Effect of dietary supplementation with a fish oil concentrate on the alkenylacyl class of ethanolamine phospholipid in human platelets

H. M. Aukema and B. J. Holub

Department of Nutritional Sciences, Univeristy of Guelph, Guelph, Ontario, Canada N1G 2W1

Abstract It has been demonstrated that the alkenylacyl class of ethanolamine phospholipid (PE) represents one of the major forms of eicosapentaenoic acid (EPA)-containing phospholipid in the circulating platelets isolated from human subjects consuming a fish oil concentrate. Since the alkenylacyl PE from human platelets is enriched in the eicosanoid precursor arachidonic acid (AA) and the n-6 polyunsaturate adrenic acid (AdA), it was of interest to study changes in alkenylacyl PE fatty acid composition upon fish oil supplementation. Healthy volunteers were given 20 capsules of MaxEPA daily (3.6 g of EPA plus 2.4 g of docosahexaenoic acid, DHA) for 6 weeks followed by a 6-week recovery period. Washed platelet suspensions were prepared and the fatty acid compositions of the phospholipid components were evaluated by thin-layer and gas-liquid chromatography at weeks 0, 3, 6, 9, and 12. Fatty acid composition changes were more pronounced in the alkenylacyl PE than in other platelet phospholipids as a result of fish oil consumption. The alkenylacyl PE exhibited a greater drop (by 20.3 mol%, i.e., from 72.0 to 51.7 mol%) in AA than diacyl PE (by 1.6 mol%) or total (predominantly diacyl) choline phospholipids (PC) (by 4.5 mol%). In alkenylacyl PE, the predominant reservoir of AdA in human platelet phospholipid, a dramatic reduction in the level of AdA also resulted with MaxEPA supplementation (from 7.9 to 3.1 mol%); diacyl PE and total PC decreased by 0.6 and 0.3 mol%, respectively. With respect to the n-3 fatty acids, EPA rose by 12.5 mol% in alkenylacyl PE, compared to only 3.8 and 2.5 mol% in diacyl PE and total PC, respectively. The 22-carbon n-3 fatty acids, docosapentaenoic acid (DPA) and DHA, also exhibited pronounced fatty acid changes in alkenylacyl PE. DPA increased by 4.3 mol% in the alkenylacyl PE, while in diacyl PE and total PC the increases were 1.5 and 0.5 mol%, respectively. DHA increased by 2.1 mol% in alkenylacyl PE, 1.5 mol% in diacyl PE, and 0.5 mol% in total PC after 6 weeks of fish oil supplementation. It was estimated that, of the total mass AA decrease in platelet phospholipid upon fish oil consumption, 37% was accounted for by the alkenylacyl PE. Approximately 71% of the EPA mass rise was calculated to be represented by the alkenylacyl PE plus diacyl PC, with each contributing about equally. The fatty acid compositions were restored to initial values by week 12 and, with the exception of AA in PC, were essentially normalized by week 9. The marked enrichment of the alkenylacyl PE in EPA as well as DPA and DHA at the expense of AA and AdA may be important in relation to the putative beneficial effects of fish oil on platelet reactivity and eicosanoid biosynthesis in human platelets.—**Aukema, H. M., and B. J. Holub.** Effect of dietary supplementation with a fish oil concentrate on the alkenylacyl class of ethanolamine phospholipid in human platelets. *J. Lipid Res.* 1989. **30:** 59-64.

Supplementary key words human platelet phospholipid • eicosapentaenoic acid • arachidonic acid • adrenic acid • ethanolamine phospholipids

The consumption of n-3 polyunsaturated fatty acids (PUFAs) derived from seafood appears to offer a means of ameliorating cardiovascular disease risk. There is considerable evidence that the ingestion of n-3 fatty acids can potentially decrease arterial thrombosis by reducing platelet aggregation and platelet-blood vessel wall interactions (for reviews, see refs. 1-3).

