Modification of spleen phospholipid fatty acid composition by dietary fish oil and by n–3 fatty acid ethyl esters

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Abstract We have compared the effects of diets containing purified ethyl esters of either eicosapentaenoic acid, docosahexaenoic acid, or a mixture of both of these compounds, with diets containing either purified fish oil or beef tallow on spleen phospholipid fatty acid composition. Autoimmune mice, the (NZB × NZW)F1 strain, were fed with experimental diets for 14 weeks, after which spleen phospholipids were extracted and separated into classes by HPLC, and the alkenylacyl, alkylacyl, and diacyl subclasses of glycerylphosphatidylethanolamine and glycerylphosphatidylcholine were resolved as their benzoyl esters by HPLC. Fatty acids were analyzed by capillary gas–liquid chromatography of their methyl esters. Each of the marine lipid diets suppressed n-6 fatty acids and elevated n-3 fatty acids in all phospholipids. The eicosapentaenoic acid ethyl ester diets led to high levels of eicosapentaenoic acid and docosapentaenoic acid (C22:5n-3), but little or no increase in docosahexaenoic acid. The docosahexaenoic acid ethyl ester diets elevated docosahexaenoic acid, docosapentaenoic acid, and eicosapentaenoic acid in all phospholipids, indicating that extensive retroconversion of 22 carbon n-3 fatty acids had occurred. These results document changes in the fatty acid composition of mammalian phospholipids that are induced by dietary fish oil triglycerides and by dietary long chain n-3 fatty acid ethyl esters. —**Robinson, D. R., L-L. Xu, C. T. Knoell, S. Tateno, and W. Olesiak.** Modification of spleen phospholipid fatty acid composition by dietary fish oil and by n-3 fatty acid ethyl esters. *J. Lipid Res.* 1993. **34:** 1423–1434.

Supplementary key words eicosapentaenoic acid • docosahexaenoic acid • retroconversion

Dietary marine lipids alleviate inflammatory autoimmune diseases in experimental animals (1–6), and clinical trials have documented therapeutic effects of dietary supplements of marine lipids in the human autoimmune disease, rheumatoid arthritis (7–9). The production of inflammatory eicosanoids derived from arachidonic acid in human leukocytes is reduced by marine lipids, and n-3 analogues of certain eicosanoids are less active than their n-6 analogues (10–13). Although changes in eicosanoid syntheses may contribute to the apparent anti-inflammatory effects of fish oils, the mechanisms of these anti-inflammatory effects remain uncertain and several other possible mechanisms exist. Incorporation of n-3 fatty acids leads to alterations in many functions of cell membranes (14–16).

Although it is logical to assume that the biological effects of fish oils are due to n-3 fatty acids, there has been little work comparing the effects of individual n-3 fatty acids with the effects of fish oil triglycerides, or on the differences in the abilities of the individual n-3 fatty acids and fish oils to modify the fatty acid composition of tissue lipids. In order to help clarify the mechanisms of the anti-inflammatory effects of marine lipids in autoimmune diseases, we have carried out experiments comparing the effects of purified preparations of two n-3 fatty acid ethyl esters, eicosapentaenoic acid (20:5n-3, EPA-E) and docosahexaenoic acid (22:6n-3, DHA-E), with a refined fish oil triglyceride preparation and with beef tallow. In this report, we compare fatty acid compositions of the major phospholipid classes, including the ether-linked phospholipid subclasses, from spleens of (NZB × NZW)F1 (NZB/NZW) mice fed with experimental diets containing these lipids for 13 weeks. We have also compared the effects of these same diets on the severity of the autoimmune glomerulonephritis in these experiments, and these results are presented in the accompanying manuscript (17).

Abbreviations: PUFA, polyunsaturated fatty acids; FAME, fatty acid methyl ester; EPA and EPA-E, eicosapentaenoic acid (20:5n-3) and its ethyl ester; DHA and DHA-E, docosahexaenoic acid (22:6n-3) and its ethyl ester; AA, arachidonic acid (20:4n-6); NZB/W, New Zealand Black-White F1 hybrid mice, (NZB × NZW)F1; BT, beef tallow; FO, fish oil; HPLC, high performance liquid chromatography; GPC, *sn*-glycero-3-phosphocholine; GPE, *sn*-glycero-3-phosphoethanolamine; GPI, *sn*-glycero-3-phosphoinositol; GPS, *sn*-glycero-3-phosphoserine; alkylacyl, 1-O-alkyl-2-.

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EXPERIMENTAL PROCEDURES

Materials

High performance liquid chromatography (HPLC) grade methanol, hexane, cyclohexane, chloroform stabilized with 1% (v/v) ethanol, 2-propanol, acetic acid, and water were obtained from Fisher Scientific (Medford, MA). Ethanol was purchased from Pharmaco (Dayton, NJ); 30% ammonium hydroxide and HPLC glacial acetic acid were from J. T. Baker (Phillipsburg, PA). Benzene and diethyl ether (HPLC grade) were from Burdick & Jackson (Muskegon, WI). Phospholipase C, Type V (from *Bacillus cereus*), potassium phosphate, silicic acid, benzoic anhydride, 4-dimethylamino-pyridine, and anhydrous sodium sulfate, were purchased from Sigma (St. Louis, MO). The *t*-butylhydroquinone was from Aldrich (Milwaukee, WI). Components of the experimental diets: beef tallow (BT), safflower oil, the fat-free diet, and α-tocopherol were obtained from ICN Nutritional Biochemicals (Cleveland, OH).

Methods

Female NZB/W mice were obtained from the Jackson Laboratories (Bar Harbor, ME) and housed 4–5 per cage under standard conditions. The mice were fed ad libitum with normal laboratory chow (Purina No 5015, 11% fat) until age 22 weeks, when they were randomly assigned to one of the experimental diets, and fed the experimental diets for the next 14 weeks. Experimental diets were formulated with the fat-free diet which contained 21% casein, 15.6% cellulose, 58.5% sucrose, 4% USP XIV balanced salt mixture (all % wt), vitamins, and essential trace minerals (ICN Nutritional Biochemicals, Cat. No. 02-999999, Quote BS931065). All of the dietary oils were stabilized with 0.02% α-tocopherol. All diets contained 88% of the fat-free mixture blended with 12% fat, of which 2% was safflower oil to prevent essential fatty acid deficiency and 10% was either an n-3 fatty acid-enriched preparation, beef tallow (BT), or a mixture of the two. Diets containing less than 10% of an n-3 fatty acid preparation had added BT such that the sum of the n-3 fatty acid preparation and the BT was 10%. The quantities of the lipids in all diets are given as wt% of the total diet. The dietary marine lipid preparations, generously provided by Nippon-Suisan Kaisha Ltd., Tokyo, Japan, were Nissui-28, a refined fish oil triglyceride (FO), and two purified n-3 fatty acid ethyl esters, 5,8,11,14,17, all *cis*-eicosapentaenoic acid (EPA-E), and 4,7,10,13,16,19, all *cis*-docosahexaenoic acid (DHA-E). All n-3 fatty acid preparations were stored at 4°C under N_2 in the dark in airtight containers, after addition of 0.02% *t*-butylhydroquinone. The fatty acid compositions of the dietary lipids are given in **Table 1.**

