Influence of dietary n–3 fatty acids on macrophage glycerophospholipid molecular species and peptidoleukotriene synthesis

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Abstract This study examined the ability of dietary n-3 fatty acids to modify mouse peritoneal macrophage glycerophospholipid molecular species and peptidoleukotriene synthesis. After a 2-week feeding period, fish versus corn oil feeding significantly ($P < 0.01$) lowered n-6 polyunsaturated fatty acid (PUFA) mol % levels, i.e., arachidonic acid (20:4n-6) in diacylphosphatidylserine (PtdSer), diacylphosphatidylinositol (PtdIns), diacylglycerophosphoethanolamine (PtdEtn), alkenylacylglycerophosphoethanolamine (PlsEtn), and diacylglycerophosphocholine (PtdCho). A notable exception was alkylacylglycerophosphocholine (PakCho), where only moderate decreases in 16:0-20:4n-6 and 18:0-20:4n-6 species were observed after fish oil supplementation. The predominant n-3 PUFA in macrophage phospholipid subclasses was docosapentaenoic acid (22:5n-3). The major n-3 species were 18:0-22:5n-3 in PtdIns, PtdSer, glycerophosphoethanolamines (EtnGpl) and 16:0-22:5n-3 in PtdCho and PlsEtn. The major n-3-containing species in PakCho were 16:0-20:5n-3 and 18:1-22:6n-3. These findings indicate that n-3 PUFA are differentially incorporated into macrophage phospholipid subclasses after dietary fish oil supplementation, and suggest that phospholipid remodeling enzymes selectively discriminate between substrates based on compatibilitiy of *sn*-1 covalent linkage and the composition of the *sn*-1 and *sn*-2 aliphatic chains. Macrophage peptidoleukotriene synthesis was also strongly influenced after fish oil feeding; the LTC_5/LTC_4 ratio was significantly higher ($P < 0.01$) in fish oil-fed animals than in corn oil-fed animals, 0.85 versus 0.01, respectively. These ratios were subsequently compared to phospholipid molecular species 20:5n-3/20:4n-6 ratios in order to determine potential sources of eicosanoid precursors.—**Chapkin, R. S., C. C. Akoh, and C. C. Miller.** Influence of dietary n-3 fatty acids on macrophage glycerophospholipid molecular species and peptidoleukotriene synthesis. *J. Lipid Res.* 1991. **32:** 1205-1213.

Supplementary key words mouse peritoneal macrophage • phospholipid • fish oil

Macrophages are a highly diverse population of cells that are capable of influencing numerous aspects of immune and inflammatory responses (1). Interestingly, dietary fish oil has been reported to attenuate many of these same resposnes (2, 3). A mechanism by which fish oil constituents are believed to exert their effect is by altering membrane phospholipid polyunsaturated fatty acid (PUFA) composition, such that the profile and character of the biologically active products derived from these phospholipids are altered. Indeed, incorporation of fish oil-derived PUFAs into membrane phospholipids alters the quantitative release of phospholipid-derived platelet activating factor (PAF) and arachidonic acid (20:4n-6)-derived eicosanoids (4-7), metabolites that are putative mediators of immune and inflammatory events (8-10). The modified release of these products from fish oil-enriched cells presumably leads to a diminished macrophage response as evidenced by studies showing that fish oil-derived PUFAs can, in fact, influence macrophage function (11, 12).

To better understand the molecular basis for this effect, it is imperative that a study be undertaken to describe in detail the effects of dietary fish oil on the fatty acid make-up of macrophage membrane phospholipids. Phospholipids are broadly categorized according to their *sn*-3-phosphoglycerol backbone into four major classes: glycero-

Abbreviations: PtdIns, diacylphosphatidylinositol; PtdSer, diacylphosphatidylserine; ChoGpl, glycerophosphocholines; PakCho, alkylacylglycerophosphocholine; PtdCho, diacylglycerophosphocholine; EtnGpl, glycerophosphoethanolamines; PlsEtn, alkenylacylphosphoethanolamine; PtdEtn, diacylglycerophosphoethanolamine; PUFAs, polyunsaturated fatty acids; PAF, 1-O-alkyl-2-acetyl-*sn*-glycero-3-phosphocholine; LTC_4, 20:4n-6 derived peptidoleukotriene C_4; LTC_5, 20:5n-3 derived peptidoleukotriene C_5; TLC, thin-layer chromatography; HPLC, high performance liquid chromatography; RIA, radioimmunoassay. The *sn* configuration refers to the position of the aliphatic chain along the glycerol backbone. PUFA nomenclature describes (the number of carbon atoms in the fatty acid backbone):(the number of double bonds) n-(the position of the first double bond from the methyl end). PUFAs of the n-3 family are primarily of marine origin while members of the n-6 family are primarily of terrene plant origin.

phosphocholines (ChoGpl), glycerophosphoethanolamines (EtnGpl), diacylphosphatidylserine (PtdSer), and diacylphosphatidylinositol (PtdIns). These glycerophospholipids are predominantly in an *sn*-1,2-diacyl configuration; however, ChoGpl and EtnGpl also contain important ether-linked subclasses, 1-alkyl-2-acylglycerophosphocholine (PakCho) and 1-alkenyl-2-acylglycerophosphoethanolamine (PlsEtn), respectively (13, 14). It is becoming increasingly apparent that it is also useful to determine the composition of molecular species, i.e., quantitation of phospholipids according to specific *sn*-1 and *sn*-2-aliphatic moieties. The significance of molecular species composition relates to the substrate specificities of enzymes involved in phospholipid and eicosanoid metabolism (13, 15). For example, there is evidence that certain phospholipid remodeling enzymes selectively discriminate between substrates based on both the *sn*-1 covalent linkage and composition of the *sn*-1 and *sn*-2 moieties (13, 15–18).

