-monoacylglycerol level.

Monoacylglycerol pathway

The present study shows that the chylomicron triacylglycerols derived from menhaden oil feeding retain much of the fatty acid composition of the *sn*-2 position, as already demonstrated by previous workers with a variety of dietary fats and oils (30). The estimated retention of a maximum of 85% of the 2-monoacylglycerol structure is higher than previously reported because the present values are based on the composition of the 2-monoacylglycerols in the villus cell rather than in the intestinal lumen or in the original oil. The fatty acid compositions of the *sn*-1 and *sn*-3 positions of the lymph triacylglycerols from the oil-fed rat, however, are markedly different and clearly do not represent a random acylation of the 2-monoacylglycerols as concluded from in vitro

TABLE 5. Positional distribution and overall composition of fatty acids in lymph chylomicron triacylglycerols from a rat fed menhaden oil fatty acids as ethyl esters

Fatty Acids	Meal 1[a]				Meal 2[a]			
	sn-1-	*sn*-2-	*sn*-3-	TG[b]	*sn*-1-	*sn*-2-	*sn*-3-	TG[b]
	mole %							
14:0	7.9	9.6	8.1	8.2	5.6	10.5	6.3	7.7
15:0	0.5	1.0	0.7	0.6	0.4	1.1	0.3	0.6
16:0	23.0	19.9	9.7	16.8	23.2	21.6	11.4	18.0
16:1n-7	11.0	17.8	10.8	12.9	8.9	17.6	8.7	12.0
17:0	0.6	0.6	0.2	0.6	1.3	0.5	0.9	0.6
16:2n-4	1.3	3.4	2.2	2.3	1.0	3.4	1.7	2.1
18:0	7.3	0.9	1.2	3.1	8.1	0.8	1.8	3.2
16:3n-4	– 0.2	3.6	3.0	2.3	0.3	1.9	2.4	2.3
18:1n-9	8.5	13.8	5.2	8.9	9.1	13.5	6.0	9.0
18:1n-7	6.0	1.7	3.0	3.5	5.5	1.7	2.9	3.4
16:4n-1	0.0	2.0	3.5	2.0	0.0	2.0	3.3	1.8
18:2n-6	4.4	13.3	4.0	6.7	4.7	14.7	4.7	7.7
18:3n-6	0.2	0.5	0.3	0.3	0.2	0.5	0.3	0.3
20:0	0.2	0.1	0.1	0.2	0.3	0.1	0.1	0.2
18:3n-3	0.6	1.7	1.1	1.2	0.6	1.6	1.1	1.1
20:1n-9	1.3	0.1	1.0	0.8	1.5	0.2	0.7	0.8
20:1n-7	2.4	0.2	1.7	1.4	1.9	0.2	1.2	1.1
18:4n-3	2.2	2.9	5.9	3.7	1.8	3.0	5.3	3.4
20:3n-6	2.2	– 1.8	2.2	0.9	0.2	0.1	0.3	0.2
20:4n-6	1.5	2.6	1.9	1.9	1.9	2.1	2.5	2.2
22:1n-11	0.3	0.1	0.2	0.2	0.4	0.1	0.2	0.2
20:4n-3	1.6	0.2	1.9	1.3	1.2	0.3	1.7	1.2
20:5n-3	11.7	3.3	21.2	12.9	13.0	2.2	22.2	12.8
22:4n-6 + 21:5n-3	0.1	0.9	0.5	0.7	0.9	0.0	1.3	0.8
22:4n-3	– 0.4	0.7	– 0.4	0.2	0.4	– 0.1	0.5	0.4
22:5n-3	0.9	0.7	2.3	1.4	1.3	0.5	2.6	1.5
22:6n-3	4.8	0.6	8.7	5.1	6.2	0.1	9.7	5.5

[a]Meals 1 and 2 as described in Table 2. The fatty acid compositions of *sn*-1, *sn*-2, and *sn*-3 positions were determined by subtraction of the fatty acid values in Table 3 and as described in the text.
[b]Original composition of the chylomicron triacylglycerols.

studies (31). This is probably due to the much larger variety of fatty acids available for selection by the acyltransferases. Whether the acylation proceeded via both *sn*-1,2- and *sn*-2,3-diacylglycerols as intermediates and to what extent this may have contributed to the difference in the composition of the outer positions of the final triacylglycerols was not critical at this time. There is clearly a preference for the introduction of the saturated and monounsaturated fatty acids in the *sn*-1 position as observed for the chylomicron triacylglycerols from mustardseed oil feeding. As a result, the bulk of the polyunsaturated fatty acids became esterified to the *sn*-3 position. The triacylglycerol end products of the monoacylglycerol pathway are therefore highly asymmetric, much like the original dietary menhaden oil (29).