TABLE 1. Fatty acid composition of dietary lipids[a]

FAME	Beef Tallow	Fish Oil	EPA-E[b]	DHA-E[c]	Safflower Oil
14:0	3.0 ± 0.1	6.0 ± 0.1			0.1 ± 0.0
16:0	25.4 ± 0.1	8.3 ± 0.1			6.8 ± 0.0
18:0	19.5 ± 0.1	0.8 ± 0.0			2.7 ± 0.0
16:1n-7	2.6 ± 0.0	9.5 ± 0.1			0.1 ± 0.0
18:1n-9	37.3 ± 0.1	7.4 ± 0.1			11.5 ± 0.0
18:1n-7	6.4 ± 0.0	3.0 ± 0.0			0.7 ± 0.0
18:2n-6	4.7 ± 0.1	1.4 ± 0.0			76.4 ± 0.0
20:4n-6		1.3 ± 0.0	4.8 ± 0.0		
22:5n-6		0.3 ± 0.1		2.8 ± 0.0	
18:3n-3	0.3 ± 0.0	1.1 ± 0.0			0.1 ± 0.0
18:4n-3	0.4 ± 0.0	5.1 ± 0.0	2.3 ± 0.0		
20:5n-3		30.9 ± 0.1	90.9 ± 0.1	0.9 ± 0.0	0.1 ± 0.0
22:5n-3		2.9 ± 0.0		3.6 ± 0.0	0.2 ± 0.0
22:6n-3		12.6 ± 0.0		89.6 ± 0.1	0.2 ± 0.0
Saturated	47.9	15.2	0.4	1.9	10.0
Monoene	46.7	25.1	0.6	0.1	12.8
n-6 PUFA	4.7	3.5	5.1	3.4	76.5
n-3 PUFA	0.7	55.2	93.9	94.7	0.6
Total PUFA	5.4	59.8	99.0	98.1	77.1

[a]See the Methods section for details. Each value is the wt % of the total FAME, calculated from the area of each FAME divided by the sum of the integrated areas of all FAMEs identified on gas-liquid chromatography. The data are the means ± SEM of three determinations. Only fatty acids comprising >2.0 of the total fatty acid content in one of the lipid preparations are listed individually, except for 18:3n-3.

[b]Eicosapentaenoic acid ethyl ester.

[c]Docosahexaenoic acid ethyl ester.

Diets were prepared weekly and stored at 4°C under N_2 in sealed glass containers. The diets were dispensed in clean glass dishes every other day. Animals were weighed weekly and the mean weights of all groups were maintained within 5% for each experiment, by small adjustments in the quantities of the diets given. Experimental diets were given for 13 weeks at which time the experiment was terminated. Not all groups were studied contemporaneously, and to assure that groups studied during different time periods were comparable, studies of three groups each of BT- and FO-fed mice were carried out over the duration of these experiments. Each of these groups was analyzed for all of the phospholipid classes and subclasses reported here. There were no significant differences in fatty acid composition between the three BT groups or the three FO groups. Mice were killed with ether after rapid removal of organs under ether anesthesia. Spleens were immediately frozen in liquid nitrogen and stored at −20°C under nitrogen in sealed containers until lipids were extracted. Kidneys were removed and stored in 10% aqueous formalin for histological analyses.

Lipids were extracted from mouse spleen using a slightly modified Folch method (18). Briefly, spleens from 4–5 mice were homogenized in 10 ml chloroform–methanol 2:1 (v/v) per g tissue, containing 25 mg/l butylhydroxytoluene, and allowed to incubate at room temperature overnight. The homogenate was filtered with Whatman No. 1 filter paper, and the filtrate was washed twice with 0.88% KCl (1 ml per 4 ml of filtrate) and dried under nitrogen. Lipid extracts were stored in chloroform (1 ml/g of tissue) under nitrogen at −20°C. Lipid extracts were separated into phospholipid classes by HPLC using a modification of the method of Patton, Fasulo, and Robins (19). Extracts were first filtered using a Millex-HV 0.45 mM filter unit (Millipore, Corp., Bedford, MA). They were then dried under nitrogen and resuspended in hexane–isopropanol–water 40:54:6. Separation into phospholipid classes was achieved using a 4.6 × 250 mm column packed with Lichrosphere Si-100 (EM Reagents, Germany). The separation was performed isocratically using a Perkin-Elmer Series 410 LC pump (Norwalk, CT) equipped with a Rheodyne 7125 Injector (Cotati, CA) and a Perkin-Elmer LC-90 UV spectrophotometer at 205 nm. The eluting solvent was prepared by first mixing 490 ml 2-propanol with 52 ml 25 mM potassium phosphate buffer (pH 7.0) and then adding 367 ml hexane and 100 ml absolute ethanol. The solvent was stored overnight at 4°C, and then filtered using a 0.5-mm Millipore FH filter. To the filtrate were added 2 ml water and 0.6 ml glacial acetic acid making the final composition of the mobile phase 2-propanol–hexane–ethanol–buffer–water–acetic acid 490:367:100:52:2:0.6. The quantities of phospholipid classes were determined by phosphorus analyses (20).

Separation of GPE and GPC fractions into subclasses was performed using the method of Blank, Cress, and Snyder (21). The lipid classes were first hydrolyzed with phospholipase C yielding diradylglycerols. Up to 2 mg of diradylglycerols was subsequently benzoylated using 10 mg benzoic anhydride and 4 mg 4-dimethylaminopyridine dissolved in 0.3 ml of benzene. After 1 h, the benzoylation reaction was terminated by the addition of 1 ml 30% ammonium hydroxide. The resulting diradylglycerol benzoates were extracted three times using 2 ml hexane. Extracts were then washed with 1 ml water and dried overnight with anhydrous sodium sulfate. The samples were then dried under nitrogen and reconstituted in 1 ml chloroform. Removal of the 4-dimethylaminopyridine was accomplished using 0.5 cm × 2 cm silicic acid columns, eluting the diradylglycerobenzoates with 11–12 ml chloroform. Samples were then dried under nitrogen and reconstituted in 1 ml cyclohexane.

Separation of diradylglycerobenzoates into diacyl-, alkylacyl-, and alkenylacyl- subclasses was accomplished by straight-phase HPLC using a 4.6 × 250 mm Ultrasphere-Si column (Beckman, San Ramon, CA) eluted isocratically with cyclohexane–diethyl ether–acetic acid 970:30:0.7 at a flow rate of 1.0 ml/min. Subclasses were quantitatively measured at 230 nm using a Perkin-Elmer LCI-100 laboratory integrator. The order of elution of the subclasses was alkenylacyl-, (7 min), alkylacyl-, (10 min), and diacylglycerobenzoate (20 min).