Over 95% of the major n-3 fatty acid, EPA (20:5, eicosapentaenoic acid), found in the platelets of fish oil consumers, is associated with the choline (PC) and ethanolamine (PE) phospholipids (4, 5). These phospholipids are composed of three different classes in human platelets: diacyl, and the ether-containing species, alkylacyl and alkenylacyl. Analysis of platelets isolated from individuals taking a fish oil concentrate revealed that the class of alkenylacyl PE represents a major form of the EPA-containing phospholipid (6). The alkenylacyl PE class is reported to be 45-60% of the total PE (7, 8) which represents only 11-15% of total phospholipid. The remainder of the PE is represented mainly by diacyl species, and less than 4% is the alkylacyl species (7, 8). It can also be calculated that the alkenylacyl PE class contains the largest pool of 22:4(n-6) (adrenic acid, AdA) in normal human platelets (5, 8); any alterations in the AdA content

Abbreviations: PUFA, polyunsaturated fatty acid; PE, ethanolamine phospholipids; EPA, eicosapentaenoic acid; AA, arachidonic acid; AdA, adrenic acid; DHA, docosahexaenoic acid; DPA, docosapentaenoic acid; PC, choline phospholipids; PS, phosphatidylserine; PI, phosphatidylinositol; FAME, fatty acid methyl ester.

in the human platelet phospholipid of fish oil consumers may also be significant since AdA has the potential to modulate arachidonic acid (20:4(n-6) (AA) metabolism (9). Although numerous investigations have documented the changes in n-6/n-3 fatty acids in total platelet phospholipid upon fish oil consumption (for reviews, see refs. 1-3), the progressive alterations in the fatty acid compositions of the alkenylacyl PE in human platelets have not been reported in this regard to date. Also, in the few trials where individual phospholipids, i.e., total PC/PE/phosphatidylserine (PS) /phosphatidylinositol (PI), have been examined (4, 5, 10, 11), the alkenylacyl subclass of PE was not studied.

The purpose of the present investigation was to monitor the alterations that occur in the fatty acid compositions of the alkenylacyl PE in the platelets of human subjects given a fish oil concentrate (as MaxEPA), including the reversibility of these changes following a recovery period. The very marked alterations obseved in the alkenylacyl PE were found to be significantly more pronounced than those seen in diacyl PE or total PC.

MATERIALS AND METHODS

Materials

MaxEPA capsules were provided by Seven Seas Health Care Ltd., Marfleet, U. K. Heparin and 2', 7'-dichlorofluorescein were obtained from Sigma Chemical Co. (St. Louis, MO). Bovine serum albumin (fraction V, fatty acid-free) was from Boehringer Mannheim Canada (Dorval, QUE). Apyrase was isolated from potatoes (12) and dissolved in 0.9% NaCl. Merck silica gel 60 HR plates were from British Drug House (Toronto, ONT). Gas-liquid chromatographic reference standards and monopentadecanoin were from Nu-Chek-Prep, Inc. (Elysian, MN). Siliconized glassware or polypropylene centrifuge tubes were used throughout the platelet isolation. All reagents used were of analytical grade.

Dietary protocol and platelet isolation

The experimental protocol was approved by the Human Subjects Committee of the University of Guelph. Informed written consent was obtained from five male volunteers whose mean age was 26.4 years and mean weight was 72.7 kg. Subjects were asked to refrain from consuming seafood for 2 weeks prior to and during the entire experimental period. They consumed 20 MaxEPA capsules per day with their meals for 6 weeks, but otherwise maintained their normal diets. This level of supplementation represented an intake of 3.6 g of EPA and 2.4 g of 22:6(n-3) (docosahexaenoic acid, DHA) per day. The supplementation period was followed by a recovery period of 6 weeks during which no MaxEPA or seafood was ingested. After 0, 3, and 6 weeks of supplementation, and 3 (week 9) and 6 (week 12) weeks after supplementation ceased, blood samples from each subject were drawn from an antecubital vein into Vacutainer tubes containing 1/6 volume of acid-citrate- dextrose anticoagulant (13). Washed platelet suspensions were prepared according to the method of Mustard et al. (14).

Separation of phospholipid classes

Lipids were extracted by the method of Bligh and Dyer (15) from 1 ml of platelet suspension adjusted to contain 2×10^9 platelets. Platelets were counted using a Coulter Counter model ZM, Coulter Electronics of Canada Ltd. (Burlington, ONT). The lipid extract was then dried under nitrogen so as to coat the bottom of a 20-ml glass vial. The lipid was then exposed for 5 min to HCl fumes at a distance of 5-6 cm to hydrolyze the aldehyde from the 1-position of alkenylacyl phospholipids (16). After flushing with nitrogen, the resulting lysophospholipid and unreacted phospholipids were dissolved in solvent and separated by a modification of the two-dimensional thin-layer chromatography system developed by Mitchell, Ferrell, and Huestis (17). The solvent system in the first dimension consisted of chloroform-methanol-concentrated ammonium hydroxide 65:35:5.5 (v/v/v); the second system consisted of chloroform-methanol-88% formic acid 55:25:5 (v/v/v). Lipid classes were visualized under ultraviolet light after the plates had been sprayed with 2', 7'-dichlorofluorescein in methanol-water 1:1 (v/v) and exposed to ammonia vapor. The area representing PC contained approximately 95% of the total PC (including the predominant diacyl PC plus minor alkylacyl PC); approximately 88, 7, and 5% of the PC exists as the diacyl, alkylacyl, and alkenylacyl species, respectively, in human platelets (7, 8). The area representing PE will be referred to as diacyl PE since it contains mainly diacyl PE (ca. 94%) with little alkylacyl PE (ca. 6%) (7, 8). The lyso PE area is the 1-lyso (2-acyl) PE derived from the acid hydrolysis of alkenylacyl PE; endogenous lyso PE has been reported, and was confirmed herein, to occur only in trace amounts in resting platelets (18). Since over 95% of the EPA found in the platelets of EPA consumers is found in PC and PE (6), these three classes, total PC, diacyl PE, and alkenylacyl PE (as lyso PE), were analyzed.

