Fish Oil: A Potent Inhibitor of Platelet Adhesiveness

By Xiaolin Li and Manfred Steiner

The effect of fish oil administration on platelet function was studied in eight normal individuals, four men and four women, who received fish oil equivalent to 6 g eicosapentaenoic acid per day for a period of 25 days. Platelet aggregation, platelet adhesion, phospholipid and fatty acid distribution were measured at periodic intervals before, during, and after the period of fish oil administration. Platelet aggregation induced by arachidonic acid, adenosine diphosphate, and collagen showed a moderate increase in ED50 in response to the administration of fish oil. Conversely, platelet adhesion to fibrinogen and collagen I, which was studied at low shear rates in a laminar flow chamber, showed a striking 60% to 65% decrease after fish oil supplementation of the diet. The change in adhesiveness could be correlated with the pseudopodia formed in response to agonistic stimulation. Scanning electron microscopic examination of adherent platelets showed an overall reduction of pseudopodia that appeared short and stubby on fish oil administration. The profile of the fatty acids extracted from plasma confirmed compliance of the volunteers with their dietary supplements. Analysis of phospholipids showed changes in sphingomyelin, lysophosphatidylcholine, and phosphatidylcholine between pseudopodia and platelet cell bodies. Fish oil administration did not affect their overall distribution except for a moderate decrease in phosphatidylethanolamine in platelet pseudopodia. Changes were also recognized in the total fatty acids extracted from platelets, affecting primarily arachidonic acid, eicosapentaenoic acid, and docosahexaenoic acid. There were no changes in platelet adhesiveness in a group of five normal individuals who received a vegetable oil supplement of equal dose and duration as that of the fish oil. We conclude from these studies that fish oil, at least when administered over a limited period of time, is an effective inhibitor of platelet adhesiveness.

© 1990 by The American Society of Hematology.

OBSERVATIONS IN Greenland Eskimos led Dyerberg et al1 to conclude that a diet rich in (n-3) long-chain fatty acids has profound effects on hemostasis and lipoprotein metabolism. They postulated that such effects accounted for the apparent rarity of thrombo-occlusive vascular disease in the aforementioned population group.2-4 Controlled experiments in many laboratories5-15 have subsequently provided direct proof that fish oils affect platelet number, composition, and function. Although there have been some discrepant results, it appears that platelet aggregation is only mildly affected by the ingestion of 5,8,11,14,17-eicosapentaenoic acid (20:5 [n-3], EPA) in various forms of purity (up to 85%).14 Doses of less than 3 to 4 g EPA/d were reported14,12,13,16 to produce some decrease in platelet aggregation, especially with collagen as the stimulus. Higher doses of fish oil, equivalent to 6 g EPA or more,12-14 are needed to affect more definite changes in the aggregatory response of platelets. But even at very high intake levels of EPA, the effect of fish oil never equals or comes close to the inhibition that acetylsalicylic acid produces.

There are as yet no studies on the effect of dietary fish oil supplementation on platelet adhesiveness. The bleeding time that has been considered to be closely related to platelet adhesiveness has been prolonged to a variable degree by the administration of moderate to high doses of (n-3) polyunsaturated fatty acids.1-6 For this reason we have investigated the adhesive behavior of platelets. A group of eight normal individuals was tested before and after supplementation of their usual diet with fish oil equivalent to 6 g EPA/d. The results of our study show a dramatic reduction in platelet adhesiveness to collagen and fibrinogen when tested in a laminar flow chamber under controlled flow conditions. This may be due to a fish oil induced decrease of pseudopodial processes in stimulated platelets. A delayed onset and prolonged washout period characterized the response.

MATERIALS AND METHODS

Experimental design. A total of eight normal, healthy volunteers (four men and four women), all nonsmokers, aged 25 to 56 years were studied. They abstained from all medications for the entire 37-day period of the investigation. All participants in the study were advised to continue their regular (average American, mixed) diet and to refrain from alcoholic beverages for the duration of the study. A dietician carefully interviewed the volunteers at the time they donated blood to ensure that the individuals were continuing their usual dietary habits while taking the supplementation. Fish oil was administered in the form of 1 g capsules (Max EPA). The total amount was equivalent to 6 g EPA. Analysis of representative examples of the fish oil capsules showed an EPA content between 17.4% and 18.3% and a DHA content (4,7,10,13,16,19-docosahexaenoic acid, C22:6 [n-3]) varying between 11.2% and 12.3% of the total lipids present. Baseline investigations included complete blood and platelet counts, platelet aggregation and adhesion measurements, quantification of platelet pseudopodia and lipid analyses, including distribution of phospholipids and fatty acids. Measurements of these parameters were repeated at intervals during the 25-day period of fish oil supplementation and the week after discontinuation (usually day 30) of the fish oil regimen. In addition, a group of five normal individuals (three men and two women) aged 36 to 62 years were placed on a vegetable oil supplement for the same period of time and in the same amount as the fish oil was given. The vegetable oil consisted of a 1:1 mixture of palm oil and corn oil and was administered in liquid form. Both fish and vegetable oil contained 1 mg α-tocopherol/g of oil. Informed consent was obtained from all volunteers participating in this study.
FISH OIL AND PLATELET ADHESIVENESS