Fatty acid methyl esters (FAME) were prepared by acid-catalyzed transesterification of phospholipids and diradylglycerobenzoates using Instant Methanolic HCl Kits (Cat. No. 18053, Alltech/Applied Science, Deerfield, IL). The fatty acid composition was analyzed by gas-liquid chromatography of the FAME using a Perkin-Elmer 8500 gas chromatograph and a SUPELCOWAX 10 flexible fused silica column, 0.25 mm I.D. × 30 m (Supelco Inc., Bellefonte, PA). Split injection was used with a ratio of 1:25 with the injector at 300°C. Initial oven temperature was 172°C for 3 min following which the temperature was increased 3°C per min to a final temperature of 235°C (22). A flame ionization detector was used at 300°C and the detector signals were quantitated with a Perkin-Elmer 8500 Data Handling System. The FAME composition was calculated by dividing the integrated area of each peak by the total area of all identified FAME peaks on each chromatogram. In general, the area of the total identified peaks accounted for approximately 95% of the total integrated area of each run, after the initial solvent peak. Identification of each FAME peak was based on comparison of relative retention time of FAME in PUFA I and PUFA II standards (Supelco Inc.). In all cases, replicate determinations represent analyses of fractions from different HPLC class separations.

TABLE 2. Fatty acid composition of spleen 1,2 diacyl-*sn*-glycero-3-phosphoethanolamines from NZB/W mice fed either beef tallow or 10% marine lipid diets[a]

FAME	BT	FO 10%	EPA-E 10%	DHA-E 10%
16:0	8.5 ± 0.1	12.0 ± 1.4	9.5 ± 0.2	14.5 ± 0.5
18:0	31.0 ± 0.1	31.3 ± 1.1	31.2 ± 0.5	27.6 ± 0.1
16:1n-9	0.1 ± 0.1			0.1 ± 0.0
16:1n-7	0.5 ± 0.0	1.0 ± 0.2	0.5 ± 0.0	0.8 ± 0.1
18:1n-9	12.5 ± 0.4	8.4 ± 0.5	6.9 ± 0.1	8.0 ± 0.1
18:1n-7	3.4 ± 0.1	3.8 ± 0.3	2.3 ± 0.0	2.6 ± 0.0
20:1	0.3 ± 0.2			
18:2n-6	4.1 ± 0.1	5.3 ± 0.5	3.7 ± 0.0	6.8 ± 0.1
20:2n-6	0.7 ± 0.0	0.7 ± 0.1	0.5 ± 0.0	0.7 ± 0.0
20:3n-6	1.0 ± 0.0	0.9 ± 0.0	0.5 ± 0.0	1.1 ± 0.0
20:4n-6	25.9 ± 0.5	10.4 ± 0.1	14.7 ± 0.1	7.6 ± 0.5
22:4n-6	5.4 ± 0.0	0.6 ± 0.0	0.9 ± 0.0	0.3 ± 0.0
22:5n-6	3.1 ± 0.1		0.8 ± 0.2	
20:5n-3		9.0 ± 0.3	11.5 ± 0.1	5.4 ± 0.0
22:5n-3	0.3 ± 0.0	6.4 ± 0.4	14.2 ± 0.2	2.5 ± 0.0
22:6n-3	3.2 ± 0.3	10.3 ± 1.0	3.6 ± 0.1	21.4 ± 0.1
Saturated	39.5	43.3	40.7	42.1
Monoene	16.9	13.2	9.8	11.5
n-6 PUFA	40.1	17.8	20.3	17.2
n-3 PUFA	3.5	25.7	29.3	29.3
Total PUFA	43.7	43.5	49.6	46.5

[a]The data are the means ± SEM of two determinations. See footnote to Table 1 for details.

RESULTS

All experimental diets contained 12 wt% lipid, of which 2 wt% was safflower oil, in order to provide adequate quantities of linoleic acid. Diets with less than 10% marine lipid had sufficient beef tallow added to bring the total lipid content to 12%, including the safflower oil. The fatty acid compositions of the lipid preparations used in these diets are given in Table 1.[2] Nearly 95% of the fatty acids in BT were either saturated or monoenoic, in nearly equal proportions. The FO preparation contained 55% n-3 fatty acids and 3.5% n-6. The EPA-E contained 91% EPA and 4.8% arachidonic acid, and the DHA-E contained 90% DHA and small quantities of other PUFA, primarily 22:5n-6, and 22:5n-3.

The distribution of spleen phospholipids into classes and subclasses did not differ significantly among any of the different dietary groups. Five phospholipid classes were isolated from spleen lipid extracts by HPLC, and the quantities of each class were measured by phosphorus determinations. The percentage of each class was calculated from the percent of the total phosphorus measured in the five classes. Sphingomyelin was estimated to comprise <5% of total phospholipid, based on the absorbance of the HPLC tracings in the area corresponding to sphingomyelin, and this class was not analyzed further. The distribution of phospholipids into the five classes for the BT group was: GPE 32%, GPI 12%, GPS 16%, cardiolipin 2.5%, and GPC 37%. Since the class distribution of phospholipids was similar for all the dietary groups, the remaining distribution data are not presented. The distribution of glycerophosphoethanolamine (GPE) and glycerophosphocholine (GPC) into subclasses was determined from the absorbance of their diradyl benzoates at 230 nm during separation of the subclasses by reverse phase HPLC. The integrated absorbencies of the three subclasses were taken as 100%, and the quantities of each subclass were calculated from its absorbance. For the BT group, the subclass distribution of GPE was: alkenylacyl 31%, alkylacyl 9%, and diacyl 60%. The subclass distribution of GPC for the BT group was: alkenylacyl 1.7%, alkylacyl 16%, and diacyl 82%. Again, the subclass distribution of the other dietary groups is not presented since it was nearly the same in all cases.

Fatty acid analyses of NZB/W mouse spleen phospholipids. The results of determinations of the fatty acid compositions of the diacyl fractions of *sn*-glycero-3-phosphoethanolamine (GPE) and *sn*-glycero-3-phosphocholine (GPC) are given in **Table 2 and Table 3,** respectively, for

[2]In reporting the quantities of fatty acids, we have assumed that the oils consist entirely of fatty acids. Neglecting the contribution of the glycerol and ethanol components of the oils introduces errors in the fatty acid contents of the diets ranging from approximately 5% for the beef tallow and the fish oil preparations to 8% for the EPA-E.

