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Lipid structure of rat adipocyte plasma membranes following dietary lard and fish oil

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Abstract

We have determined the changes in the lipid structure of the adipocyte plasma membranes of rats receiving lard or fish oil in their diet. For this purpose, mature Wistar rats were fed 20% (w/w) lard or fish oil diets for 22 days, when the plasma membranes of the epididymal and perirenal adipocytes were prepared. Detailed analysis of the membrane lipids by chromatographic methods showed that dietary fat exerted a major effect on the lipid class and molecular species composition of the phospholipids. As a result of fish oil feeding, significant increases in the 20:5($n-3$), 22:5($n-3$) and 22:6($n-3$) were detected in all glycerophospholipid classes, while the 18:1($n-9$) and 18:2($n-6$) and to a lesser extent 20:4($n-6$) decreased. Incorporation of $n-3$ fatty acids increased the phosphatidylcholine/sphingomyelin ratio without changing the total phospholipid or free cholesterol content of the membrane. Fish oil feeding also caused a marked increase in the proportion of 24:1 in sphingomyelins, which occurred mainly at the expense of 18:0 and 24:0. New $n-3$ fatty acid-containing species appeared in the choline and ethanolamine glycerophospholipids, when compared to membrane lipids from lard-fed rats. Membranes from fish oil fed rats also had moderately higher levels of ether lipids. Few differences were seen between the membranes of the epididymal and perirenal adipocytes. It is concluded that dietary fish oils modify the lipid structure of rat adipocyte plasma membranes by increasing the ratio of phosphatidylcholine to sphingomyelin and by increasing the proportion of molecular species with polyunsaturated fatty acids, which would be anticipated to increase the fluidity of the lipid bilayer of adipocyte plasma membranes.

Keywords: Lipid class; Fatty acid, $n-3$; Molecular species

1. Introduction

Many of the general beneficial effects of dietary intake of marine $n-3$ fatty acids have been attributed to their incorporation into membrane phospholipids. Such effects include the antithrombic inhibition of platelet aggregation [1], the hypotriglyceridemic inhibition of VLDL secretion [2], improved membrane fluidity in diabetics [3] and prevention of

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insulin resistance induced by high fat feeding [4]. We have observed specific changes in adipocyte function with fish oil feeding, including modification of HDL binding to plasma membranes, enhancement of hormone-stimulated lipolysis and limitation of adipocyte hypertrophy [5–7]. On the basis of physico-chemical studies with artificial lipid membranes, these changes in function of cells and tissues could have resulted from alterations in the lipid composition of the cell membranes caused by fish oil consumption. The present study provides the first detailed description of the molecular species of phospholipids in adipocyte plasma membranes, and a documentation of the effects of dietary fish oils on the molecular structure of adipocyte plasma membranes.

2. Materials and methods

2.1. Animals and diets

Seven male Wistar rats were obtained every two weeks from Charles River (Saint Constant, Quebec) over a period of 6 weeks. The three groups of 7 rats were fed laboratory chow (Purina Mills, St. Louis, MO) until the average weight of the rats in each group was close to 250 g. Each group was then transferred to a Teklad (Madison, WI) powder-based diet for 3 weeks. Three rats in each group were fed a 20% lard supplemented diet; the remaining four rats in each group were fed a 20% fish oil (MaxEPA: R.P. Scherer, Windsor, Ontario) diet. The fatty acid composition of the diets is given in Table 1. To minimize oxidation, the commercial fish oil preparation was stored in the dark, at 4°C, under nitrogen, and in separate containers, and diets were freshly mixed three times weekly.

Table 1
Fatty acid composition (mol%) of the fish oil and lard supplemented diets

