Dietary Fish Oil Accelerates the Response to Coronary Thrombolysis With Tissue-Type Plasminogen Activator
Evidence for a Modest Platelet Inhibitory Effect In Vivo

Gregory A. Braden, MD, Howard R. Knapp, MD, PhD,
Desmond J. FitzGerald, MD, and Garret A. FitzGerald, MD

To assess the platelet inhibitory effect of high doses of fish oils and relate it to alterations in eicosanoid synthesis, we used a canine model in which coronary thrombosis, the time to reperfusion induced by recombinant tissue-type plasminogen activator (rt-PA), and the rate of spontaneous reocclusion are sensitive to platelet inhibition. In the animals fed fish oil, the time to rt-PA induced thrombolysis was accelerated (mean, 63 vs. 27 minutes; p<0.003). The time to thrombotic occlusion and the rate of reocclusion were unaltered. The ratio of eicosapentaenoic acid (EPA) to arachidonic acid rose in platelet and endothelial cell membranes, whereas serum thromboxane (Tx) B levels fell a mean 86%, and basal excretion of 2,3-dinor-TxB2 (TXA2-M) declined. Basal prostaglandin (PG) I2 formation was unaltered, whereas biosynthesis of EPA-derived TXA2 and PGI3 increased. In control animals, TXA2 formation increased during thrombosis; there was a further, more marked rise during reperfusion. PGI3 formation also increased, probably as a response to platelet-vascular interactions. Stimulated production of both eicosanoids was strikingly suppressed in the animals fed fish oil. Fish oils significantly enhance the efficacy of rt-PA in vivo, albeit to a modest extent. Because the time to reperfusion is highly sensitive to Tx-dependent platelet activation, this effect is likely to reflect the demonstrated suppression of TXA2 biosynthesis by fish oils. (Circulation 1990;82:178–187)

Supplementation of the Western diet with marine lipids may serve to reduce the incidence of thrombotic disease.1,2 Although this concept has gained much credibility, the data that support the antithrombotic efficacy of fish oils are indirect. Thus, the incidence of thrombotic coronary vascular disease has been inversely related to the dietary consumption of fish.3,4 However, this has been true of some, but not all,5,6 such studies, and in one instance where such a relation has been apparent, the fish consumed had a low content of n-3 fatty acids.3 It is the high amounts of these fatty acids, particularly eicosapentaenoic acid (EPA), in the fish and marine mammals consumed by Greenland Eskimos that are thought to explain the “antithrombotic tendency” of this population.1,7,8 EPA is metabolized to oxygenated derivatives analogous to those of the n-6 fatty acid, arachidonic acid (AA), which can replace in cell membranes.9 However, the EPA metabolite, thromboxane (TX) A2, is produced in lesser amounts in vivo than the proaggregant, vasocostrictor AA metabolite TXA2 and may lack the biological potency of the dienoic compound.10 In contrast, under physiological conditions, AA and EPA appear to be comparable substrates for the formation of platelet inhibitory, vasodilator prostacyclins (PGIs), and their respective products, PGI3 and PGF3, are of similar biological potency.10,11

The most direct attempts to measure the antithrombotic effects of dietary fish oils in populations such as the Greenland Eskimos and in studies of dietary supplementation have involved the measurement of platelet aggregation ex vivo and the bleeding time. These surrogate variables suggest an effect that is much less pronounced than that of standard platelet inhibitors, such as aspirin.12,13 This would be consistent with the substantial, but incomplete, inhibition of

From the Divisions of Clinical Pharmacology and Cardiology, Vanderbilt University, Nashville, Tennessee.

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Address for correspondence: Garret A. FitzGerald, MD, Division of Clinical Pharmacology, Vanderbilt University, Nashville, TN 37232.

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endogenous Tx biosynthesis achieved by even very high doses of fish oils and the nonlinearity of the relationship between Tx biosynthesis and Tx-dependent platelet activation. However, the potential of fish oils as an approach to the prevention of thrombosis is widely accepted, despite these observations and the lack of a direct assessment of their antithrombotic effects in vivo.