Fatty acid analysis

The separated classes of lipid were transmethylated in the presence of monopentadecanoin as internal standard (19) and the resulting fatty acid methyl esters (FAMEs) were extracted with petroleum ether, dried, and immediately redissolved in hexane. The FAMEs were analyzed (19) using a DB-225 megabore column (Chromatographic Specialties Inc., Brockville, ONT) installed on a Hewlett-Packard 5890 gas-liquid chromatograph with flame ionization detection. Fatty acids were identified by comparison of peak retention times to those of known standards. Gel scrapings from blank regions of the thin-layer plates were also treated as above and the minor peak values obtained were subtracted

from sample peak values to correct for any background contamination.

Statistical analysis

Results were analyzed as a block design, using individual subjects as blocks. Significant differences in fatty acid compositions between sampling times were established by analysis of variance followed by Tukey's Studentized range test (20).

RESULTS

Table 1 gives the fatty acid composition of PC over the 12-week experimental period. The changes in fatty acid composition in PC upon fish oil supplementation are in general agreement with those reported by others (4, 11, 21). Data for diacyl PE and alkenylacyl PE (lyso PE) are given in **Table 2** and **Table 3**, respectively. At week 0, less than 2% of the fatty acyl chains in the alkenylacyl PE were represented by saturated fatty acids as compared to 43 and 45% for diacyl PE and total PC. These profiles are in general agreement with a published report on normal human volunteers (8); the predominance of PUFAs in this locale is consistent with the enrichment of PUFAs found in the 2-position of platelet phospholipids.

The major changes upon fish oil supplementation occurred in the long-chain PUFAs and, as can be seen from Table 3, the alkenylacyl PE class in human platelets is particularly enriched in the long-chain PUFAs. As a result, the most dramatic changes in fatty acid composition after 3–6 weeks of fish oil supplementation occurred in the alkenylacyl PE class. The fatty acid changes in week 6 were slightly greater than in week 3, but there were no significant differences between any of the fatty acid values in weeks 3 and 6; reports on subjects on long-term fish oil feeding indicate that fatty acid changes are minimal after 6 weeks (4, 22). Moreover, the rate at which the different phospholipid classes reached equilibrium was not markedly different. At week 6, AA had decreased by 4.5 and 1.6 mol% in total PC and diacyl PE, respectively, while in alkenylacyl PE the decrease was by 20.3 mol%. The other major n-6 PUFA, AdA, dropped by a 4.8 mol% in the alkenylacyl PE class, while in diacyl PE and total PC the declines wre only 0.6 and 0.3 mol% respectively. In relative percentage terms, the changes in the n-6 fatty acids were also greater in the alkenylacyl PE class (28% for AA and 61% for AdA) compared to the diacyl PE class (7% for AA and 40% for AdA). The relative percentage decreases in n-6 fatty acids in total PC (36% for AA and 50% for AdA) were generally similar to the changes in the alkenylacyl PE class. The n-3 fatty acids showed the largest mol% increases in the alkenylacyl PE class as well. EPA rose by 12.5 mol% in alkenylacyl PE, but only by 3.8 and 2.5 mol% in diacyl PE and total PC, respectively. Docosapentaenoic acid (DPA), 22:5(n-3), increased by 4.3 mol% in the alkenylacyl PE compared to only 1.5 mol% in diacyl PE and 0.5 mol% in PC. DHA increased by 2.1 mol% in alkenylacyl PE, 1.5 mol% in diacyl PE, and 0.5 mol% in total PC after 6 weeks of fish oil supplementation.