Fatty acids	Lard diet	Fish oil diet
14:0	2.0 ± 0.12	11.3 ± 0.21
15:0		0.8 ± 0.06
16:0	27.8 ± 2.26	24.5 ± 0.65
16:1($n-7$)	2.2 ± 0.47	11.1 ± 1.07
16:3($n-4$)		1.3 ± 0.09
16:4($n-1$)		2.4 ± 0.06
18:0	16.6 ± 0.17	4.0 ± 0.10
18:1($n-9$)	39.4 ± 0.51	9.6 ± 0.12
18:1($n-7$)		3.5 ± 0.10
18:2($n-6$)	10.7 ± 2.10	1.6 ± 0.06
18:3($n-3$)	0.4 ± 0.06	0.6 ± 0.06
18:4($n-3$)		2.7 ± 0.18
20:0	0.2 ± 0.17	
20:1($n-9$)	0.7 ± 0.15	1.4 ± 0.26
20:4($n-6$)		0.6 ± 0.06
20:4($n-3$)		0.6 ± 0.06
20:5($n-3$)		14.2 ± 0.76
21:5($n-3$)		0.6 ± 0.04
22:1($n-11$)		0.4 ± 0.03
22:4($n-3$)		0.3 ± 0.02
22:5($n-3$)		1.2 ± 0.08
22:6($n-3$)		7.1 ± 0.20
24:1		0.3 ± 0.06

2.2. Isolation of adipocytes

After 22 ± 3 days on the 20% fat diets the rats were anaesthetized with diethyl ether and the epididymal and perirenal fat pads were removed. The fat from the lard-fed rats in each group was pooled, as was the fat from the fish oil fed rats in each group. Thus a total number of twelve adipose samples were obtained from the 21 rats: 3 per fat pad site per diet. Adipocytes were released from these samples by incubating with collagenase from *Clostridium histolyticum* in Krebs–Ringer bicarbonate buffer for 1 h at 37°C [8].

2.3. Preparation of plasma membranes

Adipocyte plasma membranes from epididymal and perirenal adipocytes were isolated, purified and assayed with procedures routinely used for human and rat adipocyte plasma membranes [8,9]. Such procedures ensure negligible mitochondrial, lysosomal, and microsomal contamination of membrane preparations. For the membranes whose lipid composition is described here there was a 5.1 ± 0.4 fold enrichment in 5′-nucleotidase activity by comparison with the cell homogenate. Literature values for the purification ratios of adipocyte plasma membranes range from 3- to 16-fold depending on the isolation procedure and the plasma membrane biochemical marker assayed [8–12].

2.4. Lipid extraction

5 μg of the internal standard, tridecanoin [13] was added to a measured amount of membrane (about 100

μg of membrane protein) from each of the twelve samples. The sample and internal standard were then extracted together in 4 ml of cold $CHCl_3$-methanol (2:1). 1 ml of cold 0.9% NaCl in water was added to the extract and the $CHCl_3$ layer was removed and evaporated under nitrogen at 25°C.

2.5. Lipid class separation and quantification

The lipid extract was redissolved in 50 μl of $CHCl_3$ and spotted on an activated silica gel H coated plate. The vial containing the extract was then rinsed with 50 μl of $CHCl_3$-methanol (2:1) and this was also spotted on the same band. Thin layer chromatographic (TLC) separation of lipid classes was effected by developing in $CHCl_3$-methanol-acetic acid-water (58:27:11:4, by vol.). Dichlorofluorescein was used to visualize the neutral lipid band and the four major phospholipid bands, phosphatidylethanolamine (PE), phosphatidylcholine (PC), sphingomyelin (Sph), phosphatidylserine (PS) and phosphatidylinositol (PI). The phospholipid classes were quantified by measuring the fatty acid content of each band.

2.6. Preparation and measurement of fatty acids and dimethylacetals

After TLC separation, 5 μg of the internal standard, heptadecanoic acid, was added to each of the phospholipid bands in 5 μl of hexane. The bands were then scraped into individual tubes and fatty acid methyl esters (FAME) were prepared by transmethylation with 6% (v/v) H_2SO_4 in methanol [14]. The FAME were extracted into hexane and separated by gas chromatography (GC) on polar (RTX2330) and non-polar (5% phenyl methyl silicone) capillary columns. The second GC analysis was performed to compensate for any injector discrimination against higher molecular weight FAME in the first GC analysis.

2.7. Membrane cholesterol determination

The neutral lipid TLC band was scraped into a tube and extracted three times with 1 ml of $CHCl_3$. After silylation of the neutral lipids a lipid profile [13] was determined by gas chromatography on a non-polar (5% phenyl methyl silicone) capillary column.