TABLE 3. Fatty acid composition of spleen 1,2-diacyl-*sn*-glycero-3-phosphocholine from NZB/W mice fed either 10% beef tallow or 10% marine lipid diets[a]

FAME	BT	FO 10%	EPA-E 10%	DHA-E 10%
14:0	0.4 ± 0.0	0.4 ± 0.0	0.2 ± 0.0	
16:0	33.8 ± 0.3	41.4 ± 0.8	39.0 ± 0.0	30.8 ± 0.6
18:0	11.3 ± 0.1	10.1 ± 0.2	10.6 ± 0.0	13.1 ± 0.3
16:1n-9	0.9 ± 0.1	1.1 ± 0.0	1.1 ± 0.0	0.7 ± 0.0
16:1n-7	1.6 ± 0.0	2.7 ± 0.1	2.3 ± 0.0	1.4 ± 0.0
18:1n-9	16.8 ± 0.4	11.7 ± 0.3	10.7 ± 0.1	11.5 ± 0.1
18:1n-7	4.3 ± 0.1	5.1 ± 0.1	2.8 ± 0.0	4.0 ± 0.0
20:1	1.5 ± 0.1	0.3 ± 0.2	0.3 ± 0.0	0.3 ± 0.2
18:2n-6	7.6 ± 0.2	10.2 ± 0.0	8.0 ± 0.1	16.2 ± 0.1
20:2n-6	1.2 ± 0.2	1.0 ± 0.0	0.6 ± 0.1	1.3 ± 0.1
20:3n-6	1.7 ± 0.2	0.9 ± 0.1	0.6 ± 0.0	1.7 ± 0.1
20:4n-6	16.1 ± 0.2	4.5 ± 0.0	9.0 ± 0.2	4.6 ± 0.2
22:4n-6	1.4 ± 0.0		0.3 ± 0.0	
22:5n-6	0.8 ± 0.1			
20:5n-3		5.9 ± 0.0	8.7 ± 0.2	4.6 ± 0.1
22:5n-3		2.3 ± 0.0	4.8 ± 0.1	1.6 ± 0.4
22:6n-3	0.8 ± 0.1	2.3 ± 0.0	0.9 ± 0.0	8.1 ± 0.2
Saturated	45.4	51.9	49.8	43.9
Monoene	25.1	20.9	17.1	17.7
n-6 PUFA	28.8	16.7	18.4	23.8
n-3 PUFA	0.8	10.6	14.4	14.3
Total PUFA	29.6	27.3	32.8	38.1

[a]Means ± SEM of two determinations.

mice fed the 10% BT and 10% marine lipid diets. The fatty acid compositions of inositol glycerophospholipids (GPI) and serine glycerophospholipids (GPS) are given in **Table 4 and Table 5,** respectively, for 10% BT and 10% marine lipid diets. The n-3 fatty acids from both FO and the n-3 fatty acid ethyl esters are extensively incorporated into all four of the major classes of diacyl phospholipids, with suppression of arachidonic acid and the 22 carbon n-6 fatty acids. The ether-linked phospholipids, 1-O-alk-1'-enyl-PE and 1-O-alkyl-PC have more extensive incorporation of n-3 fatty acids by the marine lipid diets and greater replacement of the large quantities of n-6 fatty acids which are present in the BT group (**Table 6 and Table 7**), than the diacyl phospholipids.

In order to evaluate the effects of varying quantities of dietary n-3 fatty acids on phospholipid fatty acid composition, we analyzed tissues from groups of mice fed diets containing less than 10% marine lipids. The fatty acid composition of diacyl PE and 1-O-alk-1'-enyl PE were determined from diets containing 3% and 6% levels of both EPA-E and DHA-E (**Table 8 and Table 9**). Extensive n-3 fatty acid incorporation into these phospholipids occurs at all levels of dietary marine lipids, but there are somewhat lower contents of n-3 fatty acids seen with the 3% diets than with either the 6% or the 10% n-3 fatty acid ethyl ester diets.

Because EPA-E and DHA-E apparently had synergistic effects in alleviating autoimmune glomerulonephritis (17), we determined the fatty acid composition of spleen phospholipids in mice fed a diet containing a mixture of 2.8% EPA-E and 0.8% DHA-E. For example, the total n-3 fatty acid contents of alkenylacyl GPE and diacyl GPE were 52.4% and 20.8%, respectively, for the mixed n-3 fatty acid diet. These values may be compared with corresponding values of 55.7% and 20.0% for the 3% EPA-E group (Tables 8, 9 and **Table 10**). Similar comparisons of the mixed n-3 diet with the 3% DHA-E diet reveal somewhat greater n-3 fatty acid content associated with the latter diet. Thus there was no significant enhancement of incorporation of n-3 fatty acids or greater reductions in n-6 fatty acids with the mixed fatty acid diet compared to diets containing a single n-3 fatty acid ethyl ester preparation. The synergistic effects of mixtures of the two n-3 fatty acids on the suppression of autoimmune disease in NZB/NZW mice cannot be accounted for on the basis on greater changes in fatty acid composition produced by the mixed n-3 fatty acid diets.

DISCUSSION

We carried out the present experiments to compare the effects of dietary fish oils and purified n-3 fatty acid ethyl esters on the severity of an autoimmune disease and on

TABLE 4. Fatty acid composition of spleen inositol glycerophospholipids from NZB/W mice fed either 10% beef tallow or 10% marine lipid diets

FAME	BT[a]	FO 10%[a]	EPA-E 10%[b]	DHA-E 10%[a]
14:0		0.3 ± 0.2	0.2 ± 0.1	0.3 ± 0.1
16:0	3.8 ± 0.2	6.9 ± 0.4	6.5 ± 0.5	8.1 ± 0.6
18:0	40.5 ± 0.7	43.4 ± 2.7	39.9 ± 0.8	43.2 ± 2.3
16:1n-9	0.2 ± 0.1	0.6 ± 0.4	0.4 ± 0.1	0.2 ± 0.1
16:1n-7	0.1 ± 0.1	0.4 ± 0.2	0.2 ± 0.1	0.5 ± 0.2
18:1n-9	5.6 ± 0.4	4.5 ± 0.3	4.0 ± 0.1	5.0 ± 0.3
18:1n-7	1.5 ± 0.1	1.7 ± 0.1	1.1 ± 0.1	1.6 ± 0.1
20:1	0.1 ± 0.1			
18:2n-6	1.6 ± 0.1	2.1 ± 0.2	1.9 ± 0.1	3.5 ± 0.2
20:2n-6	0.3 ± 0.1		0.2 ± 0.1	
20:3n-6	1.0 ± 0.1	1.8 ± 0.2	0.8 ± 0.1	2.7 ± 0.3
20:4n-6	41.4 ± 0.7	20.4 ± 0.6	25.1 ± 0.1	17.5 ± 0.9
22:4n-6	2.5 ± 0.1	0.2 ± 0.1	0.4 ± 0.0	0.3 ± 0.1
22:5n-6	0.5 ± 0.1		0.1 ± 0.1	0.5 ± 0.0
20:5n-3		10.6 ± 0.5	11.9 ± 0.4	7.4 ± 0.7
22:5n-3		4.6 ± 0.4	6.6 ± 0.1	2.8 ± 0.3
22:6n-3	0.9 ± 0.1	2.6 ± 0.4	0.6 ± 0.1	6.6 ± 0.7
Saturated	44.3	50.5	46.6	51.5
Monoene	7.5	7.3	5.8	7.3
n-6 PUFA	47.2	24.5	28.5	24.5
n-3 PUFA	0.9	17.7	19.1	16.7
Total PUFA	48.2	42.2	47.6	41.2

[a]Means ± SEM of determinations.
[b]Means ± SEM of five determinations.