This study was designed to determine whether fish oils inhibited platelet-dependent events in vivo. We selected a canine model in which thrombosis induced by electrical injury to the coronary endothelium is subject to therapeutic lysis with recombinant tissue-type plasminogen activator (rt-PA). In this setting, the time to thrombosis, the time to lysis with rt-PA, and the incidence of vascular reocclusion after thrombolysis have all been shown to be platelet dependent. Furthermore, we related the functional and biochemical effects of fish oil to each other by measurement of major urinary metabolites of both EPA- and AA-derived Tx and PGI by sensitive and specific methods. These studies suggest that very high doses of fish oil enhance the efficacy of rt-PA. The results are consistent with a modest platelet inhibitory effect of fish oils in vivo and support the hypothesis that this reflects a modification of eicosanoid formation in vivo.

Methods

Study Design

All studies were reviewed and approved by the animal care committee at Vanderbilt University. Male mongrel dogs weighing 16–24 kg were randomized to receive either standard canine lab chow or a special low-fat lab chow (Purina Mills, Richmond, Ind.) supplemented with fish oil (Max EPA: 18% EPA; 12% docosahexaenoic acid [DHA] kindly provided by Dr. Desmond Davies, R.P. Scherer Co., Troy, Mich.) to supply one third of the total daily calories. This corresponded to roughly 10 g EPA/day (mean, 0.5 g/kg) and 6.7 g DHA/day (mean, 0.33 g/kg). All animals were fed so that 125% of their daily caloric requirements were supplied.

After 3 weeks of feeding, the dogs were anesthetized with 30 mg/kg pentobarbital and ventilated with a Harvard respirator (Harvard Apparatus Co., South Natick, Mass.). The left circumflex coronary artery was isolated through a left thoracotomy. All branches were ligated down to the first obtuse marginal branch. A needle electrode was placed into the lumen of the vessel and secured with a suture. The needle electrode consisted of a 30-gauge monofilament insulated wire onto which was crimped the distal 3-mm segment of a 25-gauge hypodermic needle. An ultrasonic flow probe (Crystal Biotech, Holliston, Mass.) was secured just proximal to the needle electrode. Their respective leads were brought through the chest wall and buried in a subcutaneous pouch. The wound was closed and allowed to heal for 5–7 days, a period that permits the urinary eicosanoids to fall to preoperative levels. The postoperative treatment consisted of heparin (100 IU/kg subcutaneously every 8 hours for 48 hours) and prophylactic antibiotics (Combicet; Pfizer, New York).

After 4 weeks of feeding, the dogs were sedated with acepromazine 1 mg/kg and morphine sulphate 1 mg/kg on the day of the experiment. After local infiltration with 1% lidocaine, the flow probe and electrode leads were externalized through a small skin nick. The flow probe leads were connected to a directional Doppler flowmeter (545-C, Biomedical Engineering, Iowa City, Iowa) and coronary flow velocity recorded continuously with a strip chart recorder. Systemic hemodynamics were not monitored to minimize the likelihood of catheter-induced artifacts on eicosanoid formation. Coronary thrombosis was induced by endothelial damage from a 200 μA direct current through the needle electrode. Occlusion and reocclusion were defined as sustained zero blood flow velocity. Once complete coronary occlusion had persisted for 2 hours, thrombolysis was achieved by the infusion of 10 μg/kg/min rt-PA (Genentech, South San Francisco, Calif.), which continued for 10 minutes after reperfusion. A bolus infusion of rt-PA was not used, because the short half-life of the compound in the dog (mean, 2.4 minutes) indicates the rapid achievement of steady-state concentrations during a sustained infusion. The time to reperfusion was defined as the time from the onset of rt-PA administration until reperfusion flow velocity equaled or exceeded baseline. The animals were observed for 3 hours after reperfusion. The time to complete coronary occlusion, the time to rt-PA–induced reperfusion, and the time to spontaneous reocclusion were recorded.