2.8. Analysis of molecular species

A second sample of each of the adipocyte plasma membrane preparations was extracted in cold $CHCl_3$-methanol (2:1) as above except no internal standard was added. After TLC separation the phosphatidylethanolamine and phosphatidylcholine bands were extracted with $CHCl_3$-methanol and then $CHCl_3$. The triplicate samples from the three groups were then combined to ensure sufficient sample for subsequent analyses [14]. The combined phospholipid class samples were dried and redissolved in 20 μl of methanol. 2 ml of diethyl ether was then added to each sample followed by 10 IU of phospholipase C from *Bacillus cereus* (Sigma type XIII) in 1 ml of 250 mM Tris-HCl buffer (pH 7.7). To this was added 1 ml of 20 mM $CaCl_2$ in Tris-HCl. The mixture was incubated under nitrogen for 2 h at 29°C. The hydrolysed lipids were extracted twice with $CHCl_3$ and, after solvent removal, converted into trimethylsilyl ethers by reacting for 30 min at room temperature with hexamethyldisilazane and trimethylchlorosilane in pyridine [14].

Each sample was analyzed by gas chromatography with flame ionization detection (FID) or GC with mass spectrometry (MS). GC/FID separations were performed on polar (RTx2330) and non-polar (SE-54) capillary columns [14,15]. GC/MS was performed on a Hewlett–Packard Model 5985B quadrupole mass spectrometer directly coupled to a Hewlett–Packard Model 5840A gas chromatograph equipped with a non-polar (SE-54) capillary column [14].

The diradylglycerol peaks obtained by separation on the polar column were identified by comparing with standards and calculation of retention factors [16], and by ammonia or methane chemical ionization mass spectrometry of the components resolved on the non-polar column. To compensate for any injector discrimination the sum of the species with a given carbon number was corrected to the area obtained for that carbon number after GC analyses on the non-polar column. Peak identification and quantitation was also verified by comparing with fatty acid methyl ester and dimethylacetal data from the same samples. The calculated molar distribution of fatty acid methyl

esters and dimethylacetals differed from the observed distribution usually by less than 2 mol%.

2.9. Statistics

Data are reported as mean ± standard deviation; Student's *t*-tests were used to compare means.

3. Results and discussion

3.1. Effect of diet on lipid class composition

Lipid class composition of membranes is an important determinant of physical structure [17]. Table 2 gives the content of phospholipids and free cholesterol of rat adipocyte plasma membranes following lard and fish oil feeding. The latter increased the proportion of phosphatidylcholine and the phosphatidylcholine/sphingomyelin ratio in adipocyte plasma membranes without significantly altering the lipid class/protein ratios. Cholesterol and sphingomyelin generally have a condensing effect on membrane bilayers [18]. Hence membrane lipids from fish oil fed rats would be expected to contain more free cholesterol and sphingomyelin than the corresponding membranes from lard-fed rats, in order to compensate for any loosening of the bilayer due to increased content of polyunsaturated fatty-containing molecular species of glycerophospholipids [19]. This was not observed here; however, the increased phosphatidylcholine proportion and phosphatidylcholine/sphingomyelin ratio are in agreement with increased proportions of phosphatidylcholine in human platelets following fish oil supplementation [20] which was attributed to increased synthesis. Since previous work [17] has shown that red blood cell shape is influenced by changes in phosphatidylcholine/free cholesterol and phosphatidylcholine/sphingomyelin ratios, it is possible that fish oil feeding may also have brought about changes in physico-chemical properties of adipocyte plasma membranes.

In phosphatidylethanolamine and phosphatidylcholine from epididymal and perirenal membranes the level of the plasmalogen subclass was 2–3 mol% higher in samples from fish oil fed rats (Tables 3 and 4). This modest increase in total plasmalogens may also help impart physical and chemical stability [21].

3.2. Effect of dietary fat on fatty acid and dimethylacetal composition

The different glycerophospholipid classes in the adipocyte plasma membranes differ markedly in their fatty acid composition, as well as in their response to different dietary fats. The largest change took place in the content of 20:5($n-3$) and 22:6($n-3$) acids.

