Effect of a fish oil diet on the composition of rat neutrophil lipids and the molecular species of choline and ethanolamine glycerophospholipids

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Abstract When rats were fed a corn oil versus a corn oil-fish oil diet the overall phospholipid content and composition as well as the subclass distribution of the choline- and ethanolamine-containing glycerophospholipids from neutrophils were not altered. The serine-containing glycerophospholipids were characterized by high levels of stearic and oleic acids. When fish oil was added to the diet it replaced some of the arachidonate in both the inositol- and the serine-containing glycerophospholipids. In the corn oil-fed animals, 25.2 and 33.6 mole %, respectively, of the molecular species of 1,2-diacyl- and 1-O-alkyl-2-acyl-*sn*-glycero-3-phosphocholine contained arachidonate. The values for 1,2-diacyl and 1-O-alk-1'-enyl-2-acyl-*sn*-glycero-3-phosphoethanolamine were, respectively, 41 and 55.8 mole %. When half of the 5% corn oil in the diet was replaced by fish oil, there was a 53, 38, 27, and 25% reduction, respectively, in the level of arachidonate in these four lipid subclasses. The amount of 5,8,11,14,17-eicosapentaenoic acid incorporated into these four subclasses was always less than the decline in arachidonic acid. This was due, in part, to the acylation of small amounts of 22-carbon (n−3) acids into these lipids. Molecular species analysis demonstrated that 5,8,11,14,17-eicosapentaenoic acid paired with the same components at the *sn*-1 position, and in the same ratio, as did arachidonic acid. The amounts of 16- and 18-carbon saturated and unsaturated fatty acid at the *sn*-2 position were not altered by dietary change. Collectively, these findings suggest that 5,8,11,14,17-eicosapentaenoic and arachidonic acids are metabolized in a similar way by neutrophils. These studies also support the concept that neutrophils contain two metabolic pools of phospholipids. One pool is altered by dietary fat change while the pool containing 16- and 18-carbon acids is resistant to change when fish oil is included in the diet. —**Careaga-Houck, M., and H. Sprecher.** Effect of a fish oil diet on the composition of rat neutrophil lipids and the molecular species of choline and ethanolamine glycerophospholipids. *J. Lipid Res.* 1989. **30:** 77–87.

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When neutrophils are treated with various agonists there is rapid release of arachidonic acid from membrane lipids followed by its conversion to 5-hydroperoxyeicosatetraenoic acid (5-HPETE). 5-HPETE is then converted to leukotriene A_4 and then into leukotriene B_4 (1–3). Leukotriene B_4 is a potent chemotactic agent for neutrophils (4–6). Neutrophils are also able to metabolize 20:5(n−3) into leukotriene B_5 (7–10). Leukotriene B_5 is only about 10% as active in recruiting neutrophils as is leukotriene B_4 (8, 9, 11). These findings suggest that the inflammatory response can be altered by replacing some of the arachidonic acid in neutrophil phospholipids with 20:5 (n−3).

When neutrophils are exposed to various stimuli they also produce PAF (12). PAF is a potent bioactive phospholipid with a wide range of biological activities (13). Recent studies suggest that 1-O-alkyl-2-arachidonyl-GPC serves as a common precursor for both PAF and leukotriene B_4 (14–16). Agonist-induced activation of phospholipase A_2 specifically releases arachidonic acid from this lipid for subsequent metabolism to leukotriene B_4. Acetylation of the resulting 1-O-alkyl-2-lyso-GPC yields PAF.

In order to more precisely define how dietary fat change mediates neutrophil function, it is important to define how and where (n−3) acids are incorporated into neutrophil phospholipids. Elicited rat peritoneal neutrophils retain their ability to synthesize both PAF (17) and leukotrienes (18, 19). In this study we show that 20:5 (n−3) specifically replaces arachidonic acid in neutrophil lipids when the diets of rats are supplemented with fish oil.

EXPERIMENTAL PROCEDURES

Materials

Oyster glycogen and phospholipase C (*Bacillus cereus*) were purchased from Sigma Chemical Co., St. Louis, MO. Benzoic anhydride, 4-dimethylaminopyridine, and methanesul-

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Abbreviations: GPC, *sn*-glycero-3-phosphocholine; GPE, *sn*-glycero-3-phosphoethanolamine; PAF, 1-O-alkyl-2-acetyl-*sn*-glycero-3-phosphocholine; TLC, thin-layer chromatography; HPLC, high performance liquid chromatography; GLC, gas-liquid chromatography.

fonyl chloride were from Aldrich Chemical Co., Inc., Milwaukee, WI. Heparin was a product of Organon Inc., West Orange, NJ. Aquasil was used as a siliconizing agent and was obtained from Pierce Chemical Co., Rockford, IL. Phospholipid standards were purchased from Avanti Biochemicals Inc., Birmingham, AL. Methyl esters and long chain alcohols were from Nu-Chek Prep. Inc., Elysian, MN. Whatman LK5 and LK6 thin-layer plates were obtained from Whatman, Inc., Clifton, NJ. All solvents were either reagent or HPLC grade.

Animals and diets

Male weanling Sprague-Dawley rats were divided into two groups. One group was fed the AIN-76 diet which contained 5% corn oil (ICN Biomedicals, Inc., Cleveland, OH). The second group received a modified AIN-76 diet in which 50% of the corn oil was replaced by menhaden oil. The oil was a generous gift from Dr. Tony Bimbo, Zapata Haynie Corporation, Reedville, VA. This diet was prepared by ICN Biomedicals and was shipped to us in a container with dry ice. The diet was stored at – 70°C and rats were fed daily. The animals were maintained on these diets for 6 weeks. The entire protocol was carried out twice. In each experiment 32 rats were maintained on each of the two diets.