TABLE 5. Fatty acid composition of spleen serine glycerophospholipids from NZB/W mice fed either 10% beef tallow or 10% marine lipid diets[a]

FAME	BT	FO 10%	EPA-E 10%	DHA-E 10%
14:0		0.2 ± 0.1	0.2 ± 0.1	0.1 ± 0.0
16:0	2.9 ± 0.3	5.5 ± 0.4	5.1 ± 0.5	5.6 ± 0.5
18:0	45.3 ± 0.3	49.7 ± 2.2	45.0 ± 0.9	49.2 ± 2.3
16:1n-9	0.3 ± 0.1	0.1 ± 0.1	0.3 ± 0.1	0.1 ± 0.1
16:1n-7	0.1 ± 0.1	0.8 ± 0.2	0.4 ± 0.1	0.4 ± 0.0
18:1n-9	7.4 ± 0.1	6.0 ± 0.3	5.2 ± 0.2	4.8 ± 0.1
18:1n-7	1.7 ± 0.0	1.6 ± 0.1	1.0 ± 0.0	1.2 ± 0.1
20:1	0.3 ± 0.2			
18:2n-6	3.8 ± 0.2	4.8 ± 0.3	3.9 ± 0.1	5.8 ± 0.1
20:2n-6		0.1 ± 0.1	0.2 ± 0.1	0.0 ± 0.0
20:3n-6	2.3 ± 0.1	1.8 ± 0.2	1.0 ± 0.1	1.9 ± 0.2
20:4n-6	16.7 ± 0.4	3.6 ± 0.3	6.3 ± 0.2	2.3 ± 0.1
22:4n-6	8.1 ± 0.4	0.3 ± 0.1	0.8 ± 0.0	0.1 ± 0.1
22:5n-6	4.4 ± 0.3	0.3 ± 0.1	0.2 ± 0.1	0.7 ± 0.1
20:5n-3	0.1 ± 0.1	4.2 ± 0.4	5.6 ± 0.1	1.8 ± 0.1
22:5n-3	0.7 ± 0.0	9.4 ± 0.8	20.4 ± 0.5	3.1 ± 0.3
22:6n-3	6.3 ± 0.2	13.4 ± 1.5	4.6 ± 0.1	23.0 ± 2.2
Saturated	48.2	55.5	50.3	54.8
Monoene	9.8	8.5	7.01	6.5
n-6 PUFA	35.3	10.9	12.3	10.8
n-3 PUFA	7.1	27.0	30.5	27.9
Total PUFA	42.4	37.9	42.8	38.7

[a]Mean ± SEM wt % of five determinations.

TABLE 6. Fatty acid composition of spleen 1-O-alk-1'-enyl-2-*sn*-acyl-glycero-3-phosphoethanolamines from NZB/W mice fed either beef tallow or 10% marine lipid diets[a]

FAME	BT	FO 10%	EPA-E 10%	DHA-E 10%
14:0	0.2 ± 0.2			
16:0	5.3 ± 2.3	6.2 ± 1.2	4.4 ± 0.7	2.5 ± 0.3
18:0	1.7 ± 0.3	3.5 ± 0.4	2.1 ± 0.2	3.3 ± 0.4
16:1n-9	0.1 ± 0.1		1.0 ± 0.4	
16:1n-7	0.1 ± 0.1	2.5 ± 0.4	1.2 ± 0.5	1.0 ± 0.1
18:1n-9	2.6 ± 0.1	3.1 ± 0.5	2.2 ± 0.0	2.5 ± 0.2
18:1n-7	0.7 ± 0.0	0.9 ± 0.1		
18:2n-6	1.2 ± 0.1	1.9 ± 0.2	1.2 ± 0.0	2.2 ± 0.0
20:3n-6				0.5 ± 0.0
20:4n-6	52.2 ± 1.0	18.1 ± 0.7	24.6 ± 0.3	10.5 ± 0.4
22:4n-6	18.7 ± 0.7	1.3 ± 0.0	1.9 ± 0.0	0.6 ± 0.0
22:5n-6	6.3 ± 0.0			0.7 ± 0.0
20:5n-3	0.2 ± 0.0	18.7 ± 0.5	21.7 ± 0.4	12.1 ± 0.1
22:5n-3	1.1 ± 0.0	19.0 ± 0.8	33.5 ± 1.0	7.1 ± 0.1
22:6n-3	9.7 ± 0.1	25.0 ± 2.2	6.3 ± 0.4	57.1 ± 1.1
Saturated	7.2	9.6	6.5	5.8
Monoene	3.5	6.5	4.4	3.5
n-6 PUFA	78.4	21.3	27.7	14.4
n-3 PUFA	10.9	62.7	61.5	76.3
Total PUFA	89.3	84.0	89.2	90.7

[a]Mean ± SEM of two determinations.

the fatty acid composition of phospholipids. We chose to use the spleen for fatty acid analyses as this organ contains large quantities of lymphocytes, macrophages, and vascular tissue, all of which are important in chronic autoimmune disease (23). Although one of the aims of these experiments was to compare the severity of the renal pathology in the different dietary groups of mice, we chose not to analyze kidney phospholipids, since differences in pathologic changes between different dietary groups could conceivably influence the fatty acid compo-

TABLE 7. Fatty acid composition of spleen 1-O-alkyl-2-acyl-*sn*-glycero-3-phosphocholine from NZB/W mice fed either 10% beef tallow or 10% marine lipid diets[a]

FAME	BT	FO 10%	EPA-E 10%	DHA-E 10%
14:0	0.8 ± 0.0	0.4 ± 0.3	0.6 ± 0.4	
16:0	32.3 ± 0.7	36.0 ± 0.4	32.1 ± 8.2	26.4 ± 0.4
18:0	3.5 ± 0.0	3.6 ± 0.7	3.3 ± 0.6	11.8 ± 0.2
16:1n-9	0.9 ± 0.1	1.3 ± 0.1		
16:1n-7	1.4 ± 0.1	2.3 ± 0.1	1.7 ± 0.5	0.6 ± 0.4
18:1n-9	3.8 ± 0.3	3.5 ± 0.1	2.6 ± 0.2	5.7 ± 0.1
18:1n-7	1.7 ± 0.3	1.4 ± 0.0	0.4 ± 0.3	2.3 ± 0.1
18:2n-6	3.3 ± 0.0	6.0 ± 0.1	5.0 ± 0.1	9.2 ± 0.5
20:3n-6	1.3 ± 0.2	1.5 ± 0.1	1.0 ± 0.7	
20:4n-6	42.3 ± 0.0	12.1 ± 0.2	19.9 ± 2.0	8.8 ± 0.2
22:4n-6	4.6 ± 0.1		0.4 ± 0.3	
22:5n-6	1.6 ± 0.0			
20:5n-3		16.1 ± 0.4	19.4 ± 3.3	11.9 ± 0.4
22:5n-3	0.3 ± 0.0	9.1 ± 0.0	11.9 ± 3.0	5.0 ± 0.1
22:6n-3	2.2 ± 0.1	6.9 ± 0.1	1.8 ± 0.4	18.3 ± 0.6
Saturated	36.5	40.0	36.0	38.2
Monoene	7.9	8.4	4.7	8.6
n-6 PUFA	53.2	19.6	26.3	18.1
n-3 PUFA	2.5	32.1	33.1	35.2
Total PUFA	55.7	51.6	59.4	53.3

[a]Mean ± SEM of two determinations.