Urine was collected for analysis of eicosanoid metabolites at the time of randomization, just before starting the current to induce coronary thrombosis and hourly during the experimental protocol period. Blood was collected as previously described for measurement of serum Tx at the time of randomization and at the end of the feeding period. Platelet-rich plasma was prepared by centrifuging a citrated blood sample for 50 seconds at 3,000 rpm, at the time of randomization and at the end of the feeding period. A platelet pellet was obtained by centrifuging the platelet-rich plasma; it was washed with isotonic saline and extracted for phospholipid analysis. At the conclusion of the experiment, the dog was killed by an overdose of pentobarbital, and samples of aorta were obtained for phospholipid analysis.

Synthesis of Prostacyclin and Thromboxanes

All measurements of in vivo formation of eicosanoids were determined with stable isotope dilution assays with negative ion, chemical ionization gas chromatography–mass spectrometry (GC-MS) methods as previously described. We selected the dinor metabolites of PGI and TXA as our analytical targets, because they are major urinary metabolites of these eicosanoids in the dog. Indeed, in the case of TXA, the dinor metabolite is even more abundant than the
11-dehydro metabolite in canine urine, in contrast to the case in humans.\textsuperscript{21} Briefly, the concentrations of excreted 2,3-dinor-6-keto-PGF\textsubscript{1a} (PGI\textsubscript{2}-M) and 2,3-dinor-TxB\textsubscript{2} (TxA\textsubscript{2}-M) metabolites were determined by adding known amounts of internal standards to 3 ml urine, which was purified by sequential steps of column and thin-layer chromatography. The purified compounds were derivatized and analyzed by selected-ion monitoring, at m/z (mass over charge) 586 for the endogenous compounds and m/z 590 for the tetradeuterated standards. To quantify the trienoic metabolites of PGI\textsubscript{3} (2,3-dinor-6-keto-17-ene-PGF\textsubscript{1a}) and TxA\textsubscript{3} (2,3-dinor-17-ene-TxB\textsubscript{2}) derived from EPA, we conducted monitoring at two mass units lower (for the double bond) to detect a peak with a shape identical to and a slightly longer retention time than the shape and retention time of the analogous dienoic metabolites as described elsewhere.\textsuperscript{22} This approach has been shown to reflect the biosynthesis of these trienoic eicosanoids in vivo.\textsuperscript{22,23}

Serum immunoreactive TxB was determined by radioimmunoassay.\textsuperscript{19} The formation of TxB\textsubscript{3} was confirmed by GC-MS with a di-O\textsubscript{18} labeled internal standard as previously described.\textsuperscript{23} The cross-reactivity of the anti-TxB\textsubscript{2} antibody with the TxB\textsubscript{2} was determined by displacement of tritiated TxB\textsubscript{2} by cold TxB\textsubscript{2} and TxB\textsubscript{3}.

\textit{Analysis of Platelet and Vascular Membrane Phospholipids}

Membrane phospholipids were extracted from washed platelets and homogenized aorta according to the procedure by Folch et al.\textsuperscript{24} and were separated by a modification of the method of Skipski et al.\textsuperscript{25} Fatty acid methyl esters were analyzed by gas chromatography (Varian 2100, Varian Corp., Palo Alto, Calif.).

\textit{Statistical Analysis}

Nonparametric methods were used to avoid assumptions as to the distribution of the variables under study. Changes in metabolite formation were first assessed by Friedman’s analysis of variance.\textsuperscript{26} Pairwise comparisons were performed as appropriate by the method of Lord.\textsuperscript{27} Data are expressed as the mean±SEM.

\textbf{Results}

All animals tolerated the fish oil supplementation without adverse effects and consumed the diet in its entirety each day.

\textit{Platelet Inhibitory Effects of Fish Oil In Vivo}

The platelet count tended to rise during the fish oil (227,000±26,000 versus 285,000±34,000/mm\textsuperscript{3}) and control diets (258,000±38,000 versus 346,000±50,000/mm\textsuperscript{3}). However, no significant difference was apparent either within the groups over time or between the two groups at the end of the feeding period.