Neutrophil isolation

Rats received an intraperitoneal injection of 20 ml of 0.2% oyster glycogen in 0.15 M NaCl. Four hours later the rats were anesthetized with diethyl ether, and 40 ml of phosphate buffer, pH 7.4, containing 20 units/ml of heparin was injected into the peritoneal cavity. A needle was inserted into the peritoneal cavity and the peritoneal exudate was drawn into a plastic syringe. The cells were pelleted by centrifuging at 200 *g* for 10 min. The supernatant was discarded and the small number of contaminating erythrocytes was removed by lysis for 10 min at room temperature with 10 ml of a solution containing 8.29 g NH_4Cl, 1 g $KHCO_3$, and 0.037 g Na_2EDTA/l (20).

Lipid isolation and separation

Lipids were extracted by the procedure of Bligh and Dyer (21). The organic phase was taken to dryness under a stream of N_2 at room temperature and the lipid residue was dissolved in a small volume of $CHCl_3$–MeOH 2:1 (v/v). Lipids were separated by one-dimensional TLC on Whatman LK5 plates using $CHCl_3$–MeOH–40% methylamine 60:20:5 (v/v/v) as the developing solvent (22). Individual phospholipids were localized by spraying the plates with 0.1% (w/v) 2', 7'-dichlorofluorescein in ethanol. Individual components were transferred to screw-cap vials and extracted twice with 5.0 ml of $CHCl_3$–MeOH–H_2O 5:5:1 (v/v/v) followed by centrifugation. The 10 ml of extract was washed by addition of 4.5 ml of $CHCl_3$ and 2 ml of H_2O. The upper aqueous layer was discarded and the solvent from the lower phase was removed under a stream of N_2. The lipids were redissolved in $CHCl_3$–MeOH 2:1 (v/v) and stored at – 70°C.

Phosphorus analysis was carried out as described by Rouser, Fleischer, and Yamamoto (23) after scraping aliquots of individual phospholipids from thin-layer plates into test tubes.

Separation of molecular species of choline and ethanolamine glycerophospholipids

The conditions for treating phospholipids with phospholipase C were basically those described by Mavis, Bell, and Vagelos (24). Lyophilized *Bacillus cereus* phospholipase C (100 units) was dissolved in 1 ml 0.1 M Tris-HCl, pH 7.4. The solvent from choline and ethanolamine glycerophospholipids was removed under N_2 and each lipid was dissolved in 3 ml of diethyl ether. One ml of Tris-HCl, pH 7.4, containing 10 mM $CaCl_2$ was added and the reaction was initiated by the addition of 50 units of phospholipase C. After stirring for 3 hr, a 10-μl aliquot of the ether layer was applied to a Whatman LK6 plate that was developed in hexane–ethyl ether–acetic acid 80:20:2 (v/v/v). More enzyme was added if the reaction had not gone to completion. When the reaction was complete, the ether was removed under N_2 and the diradylglycerols were recovered by extraction (21).

Diradylglycerols were converted to benzoates as described by Blank et al. (25) except that the reactions were stopped by addition of 2 ml of hexane and 1 ml of concentrated NH_4OH (26). The capped vials were vortexed until both phases were clear. The upper aqueous layer was extracted with hexane three times and the pooled hexane extracts were washed once with 1 ml of H_2O. The solvent was removed under N_2 and diradylglycerobenzoates were separated into classes by TLC on LK6 plates using benzene–hexane–ethyl ether 50:45:4 (v/v/v) as solvent (27). The 1-O-alk-1'-enyl-2-acyl-, 1-O-alkyl-2-acyl-, and 1,2-diacylglycerobenzoates were visualized by spraying the plates with 2', 7'-dichlorofluorescein. Each component was scraped into a screw-cap test tube that contained 2.5 ml of EtOH. The tubes were vortexed, and 2.5 ml of water and 5 ml of hexane were added. The samples were vortexed and the hexane was removed. Each sample was extracted five times with hexane. The pooled hexane extracts were dried under a stream of N_2 and the samples were dissolved in ethanol. The amount of each subclass was determined from the absorbance at 230 nm using $\xi = 13{,}175$ (27). Separation of molecular species of each subclass of diradylglycerobenzoates was accomplished with a DuPont HPLC system that consisted of an 870 pump, 8800 gradient controller, a column oven set at 35°C, and a variable wavelength detector set at 230 nm. Chromatography was carried out using a Zorbax 10 μm ODS Column (0.46 × 25 cm) preceded by a guard column. The 1,2-diacyl- and 1-O-alk-1'-enyl-2-acylglycerobenzoate fractions were separated by isocratic elution with acetonitrile–2-propanol 80:20 (v/v). For 1-O-alkyl-2-acylglycerobenzoates, the ratio of solvents was 72:28 (v/v). The column flow rate was always 1 ml/min. Integration was carried out with a Varian 4290 integrator. The detector response was linear up to at least 40 nmol/component as defined by calibrating the system with the benzoate derivative of 1,2-diolein.

Gas–liquid chromatography and gas–liquid chromatography–mass spectrometry

Individual lipid fractions were reacted with 3% (w/v) anhydrous HCl in methanol for 60 min at 80°C in a screw-cap vial. After 1 hr 1 ml of water was added and the products were extracted three times with 1 ml of hexane. Aliquots of hexane were injected into a Varian Vista 6000 gas chromatograph equipped with a 10 ft by 2 mm i.d. glass column packed with 10% SP-2330 on 100/120 mesh Supelcoport (Supelco, Bellefonte, PA). Helium was the carrier gas (30 ml/min) and the temperatures of the injector and detector were 240°C and 250°C, respectively. The oven temperature was held at 180°C for 17 min and then increased at 2°C/min to 190°C where it was maintained until the methyl ester of 22:6 (n–3) eluted. Methyl esters were identified by comparing retention times with authentic standards. The dimethylacetals of 16:0, 16:1, 18:0, and 18:1 aldehydes were prepared by making the mesylate of the corresponding long chain alcohols (28). The mesylates were then oxidized to the aldehydes by heating with $NaHCO_3$ in dimethylsulfoxide (29). The resulting aldehydes were then converted to dimethylacetals by reaction with anhydrous HCl in methanol. The structures of the dimethylacetals were confirmed by mass spectrometry using a Hewlett Packard 5970A mass selective detector and a 5790 gas chromatograph containing a 15 m × 0.25 mm i.d., DB-1 J and W capillary column. Injections were made in isooctane in the splitless mode at 70°C. After 1 min the oven was programmed to 240°C at 30°C/min. The base peak of all spectra was at m/z = 75 which corresponds to an ion with the composition of $CH_2O{=}CH{-}OCH_3^+$. In addition, all spectra contained a prominent ion 15 mass units less than the calculated molecular weight. These compounds were used as standards to identify the dimethylacetals that were formed when the composite 1-O-alk-1'-enyl-2-acylglycerobenzoates and individual molecular species were analyzed by GLC after conversion to methyl esters and dimethylacetals.