Preparation of platelets. Blood was obtained from the antecubital vein and collected into 1/10 vol of 3.8% sodium citrate. Platelet-rich plasma (PRP) was prepared as previously described. The platelet count of the PRP was adjusted to 300,000 to 350,000/μL.

For the measurement of platelet pseudopodia, platelets were separated from PRP by centrifugation at 800g and the platelet pellet was resuspended in 3 mmol/L (N-morpholino) ethane sulfonic acid containing 0.147 mmol/L NaCl, 3 mmol/L KCl, and 4.8 mmol/L D-glucose (MES buffer) containing 10% ACD. The pH of this buffer was adjusted with Tris base to pH 7.2. After 2 washes with this buffer, the platelets were resuspended in MES buffer and their number adjusted to 1 x 10⁶/mL.

Evaluation of platelet functions. Platelet aggregation was performed as previously described. Minimal effective concentrations to achieve complete aggregation were determined for arachidonic acid, adenosine diphosphate (ADP), epinephrine, and collagen I. Platelet aggregation was evaluated with respect to the initial slope and the maximal height of the aggregation curve achieved (percent optical transmission: 0% PRP, 100% platelet-poor plasma [PPP]). The dose-response relation between concentration of platelet agonist and aggregation response was evaluated by plotting the log dose.
developed photomicrographs resulting from each experiment. For
occupied sites that had never been occupied until the present time;
and (4) cumulative sites giving a running total of the fresh adhesion
control. The differences in the cumulative
platelets; (2) fresh adhesion sites, ie, the number of currently
man fibrinogen and collagen I. Time resolved measurements were
previously described. Two adhesive surfaces were examined, hu-
were calculated by paired t-tests.
(1) Occupied sites, ie, the total number of sites presently occupied by
Platelet adhesion was measured in a laminar flow chamber as
previously described. Two adhesive surfaces were examined, hu-
man fibrinogen and collagen I. Time resolved measurements were
made over a 13-minute period at 30-second intervals in a 71,000
μmol/L)² area at a shear rate ranging between 20 and 25 s⁻¹. Shear
rates were calculated according to methods described by Batchelor
for Hele-Shaw cells.
Computer-aided analysis of the data was performed on a series of
developed photomicrographs resulting from each experiment. For
each discrete time period, the following parameters were evaluated:
(1) Occupied sites, ie, the total number of sites presently occupied by
platelets; (2) fresh adhesion sites, ie, the number of currently
occupied sites that were not occupied during the immediately
preceding time period; (3) new sites, ie, the number of currently
occupied sites that had never been occupied until the present time; and
(4) cumulative sites giving a running total of the fresh adhesion
sites. From these data we determined the adhesion rate, which is
represented by the slope of the linear regression of a plot relating
cumulative sites versus time, and the reuse of sites index represented
by the slope of the least square linear regression line for a plot of the
natural logarithm of the total number of sites occupied by platelets
versus the different number of times such occupations occurred.
Isolation of platelet pseudopodia. After careful elimination of
all contaminating red blood cells by repeated centrifugation (580g),
the platelets were sedimented from the PRP, washed twice with
MES buffer containing 10% ACD, and finally resuspended in a small
volume of this buffer without ACD. The platelet concentration was
adjusted to approximately 3 x 10⁷/mL, and the pH of the platelet
suspension was brought up to pH 7.2 using Tris base. The platelets
were allowed to remain for 15 minutes at 37°C and were then
stimulated with bovine thrombin (0.5 U/mL). During the next 5
minutes, the platelets were gently inverted x 2. Shape change was
verified by phase microscopy. The platelet suspension was then
subjected to homogenization in a glass/Teflon tissue grinder with a
clearance of 0.056 mm. After 5 up and down strokes, the platelet
suspension was centrifuged at 950g for 25 minutes. The supernatant
was taken off and centrifuged at 2,300g for 25 minutes. The pellets
of the two centrifugations, representing cell bodies and pseudopodia,
respectively, were resuspended in a small volume of MES buffer and
were then ready for lipid extraction, protein analysis, or inorganic
phosphorus measurement. The purity of the separated fractions was
also determined by electron microscopy (Fig 1). The pseudopodia
preparations (Fig 1A) showed a fairly uniform picture of small
vesicle-like structures, most of which contained material of similar
appearance as that of the cytoplasm of platelets. The platelet bodies
(Fig 2) by and large displayed rounded shapes with subcellular
granules dispersed throughout the cell.
Lipid analysis. Washed suspensions of platelets or PPP were
extracted with chloroform/methanol (1:2, vol/vol) containing buty-
lated hydroxytoluene (BHT), 50 μg/mL. To 1 vol cell extract or PPP
were added 1/10 vol 0.2 mol/L EDTA, 1 drop 88% formic acid, and
3.7 vol chloroform/methanol. An additional 1.25 vol 2 mol/L KCl
and 1.25 vol chloroform with BHT were added. The lipid extract was
recovered and the remainder was reextracted with 1.5 vol chloroform
containing BHT. The combined extracts were concentrated under
N₂ and redissolved in a small amount (usually 0.2 mL) chloroform/
methanol. Platelet phospholipids were analyzed by thin layer chroma-
tography as previously described. Lipid phosphorus was measured
according to Chen et al. A standard curve was prepared using
phingomyelin.
To analyze fatty acids from whole platelets or PPP, an internal
standard, 5 μg heptadecanoic acid, was added to each lipid extract.
Fatty acids were transesterified with boron trifluoride (at 100°C for
90 minutes). The fatty acid methyl esters were extracted twice with
petroleum ether. The extracts were combined, evaporated, and
dissolved in 25 to 50 μL methylene chloride containing BHT (5
mg/mL). Gas chromatography was performed in a model 8500
Perkin-Elmer gas chromatograph using a capillary column SP-2230