With respect to recovery (weeks 6–12), alterations in fatty acid composition due to MaxEPA supplementation were restored to baseline levels by week 12. With the exception of AA in PC, fatty acid values at week 9 were not markedly different from week 12 values. Quadratic equations (20), when fitted to the data, indicate that the various fatty acid profiles return to basal levels between weeks 10 and 12.

DISCUSSION

The pronounced increases in the n-3 PUFAs, namely EPA, DPA, and DHA, and decreases in the n-6 PUFAs, AA, and AdA, in the alkenylacyl PE as compared to di-

TABLE 1. Fatty acid composition of PC in platelets of five subjects consuming MaxEPA for a 6-week period followed by a 6-week recovery period

Fatty Acid	Week 0	Week 3	Week 6	Week 9	Week 12
16:0	30.1 ± 0.7^{a}	31.8 ± 1.1^{ab}	32.3 ± 0.9^{b}	31.7 ± 0.6^{ab}	30.5 ± 0.7^{ab}
18:0	13.5 ± 0.5	12.8 ± 0.4	12.6 ± 0.4	13.6 ± 0.8	13.6 ± 0.6
18:1	23.5 ± 0.6	24.1 ± 1.1	24.6 ± 0.9	22.7 ± 1.2	22.5 ± 0.6
18:2(n-6)	7.5 ± 0.7^{ab}	6.3 ± 0.5^{b}	6.9 ± 0.6^{ab}	7.8 ± 0.9^{ab}	8.5 ± 0.8^{a}
20:0	1.0 ± 0.1	1.0 ± 0.1	1.0 ± 0.0	1.0 ± 0.1	1.0 ± 0.0
20:1	1.3 ± 0.1	1.1 ± 0.1	0.8 ± 0.2	1.2 ± 0.1	1.0 ± 0.3
20:3(n-6)	1.6 ± 0.1^{a}	0.9 ± 0.0^{b}	0.7 ± 0.2^{b}	1.5 ± 0.1^{a}	1.5 ± 0.1^{a}
20:4(n-6)	12.4 ± 0.5^{a}	8.2 ± 0.7^{c}	7.9 ± 0.7^{c}	9.2 ± 0.6^{c}	10.9 ± 0.7^{b}
20:5(n-3)	0.3 ± 0.1^{a}	2.4 ± 0.1^{b}	2.8 ± 0.3^{b}	0.6 ± 0.1^{a}	0.4 ± 0.1^{a}
22:4(n-6)	0.6 ± 0.1^{a}	0.2 ± 0.1^{c}	0.3 ± 0.1^{bc}	0.5 ± 0.1^{ab}	0.6 ± 0.1^{a}
22:5(n-3)	0.6 ± 0.1^{a}	0.9 ± 0.1^{b}	1.1 ± 0.1^{b}	0.6 ± 0.1^{a}	0.6 ± 0.0^{a}
22:6(n-3)	0.8 ± 0.2^{ab}	1.2 ± 0.1^{ac}	1.3 ± 0.1^{c}	0.9 ± 0.1^{ab}	0.8 ± 0.1^{b}

Data are given in mol% as means ± SE.
^{a,b,c}Values across each row having different superscripts are significantly different from each other ($P < 0.05$).

TABLE 2. Fatty acid composition of diacyl PE in platelets of five subjects consuming MaxEPA for a 6-week period followed by a 6-week recovery period

Fatty Acid	Week 0	Week 3	Week 6	Week 9	Week 12
16:0	7.1 ± 0.5	9.4 ± 1.5	7.7 ± 0.5	7.6 ± 0.6	8.4 ± 0.7
18:0	36.2 ± 2.2	32.6 ± 2.4	31.1 ± 2.0	33.9 ± 2.4	32.9 ± 1.7
18:1	13.3 ± 0.4	15.5 ± 0.8	14.1 ± 1.0	15.2 ± 1.2	14.0 ± 1.0
18:2(n-6)	3.7 ± 0.4	3.8 ± 0.4	4.1 ± 0.6	4.1 ± 0.5	4.1 ± 0.5
20:0	1.2 ± 0.1	1.2 ± 0.1	1.2 ± 0.1	1.4 ± 0.2	1.2 ± 0.1
20:1	1.0 ± 0.1	1.0 ± 0.0	1.0 ± 0.1	0.8 ± 0.2	1.0 ± 0.1
20:3(n-6)	1.1 ± 0.2	0.8 ± 0.1	0.8 ± 0.2	1.1 ± 0.2	1.0 ± 0.1
20:4(n-6)	24.6 ± 2.1	22.7 ± 2.3	23.0 ± 1.6	24.0 ± 2.7	26.6 ± 2.0
20:5(n-3)	0.2 ± 0.2[a]	2.8 ± 0.3[b]	4.0 ± 0.6[b]	0.5 ± 0.1[a]	0.4 ± 0.1[a]
22:4(n-6)	1.5 ± 0.3[a]	0.6 ± 0.1[b]	0.9 ± 0.1[ab]	1.0 ± 0.1[ab]	1.4 ± 0.2[a]
22:5(n-3)	1.1 ± 0.2[a]	2.1 ± 0.5[ab]	2.6 ± 0.3[b]	1.4 ± 0.2[a]	1.3 ± 0.2[a]
22:6(n-3)	1.2 ± 0.3[a]	2.3 ± 0.4[b]	2.7 ± 0.3[b]	1.8 ± 0.3[ab]	1.7 ± 0.3[ab]