The composite 1-O-alkyl-2-acylglycerobenzoate fraction was also reacted with anhydrous HCl in methanol. Under the conditions used for GLC analysis the resulting 1-O-alkylglycerol derivatives are retained on the column. Thus, this analysis defines only the fatty acid composition at the *sn*-2 position. When the 1-O-alkyl-2-acylglycerobenzoate fraction was resolved into molecular species, each fraction was also treated with anhydrous HCl in methanol. An aliquot of this fraction was analyzed by GLC to define the fatty acid composition at the *sn*-2 position. The remainder of the hexane was removed under N_2 and the residual lipid was reacted with 0.1 ml of 2,2-dimethoxypropane and 0.1 ml of acetone containing 0.75% (v/v) concentrated HCl (30). After the mixture stood at room temperature for 30 min the solvent was removed under N_2 and the sample was dissolved in isooctane. The isopropylidene derivatives were then identified by GLC–mass spectrometry. All isopropylidene derivatives had a base peak at m/z = 101 which corresponds to an ion formed by cleavage between the *sn*-1 and *sn*-2 carbon atoms. In addition, each spectrum contained a major ion at M–15 which was used to identify the component at the *sn*-1 position (30).

RESULTS

Table 1 shows that dietary fat change did not alter the phospholipid composition of rat polymorphonuclear leukocytes. Cells from both dietary groups contained 6.4 ± 1.2 (n = 6) μmol of lipid phosphorus/10^9 cells. **Table 2** shows the subclass composition of the choline- and ethanolamine-containing glycerophospholipids from the two dietary

TABLE 1. Phospholipid composition of rat polymorphonuclear leukocytes

	Diet	
Phospholipid	Corn Oil	Corn Oil + Fish Oil
	mol % phosphorus	
Choline-containing glycerophospholipids	42.0 ± 0.8	38.7 ± 0.9
Ethanolamine-containing glycerophospholipids	30.6 ± 0.2	26.6 ± 0.8
Sphingomyelin	12.7 ± 0.3	16.0 ± 0.3
Inositol-containing glycerophospholipids	5.9 ± 0.2	6.9 ± 0.4
Serine-containing glycerophospholipids	7.8 ± 0.1	11.9 ± 0.7

Values are means of three separate determinations from each of two cell preparations ± SE.

TABLE 2. Subclass composition of choline- and ethanolamine-containing glycerophospholipids of rat polymorphonuclear leukocytes

	Choline-Containing Glycerophospholipids		Ethanolamine-Containing Glycerophospholipids	
	Corn Oil	Corn Oil + Fish Oil	Corn Oil	Corn Oil + Fish Oil
		mol %		
1-O-Alk-1'-enyl-2-acyl	6.3 ± 0.9	8.2 ± 4.6	58.2 ± 8.9	58.4 ± 7.3
1-O-Alkyl-2-acyl	50.8 ± 0.1	48.9 ± 4.9	10.5 ± 3.8	13.1 ± 5.9
1,2-Diacyl	42.9 ± 0.9	42.9 ± 0.3	31.3 ± 5.1	28.6 ± 1.4

Values are means of three separate determinations from each of two cell preparations ± SE.

groups. The choline-containing lipids are characterized by their high content of the 1-O-alkyl-2-acyl subclass while about 60% of the ethanolamine glycerophospholipids are plasmalogens. Again, dietary fat change did not alter the subclass distribution of either glycerophospholipid.

The serine-containing glycerophospholipids from both dietary groups are characterized by high levels of stearic and oleic acids (**Table 3**). This lipid from corn oil-fed rats contained only relatively small amounts of linoleic and arachidonic acids. When fish oil was added to the diet the oleate and linoleate levels were not altered. Fish oil supplements did depress the arachidonate content by about 30% but the lipid now contained only 0.5% 20:5(n-3). There were small increases in the amounts of long chain (n-3) acids which were accompanied by a decline of 22:4(n-6).

Table 3 also defines how dietary fish oil modified the fatty acid composition of the inositol-containing glycerophospholipids. Palmitic and stearic acids together comprise about 50% of the fatty acids in this phospholipid from both dietary groups. Again, when fish oil was added to the diet there was about a 30% reduction in the level of arachidonic acid which was accompanied by the acylation of small amounts of 20:5(n-3), 22:5(n-3), and 22:6(n-3).