![Fig 2. Platelet adhesion to fibrinogen. Cumulative number of
adhesion sites over a 13-minute period are shown. Platelet
adhesion was measured at baseline (○), after 2 weeks (●), after 25
days (□), and on day 30 (○) after fish oil was started. Each point
represents the mean of the results obtained from eight individuals
whose platelets were studied. The differences in the cumulative
number of adhesion sites between baseline and study period
measurements were significant at P < .025 at 6 minutes. The
significance increased to P < .005 at 12 minutes. All differences
were calculated by paired t-tests.](#)

Table 1. ED 50 of Platelet Aggregation Before and After Fish Oil Supplementation

<table>
<thead>
<tr>
<th>Agonist</th>
<th>Baseline</th>
<th>Day 14</th>
<th>Day 25</th>
<th>Day 30</th>
</tr>
</thead>
<tbody>
<tr>
<td>Arachidonic acid (μM)</td>
<td>-3.34 ± 0.12</td>
<td>-3.29 ± 0.08</td>
<td>-2.56 ± 0.29†</td>
<td>-3.2 ± 0.07</td>
</tr>
<tr>
<td>Epinephrine (μM)</td>
<td>-5.85 ± 0.62</td>
<td>-5.87 ± 0.86</td>
<td>-4.94 ± 0.57</td>
<td>-6.86 ± 0.71</td>
</tr>
<tr>
<td>ADP (μM)</td>
<td>-6.78 ± 0.38</td>
<td>-6.66 ± 0.28</td>
<td>-4.52 ± 0.34‡</td>
<td>-5.65 ± 0.32</td>
</tr>
<tr>
<td>Collagen (μg/mL)</td>
<td>-6.04 ± 0.36</td>
<td>-6.00 ± 0.26</td>
<td>-4.74 ± 0.23‡</td>
<td>-5.99 ± 0.18</td>
</tr>
</tbody>
</table>

*Mean ± 1 SD of eight individuals tested.
†P < .05.
‡P < .025.
Table 2. Effect of Fish Oil Administration on Total Number of Adhesion Sites

<table>
<thead>
<tr>
<th>Adhesive Protein</th>
<th>Study Day*</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Baseline</td>
</tr>
<tr>
<td>Fibrinogen</td>
<td>560 ± 120</td>
</tr>
<tr>
<td>Collagen I</td>
<td>277.4 ± 125</td>
</tr>
</tbody>
</table>

*Mean ± 1 SD of eight individuals.
†P < .0005.
‡P < .01.