Table 2
Effect of dietary fat on lipid class composition of 12 samples of adipocyte plasma membranes prepared from 21 rats

Lipid class	Epididymal		Perirenal	
	lard-fed	fish oil-fed	lard-fed	fish oil-fed
	nmol/μg protein			
Cholesterol	0.39 ± 0.08	0.37 ± 0.02	0.40 ± 0.08	0.33 ± 0.05
Phospholipid	1.29 ± 0.31	1.28 ± 0.09	1.32 ± 0.37	1.32 ± 0.31
	mol% total phospholipid			
PC	34.9 ± 4.3 [a]	42.3 ± 4.0 [b]	37.8 ± 6.4	42.4 ± 2.8 [b]
PE	23.1 ± 0.6	22.6 ± 1.6	23.7 ± 1.4	25.0 ± 1.6
PS + PI	15.5 ± 1.3 [a]	14.4 ± 0.6	15.0 ± 1.9	12.8 ± 1.0 [b]
SPH	26.6 ± 3.6	20.6 ± 2.0	23.4 ± 4.0	19.8 ± 2.8
	mol/mol			
PL/FC	3.5 ± 1.1	3.4 ± 0.2 [a]	3.4 ± 0.8	3.9 ± 0.4 [b]
PC/FC	1.2 ± 0.5	1.5 ± 0.2	1.3 ± 0.5	1.7 ± 0.2
PC/SPH	1.3 ± 0.3 [a]	2.1 ± 0.4 [b]	1.7 ± 0.5 [a]	2.2 ± 0.5

Means in the same row with different superscripts are significantly different ($P < 0.05$). FC: free cholesterol.

Table 3
Molecular species (polar capillary GLC) of phosphatidylethanolamine in rat adipocyte plasma membranes following dietary lard and fish oil

Molecular species	Peak #	Epididymal		Perirenal	
		lard	fish oil	lard	fish oil
14:-16: *			0.07		0.04
16:0-16:0	1	0.53	0.28	0.58	0.75
16:0-16:1($n-7$)	2	0.15	0.29	0.37	0.25
16:0″-20:4($n-6$)	4	10.27	5.13	7.98	3.77
16:0-18:1($n-9$)	5	3.66	1.99	5.23	2.82
16:0″-20:5($n-3$)	6		3.04		3.13
16:0-18:2($n-6$)	7	4.74	2.76	4.06	2.18
18:0″-20:4($n-6$)	9	3.33	1.24	2.43	0.72
18:1″-20:4($n-6$)	10	2.23	0.40	1.42	0.24
18:0-18:1($n-9$)	11	4.90	4.45	5.54	4.37
18:1($n-9$)-18:1($n-9$)	12	8.65		10.22	
16:0″-22:5/22:6($n-3$)	12		5.51		4.43
18:0-18:2($n-6$)	13	10.51	4.97	10.10	4.37
16:0-20:4($n-6$)	14	4.94	5.58	4.28	4.28
18:1($n-9$)-18:2($n-6$)	15	6.41		7.92	
16:1($n-7$)-20:4($n-6$)	16	1.82	2.22	1.76	2.55
16:0-20:5($n-3$)	17		3.53		3.57
18:2($n-6$)-18:2($n-6$)	17	1.66		1.93	
18:0″-22:5/22:6($n-3$)	20		2.45		2.76
18:0-20:4($n-6$)	22	19.43	16.62	18.69	12.97
18:1($n-9$)-20:4($n-6$)	23	9.31	3.02	9.42	2.99
18:1($n-7$)-20:4($n-6$)	24	1.89		1.83	
18:0-20:5($n-3$)	25		5.91		6.36
16:0-22:5/22:6($n-3$)	26	1.43	8.02	1.24	9.46
18:1($n-9$)-20:5($n-3$)	27		0.80		1.74
18:1($n-7$)-20:5($n-3$)	28		0.48		0.80
16:1($n-7$)-22:5/22:6($n-3$)	29		0.75		1.69
18:0-22:5/22:6($n-3$)	30	1.80	11.12	2.34	12.85
18:1($n-9$)-22:5/22:6($n-3$)	31		2.25		3.18
18:1($n-7$)-22:5/22:6($n-3$)	32		1.07		1.09
20:-22: *		0.12	0.52	0.19	0.54
Other		2.22	5.53	2.47	6.10

Data are mol% composition of membranes pooled from 9 lard fed rats and 12 fish oil fed rats. Peak # refers to separations on the RTX2330 capillary column (Fig. 2); C30 and C42 species (*) were identified on a non-polar column only. ″ designates the alk-1-enyl moiety in plasmalogens.

Rats fed fish oil-supplemented diets had higher contents of 20:5($n-3$) and 22:6($n-3$) compared with rats fed lard as a control, especially in phosphatidylethanolamine (Fig. 1a), which took up more of these fatty acids than other phospholipid classes (Fig. 1). This change took place largely at the expense of the 18:1($n-9$) and 18:2($n-6$) fatty acids, with less alteration being seen in the proportion of 20:4($n-6$). Similar changes were seen in glycerophospholipid classes of perirenal membranes.