Table 4 shows the fatty acid composition of the two 1,2-diacyl-GPC fractions. **Fig. 1** depicts the resolution that was obtained when these 1,2-diacylglycerobenzoates were separated by reverse phase HPLC. As shown in Table 4 the fish oil did not alter the level of either palmitate or linoleate in this lipid subclass. The analysis of molecular species (**Table 5**) showed that there was no altered pairing of these acids since the molar amounts of 16:0-18:2, 18:0-18:2, and 16:0-16:0 were not altered by dietary change. Conversely, when fish oil was added to the diet the molar percent of arachidonate declined from 12.6 to 5.9% (Table 4). The molar amounts of 16:0-20:4 and 18:0-20:4 both

TABLE 3. Fatty acid composition of inositol- and serine-containing glycerophospholipids from rat polymorphonuclear leukocytes

	Serine Glycerophospholipids		Inositol Glycerophospholipids	
Fatty Acid	Corn Oil	Corn Oil + Fish Oil	Corn Oil	Corn Oil + Fish Oil
		mol % ± SE		
16:0	9.9 ± 1.8	9.2 ± 2.7	15.6 ± 0.7	16.0 ± 3.2
16:1 (n-7)	1.0 ± 0.2	2.7 ± 1.7		
18:0	45.9 ± 0.4	45.4 ± 0.9	37.6 ± 0.7	36.2 ± 3.4
18:1 (n-9)	28.4 ± 1.5	30.6 ± 1.6	8.0 ± 0.3	12.5 ± 1.4
18:2 (n-6)	2.8 ± 0.3	3.4 ± 0.1	7.3 ± 0.1	6.0 ± 1.9
18:3 (n-3)		0.3 ± 0.1	0.8 ± 0.1	< 0.2
20:3 (n-6)	1.7 ± 0.3	1.9 ± 0.1	3.3 ± 0.3	4.4 ± 1.1
20:4 (n-6)	7.6 ± 0.3	5.2 ± 0.8	21.5 ± 1.1	14.1 ± 2.1
20:5 (n-3)	0.2 ± 0.1	0.5 ± 0.1	0.4 ± 0.1	1.6 ± 0.1
22:4 (n-6)	2.1 ± 0.1	1.1 ± 0.2	4.1 ± 0.1	2.7 ± 0.7
22:5 (n-6)	0.2 ± 0.1	0.5 ± 0.1	0.3 ± 0.1	1.4 ± 0.5
22:5 (n-3)		1.2 ± 0.3	1.5 ± 0.1	2.3 ± 0.2
22:6 (n-3)		0.3 ± 0.1		0.3 ± 0.1

Composition was determined by duplicate analyses of two cell preparations.

TABLE 4. Fatty acid composition of the 1,2-diacyl-*sn*-glycero-3-phosphocholines

	Positions 1 and 2	
Fatty Acid	Corn Oil	Corn Oil + Fish Oil
	mol % ± SE	
16:0	35.7 ± 0.3	40.4 ± 0.4
16:1 (n-7)	3.3 ± 0.4	4.2 ± 0.5
18:0	18.0 ± 1.1	17.6 ± 2.6
18:1 (n-9)	17.7 ± 0.7	17.3 ± 0.2
18:2 (n-6)	14.0 ± 0.4	14.0 ± 1.6
20:3 (n-6)	1.5 ± 0.3	1.0 ± 0.1
20:4 (n-6)	12.6 ± 1.3	5.9 ± 0.5
20:5 (n-3)	< 0.2	2.4 ± 0.1
22:4 (n-6)	1.9 ± 0.1	2.0 ± 0.3
22:5 (n-6)	0.2 ± 0.1	
22:5 (n-3)		1.5 ± 0.4
22:6 (n-3)		0.5 ± 0.2

Composition was determined by duplicate analyses of two cell preparations.

declined by about 40%. When fish oil was included in the diet the 2.4 mol percent of 20:5 (n-3) paired with both palmitic and stearic acids. The 16:0-20:4 to 18:0-20:4 molecular species ratio was 1.2 in corn oil-fed animals and it was not altered when fish oil was included in the diet. The ratio of 16:0-20:5/18:0-20:5 was 1.4 in the fish oil-supplemented group. These findings show that, in this subclass of lipids, both arachidonate and 20:5 (n-3) are recognized in a similar way by the enzymes that synthesize individual molecular species. In addition, those molecular species that contain either 16- or 18-carbon acids at the *sn*-2 position are metabolically different than those that contain 20- or 22-carbon polyenoic acids. This conclusion is based on the findings in Table 5 which show that the molar amounts of molecular species containing long chain polyunsaturated fatty acids are, in general, altered by feeding fish oil while those that contain 16- or 18-carbon acids are resistant to change.

When this and other subclasses of lipids were separated by reverse phase HPLC, two components (i.e., fractions 20 and 21 in Fig. 1), which apparently contained only stearic acid, were always detected. This could occur by acyl migration to yield the 1,3-diacyl isomer prior to or during reaction with benzoic anhydride. This, however, appears unlikely since this type of peak splitting was not observed for other molecular species. It appears most likely that peak 21 contains a fatty acid that we could not identify.

The fatty acid composition at the *sn*-2 position of the two 1-O-alkyl-2-acyl-GPC fractions is shown in **Table 6**. Again, the inclusion of fish oil in the diet did not alter the levels of 16- and 18-carbon acids at the *sn*-2 position. The level of arachidonate declined by 38% when fish oil was included in the diet. As shown in Table 5, arachidonate pairs with linoleate, oleate, palmitate, and stearate. When fish oil was added to the diet there was a decline in the amounts of all of these molecular species. We were only able to identify 16:0-20:5 and 18:1-20:5 as well as a compound in fraction 5 that contained 20:5(n-3). When the data in Table 5 were used to calculate the fatty acid composition of the entire fraction (Table 6) we found 21.3 mol % arachidonate but only 2.9% 20:5(n-3). It is thus likely that we failed to detect some molecular species that contained 20:5(n-3). The apparent absence of 18:0-20:5 is particularly surprising.

Table 7 and **Table 8** compare the composition of the 1,2-diacyl- and the 1-O-alk-1'-enyl-2-acyl-GPE fractions. Again the level of oleate and linoleate in both these fractions was not altered by dietary change nor was there a redistribution of these fatty acids among molecular species (**Table 9**). Stearic acid is the principal saturated fatty acid in the diacyl fraction. In the corn oil-fed rats, 93% of the arachidonate was paired with stearate, while only small amounts were paired with either oleate or palmitate. When fish oil was added to the diet the arachidonate level was reduced by 27% and the diacyl fraction now contained 2.1, 4.7, and 1.4 mol %, respectively, of 20:5(n-3), 22:5(n-3), and 22:6(n-3). In the fish oil-supplemented animals, 87 mol % of the arachidonate and 90 mol % of the 20:5(n-3) paired with stearate. Both 22:5(n-3) and 22:6(n-3) also preferentially paired with stearic acid.