To date, no attempt has been made to determine the specific substitution of fish oil-derived n-3 PUFAs for 20:4n-6 in phospholipid molecular species. Therefore, the purpose of the present study was to determine the ability of dietary fish oil to modify the molecular species composition of mouse peritoneal macrophage glycerophospholipids. In addition, an attempt was made to relate these alterations with patterns of peptidoleukotriene synthesis by determining the production of pro-inflammatory peptidoleukotriene C_4 (LTC_4), a product of arachidonic acid, and of the less potent peptidoleukotriene C_5 (LTC_5) (19), a product of the fish oil-derived n-3 PUFA, eicosapentaenoic acid (20:5n-3) (20).

MATERIALS AND METHODS

Materials

Phospholipase C (*Bacillus cereus*) and zymosan were purchased from Sigma (St. Louis, MO). Benzoic anhydride and 4-dimethylaminopyridine were from Aldrich (Milwaukee, WI). Phospholipid standards were purchased from Avanti Polar Lipids, Inc. (Birmingham, AL). Thin-layer plates and silica gel G were purchased from E. Merck (Darmstadt, Germany). All tissue culture medium was from Whitaker M.A. Bioproducts (Walkersville, MD). Fetal bovine serum was from Hyclone (Logan, UT). Eicosanoid standards were purchased from Cayman Chemicals (Ann Arbor, MI). Peptidoleukotriene radioimmunoassay (RIA) kit was from Nuclear Magnetics (Cambridge, MA). C-18 solid-phase extraction (SPE) columns were obtained from J.T. Baker (Philipsburg, NJ). All solvents were of Optima grade (Fisher Scientific, Fair Lawn, NJ).

Animals and diets

Specific pathogen-free female C57BL/6NCR mice (Frederick Cancer Research Facility, Frederick, MD) weighing 15–18 g were divided into two groups consisting of 15 mice each. The animals were housed in autoclaved cages in a laminar flow hood and given autoclaved water to minimize spontaneous activation of macrophages (21). Mice were fed ad libitum, one of two semi-purified diets that were adequate in all nutrients (22). The diets varied only in the type of oil fed, i.e., either corn or a mixture of fish/corn in an 8.5:1.5 ratio at 10% of the diet by weight. The corn oil was a generous gift from Mr. Sid Tracy, Traco Labs, Champaign, IL. The menhaden fish oil was provided by the National Institutes of Health (Fish Oil Test Material Program, Washington, DC). The composition of the major dietary fatty acids, as determined by gas–liquid chromatography (22), was (weight %): corn—16:0 (11.1%), 18:0 (1.9%), 18:1n-9 (26.5%), 18:2n-6 (57.9%); fish/corn—14:0 (6.8%), 16:0 (16.9%), 18:0 (2.7%), 18:1n-9 (11.9%), 18:2n-6 (10.0%), 20:5n-3 (11.8%), 22.5n-3 (1.7%), 22:6n-3 (9.4%). The animals were maintained on these diets for 2 weeks.

Preparation of macrophages

Peritoneal macrophages were isolated by adherence from thioglycollate-elicited responsive cells as previously described (21). Specifically, peritoneal exudate cells were obtained by lavage using Hank's balanced salt solution (HBSS) supplemented with 10 U/ml heparin. These thioglycollate-elicited cells were centrifuged for 5 min at 500 *g*, rinsed with HBSS, and plated using medium A (alpha-MEM with 2 mM glutamine, 20 mM HEPES, 5 mM reduced glutathione, and 5 μg/ml gentamicin) supplemented with 5% heat-inactivated fetal bovine serum. The cells were plated at a density of 0.5×10^6 macrophages/cm^2 in 100-mm culture dishes and were 85% macrophages based on differential staining. The macrophages were allowed to adhere for 1 h after which nonadherent cells were removed by vigorous rinsing with HBSS. Greater than 95% of the adherent cells were macrophages based on the phagocytosis of opsonized sheep erythrocytes. For lipid analysis, cells from two mice were pooled (7×10^7 macrophages). For the synthesis of peptidoleukotrienes, macrophage monolayers were pre-incubated in medium A for 2 h with indomethacin (10 μM) added to minimize cyclooxygenase activity (20).

Lipid analysis

Lipids were extracted by the procedure of Folch, Lees, and Sloane Stanley (23). Phospholipid classes were separated by one-dimensional thin-layer chromatography (TLC) on silica gel-60 plates using chloroform–methanol–

acetic acid–water 50:37.5:3.5:2 (by vol) as the developing solvent (22). Since the acyl group composition of the alkylacyl and alkenylacyl lipid subclasses is derived exclusively from the *sn*-2 position, whereas the acyl groups from the diacyl subclasses represent the *sn*-1 and *sn*-2 positions, the molar amounts of the total fatty acid methyl esters from the ether-containing subclasses were multiplied by 2 for phospholipid subclass and class mass determination (14). Isolated ChoGpl, EtnGpl, PtdIns, and PtdSer fractions were converted to benzoate derivatives after phospholipase C (*Bacillus cereus*) hydrolysis as previously described (14, 24, 25). Briefly, the extracted diradylglycerols were treated with benzoic anhydride and 4-dimethylaminopyridine for 1 h at 22°C and the resultant diradylglycerobenzoates were further fractionated on silica gel G plates using benzene–hexane–diethyl ether 50:45:4 (by vol) as the developing solvent (14, 24). Component molecular species were quantitated on-line at 230 nm by reverse phase high-performance liquid chromatography (RP-HPLC) (14, 26). The diacyl, alkylacyl, and alkenylacylglycerobenzoates were separated isocratically with acetonitrile–2-propanol at 1 ml/min using ratios of 70:30, 63:37, and 65:35 (by vol), respectively. The log of the relative retention times of molecular species was determined after dividing the retention time of each peak by the retention time of the reference peak, 16:0–18:1 (26, 27). Individual molecular species were also identified by gas–liquid chromatography after conversion to their corresponding fatty acid methyl esters and dimethyl acetals (26, 28). Alkylacyl molecular species were further characterized by preparing dibenzoates of the corresponding alkylglycerols (26, 29).