FISH OIL AND PLATELET ADHESIVENESS

Table 2. Effect of Fish Oil Administration on Total Number of Adhesion Sites

<table>
<thead>
<tr>
<th>Adhesive Protein</th>
<th>Study Day*</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Baseline</td>
</tr>
<tr>
<td>Fibrinogen</td>
<td>560 ± 120</td>
</tr>
<tr>
<td>Collagen I</td>
<td>277.4 ± 125</td>
</tr>
</tbody>
</table>

*Mean ± 1 SD of eight individuals.
†P < .0005.
‡P < .01.

Materials. Human fibrinogen was purchased from Kabi Diagnostics (Uppsala, Sweden). Rat tail collagen, type I was obtained from Serva Biochemicals (Westbury, NY). Fish oil was administered in 1 g gelatin capsules obtained from Scherer Corp (Clearwater, FL) (Max EPA). Palm oil was obtained from Palmco, Inc (Portland, OR) and corn oil from Best Foods (Union, NJ). Boron trifluoride in 14% methanol was obtained from Analabs (Norwalk, CT). All other chemicals were of highest purity available.

RESULTS

Platelet aggregation performed with a set of fixed concentrations of agonists, (624 μmol/L arachidonic acid, 5 μmol/L epinephrine, 2.5 μmol/L ADP, and 1.6 μg/mL collagen) showed no statistically significant difference either comparing population means or performing individual paired t-tests. However, when ED 50 values were determined for each agonist (Table 1), moderate increases of the threshold concentrations were noted in response to fish oil administration. The changes for arachidonic acid, ADP, and collagen attained statistical significance at day 25 of the study.

In contrast to the aggregation studies, platelet adhesion was found to be drastically affected by the administration of fish oil. Measurement of platelet adhesivity to fibrinogen and collagen at low shear rate showed an impressive reduction of the cumulative number of adhesion sites (Table 2) and of the rate of adhesion (Figs 2 and 3). A 60% to 65% decrease in both of these parameters was noted within 14 days after the initiation of the fish oil administration. As previously noted by other investigators, the effect of the dietary supplement has a delayed onset and requires a washout time that exceeds 1 week. The reuse of site index also decreased (Figs 4 and 5),...
of adhesion sites at the 25-day assay. Adhesion to collagen I coated surfaces was studied.

Time-resolved analyses of platelet adhesion give important information on the reutilization of sites that have been previously occupied by platelets. A fish oil supplemented platelet formulation could represent another possibility. Adhesion studies in a group of five normal individuals on a vegetable oil supplement, given in equal amounts and for the same length of time as the fish oil, failed to show a difference between pre- (baseline) and postsupplementation samples (Table 3).

Compliance of the study subjects could be verified by the changes in the fatty acid distribution in plasma and platelets (Tables 4 and 5). There was a very marked increase in C20:5 (n-3) and C22:6 (n-3). There was also an increase in C16:1 and C24:1 concomitant with the fish oil administration. On the other hand, C18:2 and C20:4 showed a decrease over the same time interval. After fish oil was discontinued on day 25 of the study, these fatty acids began to return toward baseline, a process that was not yet completed on day 30.

In an effort to determine the reason for the reduced platelet adhesiveness after fish oil administration, we performed scanning electron microscopy of the adherent platelets. As shown in Fig 6, fish oil supplemented platelets exhibited a striking decrease in the number of pseudopodia.

In addition, the pseudopodia were shorter, wider at the base, and more rounded. To ascertain whether these differences were representative of the majority of the platelets, we obtained quantitative measurements of the pseudopodia that formed in fish oil supplemented platelets on stimulation with thrombin (Table 6). A progressive decrease in the ratio of pseudopodia to platelet cell bodies was noticeable over the entire period of the study. It is interesting to note that the magnitude of this reduction was comparable with that affecting platelet adhesiveness.

The distribution of phospholipids between pseudopodia and cell bodies showed differences in lysophosphatidylcholine, sphingomyelin, and phosphatidylcholine (Table 7). These changes were not influenced by the administration of fish oil. Only phosphatidylethanolamine decreased slightly in pseudopodia and increased somewhat in platelet cell bodies.

**DISCUSSION**

Our aggregation studies were able to show only a minor increase of the threshold concentrations of various platelet agonists after fish oil consumption. Other investigators have obtained more pronounced fish oil-induced reductions in platelet aggregability even though the supplementation level of our study was similar to that used in other studies. The reason for the discrepancy is not immediately apparent. Differences in the populations examined in the various studies could be one possible explanation. Noncompliance of the volunteers with the fish oil administration can be ruled out because of the increase of DHA and EPA in platelets and plasma.