The plasmalogen fraction of the ethanolamine glycerophospholipids contained the highest level of arachidonate of the four subclasses that were analyzed. The data in Table 8 show that 55.8 mol % of the molecular species of this fraction contain arachidonate at the *sn*-2 position. In the corn oil-fed animals arachidonate pairs with oleate, palmitate, and stearate in a ratio of 1:2.1:2.9. When fish oil was added to the diet the residual arachidonate and the 8.2 mol % 20:5:(n-3) paired with oleate, palmitate, and stearate in ratios of 1:1.9:2.5 and 1.0:2.4:3.0, respectively. Again, as in the other lipid fractions, the pairing of 20:5(n-3) is within experimental error, identical with that for arachidonate. The small amounts of 22:5(n-3) and 22:6(n-3) in this lipid fraction preferentially pair with stearate. When fractions 19 and 21 were analyzed by mass spectrometry it was possible to identify the dimethylacetal of 20:0 and 22:1 aldehydes. Due to the small amount of material it was, however, not possible to establish the nature of the fatty acid(s) in these two fractions.

DISCUSSION

The phospholipid content and the class distribution of elicited rat neutrophils are not affected by dietary fish oil and are similar to the values reported by Ramesha and Pickett (31) for rats raised on chow and fat-free diets. In fact, the phospholipid content and class distribution of rat

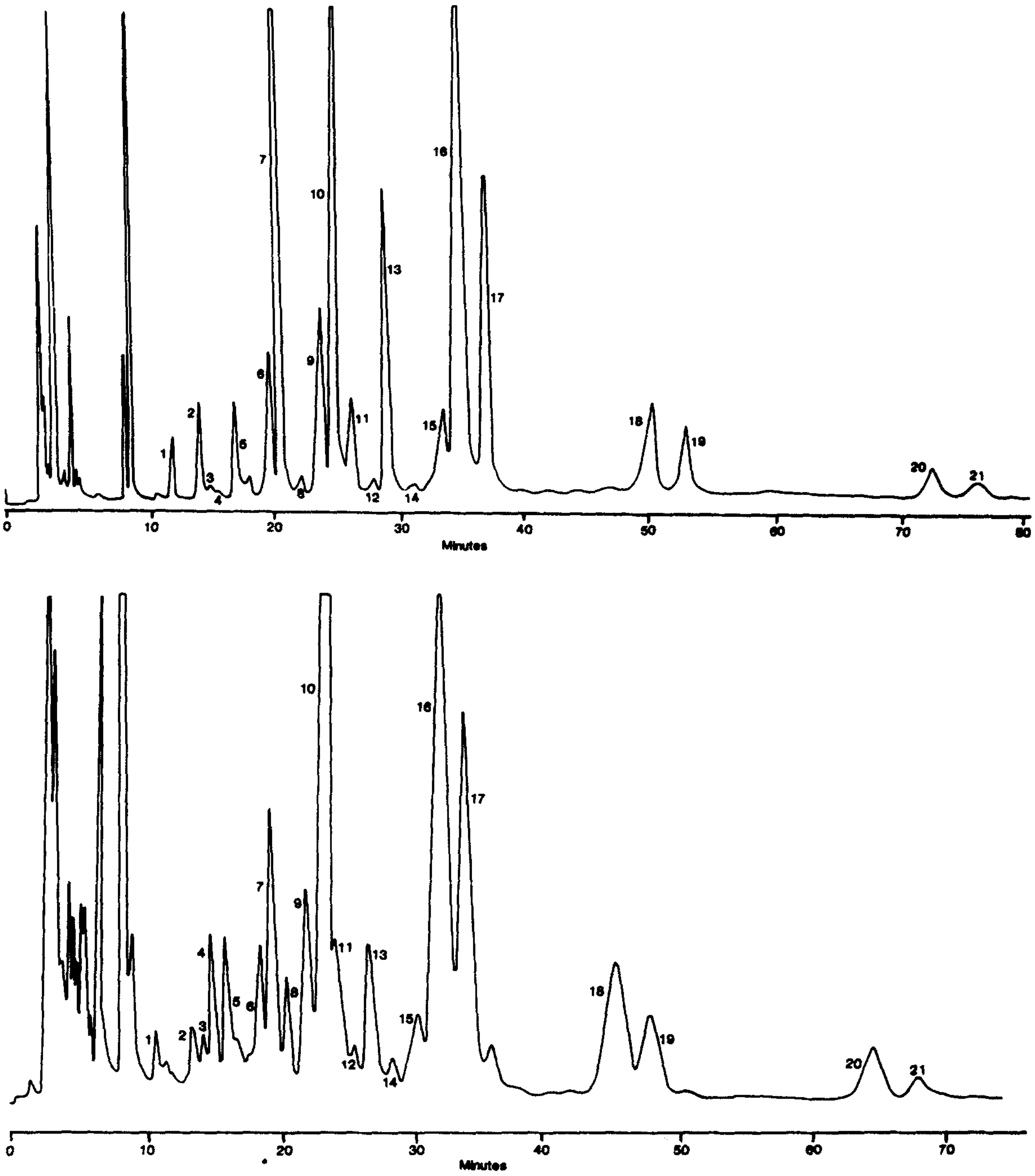

Fig. 1. HPLC separation of molecular species of 1,2-diacylglycerolbenzoates from choline-containing glycerophospholipids of polymorphonuclear leukocytes from rats fed corn oil (top) or the corn oil fish oil diet (bottom).

neutrophils are similar to those of rabbits (32), humans (33), and guinea pigs (34). In all species the choline-containing glycerophospholipids are characterized by their high ether content while 40–50% of the ethanolamine-containing glycerophospholipids are plasmalogens.