The glycerophospholipids of the adipocyte membrane were, however, quite selective in the incorporation of dietary polyunsaturated fatty acids. Thus, 16:3($n-4$), 16:4($n-1$), and 18:4($n-3$), which are present in the fish oil diet at levels above 1 mol% (Table 1), were not detected in any plasma membrane glycerophospholipids. In contrast, 16:1($n-7$) was readily incorporated (Fig. 1a, b and c), as were 18:1($n-7$) and even 18:1″($n-7$), when compared to lard-fed rats. Dietary fish oil also increased levels of monounsaturated fatty acids of the $n-7$ series in liver microsomes [22].

Sphingomyelins of epididymal and perirenal membranes were also affected, although this lipid class did not appear to take up polyunsaturated fatty acids (Fig. 1d). Interestingly, fish oil feeding led to a large increase in the 24:1 content of the sphingomyelins of the adipocyte plasma membranes, although this fatty acid was only a minor component of the fish oil. This observation is consistent with that of Ahmed and Holub [23] who found a 7% increase in 24:1 in the sphingomyelin of human platelets after ingestion of cod liver oil, which also contains little 24:1. Synthesis of 24:1 from saturated fatty acids seems unlikely, since polyunsaturated fatty acids, including those found in fish oils, selectively inhibit monoene formation [22,24]. Since fish oils contain significant amounts of monoenoic fatty acids, 24:1 could be derived from them by chain elongation [24] as well as direct incorporation [25]. Several positional isomers of 24:1 are known to occur in man, rodents and fish, of which the most prominent are 24:1($n-9$) and 24:1($n-7$) [26,27]. In earlier experiments with safflower oil fed rats [9], we found significant increases in 22:0 and 24:0 content of sphingomyelins of adipocyte plasma membranes.

The ratio of polyunsaturated to saturated fatty acids of adipocyte phospholipids has been used previously as an indicator of membrane fluidity [9,28,29]. In dietary studies with fish oils this ratio may be a poor indicator. The P/S ratio of fatty acids in diacylglycerophospholipids from fish oil fed rats was slightly reduced despite the large incorporation of 20:5($n-3$) and 22:6($n-3$) fatty acids into membrane lipids. This was due to the fact that in the calculation of P/S ratios all fatty acids with two or more double bonds are given equal weighting. When the actual number of double bonds per fatty acid molecule were considered, epididymal and perirenal membranes from fish oil fed rats possessed a much

Table 4
Molecular species (polar capillary GLC) of phosphatidylcholine in rat adipocyte plasma membranes following dietary lard and fish oil

Molecular species	Peak	Epididymal		Perirenal	
		lard	fish oil	lard	fish oil
14:-16: *		0.96	0.55	0.51	0.81
16:0-16:0	1	1.33	3.62	1.39	4.08
16:0-16:1($n-7$)	2	0.88	3.03	1.11	3.47
16:0″-20:4($n-6$)	4	0.35	1.95	0.24	2.05
16:0-18:1($n-9$)	5	13.02	10.97	17.46	13.02
16:0-18:2($n-6$)	7	11.27	8.38	12.35	9.00
18:0″-20:4($n-6$)	9	0.45	0.37	0.34	0.32
18:0-18:1($n-9$)	11	11.91	7.42	13.53	8.89
18:1($n-9$)-18:1($n-9$)	12	3.54		4.85	
16:0″-22:5/22:6($n-3$)	12		0.56		0.70
18:0-18:2($n-6$)	13	23.15	11.72	20.62	10.66
16:0-20:4($n-6$)	14	5.56	7.50	5.48	5.49
18:1($n-9$)-18:2($n-6$)	15	2.97		3.01	
16:1($n-7$)-20:4($n-6$)	16	0.74		0.13	
18:2($n-6$)-18:2($n-6$)	17	1.28		0.76	
16:0-20:5($n-3$)	17		3.74		3.67
18:0-20:4($n-6$)	22	16.07	16.74	14.71	12.69
18:1($n-9$)-20:4($n-6$)	23	2.50	1.46	1.24	1.39
18:1($n-7$)-20:4($n-6$)	24	0.99	1.00	0.34	0.74
18:0-20:5($n-3$)	25		5.09		5.84
16:0-22:5/22:6($n-3$)	26		3.97		4.69
18:1($n-9$)-20:5($n-3$)	27		0.49		0.40
18:1($n-7$)-20:5($n-3$)	28		0.46		0.40
18:0-22:5/22:6($n-3$)	30	1.16	6.52	1.16	7.58
Other		1.87	4.46	0.77	4.11