Data are given in mol% as means ± SE.
[a,b]Values across each row having different superscripts are significantly different from each other ($P < 0.05$).

acyl PE and total PC in the platelets of the MaxEPA consumers are of particular interest. These dramatic shifts in alkenylacyl PE are consistent with overall trends that have been observed previously in fish oil trials at the level of total or individual phospholipids (4, 5, 10, 11).

Fish oil supplementation has been reported not to affect the relative proportions of the individual platelet phospholipids, total PE, PC, PS, PI, and sphingomyelin (5); we also found no marked change in the relative proportions of alkenylacyl PE and diacyl PE in platelets over the 12-week experimental period (data not shown). Therefore, the mass changes in the fatty acyl moieties of the phospholipids that occurred following 6 weeks of MaxEPA supplementation can be estimated. **Table 4** provides a quantitative assessment of the contribution of the individual phospholipids to the mass AA decrease upon 6 weeks of fish oil supplementation based on the present data (in the case of total PC, diacyl PE, and alkenylacyl PE) plus literature values (4, 5, 10, 11) (in the case of PS, PI, and sphingomyelin). Although alkenylacyl PE represents only 15% of total platelet phospholipid, it contributes to 37% of the AA decrease. In contrast to this, the diacyl PE class represents 10% of total platelet phospholipid, but contributes to only 4% of the AA decrease. Total PC contributes 54% to the total AA decrease; the AA and EPA contents of PS, PI, and sphingomyelin are known to change very little upon fish oil supplementation (5, 10).

The major contributors to the mass changes in EPA are the alkenylacyl PE and total PC, followed by a lesser contribution by diacyl PE (**Table 5**). Since approximately 77% of the EPA-containing PC in fish oil consumers was found in the diacyl subclass (6), it can be estimated that 71% of the mass EPA rise is represented by the alkenylacyl PE plus diacyl PC with each providing approximately equivalent contributions.

The biochemical pathways responsible for the n-6/n-3 alterations of the alkenylacyl PE class remain to be elucidated. Biosynthetic reactions within the megakaryocyte (23) as well as within the platelet need to be evaluated with respect to their relative importance and selectivities in this regard including plasma lipoprotein involvement (24). In addition, the consumption of dietary fish oil containing EPA

TABLE 3. Composition of the fatty acids found in the 2-position of alkenyl-acyl PE in platelets of five subjects consuming MaxEPA for a 6-week period followed by a 6-week recovery period

Fatty Acid	Week 0	Week 3	Week 6	Week 9	Week 12
16:0	n.d.	0.2 ± 0.2	1.0 ± 1.0	1.1 ± 1.1	0.9 ± 0.7
18:0	0.8 ± 0.5	0.6 ± 0.3	2.2 ± 1.8	2.7 ± 1.6	0.9 ± 0.3
18:1	1.9 ± 0.6	2.8 ± 0.3	3.4 ± 0.8	3.2 ± 0.6	2.3 ± 0.3
18:2(n-6)	1.3 ± 0.2	1.4 ± 0.2	1.4 ± 0.3	1.9 ± 0.5	1.6 ± 0.2
20:3(n-6)	0.5 ± 0.2	1.1 ± 0.2	0.4 ± 0.3	0.2 ± 0.2	0.8 ± 0.4
20:4(n-6)	72.0 ± 3.1[a]	54.8 ± 2.5[bc]	51.7 ± 4.0[c]	64.9 ± 2.5[abc]	65.6 ± 2.7[ab]
20:5(n-3)	1.9 ± 0.8[a]	12.7 ± 1.2[b]	14.4 ± 1.1[b]	3.0 ± 0.7[a]	1.7 ± 0.4[a]
22:4(n-6)	7.9 ± 0.8[a]	3.9 ± 0.5[c]	3.1 ± 0.5[c]	5.4 ± 0.4[ac]	7.3 ± 0.8[ab]
22:5(n-3)	7.4 ± 1.0[a]	10.9 ± 1.1[b]	11.7 ± 1.1[b]	6.6 ± 1.0[a]	6.9 ± 0.9[a]
22:6(n-3)	5.0 ± 1.0[a]	6.5 ± 1.0[ab]	7.1 ± 0.9[b]	4.9 ± 0.8[a]	5.2 ± 0.7[ab]