The four different subclasses of choline- and ethanolamine-containing glycerophospholipids that were analyzed in this study are characterized by different levels of arachidonate. If one assumes that all of the arachidonate is esterified at the *sn*-2 position, it can be calculated that 25.2, 33.6, 41, and 55.8 mol% of the molecular species, respectively, of 1,2-diacyl-GPC, 1-O-alkyl-2-acyl-GPC, 1,2-diacyl-GPE, and 1-O-alk-1'-enyl-2-acyl-GPE contain arachidonate. When fish oil is added to the diet there is

TABLE 5. Composition of molecular species of choline-containing glycerophospholipids of rat polymorphonuclear leukocytes

Peak Number[a]	Molecular Species[b]	1,2-Diacyl: Corn Oil	1,2-Diacyl: Corn Oil + Fish Oil	1-O-Alkyl-2-Acyl: Corn Oil	1-O-Alkyl-2-Acyl: Corn Oil + Fish Oil
		mol % ± SE			
1	18:2-20:5		0.6 ± 0.1		
2	18:2-20:4	1.5 ± 0.2	0.8 ± 0.1	0.9 ± 0.1	
	16:1-22:5 (n-3)		0.2 ± 0.1		
3	18:1-20:5		0.8 ± 0.3		0.3 ± 0.1
4	X-Y	0.1 ± 0.0			
	18:1-22:6		0.1 ± 0.0		0.1 ± 0.0
	16:0-20:5		2.2 ± 0.1		1.3 ± 0.1
5	18:2-18:2	1.8 ± 0.1	1.5 ± 0.2	0.4 ± 0.1	0.8 ± 0.1
	16:0-22:6		0.9 ± 0.2		
	X-20:5		0.6 ± 0.1		1.2 ± 0.1
	16:1-22:4	0.5 ± 0.1	0.2 ± 0.1	0.1 ± 0.0	1.5 ± 0.1
6	18:1-20:4	3.2 ± 0.5	1.8 ± 0.1	8.9 ± 0.1	3.8 ± 0.1
	16:0-22:5 (n-3)		1.3 ± 0.1		2.6 ± 0.1
7	16:0-20:4	9.1 ± 0.3	5.4 ± 0.6	18.2 ± 1.7	13.6 ± 1.1
	18:2-22:4		0.2 ± 0.0		
8	18:0-20:5	0.1 ± 0.0	1.5 ± 0.4		
	16:0-22:5 (n-6)	0.3 ± 0.1			
9	18:1-18:2	5.5 ± 0.7	4.2 ± 0.1	3.7 ± 0.7	2.9 ± 0.2
	18:0-22:6		0.8 ± 0.0		0.6 ± 0.1
10	16:0-18:2	13.3 ± 1.1	12.1 ± 1.1	13.7 ± 0.4	15.1 ± 1.0
	16:1-18:1	2.1 ± 0.2	2.8 ± 0.2		
	16:0-20:3		0.8 ± 0.1	1.2 ± 0.1	2.5 ± 0.2
11	16:0-Y		4.7 ± 0.4		3.3 ± 0.3
	16:0-22:4	3.1 ± 0.3	0.2 ± 0.0		
12	X-Y	0.3 ± 0.1			
	18:0-22:5 (n-3)		0.7 ± 0.2		
13	18:0-20:4	7.3 ± 0.2	4.1 ± 0.3	6.8 ± 0.1	3.5 ± 0.1
14	X-Y	0.2 ± 0.1	0.7 ± 0.3	1.3 ± 0.1	1.1 ± 0.1
15	18:1-18:1	2.9 ± 0.7	2.3 ± 0.1	1.8 ± 0.6	1.5 ± 0.2
	16:0-Y	0.6 ± 0.1	0.5 ± 0.1		
16	16:0-18:1	17.4 ± 0.2	16.6 ± 1.5	11.8 ± 0.4	13.2 ± 0.5
	18:0-18:2	6.9 ± 0.1	6.6 ± 0.6	3.3 ± 0.1	3.4 ± 0.1
	18:0-20:3		3.3 ± 0.3		
	18:0-22:4	0.5 ± 0.0			
17	16:0-16:0	11.9 ± 0.5	12.9 ± 0.6	22.9 ± 0.7	22.3 ± 0.4
18	18:0-18:1	5.6 ± 0.6	4.8 ± 1.2	1.2 ± 0.3	1.1 ± 0.6
19	18:0-16:0	3.0 ± 0.5	2.4 ± 0.9	3.0 ± 0.3	2.3 ± 0.6
20	18:0-18:0	2.1 ± 0.7	2.6 ± 0.1		
21	18:0-18:0	0.7 ± 0.1	1.3 ± 0.3		

Values, expressed as mol % ± SE, are from two separate cell preparations.

[a] Peak numbers correspond to those shown in the HPLC tracings of Fig. 1.

[b] X, Unidentified fatty chain (*sn*-1 position); Y, unidentified fatty acid (*sn*-2 position).

a 53, 38, 27, and 25% reduction, respectively, in the level of arachidonate in these four lipids. This reduction is accompanied by the acylation of 20:5(n−3) into all four lipids. Molecular species analysis shows that 20:5(n−3) pairs in an almost identical way as does arachidonate. The amount of 20:5(n−3) incorporated into lipids is, however, always less than the arachidonate that is replaced. In part this is due to acylation of small amounts of 22-carbon (n−3) acids into phospholipids. When neutrophils are treated with various agonists both arachidonate and 20:5(n−3) are released and metabolized to LTB_4 and LTB_5, respectively (1–3, 7–10). When exogenous 22:6(n−3) is added to neutrophils in the presence of the calcium ionophore A23187 it is metabolized in small amounts to 4- and 7-hydroxy acids (35). However, there is no evidence to indicate that 22:6(n−3) is released from neutrophil phospholipids. When small amounts of 22:5(n−3) and 22:6(n−3) replace arachidonate they may have the potential of altering neutrophil function simply by reducing the amount of arachidonate that is available for agonist-induced release.