Phosphatidic acid pathway

In the absence of dietary 2-monoacylglycerols, the free fatty acids from lumenal hydrolysis of the alkyl esters of menhaden oil fatty acids are still effectively incorporated into chylomicrons. However, a precursor-product relationship between the phosphatidic acid *sn*-1,2-diacylglycerols and the chylomicron triacylglycerols has not been demonstrated. A comparison of the positional distribution of the fatty acids in the phosphatidic acids and chylomicron triacylglycerols in the present study shows significant differences that clearly exclude a direct precursor-product relationship. The absence of such a relationship between the fatty acid compositions is still compatible with the demonstrated incorporation of radiolabeled glycerol into intestinal triacylglycerols as shown by previous workers (1, 2) but these triacylglycerols may have been destined for cytoplasmic storage. The present results are best explained by a hydrolysis of such endogenous triacylglycerols to 2-monoacylglycerols and a reesterification via 2-monoacylglycerols without loss of the glycerol moiety. This possibility is supported by the similarity in the fatty acid composition between the *sn*-1 and *sn*-3 positions of the triacylglycerols resulting from the oil and ester feeding, and by the recovery of 2-monoacylglycerols from the villus cells during the assimilation of the fatty acid ethyl esters.

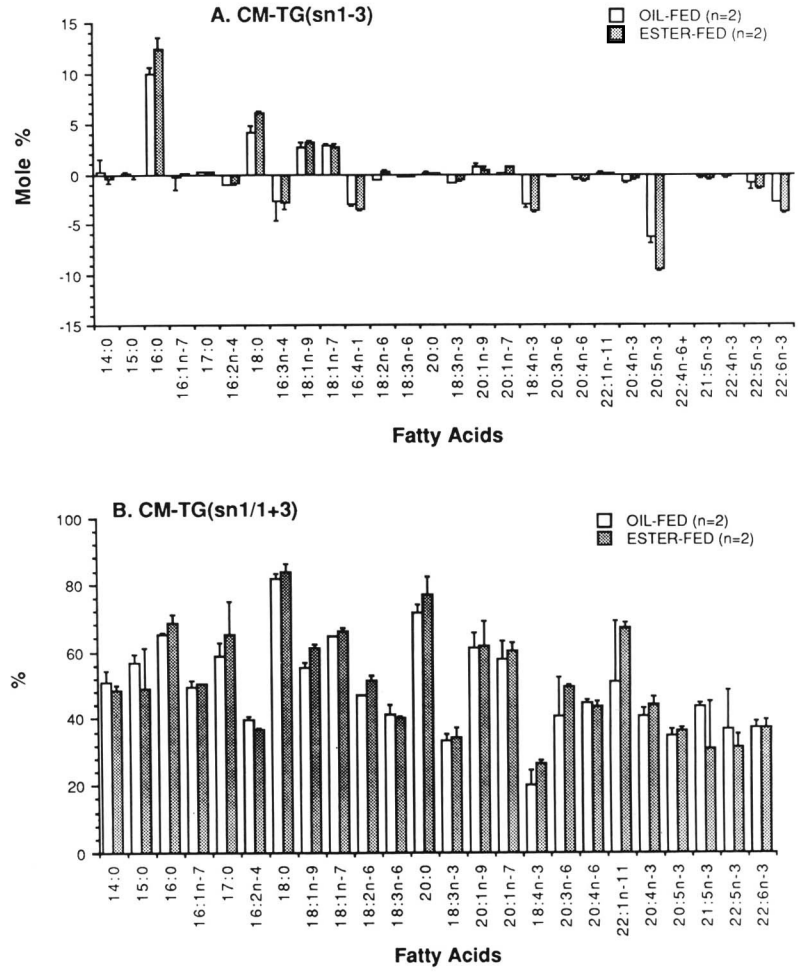

Fig. 4. Positional distribution of fatty acids in triacylglycerols. A: Differences in fatty acids between the *sn*-1 and *sn*-3 positions of chylomicron triacylglycerols from absorption of menhaden oil or its fatty acid ethyl esters. B: Ratio of fatty acids in the *sn*-1 position over the sum of fatty acids in *sn*-1 and *sn*-3 position of chylomicron triacylglycerols from oil and ester feeding; n, number of meals per animal.

There are several candidate intestinal lipases for a diacylglycerol degradation, although they attack preferentially short chain species (32). Therefore, a more likely possibility is that the diacylglycerols arising via the phosphatidic acid pathway are acylated via a diacylglycerol acyltransferase to triacylglycerols that go first into cytosolic storage and must be hydrolyzed prior to secretion as already claimed for the cytoplasmic triacylglycerols in the liver (10–14). The hepatic triacylglycerols synthesized via the phosphatidic acid pathway are believed to be hydrolyzed by an acid lipase present in the lysosomes, which participate in the secretion of VLDL triacylglycerols from the liver (14). It has been shown that triacylglycerols in the liver and intestine accumulate in the lysosomes in the absence of acid lipase (Wolman disease) (33) or in the presence of chloroquine (14, 15). The possibility that the endogenous triacylglycerols are hydrolyzed only to the diacylglycerol stage would appear to be less likely because the intestinal cells do not contain triacylglycerol lipases that yield diacylglycerols as the final reaction products (34, 35). The proposed convergence of the 2-monoacylglycerol and the phosphatidic acid pathways of intestinal triacylglycerol synthesis is consistent with the demonstration that the chylomicrons derived from oil and ester feeding possess identical surface apolipoprotein (19) and phospholipid (36, 37) composition. Furthermore, the chylomicron triacylglycerols arising from the ester feeding are smaller in size (19) apparently resulting from a slower rate of absorption (17).