Data are given in mol% as means ± SE; n.d., not detectable.
[a,b,c]Values across each row having different superscripts are significantly different from each other ($P < 0.05$).

and DHA might reduce the desaturation reactions (25) leading to reduced formation of the longer chain n-6 polyunsaturates (AA and AdA) and/or mediate altered compositions via deacylation-reacylation (26) and/or transacylation (27, 28) reactions and/or influence peroxisomal oxidation, including retroconversion of AdA to AA (29). With respect to the origin of the n-3 PUFAs appearing in platelet alkenylacyl PE with fish oil supplementation, it has been reported that dietary EPA can be converted to DPA, while dietary DHA can be retroconverted to DPA and EPA (30).

The fatty acid alterations in alkenylacyl PE and other phospholipids may have relevance to modifications in membrane fluidity and membrane-mediated processes (nutrient transport, membrane-bound enzyme activities, cell signalling, etc.) as well as the availability and release of eicosanoid precursors. With respect to the latter, the degradation of alkenylacyl PE (16, 31, 32) as well as other phospholipids (for review see ref. 33) has been documented in agonist-stimulated platelets, including the release of free AA. The increased labeling of alkenylacyl PE with radioactive AA seen in activated (pre-labeled) platelets is attributed to transacylation of fatty acid from PC (28); EPA can also actively participate in the thrombin-dependent transacylation pathway, resulting in the formation of the 1-alkenyl 2-eicosapentaenoyl PE in human platelets (34). It has been suggested that the turnover of arachidonate through alkenylacyl PE in stimulated platelets may provide AA for conversion to thromboxane A_2 (28, 35) and, therefore may have an important role in platelet aggregation. The marked suppression of AA in alkenylacyl PE with fish oil consumption would be expected to reduce the amount of AA available for metabolism via cyclooxygenase and/or lipoxygenase activities. Furthermore, it is conceivable that the alkenylacyl PE class may represent an important source of releasable EPA for both inhibition of the cyclooxygenase and dampening of thromboxane A_2 synthesis (36) as well as providing substrate for eicosanoid synthesis (3-series prostaglandins and thromboxanes, 12-heptadecatetraenoic acid, and 12-hydroxyeicosapentaenoic acid) (37, 38).

The dramatic decrease in AdA, and increases in DPA and DHA, in alkenylacyl PE as observed upon fish oil supplementation (Table 3) may also be of potential significance.

TABLE 4. Contribution of the individual phospholipids to the mass AA decrease after 6 weeks of fish oil supplementation

	% Contribution
Total PC	54
Diacyl PE	4
Alkenylacyl PE	37
PS	1-8
PI	<1
Sphingomyelin	tr

TABLE 5. Contribution of the individual phospholipids to the mass EPA increase after 6 weeks of fish oil supplementation

	% Contribution
Total PC	46
Diacyl PE	15
Alkenylacyl PE	34
PS	<4
PI	<2
Sphingomyelin	tr

With respect to AdA, previous work from our laboratory (5, 21) plus the present work on alkenylacyl PE, diacyl PE, and total PC, indicates that approximately two-thirds of the mass decrease in AdA is due to changes in the alkenylacyl PE class. The role of AdA, as well as the 22-carbon n-3 fatty acids, in the anti-thrombotic effects of fish oil remains to be elucidated.

In conclusion, the dramatic changes in the n-6/n-3 fatty acid compositions of the alkenylacyl PE class as reported herein may have a significant role in the regulation and formation of the amounts and types of AA- and EPA-derived eicosanoids and AdA metabolites in circulating human platelets. The present findings may prove to be important with respect to understanding how dietary fish oil containing n-3 PUFAs reduces platelet-blood vessel wall interactions and the overall thrombogenic index.

We would like to thank Margaret Berry for her technical assistance. This work was supported in part by a grant from the Heart and Stroke Foundation of Ontario.

Manuscript received 17 March 1988 and in revised form 14 July 1988.