Peptidoleukotriene synthesis and analysis

Macrophages were stimulated to synthesize peptidoleukotrienes by using 10 μM divalent cation ionophore A23187 in medium A for 30 min. At the end of the stimulation period, supernatants were collected and stored at −80°C until analysis. Cell protein content was determined using a modification of the Lowry assay (30). Incubation supernatants were spiked with a known amount of prostaglandin B_2 (PGB_2), applied to SPE columns, and the peptidoleukotrienes were eluted with methanol (31). Extracts were analyzed using RP-HPLC as previously described (20, 32). The identity of the peaks was confirmed by UV absorbance spectra and RIA of eluted fractions (20).

Statistical analysis

Data were analyzed using Student's *t*-test with the upper level of significance chosen at $P < 0.05$.

RESULTS

The dietary oil fatty acyl composition did not alter macrophage relative phospholipid class distribution (data not shown). However, substantial differences in molecular species profiles between corn oil and fish oil fed animals were noted (Table 1–6). In general, there was depletion at the *sn*-2 position of the n–6 PUFAs, 18:2n–6 and 20:4n–6, and substitution with the n–3 PUFAs, 20:5n–3, 22:5n–3, and 22:6n–3. Examination of the molecular species of PtdIns demonstrates how n–6 PUFAs were substituted with n–3 PUFAs (**Table 1**). For example, 18:0–20:4n–6 was the predominant 20:4n–6-containing species accounting for 65.7 mol% (mol/100 mol) of PtdIns molecular species in the corn oil-fed animals. In the animals fed fish oil, this was lowered by 42% to only 38.3 mol%. Correspondingly, n–3-containing molecular species of PtdIns were

TABLE 1. Molecular species composition of phosphatidylinositol (PtdIns)

Peak #	Molecular Species	Composition (mol/100 mol) Corn	Fish/Corn
1	18:2-20:5n-3	tr	0.7 ± 0.3
2	18:2-20:4n-6	0.6 ± 0.1	0.1 ± 0.0*
3	18:1-20:5n-3	tr	1.0 ± 0.0*
4	16:0-20:5n-3, 18:1-22:6n-3	tr	1.7 ± 0.0*
5	18:2-18:2n-6	0.8 ± 0.1	
	18:2-18:2n-6, 16:0-22:6n-3		1.4 ± 0.0*
6	x-y	tr	1.5 ± 0.0*
7	18:1-20:4n-6	5.3 ± 0.1	
	18:1-20:4n-6, 16:0-22:5n-3		6.0 ± 0.1*
8	16:0-20:4n-6	4.9 ± 0.3	3.0 ± 0.1*
9	18:0-20:5n-3	tr	11.8 ± 0.1*
10	18:1-18:2n-6	4.6 ± 0.6	tr
11	18:0-22:6n-3	tr	6.3 ± 0.3*
12	16:0-18:2n-6, 16:1-18:1n-9	0.5 ± 0.1	1.2 ± 0.1*
13	16:0-22:4n-6	1.7 ± 0.5	tr
14	18:0-22:5n-3	0.4 ± 0.3	10.7 ± 0.3*
15	18:0-20:4n-6	65.7 ± 2.6	38.3 ± 0.3*
16	18:1-18:1n-9	0.7 ± 0.4	1.8 ± 0.1*
17	16:0-18:1n-9	9.1 ± 1.1	8.6 ± 0.4
18	16:0-16:0, 18:0-18:2n-6	3.4 ± 0.9	0.3 ± 0.2*
19	18:0-20:3n-6	0.2 ± 0.1	0.5 ± 0.2
20	18:0-22:4n-6	0.4 ± 0.1	0.2 ± 0.1
21	18:0-18:1n-9	1.4 ± 0.1	3.9 ± 0.1*
22	16:0-18:0	0.2 ± 0.1	0.5 ± 0.2

The 1,2-diradylglycerobenzoates were prepared and subsequently chromatographed by isocratic reverse phase HPLC as described in the Experimental Procedures section. Peaks were detected at 230 nm with a Beckman 167 variable wavelength UV scanning detector and quantified using Beckman System Gold software. Diacyl, alkylacyl, and alkenylacyl molecular species were chromatographed using acetonitrile–2-propanol at 70:30, 63:37 and 65:35 (by vol), respectively. Values are means ± SEM (n = 4); x-y, unidentified molecular species. Peak # corresponds to the HPLC elution profile of specific phospholipid-derived diradylglycerobenzoates.

*Row values are significantly different ($P < 0.05$); tr, trace amounts, less than 0.1%.