* C30 species identified on a non-polar column only.

higher number of double bonds per fatty acid molecule than membranes from lard-fed rats (e.g., 1.83 vs. 2.20 and 1.64 vs. 2.22 for epididymal and perirenal membrane phosphatidylethanolamine). This increase, should in turn relate directly to membrane fluidity [30].

Consumption of fish oil supplemented diets increased the average chain length of the fatty acids in all glycerophospholipid classes in both types of plasma membranes, when compared to lard supplementation. The largest increase in average chain length was seen in phosphatidylethanolamine, where replacement of 18:1 and 18:2 fatty acids by 20:5($n-3$) and 22:6($n-3$) was the most extensive, rather than replacement of 20:4($n-6$) as observed in platelets of man [1,23,31]. The ethanolamine glycerophospholipids of the adipocyte plasma membrane contained significant amounts of 16:0″ along with 18:0″ and 18:1″. The 16:0″ content appeared to increase slightly upon fish oil feeding. This again indicates an increase in the plasmalogen content, which may promote membrane stabilization.

3.3. Effect of dietary fat on molecular species of glycerophospholipids

Ethanolamine glycerophospholipids exhibit the most complex profiles on account of the relatively high content of plasmalogens. Fig. 2 shows the resolution of diradylglycerol moieties of ethanolamine glycerophospholipids of epididymal plasma membranes of lard-fed (Fig. 2a) and fish oil fed (Fig. 2b) rats. Although both alkenylacyl and diacylglycerol species are present in significant amounts, most of the components are completely resolved. The peaks were identified on the basis of established retention factors [16] and knowledge of fatty acid composition of the samples. Similar elution profiles were obtained for molecular species of the diacyl- and alkenylacylglycerols of ethanolamine phosphatides of plasma