Our studies showed a fish oil-induced decrease in all the adhesion parameters that we determined. Measurement of adhesion in a laminar flow chamber has been well-characterized. Time-resolved analyses of platelet adhesion give important information on the reutilization of sites that had been used before for adhesion of platelets. It is surprising that more than half of the adhesion events take place at sites that have been previously occupied by platelets. A fish oil-induced reduction of the reutilization index was clearly evident in our studies. As we have not yet found a satisfactory explanation for the preferred utilization of previously occupied adhesion sites, it may be presumptuous to offer an explanation of the change in this behavior brought about by the administration of fish oil. However, the ultrastructural morphology of platelets enriched with (n-3) polyunsaturated fatty acids is very persuasive evidence for the importance of pseudopodia in this phenomenon.

The overall adhesion represented by the cumulative adhesion count and the rate of adhesion showed drastic reductions when fish oil was administered. These results are very similar to those obtained with platelets of individuals on supplemen-

**Table 3. Effect of Vegetable Oil Administration on Total Number of Adhesion Sites**

<table>
<thead>
<tr>
<th>Adhesive Protein</th>
<th>Study Day*</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Baseline</td>
</tr>
<tr>
<td>Fibrinogen</td>
<td>548.8 ± 67.7</td>
</tr>
<tr>
<td>Collagen I</td>
<td>344.4 ± 92.4</td>
</tr>
</tbody>
</table>

*Mean ± 1 SD of five individuals.
FISH OIL AND PLATELET ADHESIVENESS

a-tocopherol supplementation. Platelets from fish oil-supplemented individuals displayed

\[ C_{24:0} \] decreased from 1.2 to 0.8, \[ C_{22:6} \] increased from 1.0 to 1.2, and \[ C_{18:1} \] increased from 17.4 to 17.6.

Table 4. Total Fatty Acid Distribution (%) of Plasma Before and After Fish Oil Administration

<table>
<thead>
<tr>
<th>Fatty Acid</th>
<th>Baseline</th>
<th>Day 10</th>
<th>Day 17</th>
<th>Day 25</th>
<th>Day 29</th>
</tr>
</thead>
<tbody>
<tr>
<td>C 16:0</td>
<td>26.1 ± 2.5</td>
<td>26.2 ± 2.8</td>
<td>24.8 ± 2.8</td>
<td>25.3 ± 3.1</td>
<td>26.2 ± 3.0</td>
</tr>
<tr>
<td>C 16:1</td>
<td>1.1 ± 0.4</td>
<td>3.6 ± 1.5†</td>
<td>3.5 ± 0.9†</td>
<td>3.1 ± 0.8‡</td>
<td>1.8 ± 0.8</td>
</tr>
<tr>
<td>C 18:0</td>
<td>11.0 ± 1.2</td>
<td>11.3 ± 1.6</td>
<td>10.1 ± 1.8</td>
<td>11.5 ± 1.9</td>
<td>11.0 ± 1.8</td>
</tr>
<tr>
<td>C 18:1</td>
<td>19.3 ± 1.8</td>
<td>20.1 ± 2.7</td>
<td>21.8 ± 3.0</td>
<td>20.8 ± 3.3</td>
<td>18.6 ± 3.2</td>
</tr>
<tr>
<td>C 18:2</td>
<td>26.8 ± 3.6</td>
<td>18.6 ± 4.2‡</td>
<td>16.8 ± 2.8†</td>
<td>17.9 ± 3.0†</td>
<td>21.6 ± 3.4§</td>
</tr>
<tr>
<td>C 18:3</td>
<td>0.9 ± 0.2</td>
<td>1.5 ± 0.6‡</td>
<td>1.6 ± 0.9</td>
<td>1.8 ± 1.0</td>
<td>1.2 ± 0.7</td>
</tr>
<tr>
<td>C 20:0</td>
<td>0.3 ± 0.1</td>
<td>0.6 ± 0.2‡</td>
<td>0.5 ± 0.2†</td>
<td>0.2 ± 0.1</td>
<td>0.3 ± 0.1</td>
</tr>
<tr>
<td>C 20:1</td>
<td>1.1 ± 0.3</td>
<td>1.1 ± 0.3</td>
<td>1.0 ± 0.3</td>
<td>1.2 ± 0.3</td>
<td>1.1 ± 0.4</td>
</tr>
<tr>
<td>C 20:3</td>
<td>1.3 ± 0.5</td>
<td>2.1 ± 0.8§</td>
<td>2.5 ± 0.6§</td>
<td>2.2 ± 0.7§</td>
<td>1.7 ± 0.4</td>
</tr>
<tr>
<td>C 20:4</td>
<td>5.5 ± 0.9</td>
<td>3.7 ± 0.8‡</td>
<td>3.5 ± 0.8†</td>
<td>3.6 ± 0.8†</td>
<td>4.6 ± 0.9</td>
</tr>
<tr>
<td>C 22:0</td>
<td>0.3 ± 0.1</td>
<td>0.3 ± 0.1</td>
<td>0.5 ± 0.2</td>
<td>0.4 ± 0.1</td>
<td>0.4 ± 0.1</td>
</tr>
<tr>
<td>C 22:1</td>
<td>0.1 ± 0.05</td>
<td>0.2 ± 0.05</td>
<td>0.1 ± 0.05</td>
<td>0.2 ± 0.1</td>
<td>0.1 ± 0.05</td>
</tr>
<tr>
<td>C 20:5</td>
<td>0.1 ± 0.05</td>
<td>3.4 ± 1.6†</td>
<td>4.8 ± 1.7†</td>
<td>4.3 ± 1.6†</td>
<td>0.9 ± 0.3‡</td>
</tr>
<tr>
<td>C 24:0</td>
<td>1.0 ± 0.2</td>
<td>0.8 ± 0.2</td>
<td>0.7 ± 0.2</td>
<td>0.8 ± 0.2</td>
<td>1.0 ± 0.2</td>
</tr>
<tr>
<td>C 24:1</td>
<td>0.4 ± 0.1</td>
<td>1.1 ± 0.3†</td>
<td>2.8 ± 0.9†</td>
<td>2.7 ± 0.8†</td>
<td>1.8 ± 0.7†</td>
</tr>
<tr>
<td>C 22:6</td>
<td>1.6 ± 0.4</td>
<td>4.7 ± 1.8†</td>
<td>4.9 ± 1.9†</td>
<td>4.1 ± 1.7†</td>
<td>3.9 ± 1.0‡</td>
</tr>
</tbody>
</table>