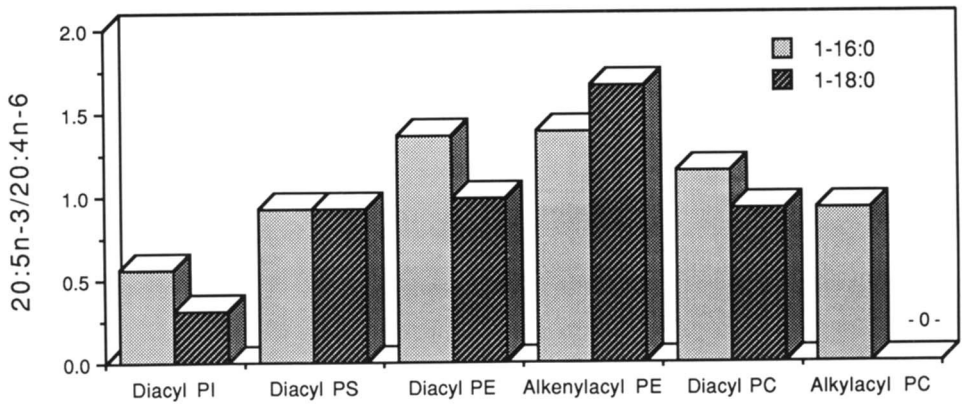

Fig. 1. Comparison of glycerophospholipid ratios of 20:5n-3 to 20:4n-6 in *sn*-1-16:0- and *sn*-1-18:0-containing molecular species in macrophages from fish oil-fed mice. Phospholipids from corn oil-fed mice contained negligible levels of 20:5n-3 (less than 0.1%). Values represent means from four separate cell preparations.

higher in fish- versus corn oil-fed animals. 18:0-20:5n-3 increased from trace amounts in the corn oil-fed animals to 11.8 mol% in the fish oil-fed animals, while 18:0-22:5n-3 and 18:0-22:6n-3 went from 0.4 mol% and trace amounts to 10.7 mol% and 6.3 mol%, respectively. Interestingly, the total mol% of *sn*-1-18:0 species remained relatively constant at 65.7 mol% in the corn oil-fed animals and 67.0 mol% in the fish oil-fed animals. *sn*-1-16:0 species of PtdIns showed similar trends with 16:0-20:4n-6 decreasing 39% from 4.9 mol% to 3.0 mol% and 16:0-20:5n-3 increasing from trace amounts to 1.7 mol%.

Comparison of PtdIns ratios of 20:5n-3 to 20:4n-6 in *sn*-1-18:0 and *sn*-1-16:0 containing species, indicated that PtdIns fatty acid remodeling may be dependent in part on the composition of the *sn*-1 moiety. For example, in the fish oil-fed animals, *sn*-1-18:0 species of PtdIns had a ratio of 20:5n-3 to 20:4n-6 of 0.31, while the ratio for *sn*-1-16:0 species was almost twice as high at 0.56 (**Fig. 1**).

The effects of dietary fish oil on the diacyl molecular species of the serine-, choline-, and ethanolamine-containing glycerophospholipids showed high variability. In general, the depletion of 20:4n-6 from PtdSer, PtdCho, and PtdEtn molecular species was considerably more extensive relative to PtdIns. Specifically, 18:0-20:4n-6 species decreased by 63, 58, and 58% in PtdSer, PtdEtn, and PtdCho, respectively, and 16:0-20:4n-6 decreased by 65, 47, and 55% (**Table 2, Table 3,** and **Table 4**). In addition, the substitution of n-3 PUFAs for 20:4n-6 was quite extensive, and unlike PtdIns, there was little difference between the 1-16:0 and 1-18:0 species with regard to the ratio of 20:5n-3 to 20:4n-6 (Fig. 1). The ratios of 20:5n-3 to 20:4n-6 in 1-16:0-containing species were 0.92, 1.36, and 1.15 for PtdSer, PtdEtn, and PtdCho, respectively, and in the 1-18:0 species were 0.93, 0.99, and 0.93.

Examination of the effects of n-3 fatty acid supplementation on 20:4n-6 levels in the ether-linked molecular species of PakCho, demonstrated an increased resistance to dietary manipulation relative to PtdCho. PakCho 18:0-20:4n-6 and 16:0-20:4n-6 decreased by only 10 and 11%, respectively (**Table 5**). The PlsEtn subclass was also more susceptible to 20:4n-6 depletion than PtdEtn, demonstrating a lowering in 18:0-20:4n-6 and 16:0-20:4n-6 species of 74 and 64%, respectively (**Table 6**). Interestingly, the extent of n-3 PUFA incorporation appeared to be dependent upon the composition of the *sn*-1 aliphatic chain (Fig. 1). For PakCho, *sn*-1-16:0 species were fairly amenable

TABLE 2. Molecular species composition of phosphatidylserine (PtdSer)

Peak #	Molecular Species	Composition (mol/100 mol) Corn	Fish/Corn
1	18:1-20:5n-3	tr	tr
2	16:0-20:5n-3, 18:1-22:6n-3	tr	0.5 ± 0.3
3	18:2-18:2n-6	0.5 ± 0.0	
	18:2-18:2n-6, 16:0-22:6n-3		1.8 ± 0.1*
4	x-y	0.3 ± 0.2	0.6 ± 0.0
5	16:0-22:5n-3	0.5 ± 0.1	4.0 ± 0.2*
6	16:0-20:4n-6	1.5 ± 0.2	0.5 ± 0.1*
7	16:0-22:5n-6	0.4 ± 0.2	tr
8	18:0-20:5n-3	tr	3.8 ± 0.1*
9	18:1-18:2n-6	5.4 ± 0.4	
	18:1-18:2n-6, 18:0-22:6n-3		10.6 ± 0.1*
10	16:0-18:2n-6, 16:1-18:1n-9	5.2 ± 0.4	3.0 ± 0.2*
11	16:0-22:4n-6	1.1 ± 0.3	tr
12	18:0-22:5n-3	1.7 ± 0.4	22.0 ± 0.4*
13	18:0-20:4n-6	11.2 ± 0.7	4.1 ± 0.1*
14	18:0-22:5n-6	2.5 ± 0.6	0.2 ± 0.1*
15	18:1-18:1n-9	2.0 ± 0.3	1.5 ± 0.2
16	16:0-18:1n-9	38.3 ± 1.9	26.1 ± 0.3*
17	16:0-16:0, 18:0-18:2n-6	10.5 ± 1.1	tr
18	18:0-20:3n-6	2.8 ± 0.1	2.4 ± 0.2
19	18:0-22:4n-6	2.4 ± 0.4	0.1 ± 0.0*
20	18:0-18:1n-9	13.8 ± 1.0	19.0 ± 0.7*

Refer to Table 1 for legend details.
*Row values are significantly different ($P < 0.05$); tr, trace amounts, less than 0.1%.