TABLE 8. Fatty acid composition of spleen 1,2 diacyl-*sn*-glycero-3-phosphoethanolamines from NZB/W mice fed either 3% or 6% n-3 fatty acid ethyl ester diets

FAME	EPA-E 3%[a]	EPA-E 6%[b]	DHA-E 3%[b]	DHA-E 6%[a]
16:0	13.1 ± 1.8	13.2 ± 1.3	12.1 ± 0.4	12.9 ± 0.0
18:0	36.5 ± 2.0	37.5 ± 1.8	37.6 ± 0.9	35.6 ± 0.5
16:1n-9	0.3 ± 0.1	0.3 ± 0.0	0.2 ± 0.0	0.2 ± 0.2
16:1n-7	0.4 ± 0.1	0.5 ± 0.1	0.4 ± 0.0	0.4 ± 0.0
18:1n-9	6.6 ± 0.7	5.3 ± 0.4	5.2 ± 0.4	5.9 ± 0.0
18:1n-7	1.7 ± 0.3	1.7 ± 0.2	1.6 ± 0.0	1.6 ± 0.0
20:1			0.1 ± 0.1	
18:2n-6	4.4 ± 0.3	3.9 ± 0.2	7.7 ± 0.2	6.5 ± 0.0
20:2n-6	0.4 ± 0.0	0.1 ± 0.1	0.4 ± 0.1	0.4 ± 0.0
20:3n-6	0.6 ± 0.0	0.5 ± 0.0	1.3 ± 0.0	0.8 ± 0.0
20:4n-6	15.0 ± 0.5	13.6 ± 0.2	8.3 ± 0.1	5.3 ± 0.0
22:4n-6	1.0 ± 0.1	0.7 ± 0.0	0.4 ± 0.0	0.2 ± 0.0
22:5n-6			0.4 ± 0.2	1.0 ± 0.1
20:5n-3	6.1 ± 0.0	8.6 ± 0.1	3.6 ± 0.1	3.5 ± 0.1
22:5n-3	8.3 ± 0.5	10.5 ± 0.3	2.1 ± 0.1	1.6 ± 0.0
22:6n-3	5.7 ± 0.2	3.7 ± 0.1	18.7 ± 0.7	24.0 ± 0.4
Saturated	49.5	50.6	49.6	48.5
Monoene	9.1	7.8	7.4	8.1
n-6 PUFA	21.3	18.7	18.6	14.2
n-3 PUFA	20.0	22.9	24.4	29.1
Total PUFA	41.4	41.5	43.0	43.3

[a]Mean ± SEM wt % of two determinations.
[b]Mean ± SEM of three determinations.

sition of the kidneys. On the other hand, pathological changes in the spleen of NZB/W mice are minor, and therefore the changes in fatty acid composition of the spleen that are induced by the experimental diets should not be influenced significantly by differences in the structure of the spleens among different dietary groups (24). Others have recently reported that murine kidneys affected with polycystic disease have an abnormal distri-

TABLE 9. Fatty acid composition of spleen 1-O-alk-1'-enyl-2-acyl-*sn*-glycero-3-phosphoethanolamines from NZB/W mice fed either 3% or 6% n-3 fatty acid ethyl ester diets

FAME	EPA-E 3%[a]	EPA-E 6%[b]	DHA-E 3%[b]	DHA-E 6%[a]
14:0		0.3 ± 0.2		
16:0	2.2 ± 0.2	2.1 ± 0.2	1.9 ± 0.1	1.9 ± 0.3
18:0	1.3 ± 0.3	1.4 ± 0.4	1.3 ± 0.1	1.5 ± 0.3
16:1n-9			0.3 ± 0.1	
16:1n-7	0.7 ± 0.1	1.2 ± 0.3	1.2 ± 0.2	1.4 ± 0.3
18:1n-9	2.8 ± 0.1	2.2 ± 0.1	3.1 ± 0.2	2.6 ± 0.0
18:1n-7	0.8 ± 0.2	0.6 ± 0.1	0.8 ± 0.7	0.5 ± 0.3
18:2n-6	1.6 ± 0.0	1.6 ± 0.1	2.9 ± 0.2	2.5 ± 0.1
20:3n-6	0.5 ± 0.0		0.9 ± 0.1	0.3 ± 0.2
20:4n-6	31.3 ± 0.3	27.1 ± 0.5	19.2 ± 0.3	12.2 ± 0.0
22:4n-6	3.1 ± 0.0	1.9 ± 0.0	1.1 ± 0.1	0.6 ± 0.0
22:5n-6			1.0 ± 0.1	1.1 ± 0.0
20:5n-3	14.4 ± 0.2	20.3 ± 0.2	8.8 ± 0.2	10.1 ± 0.1
22:5n-3	28.4 ± 0.4	33.1 ± 0.6	6.4 ± 0.2	5.7 ± 0.1
22:6n-3	13.0 ± 0.5	7.9 ± 0.1	51.2 ± 0.9	59.7 ± 0.1
Saturated	3.6	3.8	3.2	3.4
Monoene	4.3	4.1	5.4	4.5
n-6 PUFA	36.5	30.6	25.1	16.6
n-3 PUFA	55.7	61.3	66.4	75.6
Total PUFA	92.1	91.9	91.4	92.2

[a]Mean ± SEM wt % of two determinations.
[b]Mean ± SEM of three determinations.

TABLE 10. Fatty acid composition of spleen phospholipids from mice fed a mixed n-3 fatty acid ethyl ester (EPA-E + DHA-E) diet

FAME	Alkenylacyl-PE[a]	Diacyl-PE[a]	Alkyacyl-PC[b]	Diacyl-PC[b]
14:0			0.4 ± 0.1	0.2 ± 0.1
16:0	2.2 ± 1.0	9.9 ± 0.3	43.7 ± 2.8	42.0 ± 0.1
18:0	1.4 ± 0.2	33.5 ± 0.8	2.0 ± 0.7	11.1 ± 0.2
16:1n-9	0.1 ± 0.1	0.2 ± 0.0	1.4 ± 0.1	1.2 ± 0.1
16:1n-7	1.4 ± 0.1	0.5 ± 0.0	1.7 ± 0.1	1.6 ± 0.1
18:1n-9	2.7 ± 0.2	7.9 ± 0.1	4.9 ± 0.2	16.7 ± 0.5
18:1n-7	1.1 ± 0.1	2.4 ± 0.1	1.3 ± 0.1	4.0 ± 0.6
20:1		0.3 ± 0.0		0.5 ± 0.0
18:2n-6	1.8 ± 0.2	5.0 ± 0.0	5.3 ± 0.3	9.7 ± 0.1
20:2n-6	0.1 ± 0.2	0.4 ± 0.0	0.9 ± 0.4	0.8 ± 0.0
20:3n-6	0.4 ± 0.2	1.2 ± 0.0	0.6 ± 0.4	1.0 ± 0.0
22:4n-6	2.9 ± 0.1	1.2 ± 0.1	1.2 ± 0.1	0.3 ± 0.0
22:5n-6	0.5 ± 0.2	0.4 ± 0.0		
20:4n-6	33.1 ± 1.8	17.4 ± 0.2	20.5 ± 2.0	6.8 ± 0.4
20:5n-3	8.9 ± 0.2	3.8 ± 0.2	5.1 ± 0.9	1.5 ± 0.2
22:5n-3	16.1 ± 0.2	5.5 ± 0.4	5.7 ± 0.6	1.2 ± 0.2
22:6n-3	27.5 ± 0.6	11.5 ± 1.0	5.5 ± 0.9	1.6 ± 0.2
Saturated	3.5	43.4	46.1	53.2
Monoene	5.3	11.2	9.2	24.0
n-6 PUFA	38.7	25.5	28.5	18.6
n-3 PUFA	52.4	20.8	16.2	4.3
Total PUFA	91.1	46.3	44.7	22.9