When radioactive arachidonate is incubated with neutrophils it is initially incorporated into 1,2-diacyl-GPC.

TABLE 6. Fatty acid composition of the 1-O-alkyl-2-acyl-*sn*-2-glycero-3-phosphocholines

	2-Position	
Fatty Acid	Corn Oil	Corn Oil + Fish Oil
	mol % ± SE	
16:0	34.4 ± 2.3	32.6 ± 0.1
16:1 (n-7)	4.2 ± 0.6	6.0 ± 0.3
18:0	2.6 ± 0.7	3.1 ± 0.2
18:1 (n-9)	8.9 ± 0.1	10.8 ± 1.7
18:2 (n-6)	14.6 ± 0.6	15.0 ± 0.7
18:3 (n-3)	1.0 ± 0.7	1.7 ± 0.9
20:3 (n-6)	0.9 ± 0.3	1.5 ± 0.1
20:4 (n-6)	33.6 ± 1.1	20.9 ± 0.3
20:5 (n-3)		5.9 ± 0.4
22:4 (n-6)	1.6 ± 0.1	3.1 ± 0.5
22:5 (n-6)	2.2 ± 1.3	2.4 ± 0.1
22:5 (n-3)		1.7 ± 0.1
22:6 (n-3)		0.7 ± 0.3

Composition was determined by duplicate analyses of two cell preparations.

TABLE 7. Fatty acid composition of the 1,2-diacyl-*sn*-glycero-3-phosphoethanolamines

	Positions 1 and 2	
Fatty Acid	Corn Oil	Corn Oil + Fish Oil
	mol % ± SE	
16:0	13.8 ± 0.3	10.8 ± 2.5
16:1 (n-7)	2.6 ± 0.8	1.7 ± 0.5
18:0	43.9 ± 2.6	44.0 ± 0.7
18:1 (n-9)	13.4 ± 1.9	14.1 ± 1.0
18:2 (n-6)	4.6 ± 0.7	5.2 ± 1.0
20:3 (n-6)	1.7 ± 0.8	2.2 ± 0.6
20:4 (n-6)	20.5 ± 1.1	15.0 ± 2.8
20:5 (n-3)	< 0.2	2.1 ± 0.3
22:4 (n-6)	4.1 ± 0.5	2.1 ± 0.4
22:5 (n-6)	0.5 ± 0.1	< 0.2
22:5 (n-3)		4.7 ± 0.7
22:6 (n-3)		1.4 ± 0.3

Composition was determined by duplicate analyses of two cell preparations.

Over time there is a direct transfer of arachidonate from 1,2-diacyl-GPC to 1-O-alkyl-2-acyl-GPC (36, 37). Molecular species analysis suggests that 20:5(n−3) may be incorporated into phospholipids via an identical pathway. The fish oil that was fed contained 14.5, 1.9, and 6.6%, respectively, of 20:5(n−3), 22:5(n−3), and 22:6(n−3). The finding that phospholipids contain relatively low levels of 22:5(n−3) and 22:6(n−3) suggests that, in vivo, they may be poor substrates for incorporation into diacyl-GPC. When neutrophils are incubated with exogenous 22:6(n−3) it is incorporated into phospholipids (38). If 22:5(n−3) and 22:6(n−3) are incorporated into 1,2-diacyl-GPC in vivo, the results of Sugiura et al. (39) with macrophages suggest that 22:6(n−3) should readily be transferred to 1-O-alkyl-2-acyl-GPC. When 22:6(n−3) is fed to humans there is an increase in the level of 20:5(n−3) in platelet phospholipids (40) as well as the rapid appearance of prostaglandin I_3 in the urine (41). The amount of 22:6(n−3) available for acylation in vivo may thus be small due to its rapid and preferential metabolism to 20:5(n−3) by mitochondrial retroconversion in the liver (42).

Neutrophil phospholipids contain large amounts of palmitic, oleic, and linoleic acids at their *sn*-2 position (31–34). Dietary fish oil did not change the levels of any

TABLE 8. Fatty chain composition of 1-O-alk-1′-enyl-2-acyl-*sn*-glycerophosphoethanolamines

	Corn Oil		Corn Oil + Fish Oil	
Component	Position-1[a]	Position-2[b]	Position-1[a]	Position-2[b]
		mol % ± SE		
16:0	31.1 ± 0.1	9.1 ± 1.0	36.0 ± 1.3	3.6 ± 2.4
18:0	50.2 ± 0.1	5.3 ± 0.4	46.5 ± 1.1	5.3 ± 0.4
18:1	15.3 ± 0.1	16.4 ± 0.2	13.4 ± 0.4	17.1 ± 0.8
20:0	3.2 ± 0.1		4.1 ± 0.3	
18:2 (n-6)		8.3 ± 0.1		11.5 ± 0.9
20:4 (n-6)		55.8 ± 2.0		41.8 ± 2.0
20:5 (n-3)				8.2 ± 0.1
22:4 (n-6)		3.9 ± 0.4		3.4 ± 1.5
22:5 (n-6)		1.0 ± 0.1		1.7 ± 0.2
22:5 (n-3)				2.9 ± 0.3
22:6 (n-3)				1.7 ± 0.2

Composition expressed as mol % ± SE was determined by duplicate analyses of two cell preparations.
[a] Analyzed as dimethylacetals.
[b] Analyzed as methyl esters.