There was no change in the mean time to coronary occlusion between the fish oil group (n=9) and the control group (n=7; 175±35 versus 152±26 minutes; \textit{p}=NS). However, the mean time to rt-PA–induced coronary reperfusion was shortened by 58% from 63±8 minutes in the control group to 27±4 minutes for the fish oil group (\textit{p}<0.003). After reperfusion, the mean time to spontaneous reocclusion did not differ significantly between the groups, although it tended to be longer (Figure 1) in the animals receiving fish oil (48.4±11.9 versus 30.7±3.7 minutes; \textit{p}=0.29). The reocclusion rates were similar (eight of nine in the fish oil group; seven of seven in the control group) in both groups.

\textit{Effects on Eicosanoid Formation}

\textit{Serum TxB}. The antibody used to determine TxB in serum was found to have a 30% cross-reactivity with TxB\textsubscript{2} in competition binding experiments. The formation of TxB\textsubscript{3} was detected by GC-MS in serum obtained from the fish oil group but not in the control group or in either group before feeding. In the fish oil group, TxB\textsubscript{3} accounted for 33±6% of the total immunoreactive TxB that was formed.
Total immunoreactive serum TxB did not differ significantly between the fish oil (693±80 ng/ml) and control (831±123 ng/ml) groups of animals before the diet feeding. After 4 weeks of feeding, serum TxB had fallen to 99±18 ng/ml (p<0.001) in the fish oil group, whereas it did not change significantly in the control group (Figure 2).

Urinary metabolites of TxA and PGI. Basal state. We have previously demonstrated that rt-PA administration to control animals in the absence of coronary thrombosis does not alter urinary excretion of TxA2-M.16 In the present study, there was no significant difference in TxA2-M excretion between the groups at baseline. There was no change from the basal production of TxA2 in control animals as assessed by its urinary metabolite TxA2-M during the 4-week diet period (2,229±102 versus 2,158±184 pg/mg creatinine). In the fish oil group, there was a mean 52% decrease in TxA2-M (1,692±204 versus 804±102 pg/mg creatinine, p<0.001). Concomitant with this fall in basal production of TxA2, there was an increase in excretion of TxA3-M from 35±7.6 to 578±88 pg/mg creatinine; p<0.0001). There was no change in the TxA3 produced by the control group (30.3±8 versus 22.0±7; p=NS) (Figure 3). Again, there was no significant difference between PGI2-M excretion between the groups at baseline. In contrast to the observations with TxA2-M, no significant change from basal production of PGI2 occurred with the fish oil diet (78±9 to 57±9 pg/mg creatinine; p=NS) or in the control diet (110±31 versus 125±70 pg/mg creatinine; p=NS), during the 4-week period.
There was a significant increase in production of PGI\(_2\) during this time (from 2.0±0.8 to 30±7 pg/mg creatinine; \(p<0.005\)) in the fish oil group but no change in the trace amounts in the control group (from 2.3±1.8 to 1.5±1.2 pg/mg creatinine; \(p=NS\); Figure 4).

**Stimulated production.** TxA\(_2\) biosynthesis increased during thrombosis with a further increase after reperfusion with rt-PA, which is consistent with previous observations in this animal model\(^{16}\) and in humans.\(^{28}\) In the control group, excretion of the TxA\(_2\) metabolite increased from 2,229±302 to 4,422±798 pg/mg creatinine (\(p<0.002\)) during thrombosis and increased further to a maximum of 59,703±18,000 pg/mg creatinine (\(p<0.001\)) after administration of rt-PA (Figure 5). Trace amounts of the TxA\(_2\) metabolite were evident in the control animals; these increased to low, but clearly detectable, levels during thrombosis (Figure 5).