[a]Means ± SEM wt % of two determinations.
[b]Means ± SEM of three determinations.

bution of phospholipid classes, and that phospholipids from affected kidneys had reduced quantities of DHA and elevated quantities of adrenic acid (22:4n-6) (25).

The distribution of phospholipid classes and subclasses did not differ significantly among all dietary groups, consistent with previous studies (26, 27). In addition, the contents of total saturated, total monoenoic, and total polyunsaturated fatty acids remain relatively constant for all diets within any given class and subclass. This constancy is noteworthy in view of the wide variation in the contents of saturated and unsaturated fatty acids in the experimental diets. For example, the total PUFA in the BT is 5.4% whereas the PUFA contents of the DHA-E and EPA-E diets are each > 95%. The large differences between the PUFA contents of the BT and n-3 fatty acid diets are only slightly reduced by taking into account the PUFA content of the 2% safflower oil which is present in all of the diets.

There are major differences in the PUFA composition of phospholipids associated with the different diets. As expected, all of the n-3 fatty acid diets elevate the content of n-3 PUFA associated with reductions in the contents of n-6 PUFA, but the quantities of individual PUFAs vary with the different diets. The C_{22} n-6 PUFA are nearly eliminated by all of the n-3 fatty acid diets, as illustrated by the alkenylacyl GPE from mice fed 10% BT and 10% marine lipid diets (Table 3). Here the C_{22} n-6 PUFA comprise 25% of fatty acids in the BT group and <2% in each of the three n-3 fatty acid diets. It is unlikely that the reductions in the C_{22} n-6 PUFA are explained by suppression of synthesis of these compounds from arachidonic acid, since elongation of EPA to 22:5n-3 is apparently extensive, such that the quantities of 22:5n-3 incorporated into phospholipids in mice receiving the EPA-E diets exceed the quantities of EPA incorporated in some subclasses, while levels of the C_{22} n-6 PUFA are nearly absent, suggesting that the high levels of 22:5n-3 are related to preferential incorporation into phospholipids of this fatty acid over the C_{22} n-6 PUFA. Arachidonic acid (AA) levels are markedly suppressed by the n-3 fatty acids, but not to the extent seen with the C_{22} PUFA. Suppression of arachidonic acid levels by the n-3 fatty acids occurs to varying degrees throughout all PL classes, but the EPA-E diets consistently suppress the AA levels less than either FO or DHA-E diets. While this observation indicates that EPA and DHA differ in their ability to suppress the AA content of phospholipids, it is possible that a small quantity of AA in the EPA-E preparation (4.8%) might oppose the reductions in AA by the EPA-E. Therefore, we cannot determine definitely whether or not tissue levels of AA are reduced to a greater extent by DHA-E than EPA-E, but is clear that both of these n-3 fatty acids reduce the levels of AA in phospholipids. In contrast to the C_{20} and C_{22} PUFA, linoleic acid is slightly

increased with each of the n-3 fatty acid diets compared to the BT diet, in several phospholipid classes, even though the linoleic acid content of the beef tallow diet is greater than that of any of the marine lipid diets.

The quantities of individual n-3 fatty acids in phospholipids vary with the different n-3 fatty acid preparations. The n-3 fatty acid diets only increase the quantities of long chain (> 20 carbon) n-3 fatty acids; there are no significant quantities of C_{16} or C_{18} n-3 fatty acids in phospholipids associated with any of the marine lipid diets, even with the FO which contains 5.1% 18:4n-3 and 1.1% 18:3n-3. Therefore, the n-3 fatty acids do not lead to elevated levels of C_{16} or C_{18} n-3 fatty acids either through direct incorporation or through the retroconversion of longer chain fatty acids.

The composition of n-3 fatty acids differed with each of the experimental diets. The EPA-E diets elevated the contents of EPA and 22:5n-3 in all PL subclasses. There were no increases in the levels of DHA in any of the phospholipids in groups fed with either the 10% or 6% EPA-E diets, compared to the BT diet, but the 3% EPA-E diet was associated with a small degree of elevation of DHA in both diacyl and alkenylacyl subclasses (Tables 8 and 9). These results show that in the presence of high levels of dietary EPA, elongation of EPA to 22:5n-3 is extensive but there is no significant conversion to DHA. However, when the dietary EPA-E is reduced to 3% EPA-E, elevations of DHA levels occur. With the EPA-E diets, the proportion of EPA to 22:5n-3 varies with different PL classes. In diacyl PC and alkenylacyl PE, the quantities of 22:5n-3 exceed 20:5n-3, whereas the converse is true for diacyl PC and alkenylacyl PC.

These results suggest that the high levels of n-3 fatty acids in our experiments suppressed the conversion of 22:5n-3 to DHA, a reaction that has often been attributed to 4-desaturase activity. However, recent evidence indicates that this reaction is carried out by a pathway independent of 4-desaturase. Rat hepatocyte microsomes convert 22:5n-3 to DHA by elongating 22:5n-3 to 24:5n-3, followed by desaturation at the 6 position to 24:6n-3. The latter product is then converted to DHA by β-oxidation (28). It has not been established whether or not the 6-desaturase that generates 24:6n-3 is the same enzyme that carries out 6-desaturation of C_{18} fatty acids. Inhibition of one or more 6-desaturases by high levels of dietary n-3 fatty acids could account for both the lack of elevation of 22:6n-3 in mice fed the EPA-E diets, as well as the higher levels of 18:2n-6.[3]

The DHA-E diets are associated with elevated levels of the three major long chain n-3 fatty acids in all PL classes, compared to the BT-fed animals. The DHA preparation contains only small quantities of EPA (<1%) and small quantities of 22:5n-3 (3.6%). However, the latter is similar to the level of 22:5n-6 in the DHA-E preparations (2.8%) and no significant incorporation into phospholipids of this n-6 fatty acid occurs. Thus, the substantially elevated levels of EPA in mice fed the DHA-E diets almost certainly occur secondary to retroconversion of DHA, and elevated levels of 22:5n-3 may result from retroconversion as well. There is little previously published work that documents retroconversion of dietary PUFA. Retroconversion has been demonstrated in rats based on feeding of tracer doses of radioactive DHA and measuring radioactivity in EPA and 22:5n-3 (29). In tissue culture, DHA was retroconverted to EPA in retinoblastoma cells (30). Dietary DHA-E in humans also increased EPA levels in phospholipids in plasma but not in platelets (31). To our knowledge, incorporation of retroconversion products from dietary n-3 fatty acids into ether-linked phospholipids has not been reported previously, and it is shown to occur extensively in the experiments reported here.