TABLE 9. Composition of the molecular species of ethanolamine-containing glycerophospholipids of rat polymorphonuclear leukocytes

		1,2-Diacyl		1-O-Alk-1'-enyl-2-acyl	
Peak Number	Molecular Species[a]	Corn Oil	Corn Oil + Fish Oil	Corn Oil	Corn Oil + Fish Oil
		mol % ± SE			
1	18:2-20:5			0.2 ± 0.0	0.1 ± 0.0
2	18:2-20:4			0.4 ± 0.1	0.3 ± 0.1
3	18:1-20:5				1.7 ± 0.3
4	16:0-20:5		0.4 ± 0.1		4.1 ± 0.2
	18:1-22:6		0.2 ± 0.1		1.0 ± 0.1
5	18:2-18:2	0.6 ± 0.1	0.2 ± 0.1		
	16:0-22:6		0.7 ± 0.1		1.0 ± 0.4
6	16:1-22:4	0.6 ± 0.2	0.6 ± 0.1		
7	16:0-Y	1.9 ± 0.1			
	16:0-22:5 (n-3)		0.7 ± 0.1	0.3 ± 0.0	2.2 ± 0.1
	18:1-20:4	0.3 ± 0.0	1.9 ± 0.1	9.9 ± 0.5	8.1 ± 0.1
8	X-Y		0.2 ± 0.0		
	16:0-20:4	2.7 ± 0.1	2.3 ± 0.1	20.9 ± 1.7	15.6 ± 1.0
9	18:0-20:5		3.6 ± 0.1		5.2 ± 0.1
	16:0-22:5 (n-6)	0.3 ± 0.1		0.3 ± 0.1	
10	18:1-18:2	1.5 ± 0.1	1.7 ± 0.3	1.3 ± 0.1	1.2 ± 0.1
	18:0-22:6		2.5 ± 0.4	0.2 ± 0.0	1.7 ± 0.1
11	16:0-18:2	1.5 ± 0.2	1.8 ± 0.1	3.9 ± 0.6	4.1 ± 0.2
	16:1-18:1	0.6 ± 0.1	1.9 ± 0.1		
	16:0-20:3	0.5 ± 0.1			
12	16:0-Y		0.4 ± 0.1		
	16:0-22:4	2.3 ± 0.4	0.4 ± 0.1	1.8 ± 0.1	1.2 ± 0.7
13	X-Y			1.3 ± 0.3	1.2 ± 0.2
14	X-Y			1.3 ± 0.5	
	18:0-22:5 (n-3)	2.1 ± 0.4	5.4 ± 0.2		3.2 ± 1.5
15	18:0-20:4	40.7 ± 3.1	28.0 ± 3.0	28.8 ± 1.7	21.0 ± 1.8
16	18:0-22:5 (n-6)	1.3 ± 0.4	0.4 ± 0.1	0.4 ± 0.1	
17	18:1-18:1	1.3 ± 0.1	0.7 ± 0.1	2.2 ± 0.2	2.6 ± 0.4
	16:0-Y	0.3 ± 0.0	0.7 ± 0.1		
18	16:0-18:1	5.5 ± 0.8	5.6 ± 1.1	7.0 ± 0.1	7.8 ± 0.3
	18:0-18:2	4.7 ± 0.6	6.2 ± 1.2	6.1 ± 0.1	5.4 ± 0.2
	18:0-20:3		1.5 ± 0.3		
19	18:0-22:4	7.7 ± 0.7	4.4 ± 0.8	1.8 ± 0.3	1.2 ± 0.7
	22:1-Y			1.3 ± 0.2	
20	16:0-18:0	0.3 ± 0.0	0.6 ± 0.4		
21	20:0-Y			2.0 ± 0.2	1.5 ± 0.3
22	20:0-18:1			0.4 ± 0.1	0.7 ± 0.1
23	16:0-16:0	0.5 ± 0.1			
24	18:0-18:1	20.2 ± 3.2	21.5 ± 0.1	5.6 ± 0.5	6.6 ± 0.8
25	18:0-16:0	1.8 ± 0.6	2.9 ± 1.2	0.5 ± 0.1	0.7 ± 0.2
	18:1-18:0		1.6 ± 0.7		
26	18:1-16:0		0.2 ± 0.0		
27	18:0-18:0	3.0 ± 0.2	3.8 ± 0.8		
28	18:0-18:0	1.0 ± 0.2	3.9 ± 1.1		

Values are from two separate cell preparations.

[a] X, Unidentified fatty chain (*sn*-1 position); Y, unidentified fatty acid (*sn*-2 position).

of these acids in the phospholipids nor did it alter their distribution in molecular species. Swendsen et al. (43) have recently shown that arachidonic acid is incorporated into neutrophil phospholipids by a pathway different from that used for incorporating linoleic acid and saturated fatty acids. These ex vivo studies, and the feeding studies reported here, are consistent with the concept that neutrophils contain at least two separate pools of phospholipids. One pool is resistant to dietary fat change and includes those phospholipids that contain 16- and 18-carbon acids at their *sn*-2 position. The other pool contains a fatty acid at the *sn*-2 position that can be transferred to an acceptor via the CoA independent pathway. This concept is consistent with enzymatic studies in macrophages, showing that diacyl-GPCs containing palmitic, stearic, oleic, or linoleic acid at the *sn*-2 position do not serve as substrates for the

CoA-independent transferase (39).

The fish oil-containing diet that we fed contained 2.5% corn oil. Linoleic acid is thus available for phospholipid synthesis. Chabot et al. (44) found that the level of linoleate in monkey neutrophil phospholipids was reduced when these animals were fed a fish oil diet devoid of n-6 acids. It remains to be defined whether this type of change in neutrophil phospholipid fatty acid composition alters their function by a mechanism not involving the synthesis of bioactive molecules.

Finally, it must be reemphasized that 20:5(n-3) also replaces arachidonate in 1-O-alk-1'-enyl-2-acyl-GPE. Recently Tessner and Wykle (45) have shown that 1-O-alk-1'-enyl-2-acetyl-GPE is made when neutrophils are incubated with acetate in the presence of a calcium ionophore. The physiological function of this plasmalogen analog of platelet-activating factor remains to be defined, as well as how dietary fat change modulates its synthesis. ■

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