TABLE 3. Molecular species composition of diacylglycerophosphoethanolamine (PtdEtn)

Peak #	Molecular Species	Composition (mol/100 mol) Corn	Fish/Corn
1	18:2-20:5n-3	tr	0.1 ± 0.1
2	18:2-20:4n-6	0.6 ± 0.1	0.2 ± 0.1*
3	18:1-20:5n-3	tr	0.7 ± 0.1*
4	16:0-20:5n-3, 18:1-22:6n-3	0.6 ± 0.0	4.2 ± 0.1*
5	18:2-18:2n-6	2.5 ± 0.2	
	18:2-18:2n-6, 16:0-22:6n-3		6.0 ± 0.7*
6	x-y	0.3 ± 0.2	1.6 ± 0.3*
7	18:1-20:4n-6	2.3 ± 0.1	
	18:1-20:4n-6, 16:0-22:5n-3		5.6 ± 0.4*
8	16:0-20:4n-6	5.8 ± 0.3	3.1 ± 0.1*
9	16:0-22:5n-6	1.1 ± 0.1	tr
10	18:0-20:5n-3	tr	9.4 ± 0.4*
11	18:1-18:2n-6	6.8 ± 0.2	
	18:1-18:2n-6, 18:0-22:6n-3		10.2 ± 0.2*
12	16:0-18:2n-6, 16:1-18:1n-9	5.2 ± 0.5	4.8 ± 0.4
13	16:0-22:4n-6	3.3 ± 0.6	tr
14	18:0-22:5n-3	0.9 ± 0.0	12.2 ± 0.9*
15	18:0-20:4n-6	22.2 ± 0.5	9.4 ± 0.9*
16	18:0-22:5n-6	2.8 ± 0.1	0.5 ± 0.2*
17	18:1-18:1n-9	2.9 ± 0.1	2.3 ± 0.3
18	16:0-18:1n-9	20.8 ± 1.0	17.0 ± 0.2*
19	18:0-18:2n-6	10.7 ± 0.8	tr
20	18:0-20:3n-6	2.3 ± 0.1	3.4 ± 1.4
21	18:0-22:4n-6	1.6 ± 0.1	0.1 ± 0.0*
22	18:0-18:1n-9	6.1 ± 0.2	8.0 ± 0.4*
23	16:0-18:0	1.2 ± 0.2	1.1 ± 0.1

Refer to Table 1 for legend details.
*Row values are significantly different ($P < 0.05$); tr, trace amounts, less than 0.1%.

to n-3 PUFA incorporation as evidenced by a 20:5n-3 to 20:4n-6 ratio of 0.93. This was comparable to 1.15 for *sn*-1-16:0 species of PtdCho. In addition, the *sn*-1-18:0 species of PakCho did not incorporate n-3 PUFAs to any measurable extent unlike its *sn*-1-18:0 diacyl PC counterpart (0.93). For PlsEtn, the *sn*-1-16:0 species acted much like their PtdEtn counterparts as the ratio of 20:5n-3- to 20:4n-6-containing species was 1.39 versus 1.36. However, the *sn*-1-18:0 species of PlsEtn were much more active at incorporating n-3 PUFAs as the ratio of 20:5n-3 to 20:4n-6 was increased to 1.66 versus 0.99 for PtdEtn.

It is important to note that 20:5n-3 was not the only n-3 PUFA found in macrophage phospholipids. In particular, 22:5n-3 added significantly to the total n-3 PUFA presence as was evidenced by the ratios of 22:5n-3 to 20:5n-3 in the various phospholipid subclasses (**Fig. 2**). Interestingly, PtdSer and PtdCho were particularly good stores for the longer chain n-3 PUFAs. The ratio of 18:0-22:5n-3 to 18:0-20:5n-3 was 5.73 for PtdSer and 3.93 for PtdCho. In comparison, the ratio was only 0.91 and 1.31 for PtdIns and PtdEtn, respectively. The ether-linked molecular species showed no particular preference for the longer chain n-3 PUFAs when compared with their diacyl counterparts. PlsEtn was similar to PtdEtn in terms of the ratio of 18:0-22:5n-3 to 18:0-20:5n-3 (1.16 vs. 1.31), while PakCho contained relatively low levels of the longer chain n-3 PUFAs (Table 5).

Results from peptidoleukotriene synthesis show that animals fed fish oil had distinct patterns of release when compared with the animals fed corn oil. The release of the 20:4n-6-derived LTC_4 was significantly lowered ($P < 0.01$) for the fish oil-fed animals (n=4) (27.1 ± 2.6 ng/mg protein) when compared with the corn oil-fed animals (60.3 ± 2.8 ng/mg protein). Contrarily, the release of the 20:5n-3 product LTC_5 was significantly higher ($P < 0.01$) for the fish oil-fed animals (23.1 ± 3.1 ng/mg protein) when compared with the minimal release of LTC_5 from the corn oil-fed animals (0.8 ± 0.8 ng/mg protein). Thus, the ratio of LTC_5/LTC_4 was dramatically affected, being elevated from 0.01 in the corn oil fed-animals to 0.85 in the fish oil-fed animals. This last value may have some significance with respect to a possible relationship to the 20:5n-3 to 20:4n-6 ratio of molecular species from the same subclass sharing a common *sn*-1 moiety (Fig. 1.).