*Mean ± 1 SD of eight individuals.
†P < .005.
‡P < .005.
§P < .01.
¶P < .05.
§P < .025.

Both nutritional additives reduced pseudopodia formation when stimulated by agonists. The platelets from fish oil-supplemented individuals displayed short, stubby pseudopodia that are identical in appearance to those observed in α-tocopherol-enriched platelets.24 Although in the present study only two adhesive surfaces were investigated, i.e., fibrinogen and collagen I, we believe that similar observations will hold true for other adhesive surfaces, as could be shown when we studied the effect of α-tocopherol supplementation.

The quantification of pseudopodia as a relative proportion of the platelet cell body confirmed that the observations made by scanning electron microscopy did not reflect unique and isolated examples, but rather were indicative of the overall changes induced by fish oil. The increase in inorganic phosphorus of the pseudopodial and the platelet cell body fraction indicates an increase in platelet size in response to the fish oil supplementation. Evidence of a prolonged washout time of these changes was also apparent in the ratio of pseudopodia/platelet cell bodies. In fact, at day 30 of our study this ratio was lower than before fish oil supplementation was begun. We believe that this is due primarily to a

Table 5. Total Fatty Acid Distribution (%) of Platelets Before and After Fish Oil Administration

<table>
<thead>
<tr>
<th>Fatty Acid</th>
<th>Baseline</th>
<th>Day 14</th>
<th>Day 25</th>
<th>Day 29</th>
</tr>
</thead>
<tbody>
<tr>
<td>C 16:0</td>
<td>19.8 ± 2.9</td>
<td>18.9 ± 2.6</td>
<td>21.3 ± 2.8</td>
<td>20.1 ± 2.8</td>
</tr>
<tr>
<td>C 16:1</td>
<td>1.2 ± 0.2</td>
<td>2.4 ± 0.5</td>
<td>2.3 ± 0.5</td>
<td>1.6 ± 0.4</td>
</tr>
<tr>
<td>C 16:2</td>
<td>17.6 ± 2.1</td>
<td>14.1 ± 2.2†</td>
<td>13.4 ± 2.3†</td>
<td>16.2 ± 1.9</td>
</tr>
<tr>
<td>C 18:1</td>
<td>17.4 ± 2.1</td>
<td>16.8 ± 2.3</td>
<td>17.0 ± 2.9</td>
<td>17.6 ± 2.3</td>
</tr>
<tr>
<td>C 18:2</td>
<td>8.1 ± 1.4</td>
<td>5.4 ± 1.3†</td>
<td>4.2 ± 1.2‡</td>
<td>6.8 ± 1.8</td>
</tr>
<tr>
<td>C 18:3</td>
<td>0.8 ± 0.2</td>
<td>0.9 ± 0.3</td>
<td>0.8 ± 0.3</td>
<td>0.8 ± 0.3</td>
</tr>
<tr>
<td>C 20:0</td>
<td>1.6 ± 0.4</td>
<td>1.3 ± 0.4</td>
<td>1.0 ± 0.3‡</td>
<td>1.5 ± 0.3</td>
</tr>
<tr>
<td>C 20:1</td>