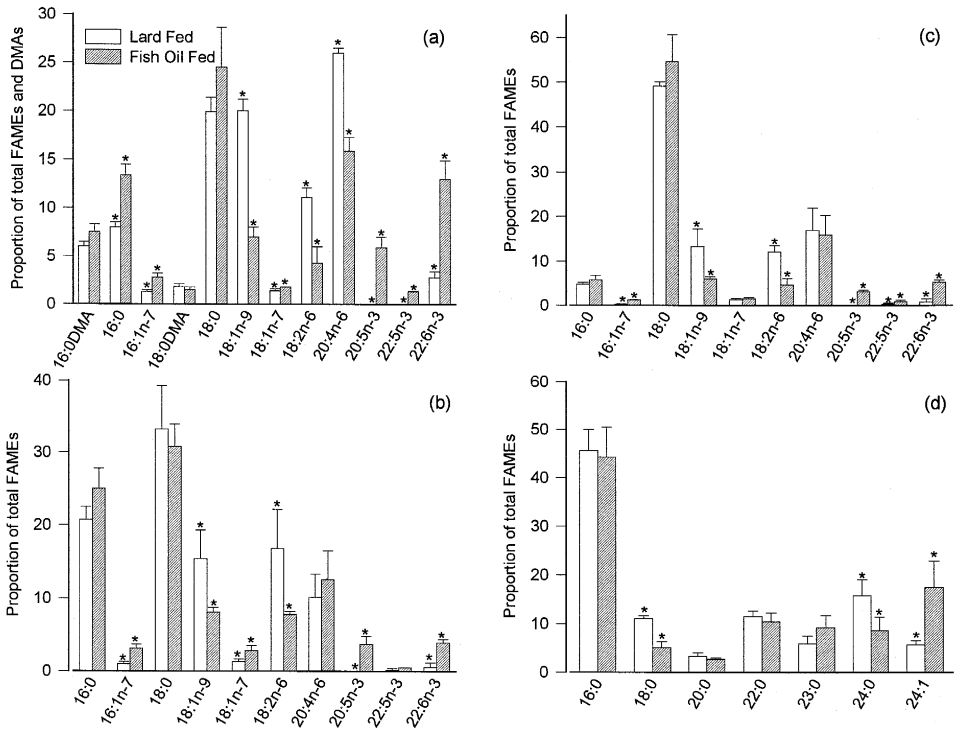

Fig. 1. Fatty acid methyl esters and dimethylacetals derived from epididymal adipocyte plasma membranes. (a) phosphatidylethanolamine, (b) phosphatidylcholine, (c) phosphatidylserine and phosphatidylinositol, and (d) sphingomyelin. FAME and DMA data are mean ± S.D. (mol%) of three separate preparations of plasma membranes for each diet. Only FAME or DMA contributing more than about 0.5 mol% to the total are included. Means for the same FAME or DMA that are significantly different ($P < 0.05$) are indicated with an asterisk.

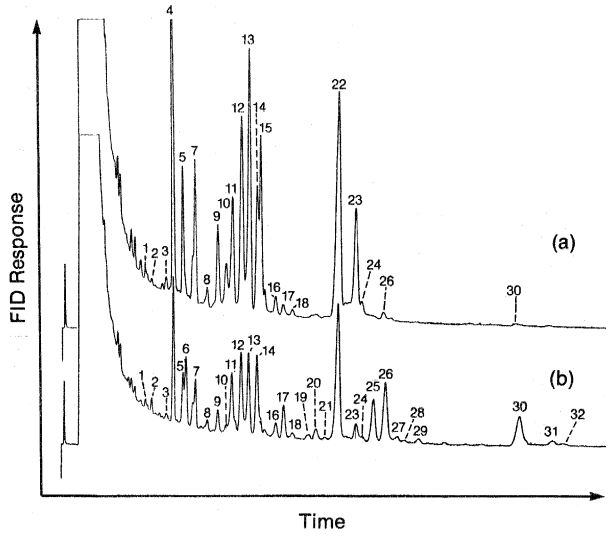

Fig. 2. Chromatograms of molecular species of adipocyte plasma membrane phosphatidylethanolamine from (a) lard-fed and (b) fish oil fed rats. Peak identities: (1) 16:0-16:0. (2) 16:0-16:1($n-7$). (3) Unknown. (4) 16:0″-20:4($n-6$). (5) 16:0-18:1($n-9$). (6) 16:0″-20:5($n-3$). (7) 16:0-18:2($n-6$). (8) Unknown. (9) 18:0″-20:4($n-6$). (10) 18:1″-20:4($n-6$). (11) 18:0-18:1($n-9$). (12) 18:1($n-9$)-18:1($n-9$)+16:0″-22:5($n-3$)+16:0″-22:6($n-3$). (13) 18:0-18:2($n-6$). (14) 16:0-20:4($n-6$). (15) 18:1($n-9$)-18:2($n-6$). (16) 16:1($n-7$)-20:4($n-6$). (17) 18:2($n-6$)-18:2($n-6$)+16:0-20:5($n-3$). (18) Unknown. (19) Unknown. (20) 18:0″-22:5($n-3$)+18:0″-22:6($n-3$). (21) Unknown. (22) 18:0-20:4($n-6$). (23) 18:1($n-9$)-20:4($n-6$). (24) 18:1($n-7$)-20:4($n-6$). (25) 18:0-20:5($n-3$). (26) 16:0-22:5($n-3$)+16:0-22:6($n-3$). (27) 18:1($n-9$)-20:5($n-3$). (28) 18:1($n-7$)-20:5($n-3$). (29) 16:1($n-7$)-22:5($n-3$)+16:1($n-7$)-22:6($n-3$). (30) 18:0-22:5($n-3$)+18:0-22:6($n-3$). (31) 18:1($n-9$)-22:5($n-3$)+18:1($n-9$)-22:6($n-3$). (32) 18:1($n-7$)-22:5($n-3$)+18:1($n-7$)-22:6($n-3$).

membranes of perirenal adipocytes. 24–31 species were identified in each sample. Relative peak areas were summed and each species was expressed as a mole percentage (Table 3). The molecular species of the diradylglycerol moieties of the choline glycerophospholipids of adipocyte plasma membranes from lard-fed and fish oil fed rats gave simpler profiles (Table 4) because of the relative absence of alkenylacylglycerol species. As a result, all species were completely resolved except for 22:5($n-3$) and 22:6($n-3$) fatty acid combinations with saturated fatty acids.