REFERENCES

1. Herold, P. M., and J. E. Kinsella. 1986. Fish oil consumption and decreased risk of cardiovascular disease: a comparison of findings from animal and human feeding trials. *Am. J. Clin. Nutr.* **43:** 566-598.
2. Leaf, A., and P. C. Weber. 1988. Cardiovascular effects of n-3 fatty acids. *N. Engl. J. Med.* **318:** 549-557.
3. Weaver, B. J., and B. J. Holub. 1988. Health effects and metabolism of dietary eicosapentaenoic acid. *Prog. Food Nutr. Sci.* **12:** 111-150.
4. Schacky, C. V., W. Siess, S. Fischer, and P. C. Weber. 1985. A comparative study of eicosapentaenoic acid metabolism by human platelets in vivo and in vitro. *J. Lipid Res.* **26:** 457-464.
5. Ahmed, A. A, and B. J. Holub. 1984. Alteration and recovery of bleeding times, platelet aggregation and fatty acid composition of individual phospholipids in platelets of human subjects receiving a supplement of cod-liver oil. *Lipids.* **19:** 617-624.
6. Holub, B. J., B. Celi, and C. M. Skeaff. 1988. The alkenylacyl class of ethanolamine phospholipid represents a major form of eicosapentaenoic acid (EPA)-containing phospholipid in the platelets of human subjects consuming a fish oil concentrate. *Thromb. Res.* **50:** 135-143.