<td>1.1 ± 0.3</td>
<td>1.8 ± 0.5†</td>
<td>2.5 ± 0.5†</td>
<td>1.8 ± 0.4†</td>
</tr>
<tr>
<td>C 20:3</td>
<td>0.4 ± 0.1</td>
<td>0.6 ± 0.2</td>
<td>0.8 ± 0.3†</td>
<td>0.5 ± 0.1</td>
</tr>
<tr>
<td>C 20:4</td>
<td>24.1 ± 3.1</td>
<td>20.2 ± 3.0§</td>
<td>17.1 ± 2.9‡</td>
<td>23.3 ± 3.0</td>
</tr>
<tr>
<td>C 22:0</td>
<td>3.2 ± 0.4</td>
<td>2.5 ± 0.3†</td>
<td>2.2 ± 0.3‡</td>
<td>3.1 ± 0.4</td>
</tr>
<tr>
<td>C 22:1</td>
<td>0.8 ± 0.2</td>
<td>1.4 ± 0.3‡</td>
<td>1.9 ± 0.4‡</td>
<td>0.9 ± 0.2</td>
</tr>
<tr>
<td>C 20:5</td>
<td>0.1 ± 0.06</td>
<td>4.8 ± 0.5‡</td>
<td>4.9 ± 0.5‡</td>
<td>1.2 ± 0.3‡</td>
</tr>
<tr>
<td>C 24:0</td>
<td>1.2 ± 0.3</td>
<td>0.9 ± 0.2</td>
<td>0.6 ± 0.1‡</td>
<td>1.0 ± 0.2</td>
</tr>
<tr>
<td>C 24:1</td>
<td>1.4 ± 0.2</td>
<td>2.3 ± 0.8‡</td>
<td>3.4 ± 0.6‡</td>
<td>1.5 ± 0.4</td>
</tr>
<tr>
<td>C 22:6</td>
<td>0.9 ± 0.2</td>
<td>5.8 ± 1.1†</td>
<td>6.0 ± 1.0‡</td>
<td>2.0 ± 0.4‡</td>
</tr>
</tbody>
</table>

*Mean ± 1 SD of eight individuals.
†P < .005.
‡P < .0005.
§P < .025.
disproportionately slow return of cell bodies to baseline values. These findings suggest that the changes affect not only the platelets in the peripheral circulation but also the megakaryocytes, thus leading to a very delayed return to presupplementation conditions. The effect of fish oil on platelet adhesion appears to be specific. An equally high intake of vegetable oil (a 1:1 mixture of palm oil and corn oil) did not alter platelet adhesion. This rules out that the increase in dietary fat was responsible for the observed results. In fact, marine and

Table 6. Identification of Platelet Pseudopodia (Ps) and Cell Bodies (CB) Before and After Fish Oil Administration

<table>
<thead>
<tr>
<th></th>
<th>Baseline</th>
<th>Day 14</th>
<th>Day 25</th>
<th>Day 30</th>
</tr>
</thead>
<tbody>
<tr>
<td>Ps</td>
<td>35.9 ± 2.4</td>
<td>40.6 ± 4.5†</td>
<td>42.3 ± 5.6‡</td>
<td>31.5 ± 3.3</td>
</tr>
<tr>
<td>CB</td>
<td>162.1 ± 9.7</td>
<td>242.3 ± 26.1§</td>
<td>236.7 ± 29.8§</td>
<td>222.6 ± 25.9§</td>
</tr>
<tr>
<td>Ps/CB</td>
<td>21.8 ± 1.9</td>
<td>17.1 ± 2.1§</td>
<td>16.5 ± 2.4§</td>
<td>14.3 ± 2.6§</td>
</tr>
</tbody>
</table>

Abbreviation: P, inorganic phosphorus.
*Mean ± 1 SD of eight individuals. For this study, platelets were stimulated with thrombin, 0.05 U/mL.
†P < .025.
‡P < .01
§P < .0005.