Limitation of study

The study could be improved by using stable isotope-labeled markers for free fatty acids and 2-monoacylglycerols in combination with gas or liquid chromatography

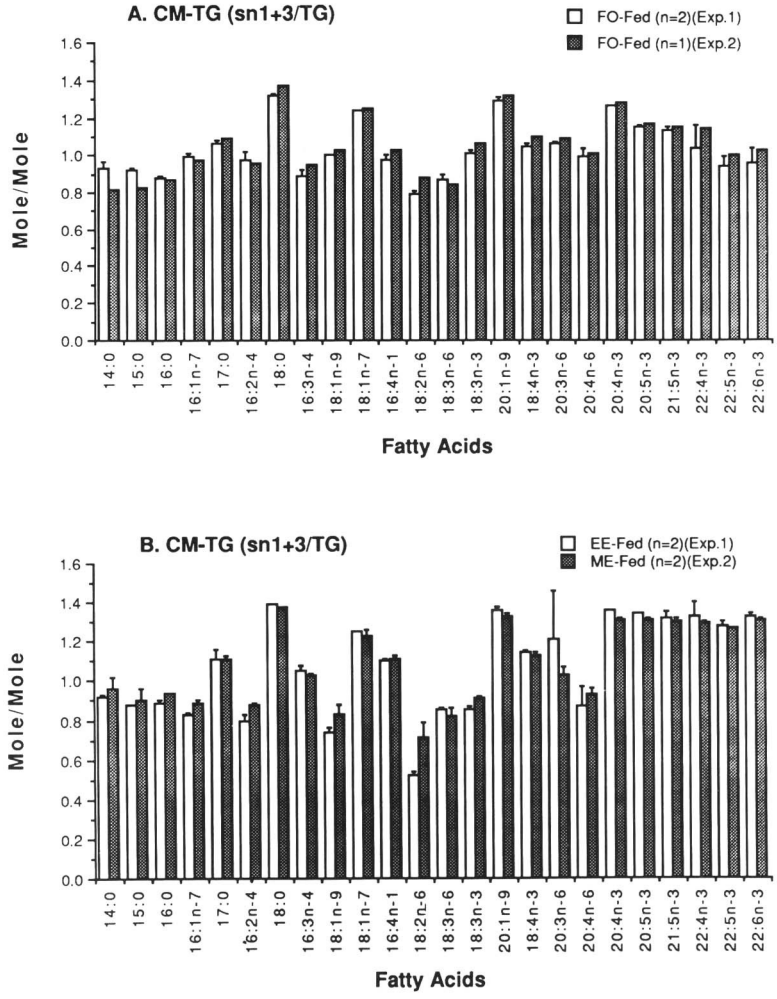

Fig. 5. Positional distribution of fatty acids in triacylglycerols. A: Ratio of fatty acids in the outer positions over the total fatty acids in chylomicron triacylglycerols during absorption of menhaden oil. The fatty acids of *sn*-2 position were determined by pancreatic lipase hydrolysis. B: Ratio of fatty acids in outer positions over fatty acids in total chylomicron triacylglycerols during absorption of menhaden oil fatty acid methyl or ethyl esters. Exp. 1 and Exp. 2, one pair of rat fed oil and esters each; n, number of meals.

and mass spectrometry. Likewise, a more extensive subcellular fractionation might have been helpful, although the separation of the different microsomal, lysosomal, and Golgi membranes is difficult. Alternatively, villus cell or intestinal tissue cultures capable of chylomicron synthesis and secretion could be considered for future work.

Conclusions

The most important aspect of the present investigation is the finding of a plausible linkage at the monoacylglycerol level of the two pathways of triacylglycerol biosynthesis, which in the past have been assumed to operate independently. The new results help to explain the similarity in the surface lipid and apolipoprotein composition of the chylomicrons generated via the phosphatidic acid and the monoacylglycerol pathways. The present data also reconcile the synthesis and secretion of the neutral lipid products of the phosphatidic acid pathway by the liver and the intestine. Finally, the present work may help to better understand Wolman's disease, which is a congenital condition lacking lysosomal acid lipase. In the past this disease has been explained on basis of lysosomal storage of cholesteryl esters, even though the storage of triacylglycerols is also very serious and occurs despite their essential absence from low density lipoproteins.

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