In the fish oil group, TxA\(_3\) biosynthesis in response to the stimuli of thrombosis and thrombolysis was markedly depressed, which is consistent with our observations under basal conditions. Thus, the increase after thrombosis (1,567±254 pg/mg creatinine) was significantly less (\(p<0.002\)) than observed in the control group. Similarly, the depression in the increase of TxA\(_2\)-M excretion (\(p<0.01\)) after thrombolysis, where the maximal values were only 7,766±3,935 pg/mg creatinine in the fish oil group, was even more striking (Figure 5). TxA\(_3\) biosynthesis was increased in the fish oil group. Correspondingly, the significant increases in excretion of this metabolite after thrombosis (from 578±88 to 1,232±280 pg/mg creatinine; \(p<0.01\)) and thrombolysis (to 5,392±1,243 pg/mg creatinine; \(p<0.001\) versus prethrombosis control) were clearly evident. However, formation of TxA\(_2\) plus TxA\(_3\) under stimulated conditions was markedly depressed by the fish oil diet. Thus, after thrombolysis, the maximal excretion of the sum of the TxA\(_2\) and TxA\(_3\) metabolites was depressed by 75±22% in the fish oil group.

Prostacyclin biosynthesis also increased, albeit to a lesser degree than Tx, in association with the induction of both coronary thrombosis and therapeutic thrombolysis (Figure 6). Thus, in the control animals, PGI\(_2\)-M excretion increased from 109±31 to 396±156 pg/mg creatinine (\(p<0.01\)) during coronary occlusion and increased further to 3,385±2,431 pg/mg creatinine (\(p<0.02\)) after thrombolysis. No alterations in the trace amounts of PGI\(_2\)-M excreted by this group were detected during coronary thrombosis or thrombolysis. As was the case with stimulated TxA\(_2\) production, the fish oil diet markedly depressed the formation of PGI\(_2\) during thrombosis and thrombolysis (Figure 6). However, in contrast to the observations with TxA\(_3\), there was no further increase in the biosynthesis of the EPA product PGI\(_3\), in response to the stimuli of thrombosis and thrombolysis (Figure 6). These data indicate that platelet activation is an insufficient stimulus to induce a detectable increment in the biosynthesis of these prostacyclins. However, the capacity to form these compounds, while depressed, remains intact, given a sufficient stimulus. Thus, transient formation of both prostacyclins was detected in association with the trauma of surgery (Table 1), 5 days before the initiation of experimental thrombosis.

**Effects on Membrane Phospholipid Composition**

A high dose of fish oil was selected for these studies. Absorption was confirmed by studies of membrane incorporation of EPA at weekly intervals during the feeding period. The degree of incorporation of n-3 fatty acids into the phospholipid components of platelet and endothelial cell membrane was most marked in the phosphatidylcholine and
STIMULATED PRODUCTION OF THROMBOXANES

**FIGURE 5.** Plots of stimulated production of thromboxane (Tx) A$_2$ and TxA$_3$ during coronary occlusion and reperfusion. Biosynthesis of these eicosanoids was assessed by production of 2,3-dinor-TxB$_2$ (TxA$_2$-M) and 2,3-dinor-17-ene-TxB$_2$ (TxA$_3$-M) metabolites in urine. Values are mean±SEM. Inset: Expanded scale of TxA$_2$-M (pg/mg creatinine) prethrombosis and during initial thrombotic coronary occlusion. Time is expressed on the x axis; each mark on this axis denotes 1 hour. Fish oil diet suppressed production of TxA$_2$ at all time points (p<0.01 for each point). TxA$_3$ production was increased over control values for each time point (p<0.01). Note that these metabolites are expressed in nanograms/milligram creatinine. Note also the difference in scales for TxA$_2$ and TxA$_3$, metabolite excretion.

phosphatidylethanolamine fractions. The relative accumulations after 4 weeks on a fish oil or control diet are depicted in Table 2. These alterations are reflected by the change in the ratio of EPA to AA. In the phosphatidylcholine fraction of platelets, for example, this ratio changed from 0.30±0.007 before feeding to 1.99±0.11 (p<0.001) after 4 weeks on the fish oil diet. Similar, but less pronounced, changes were observed in aortic tissue. Thus, in the phosphatidylcholine fraction, the EPA:AA ratio rose from 0.006±0.008 to 0.33±0.04 (p<0.001). Proportionate changes in EPA and AA were observed in the other phospholipid fractions. Accumulation of another n-3 fatty acid, DHA, paralleled that of EPA. By contrast, there was no alteration in the relative proportions of AA, EPA, and DHA in either platelets or vascular tissue in the animals fed the control diet.