DISCUSSION

The present data indicate that the relative abundance (mol%) of macrophage phospholipids was not affected by fish oil feeding (data not shown). This is consistent with

TABLE 4. Molecular species composition of diacylglycerophosphocholine (PtdCho)

Peak #	Molecular Species	Composition (mol/100 mol) Corn	Fish/Corn
1	18:2-20:5n-3	tr	0.8 ± 0.2*
2	18:2-20:4n-6	1.5 ± 0.1	0.3 ± 0.1*
3	18:1-20:5n-3	tr	0.6 ± 0.2*
4	16:0-20:5n-3, 18:1-22:6n-3	0.2 ± 0.1	4.2 ± 0.3*
5	18:2-18:2n-6	4.4 ± 0.1	
	18:2-18:2n-6, 16:0-22:6n-3		5.3 ± 0.2*
6	x-y	tr	4.4 ± 0.9*
7	18:1-20:4n-6	2.2 ± 0.1	tr
8	16:0-22:5n-3	tr	7.2 ± 0.2*
9	16:0-20:4n-6	8.0 ± 0.2	3.7 ± 0.2*
10	18:0-20:5n-3	tr	1.4 ± 0.1*
11	18:1-18:2n-6	7.7 ± 0.2	
	18:1-18:2n-6, 18:0-22:6n-3		4.7 ± 0.2*
12	16:0-18:2n-6, 16:1-18:1n-9	22.7 ± 0.1	16.9 ± 0.4*
13	18:0-22:5n-3	1.5 ± 0.1	4.1 ± 0.2*
14	18:0-20:4n-6	3.6 ± 0.2	1.5 ± 0.2*
15	18:1-18:1n-9	5.7 ± 0.1	4.5 ± 0.2*
16	16:0-18:1n-9	24.4 ± 0.5	23.6 ± 0.2
17	16:0-16:0, 18:0-18:2n-6	13.8 ± 0.2	14.4 ± 0.1
18	18:0-22:4n-6	0.1 ± 0.0	tr
19	18:0-18:1n-9	2.2 ± 0.3	3.2 ± 0.4
20	16:0-18:0	2.0 ± 0.0	2.1 ± 0.2

Refer to Table 1 for legend details.
*Row values are significantly different ($P < 0.05$); tr, trace amounts, less than 0.1%.

TABLE 5. Molecular species composition of alkyacylglycerophosphocholine (PakCho)

Peak #	Molecular Species	Composition (mol/100 mol) Corn	Fish/Corn
1	18:2-20:5n-3	tr	1.0 ± 0.2*
2	18:2-20:4n-6	1.8 ± 0.0	tr*
3	18:1-20:5n-3	tr	3.0 ± 0.1*
4	16:0-20:5n-3, 18:1-22:6n-3	1.2 ± 0.0	11.6 ± 1.6*
5	16:0-22:6n-3	tr	5.5 ± 0.1*
6	18:1-20:4n-6	0.4 ± 0.1	tr
7	16:0-22:5n-3	tr	5.1 ± 0.0*
8	16:0-20:4n-6	14.0 ± 0.3	12.4 ± 0.0*
9	18:1-18:2n-6	16.1 ± 0.4	
	18:1-18:2n-6, 18:0-22:6n-3		6.2 ± 0.3*
10	16:1-18:1n-9	tr	0.3 ± 0.3
11	16:0-18:2n-6	11.6 ± 0.6	5.5 ± 0.1*
12	16:0-22:4n-6	4.2 ± 4.2	tr
13	18:0-20:4n-6	14.3 ± 4.0	12.8 ± 0.2
14	x-y	tr	0.7 ± 0.2
15	18:0-22:5n-6	1.7 ± 0.3	0.4 ± 0.0*
16	18:1-18:1n-9	3.2 ± 0.1	2.6 ± 0.3
17	16:0-18:1n-9	23.4 ± 0.4	22.2 ± 0.8
18	16:0-16:0, 18:0-18:2n-6	6.3 ± 0.1	7.2 ± 0.4
19	18:0-18:1n-9	0.6 ± 0.0	0.6 ± 0.0*
20	16:0-18:0	1.3 ± 0.1	1.6 ± 0.5

Refer to Table 1 for legend details.
*Row values are significantly different ($P < 0.05$); tr, trace amounts, less than 0.1%.