We examined the effects of varying quantities of each marine lipid preparation on phospholipid fatty acid composition. Fatty acid analyses for a group of mice fed 5% FO are not shown here, but there were essentially no differences in the fatty acid composition of any of the phospholipids between the 10% FO and the 5% FO groups. It is possible that the changes in fatty acid composition induced by the 5% FO diet were delayed compared to the 10% FO diet but at the single feeding period of 13 weeks, no significant differences were observed. Therefore, the quantity of this preparation of FO that results in maximal incorporation of n-3 fatty acids in tissue phospholipids has not been determined but it must be no more than 5%.

A more limited set of analyses was carried out for diets containing 3% or 6% of both EPA and DHA ethyl esters, separately. The levels of EPA and 22:5n-3 increase with increasing quantities of dietary EPA-E over the range of 3-10% for both the diacyl PE and alkenylacyl PE subclasses. With the DHA-E diets, there is a slight increase in the levels DHA, 22:5n-3, and EPA in phospholipids with increasing quantities of dietary DHA-E, but the total n-3 levels are nearly maximal at the lowest quantity given (3%). These studies demonstrate that maximal or near maximal incorporation of long chain n-3 fatty acids occurs when the n-3 fatty acid content of the diet is equal or greater than that given with either the 5% FO diet or the 3% n-3 fatty acid ethyl esters. Each of the latter diets provides 2.6 wt% of n-3 fatty acids, or approximately 7% of energy intake in the form of n-3 fatty acids. Therefore, substantial quantities of n-6 PUFA, primarily AA and linoleic acid, are retained in spite of increasing the quantities of dietary n-3 fatty acids beyond 7 wt%. Thus the tissue phospholipid ratios of n-3/n-6 PUFA are not determined simply by the PUFA composition of the diet in the

[3]This concept was suggested by a referee.

experiments reported here when quantities of total n-3 PUFA of >7 wt% are ingested, although the fatty acid composition of phospholipids may reflect the n-3/n-6 ratio of the diet with lower quantities of PUFA. Others have previously reported that the ability of dietary n-3 fatty acids to alter phospholipid fatty acid composition reaches a threshold, as the quantities of dietary n-3 fatty acids are increased. Further increasing the quantities of dietary n-3 fatty acids beyond this threshold limit fails to produce greater changes in fatty acid composition (32). On the other hand, diets containing lower levels of PUFA, 18:2n-6 and 18:3n-3 in the range of 0.3-0.6 energy %, led to higher ratios of n-3/n-6 PUFA in phospholipids than diets containing higher total PUFA levels (3% energy) with similar ratios of n-3/n-6 (33-35). These studies suggest that diets with high quantities of linoleic acid, such as typical human diets in Western countries, might discriminate against incorporation of n-3 fatty acids derived from linolenic acid into phospholipids. Longer chain 20- to 22-carbon dietary n-3 fatty acids elevate tissue n-3 PUFA more effectively than does linolenic acid (34). However, increasing quantities of linoleic acid in the diet was shown to impair the incorporation of n-3 fatty acids into tissue phospholipids of marmosets by either α-linolenic acid or EPA (35), and linoleate inhibited the incorporation of EPA from dietary FO into human neutrophil phospholipids (36).

Finally, we may compare the abilities of the FO and the n-3 fatty acid ethyl ester diets to modify the fatty acid compositions of phospholipids. The increased contents of n-3 fatty acids is maximal at 5% FO, since the 5% and 10% FO diets result in essentially the same quantity of n-3 fatty acids in all phospholipid classes. In the case of the ethyl esters, the phospholipid content of n-3 fatty acids is similar for the 6% and 10% diets of both DHA-E and EPA-E, although the n-3 content of diacyl PE with the 6% EPA-E diet is slightly lower than the 10%. These results indicate that maximal effect on tissue n-3 fatty acids is seen with the 6% fatty acid ethyl ester diets, although somewhat lower quantities of n-3 fatty acids are incorporated with EPA-E than DHA-E diets. On the other hand, the 3% DHA ethyl ester diet has a quantity of n-3 fatty acids nearly identical to the 5% FO diet (2.55 vs. 2.65%, respectively) and the total n-3 fatty acid contents of the diacyl and plasmalogen PE are nearly the same for mice fed with the 3% DHA and the 5% fish oil diets. Thus, with these two diets containing nearly the same quantities of n-3 fatty acids in the form of either triglycerides or ethyl esters, nearly the same quantities of total n-3 fatty acids are incorporated into phospholipids. The EPA-E diets are slightly less effective in increasing tissue n-3 fatty acids than either the DHA-E or FO diets and these differences might be related to the presence of small quantities AA in the EPA-E preparation used in our experiments. It also seems unlikely that there are significant differences in gastrointestinal absorption between these preparations, since recent experiments in humans have demonstrated that n-3 PUFA are equally well absorbed in the form of either triglycerides or ethyl esters (37). We conclude that, under the conditions of these experiments, there are no significant differences between the abilities of n-3 fatty acid ethyl esters and triglycerides to incorporate n-3 fatty acids into tissue phospholipids.

The mixed ethyl ester diet that combines the two n-3 fatty acid ethyl esters appears to have a similar ability to modify phospholipid fatty acid composition as the n-3 esters given individually. This conclusion is based on a comparison of the total n-3 fatty acid composition of 21% and 52% for diacyl PE and alkenylacyl PE, respectively, for the mixed EPA-E/DHA-E diet (Table 10), with values of 20% and 56%, respectively, for the 3% EPA-E diet. Since the total fatty acid contents of these two diets are similar (2.7 vs. 3.2%), combining the two fatty acid ethyl esters does not potentiate their ability to modify tissue lipid composition. Therefore, the synergistic effects of EPA-E and DHA-E in alleviating the renal disease in NZB/NZW mice (see accompanying manuscript) cannot be accounted for by enhanced incorporation of n-3 fatty acids over levels that are associated with similar dietary quantities of either n-3 fatty acid alone.

The authors are grateful to several colleagues for valuable advice including Robert Ackman, Merle Blank, Ellen Williams, John Lowenstein, George Patton, and Fred Snyder. We thank Nippon-Suisan Kaisha, Ltd. for generously providing purified fish oil (Nissui 28) and the n-3 fatty acid ethyl esters used in these studies. We also appreciate the assistance of Susan Adams in preparing the manuscript. This work was supported by NIH grants AR 19427, AR 03564, AI 28465, and a grant from the Davis Family Foundation.

Manuscript received 12 November 1992 and in revised form 9 March 1993.

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