**Discussion**

Although the concept that fish oils can contribute to the prevention of occlusive vascular disease has attracted widespread credibility, there is little evidence for the efficacy of this type of intervention in the prevention of thrombosis in vivo. Rather, such an effect has been implied from the measurement of surrogate variables, such as platelet aggregation ex vivo and the bleeding time. Indeed, such results have been quite variable$^{29-32}$; even when positive results have been obtained, the effects of dietary supplementation with fish oil on these variables have been quite modest.$^{13}$ There are recent reports of the effects of fish oil on restenosis in patients who underwent coronary angioplasty.$^{33-37}$ This process reflects vascular proliferation and thrombosis, and the benefit of effective platelet inhibition remains unestablished.$^{38}$ The results of the published studies of fish oil in this setting are conflicting.

To address the platelet inhibitory potential of fish oils in vivo more directly, we sought to assess this issue in a canine model of coronary thrombosis. In this model, three distinct parameters are modified in response to platelet inhibition in vivo: the time to induction of coronary thrombosis,$^{15}$ the time to reperfusion of the obstructed vessel with rt-PA, and the time to thrombotic reocclusion of the reperfused vessel.$^{16}$ Previous studies demonstrated the importance of the contribution of platelet-derived TxA$_2$ in these phenomena.$^{15,16,39,40}$ This is of relevance,
FIGURE 6. Plots of stimulated production of prostaglandin (PG) I_2 and PGI_3 during coronary occlusion and reperfusion. Biosynthesis of these eicosanoids was assessed by production of 2,3-dinor-6-keto-PGF_1a (PGI_2-M) and 2,3-dinor-6-keto-17-ene-PGF_1a (PGI_3-M) metabolites in urine and expressed as picograms/milligram creatinine. Data are mean±SEM. Inset: Expanded scale of prethrombosis and occlusion values of PGI_2-M. Time is expressed on the horizontal axis; each mark on this axis denotes 1 hour. There is no statistical change in PGI_2 or PGI_3 production with occlusion or reperfusion in the fish oil groups. There is an increase in PGI_2 production in controls for both of the events (p<0.02). Note the difference in scales for the PGI_2 and PGI_3 metabolites.

because it has been speculated that the cardiovascular benefit from fish oil would result from suppression of the biosynthesis of this eicosanoid, coincident with augmented formation of platelet inhibitory, vasodilator prostacyclins,\textsuperscript{1,2,41} which prevent thrombosis in this model.\textsuperscript{15}

To maximize the likelihood of detecting a platelet inhibitory effect of fish oils, we selected a dose that would simulate the content of these acids in the Eskimo diet and reflect the maximum membrane incorporation likely to be achieved by palatable doses of currently available supplements in humans. Although the relation between such measurements and the availability of n-3 fatty acid substrates for mobilization by phospholipases and metabolism to eicosanoids is unknown, these measurements offer an index of the relative bioavailability of the dietary acids between studies. For example, the mean ratio of EPA to AA that we achieved in the platelet phosphatidylcholine of 1.93 in this study corresponds to reports of a ratio close to unity in Eskimos\textsuperscript{41} and in Western volunteers whose diet was supplemented with about 9 g EPA/day (50 ml Max EPA).\textsuperscript{12} This is consistent with the dietary regimen used in this study, which on the basis of body weight exceeded that consumed by Eskimos by more than threefold.

Despite this degree of incorporation, the effects of fish oils on platelet-dependent events in vivo was modest. Platelet count, which usually declines slightly

### TABLE 1. Prostacyclin Production As Stimulated by Vascular Trauma of Surgery

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<tr>
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<th>PGI_2</th>
<th>PGI_3</th>
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<tbody>
<tr>
<td>Before fish oil diet</td>
<td>76±15</td>
<td>3±1.5</td>
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<tr>
<td>Fish oil diet</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Preoperative</td>
<td>48±9</td>
<td>27±12*</td>
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<tr>
<td>Postoperative</td>
<td>777±230†‡</td>
<td>167±35†‡</td>
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Values represent urinary metabolites of prostacyclin I_2 (PGI_2) and I_3 (PGI_3) in picograms/milligram creatinine.