the minimal effect of n-3 PUFA supplementation on the relative macrophage subclass distribution of choline and ethanolamine glycerophospholipids (14). In contrast, dietary fish oil significantly altered the profile of fatty acyl moieties of macrophage phospholipids when compared to corn oil-fed animals. In general, n-6-containing species were depleted in the fish oil-fed animals (Tables 1-6). This is evidenced by dramatic decreases in the predominant 20:4n-6-containing species, 16:0-20:4n-6 and 18:0-20:4n-6 species (**Fig. 3**). As expected, the depletion of n-6 species was accompanied by increases in the corresponding n-3 species. For example, PtdIns *sn*-1-18:0-containing species demonstrated a near 1:1 substitution of the n-3 PUFAs, 20:5n-3, 22:5n-3 and 22:6n-3, for 20:4n-6 (Table 1). The ratios of 20:5n-3 to 20:4n-6 in *sn*-1-16:0 and *sn*-1-18:0 species of the various phospholipid subclasses also provide an indication of the extent of n-3 incorporation in macrophages from the fish oil-fed animals (Fig. 1). The ratios of 20:5n-3 to 20:4n-6 for these species also indicate important differences that may exist in remodeling of subclass molecular species based upon the *sn*-1 moiety. It is not uncommon that enzymes involved in phospholipid metabolism may have substrate specificities dependent in part upon the *sn*-1 covalent linkage and aliphatic chain composition (33-35). The molecular species of PakCho represent a clear example of the importance of the *sn*-1 moiety in regulating phospholipid remodeling. While *sn*-1-16:0 species of PakCho have a 20:5n-3 to 20:4n-6 ratio of 0.93, *sn*-1-18:0 species have a 20:5n-3 to 20:4n-6 ratio of zero (no detectable 20:5n-3) (Fig. 1). These results are significant because it has been shown that PLA_2, an *sn*-2 lipase responsible for liberating 20:4n-6 prior to its transformation into eicosanoids, may have a substrate specificity based in part upon both the aliphatic chain composition of the *sn*-1 moiety (36) and the acyl, alkyl, or alkenyl subclass linkage (16, 18, 37). Interestingly, PakCho has been identified as a possible source of eicosanoid precursors (9, 18, 34). Thus, if the 20:4n-6 utilized for eicosanoid synthesis is primarily derived from *sn*-1-18:0 species of PakCho, dietary fish oil would have a minimal effect upon eicosanoid synthesis. Contrarily, if the 20:4n-6 utilized for eicosanoid synthesis is primarily derived from *sn*-1-16:0 species of PakCho, dietary fish oil would have a measurable effect on eicosanoid synthesis as 20:5n-3 could compete with 20:4n-6 for eicosanoid pathways. Even if one of the other phospholipid subclasses serves preferentially as a PLA_2 substrate, there are still significant differences between 20:5n-3 incorporation into the *sn*-1-16:0 and *sn*-1-18:0 species that preferences for either of these species would differentially impact eicosanoid synthesis.

The ratio of 20:5n-3 to 20:4n-6 in the various species may also be used to indicate possible sources of peptido-

TABLE 6. Molecular species composition of alkenylacylglycerophosphoethanolamine (PlsEtn)

Peak #	Molecular Species	Composition (mol/100 mol) Corn	Fish/Corn
1	18:2-20:5n-3	tr	0.3 ± 0.1
2	18:1-20:5n-3	1.9 ± 0.1	3.4 ± 0.4*
3	16:0-20:5n-3, 18:1-22:6n-3	1.1 ± 0.1	14.7 ± 0.1*
4	16:1-22:4n-6	4.6 ± 0.2	tr*
5	16:0-22:6n-3	tr	11.5 ± 0.3*
6	x-y	tr	5.6 ± 0.2*
7	18:1-20:4n-6	9.5 ± 0.4	
	18:1-20:4n-6, 16:0-22:5n-3		24.2 ± 0.3*
8	16:0-20:4n-6	29.6 ± 0.9	10.6 ± 0.2*
9	16:0-22:5n-6	0.2 ± 0.2	tr
10	18:0-20:5n-3	tr	4.8 ± 0.0*
11	18:1-18:2n-6	1.4 ± 0.2	tr*
12	18:0-22:6n-3	tr	4.5 ± 0.1*
13	16:0-18:2n-6, 16:1-18:1n-9	4.9 ± 1.7	5.2 ± 0.0
14	16:0-22:4n-6	18.8 ± 0.2	tr*
15	18:0-22:5n-3	tr	5.6 ± 0.1*
16	18:0-20:4n-6	11.0 ± 0.4	2.9 ± 0.1*
17	18:1-18:1n-9	2.6 ± 0.3	0.4 ± 0.1*
18	16:0-18:1n-9	11.4 ± 0.3	4.7 ± 0.1*
19	16:0-16:0, 18:0-18:2n-6	0.3 ± 0.3	tr
20	18:0-20:3n-6	0.2 ± 0.1	0.1 ± 0.0
21	18:0-22:4n-6	0.7 ± 0.4	0.3 ± 0.2
22	18:0-18:1n-9	1.3 ± 0.1	0.8 ± 0.0*
23	16:0-18:0	0.5 ± 0.1	0.3 ± 0.1

Refer to Table 1 for legend details.
*Row values are significantly different ($P < 0.05$); tr, trace amounts, less than 0.1%.

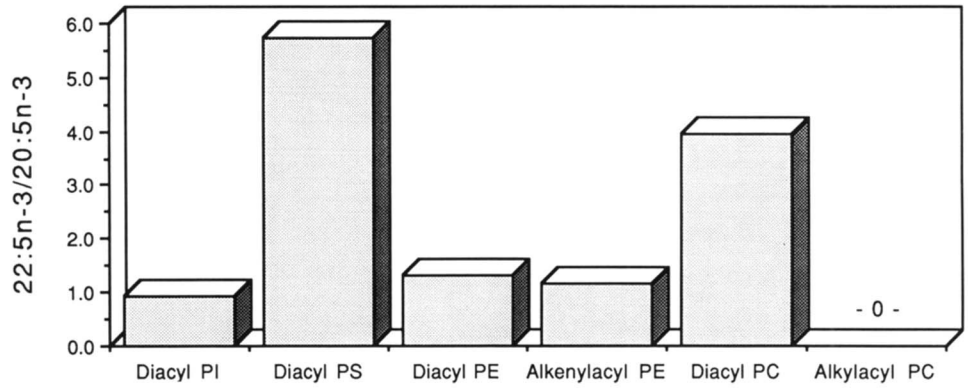

Fig. 2. Distribution of 22:5n-3 in macrophage glycerophospholipids from fish oil-fed mice. Values represent ratios of 22:5n-3 to 20:5n-3. Refer to Fig. 1 for legend details.