Alterations in fatty acid composition of adipocyte plasma membrane lipids were associated with major changes in species composition. After fish oil feeding, the major diacyl species were 18:0-20:4($n-6$), 18:0-18:2($n-6$), 16:0-18:1($n-9$), 18:0-20:5($n-3$), and 18:0-22:5/22:6($n-3$). The less abundant alkenylacyl species representing plasmalogens were 16:0″-20:4($n-6$), 16:0″-20:5($n-3$), 16:0″-22:5($n-3$)/22:6($n-3$) and 18:0″-22:5($n-3$)/22:6($n-3$). These pairings of fatty acids are similar to those observed for diradylglycerol moieties of phosphatidylethanolamine isolated from hearts of chow-fed rats [14]. Plasmalogen content of ethanolamine phospholipids from adipocyte plasma membranes varied from 10–20% and was lower than that seen for hearts and kidneys of chow-fed rats, where it averaged 25–30% [14]. Fish oil feeding resulted in the formation of species not detected in adipocyte membranes in lard-fed rats. Most new species contained 20:5($n-3$).

There were small but consistent differences in plasma membrane molecular species for the two fat pad regions. The relative level of $n-3$ acid-containing species was on average 10% higher in perirenal membranes in both choline and ethanolamine glycerophospholipids for both diets; however, plasmalogen levels were lower in perirenal membranes than in epididymal membranes.

Fatty acid composition of human adipose tissue phospholipids was previously found to be resistant to compositional changes, at least by comparison with stored triacylglycerols [28]. The apparent resistance of 16:0-20:4($n-6$) and 18:0-20:4($n-6$) species in adipocyte plasma membranes contrasts with the relative ease of displacement of 20:4($n-6$) by fish oil polyunsaturated fatty acids in human platelets, where the 20:4($n-6$) levels in phosphatidylcholine were decreased by 5 mol% [31]. The large increases upon fish oil feeding, in the content of $n-3$ fatty acid-containing species occurred largely at the expense of 18:1($n-9$) and 18:2($n-6$)-containing species, including 18:1-20:4($n-6$), while proportions of other 20:4($n-6$)-containing species were largely retained. As a result, the total level of $n-6$ fatty acid-containing species in fish oil fed rats in ethanolamine glycerophospholipids was 1/2 and in choline glycerophospholipids 3/4 of that in lard-fed rats.

Overall, major changes in proportions of fatty acids in adipocyte plasma membrane glycerophospholipids with fish oil feeding were accommodated by orderly alterations in structure of the species.

Thus, in both fish oil and lard-fed rats, the bulk of the polyunsaturated fatty acids remained paired with 16:0 and 18:0 saturated fatty acids presumably located in the *sn*-l-position. Species containing polyunsaturated fatty acids at both the *sn*-l- and *sn*-2-positions were not detected in fish oil fed rats despite a 3-fold increase in polyunsaturated fatty acid content. Furthermore, while the relative proportion of $n-6$ and $n-3$ fatty acid-containing species varied considerably, the sum of the two types of polyunsaturated species was quite similar for the diets. Likewise, the molar ratios of molecular species showed that preferential pairing of the polyunsaturated fatty acids with 18 carbon saturated fatty acids in diacylglycerophospholipids, and with 16:0″ in alkenylacylglycerophospholipids occurred following both fish oil and lard feeding. Thus, it may be concluded that incorporation of $n-3$ fatty acids into glycerophospholipids of adipocyte plasma membranes occurred in such a way as to minimize the impact on overall molecular structure.

In the absence of major compensatory changes, it would be anticipated that incorporation of $n-3$ fatty acid containing molecular species, with the greater cross-sectional area, would result in increased fluidity and permeability of adipocyte plasma membranes. It is possible therefore that these changes in the structure of the membrane lipids and physical properties are responsible for the earlier observed enhancement of HDL binding to plasma membranes and hormone stimulated lipolysis, and the limitation of adipose tissue hypertrophy [7]. It is interesting that mesenteric fat depots subsequently were found to not show limitation of hypertrophy [32,33], so that membranes isolated from these depots would provide a useful comparison. In any event, the present determination of the detailed molecular structure of adipocyte plasma membrane lipids should provide a chemical basis for a subsequent assessment of physico-chemical properties, especially membrane order [19,30], of adipocyte plasma membranes following dietary fish oil.

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