Table 7. Distribution of Phospholipids Between Platelet Pseudopodia (Ps) and Cell Bodies (CB) Before and After Fish Oil Supplementation

<table>
<thead>
<tr>
<th>Phospholipid</th>
<th>Ps</th>
<th>CB</th>
<th>Ps</th>
<th>CB</th>
<th>Ps</th>
<th>CB</th>
</tr>
</thead>
<tbody>
<tr>
<td>Lyso-PC</td>
<td>8.8 ± 2.8†</td>
<td>5.7 ± 2.5</td>
<td>11.4 ± 3.1†</td>
<td>6.8 ± 1.8</td>
<td>25.6 ± 6.0†</td>
<td>33.7 ± 7.5</td>
</tr>
<tr>
<td>SM</td>
<td>32.5 ± 5.5</td>
<td>7.1 ± 2.9</td>
<td>11.8 ± 2.7</td>
<td>11.1 ± 2.3</td>
<td>29.8 ± 7.3§</td>
<td>36.1 ± 5.1</td>
</tr>
<tr>
<td>PC</td>
<td>35.0 ± 2.2</td>
<td>12.4 ± 1.6</td>
<td>10.8 ± 2.6</td>
<td>11.6 ± 4.0</td>
<td>27.8 ± 10.2†</td>
<td>36.1 ± 5.1</td>
</tr>
<tr>
<td>PI</td>
<td>11.2 ± 2.4</td>
<td>9.6 ± 5.1</td>
<td>10.5 ± 4.2</td>
<td>11.5 ± 2.3</td>
<td>11.4 ± 2.7</td>
<td>11.3 ± 2.8</td>
</tr>
<tr>
<td>PS</td>
<td>12.1 ± 4.1</td>
<td>11.7 ± 3.8</td>
<td>12.1 ± 4.1</td>
<td>9.6 ± 5.1</td>
<td>11.4 ± 2.7</td>
<td>11.3 ± 2.8</td>
</tr>
<tr>
<td>PE</td>
<td>24.9 ± 4.2</td>
<td>21.5 ± 2.4§</td>
<td>21.5 ± 2.4§</td>
<td>26.5 ± 3.4</td>
<td>21.6 ± 1.2‡</td>
<td>25.3 ± 3.2</td>
</tr>
</tbody>
</table>

*Mean ± 1 SD of eight individuals.
†P < .025.
‡P < .005.
§P < .05.
vegetable oil were similar in degree of saturated, monounsaturated, and polyunsaturated fatty acid content (30%, 28%, and 42%, respectively, for fish oil and 29%, 35%, and 36% for the vegetable oil). Therefore, the difference is narrowed to the (n-3) polyunsaturated fatty acids, although it should be pointed out that fish oil contained cholesterol (4.5 mg/g) whereas the vegetable oil mixture did not.

We have investigated the distribution of phospholipids between pseudopodia and platelet cell bodies to find out whether fish oil induces changes in their proportion. Our results have only shown a change of phosphatidylethanolamine in response to fish oil ingestion. Pseudopodia usually have a somewhat higher proportion of lysophosphatidylcholine and sphingomyelin, but show a moderate decrease in phosphatidylethanolamine compared with platelet cell bodies. The question arises whether this change may be related to the fish oil-induced abnormalities in platelet pseudopodia. No information is available to form an opinion.

Although our study was based on a relatively small number of individuals, we are certain that our observations can be generalized since the individuals we examined comprised both sexes and a wide range of age groups. Because their original dietary habits remained undisturbed, only the effect of fish oil supplementation appears to be reflected in the results. Our study, similar to previous investigations of platelet function, used a relatively short period of dietary supplementation with fish oil. We realize that it will be important to show that long-term administration maintains the beneficial effect that fish oil has on platelet adhesiveness. Based on the present and our previous investigations of α-tocopherol, we believe that the combined administration of platelet anti-aggregating agents, eg, aspirin, and substances with anti-adhesive properties, such as fish oil or α-tocopherol, could have a very potent antithrombotic effect. However, further studies will have to be performed to establish that such a combination therapy has no undesirable side effects.

REFERENCES

Fish oil: a potent inhibitor of platelet adhesiveness [see comments]

XL Li and M Steiner