leukotriene precursors in macrophages. Experiments were undertaken to determine the effects of dietary fish oil on peptidoleukotriene synthesis in order to potentially link ratios of 20:5n-3 to 20:4n-6 with the ratios of products derived from these eicosanoid precursors. In these studies, indomethacin was utilized to shunt eicosanoid precursors away from the cyclooxygenase pathway toward the lipoxygenase pathway (10). This strategy was used because 20:5n-3 is a poor substrate for the cyclooxygenase pathway relative to 20:4n-6 (10), whereas 20:5n-3 is comparable to 20:4n-6 as a substrate for the 5-lipoxygenase pathway (38). As has been described in earlier reports (4, 6, 14), our results demonstrate a lowered synthesis of the 20:4n-6 product LTC_4 by macrophages isolated from fish oil-fed animals. This was accompanied by an increase in LTC_5 synthesis giving a ratio of LTC_5 to LTC_4 of 0.85 for the fish oil-fed animals. This ratio compares favorably with the ratios of 20:5n-3 to 20:4n-6 in a number of *sn*-1-16:0 and *sn*-1-18:0 subclass species including those of the *sn*-1-16:0 species of PakCho and the *sn*-1-16:0 and *sn*-1-18:0 species of PtdCho. Neither the *sn*-1-16:0 nor the *sn*-1-18:0 species of PtdIns appear to be likely candidates as lipoxygenase precursors according to our results in the macrophage, even though PtdIns has been implicated as a source of eicosanoid precursors in other tissues (39).

While much attention has been focused on 20:5n-3, the predominant n-3 PUFA in macrophage phospholipids after fish oil feeding is 22:5n-3, the elongase product of 20:5n-3. In fact, 18:0-22:5n-3 was the major n-3 species in PtdIns, PtdSer, and PtdEtn, and 16:0-22:5n-3 was the major n-3 species in PtdCho and PlsEtn (Tables 1-6). We have previously demonstrated that the highly active chain elongation of 20:5n-3 to 22:5n-3 by macrophage elongase, as well as a lack of detectable Δ^4 desaturase activity (capable of converting 22:5n-3 to 22:6n-3), are responsible for the accumulation of 22:5n-3 in this cell (40). The extensive elongation of 20:5n-3 may remove a potential competitive inhibitor of macrophage 20:4n-6 metabolism and thereby reduce the effects of fish oil alimentation on endogenous eicosanoid synthesis. Elongation of 20-carbon fatty acids to 22-carbon fatty acids, which are not released from cellular phospholipids in response to stimulation, has been

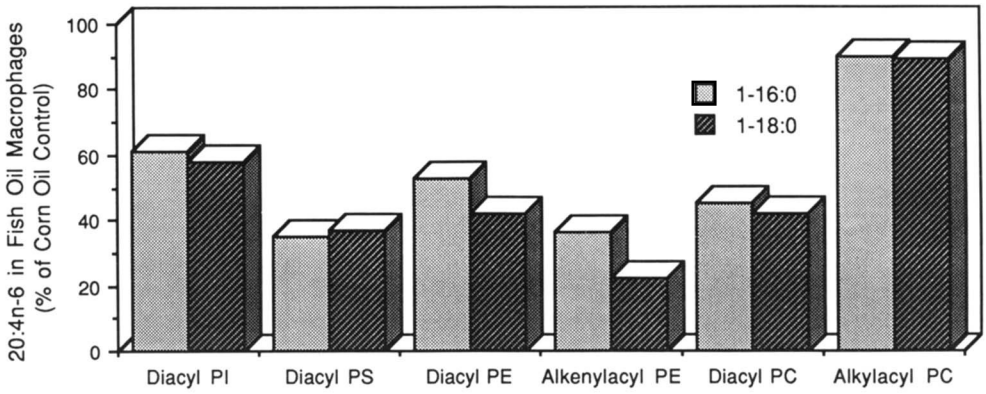

Fig. 3. Depletion of 20:4n-6 in macrophage glycerophospholipids from fish oil-fed mice. Values are expressed as percent of corn oil control cells. Refer to Fig. 1 for legend details.

demonstrated in human vascular endothelial cells (41). Our present results indicate that certain macrophage phospholipid subclasses, particularly PtdSer and PtdCho, may represent dead-end storage pools for 22:5n-3 (Fig. 2).

PakCho, which constitutes approximately 30% of ChoGpl and 15% of total peritoneal macrophage phospholipids (14), is highly enriched in 16:0-20:4n-6 and 18:0-20:4n-6 (Table 5), the common precursors to PAF. Interestingly, only moderate decreases in the 16:0-20:4n-6 and 18:0-20:4n-6 species of PakCho were observed after fish oil supplementation. In contrast, other 20:4n-6-containing species were significantly reduced by fish oil feeding. These data indicate that PakCho strongly conserves 20:4n-6 and is resistant to fat alimentation. Although the mechanisms by which PUFA are distributed among phospholipid molecular species remain poorly understood, macrophages are known to possess a CoA-independent 20:4n-6-specific acyltransferase that appears to be extremely important in the remodeling of ChoGpl molecular species (42). This may represent a means by which the macrophage preserves the availability of PAF precursors. This is consistent with recent findings in the neutrophil that dietary fish oil had little impact upon PAF production (43).

In conclusion, the present findings demonstrate that marked differences exist in the ability of macrophage phospholipid subclasses to incorporate and retain n-3 PUFAs at the expense of 20:4n-6. The results have implications for both eicosanoid and PAF synthesis and thus the ability of macrophages to function as modulators of immune and inflammatory events. Because of the complexity of phospholipid molecular species, further studies will be necessary to elucidate the remodeling mechanisms that determine the distribution of 20:4n-6 and n-3 PUFAs within macrophage phospholipids.

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