7. Natarajan, V., M. Zuzart-Augustin, H. H. O. Schmid, and G. Graf. 1983. The alkylacyl and alkenylacyl glycerophospholipids of human platelets. *Thromb. Res.* **30:** 119–125.
8. Mueller, H. W., A. D. Purdon, J. B. Smith, and R. L. Wykle. 1983. 1-O-Alkyl-linked phosphoglycerides of human platelets: distribution of arachidonate and other acyl residues in the ether-linked and diacyl species. *Lipids.* **18:** 814–819.
9. VanRollins, M., L. Horrocks, and H. Sprecher. 1985. Metabolism of 7, 10, 13, 16-docosatetraenoic acid to dihomothromboxane, 14-hydroxy-7,10,12-nonadecatrienoic acid and hydroxy fatty acids by human platelets. *Biochim. Biophys. Acta.* **833:** 272–280.
10. Brox, J. H., J. Killie, S. Gunnes, and A. Nordoy. 1981. The effect of cod liver oil and corn oil on platelets and vessel wall in man. *Throm. Haemostasis.* **46:** 604–611.
11. Mori, T., J. P. Codde, R. Vandongen, and L. J. Beilin. 1987. New findings in the fatty acid composition of individual platelet phospholipids in man after dietary fish oil supplementation. *Lipids.* **22:** 744–750.
12. Molnor, J., and L. Lorand. 1961. Studies on apyrase. *Arch. Biochem. Biophys.* **93:** 353–363.
13. Aster, R. H., and J. H. Jandl. 1964. Platelet sequestration in man. I. Methods. *J. Clin. Invest.* **43:** 843–855.
14. Mustard, J. G., D. W. Perry, N. G. Ardlie, and M. A. Packham. 1972. Preparation of suspensions of washed platelets from humans. *Br. J. Haematol.* **22:** 193–204.
15. Bligh, E. G., and W. J. Dyer. 1959. A rapid method of total lipid extraction and purification. *Can. J. Biochem. Physiol.* **37:** 911–917.
16. Mueller, H. J., J. T. O'Flaherty, and R. L. Wykle. 1982. Ether lipid content and fatty acid distribution in rabbit polymorphonuclear neutrophil phospholipids. *Lipids.* **17:** 72–77.
17. Mitchell, K. T., J. E. Ferrell, Jr., and W. H. Huestis. 1986. Separation of phosphoinositides and other phospholipids by two-dimensional thin-layer chromatography. *Anal. Biochem.* **158:** 447–453.
18. Broekman, M. J., J. W. Ward, and A. J. Marcus. 1980. Phospholipid metabolism in stimulated human platelets: changes in phosphatidylinositol, phosphatidic acid, and lysophospholipids. *J. Clin. Invest.* **66:** 275–283.
19. Holub, B J., and C. M. Skeaff. 1987. Nutritional regulation of cellular phosphatidylinositol. *Methods Enzymol.* **141:** 234–244.
20. Steel, R. G. D., and J. H. Torrie. 1980. Principles and Procedures of Statistics: A Biometrical Approach. 2nd ed. McGraw-Hill Book Co., Toronto. 172–194, 452–468.
21. Skeaff, C. M., and B. J. Holub. 1987. Effect of dietary fish oil containing eicosapentaenoic acid on the fatty acid composition of platelet phospholipids and on the thrombin-stimulated phospholipid alterations in human platelets. *In* Biology of Icosanoids. M. Lagarde, editor. INSERM, Paris. **152:** 63–76.
22. Thorngren, M., and A. Gustafson. 1981. Effects of 11-week increase in dietary eicosapentaenoic acid on bleeding time, lipids, and platelet aggregation. *Lancet.* **2:** 1190–1193.
23. Schick, B. P., and P. K. Schick. 1986. Megakaryocyte biochemistry. *Semin. Hematol.* **23:** 68–87.
24. Joist, J. H., G. Dolezel, J. V. Lloyd, and J. F. Mustard. 1976. Phospholipid transfer between plasma and platelets in vitro. *Blood.* **48:** 199–211.
25. Garg, M. L., E. Sebokova, A. B. R. Thomson, and M. T. Clandinin. 1988. Δ^6-Desaturase activity in liver microsomes of rats fed diets enriched with cholesterol and/or ω3 fatty acids. *Biochem. J.* **249:** 351–356.
26. Hill, E. E., and W. E. M. Lands. 1968. Incorporation of long-chain and polyunsaturated acids into phosphatidate and phosphatidylcholine. *Biochim. Biophys. Acta.* **152:** 645–648.
27. Rittenhouse-Simmons, S., F. A. Russell, and D. Deykin. 1976. Transfer of arachidonic acid to human platelet plasmalogen in response to thrombin. *Biochem. Biophys. Res. Commun.* **70:** 295–301.
28. Kramer, R. M., and D. Deykin. 1983. Arachidonoyl transacylase in human platelets. *J. Biol. Chem.* **258:** 13806–13811.
29. Hagve, T., and B. O. Christophersen. 1986. Evidence for peroxisomal retroconversion of adrenic acid (22:4(n–6)) and docosahexaenoic acids (22:6(n–3)) in isolated liver cells. *Biochim. Biophys. Acta.* **875:** 165–173.
30. Schacky, C. V., and P. C. Weber. 1985. Metabolism and effects on platelet function of the purified eicosapentaenoic and docosahexaenoic acids in humans. *J. Clin. Invest.* **76:** 2446–2450.
31. Kambayashi, J., T. Kawasaki, T. Tsujinaka, M. Sakon, T. Oshiro, and T. Mori. 1987. Active metabolism of phosphatidyl ethanolamine plasmalogen of stimulated platelets, analyzed by high performance liquid chromatography. *Biochem. Int.* **14:** 241–247.
32. Takamura, H., H. Narita, H. J. Park, D. Tanaka, T. Matsuura, and M. Kito. 1987. Differential hydrolysis of phospholipid molecular species during activation of human platelets with thrombin and collagen. *J. Biol. Chem.* **262:** 2262–2269.
33. Mauco, G. 1987. Phospholipids: release of arachidonate for prostaglandin and thromboxane synthesis. *In* Platelet Responses and Metabolism. Volume III: Response-Metabolism Relationships. H. Holmsen, editor. CRC Press, Boca Raton, FL. 101–119.
34. Weaver, B. J., and B. J. Holub. 1987. The thrombin-dependent enrichment of alkenylacyl ethanolamine phosphoglyceride with [^{14}C]eicosapentaenoic acid and [^{3}H]arachidonic acid in prelabelled human platelets. *Biochem. Cell. Biol.* **65:** 405–408.
35. Rittenhouse-Simmons, S., F. A. Russell, and D. Deykin. 1977. Mobilization of arachidonic acid in human platelets: kinetics and Ca^{2+} dependency. *Biochim. Biophys. Acta.* **488:** 370–380.
36. Needleman, P., A. Raz, M. S. Minkes, J. A. Ferrendelli, and H. Sprecher. 1979. Triene prostaglandins: prostacyclin and thromboxane biosynthesis and unique biological properties. *Proc. Natl. Acad. Sci. USA.* **76:** 944–948.
37. Fischer, S., and P. C. Weber. 1983. Thromboxane A_3 (TxA_3) is formed in human platelets after dietary eicosapentaenoic acid. *Biochem. Biophys. Res. Commun.* **116:** 1091–1099.
38. Hamberg, M. 1980. Transformations of 5,8,11,14,17-eicosapentaenoic acid in human platelets. *Biochim. Biophys. Acta.* **618:** 389–398.