Baseline is before fish oil diet. Preoperative samples were collected just before surgery after 3 weeks of fish oil diet. Postoperative samples represent the urine collected during surgery and the first hour after operation. Differences shown reflect comparisons between the values obtained on diet, before operation vs. the prediet baseline (*p<0.02), between the preoperative and postoperative values (‡p<0.02), and between postoperative values and baseline (†p<0.001).
TABLE 2. Comparison of the Content of Arachidonic Acid, Eicosapentaenoic Acid, and Docosahexaenoic Acid in Platelet and Aortic Phospholipid Fractions After 4 Weeks of Feeding a Control Diet or Fish Oil

<table>
<thead>
<tr>
<th></th>
<th>Platelets</th>
<th>Aorta</th>
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<tr>
<td></td>
<td>Control</td>
<td>Fish oil</td>
</tr>
<tr>
<td>PC AA</td>
<td>18.5±0.8</td>
<td>7.5±0.3*</td>
</tr>
<tr>
<td>PC EPA</td>
<td>0.5±0.1</td>
<td>14.9±0.5*</td>
</tr>
<tr>
<td>PC DHA</td>
<td>0.7±0.1</td>
<td>1.7±0.2†</td>
</tr>
<tr>
<td>PE AA</td>
<td>46.6±1.0</td>
<td>17.1±0.6*</td>
</tr>
<tr>
<td>PE EPA</td>
<td>1.4±0.2</td>
<td>31.0±2.0*</td>
</tr>
<tr>
<td>PE DHA</td>
<td>0.9±0.3</td>
<td>3.4±0.4*</td>
</tr>
<tr>
<td>PS+PI AA</td>
<td>28.3±0.7</td>
<td>16.0±0.8*</td>
</tr>
<tr>
<td>PS+PI EPA</td>
<td>0.4±0.07</td>
<td>16.7±0.7‡</td>
</tr>
<tr>
<td>PS+PI DHA</td>
<td>1.3±0.3</td>
<td>0.75±0.08‡</td>
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</table>

Values are mean percent (of phospholipid fraction) ± SEM.
Phosphatidylethanolamine (PC), phosphatidylethanolamine (PE), and the combined phosphatidylserine and phosphatidylinositol (PS+PI) fractions, arachidonic acid (AA), eicosapentaenoic acid (EPA), docosahexaenoic acid (DHA).

*p<0.001, †p<0.005, ‡p<0.01.

during fish oil feeding to volunteers,12 did not alter significantly. Indeed, there was a nonsignificant tendency for platelet count to rise during the study in both groups of animals; this may have reflected an improvement in their overall nutritional status. The time to reperfusion with rt-PA, which is also modified by TXA2 antagonists,42 was significantly shortened by fish oils. However, the time to occlusive thrombosis induced by electrical damage to the vascular endothelium was unaltered by fish oil. Furthermore, fish oil failed to influence significantly either the time to, or subsequent incidence of, coronary reocclusion.

These observations would be consistent with the hypothesis that the interaction of fish oils with rt-PA is largely due to their modification of eicosanoid biosynthesis. TXA2 appears to play a more pronounced role in the platelet-dependence of the time to reperfusion than either the time to thrombosis or reocclusion after reperfusion with rt-PA. Aspirin, which has a minor and variable effect on thrombosis, has been shown to accelerate the time to reperfusion.16 Similarly, TXA2 antagonists accelerate reperfusion at doses that do not influence the time to rethrombosis.16 Even high doses are only partially effective in preventing reocclusion.40 Other agonists, in addition to TXA2, including serotonin40 and thrombin,42,43 are of major importance in the initiation of thrombosis and in the development of reocclusion after successful reperfusion. It might, therefore, be anticipated that more potent antiplatelet effects than the incomplete inhibition of TXA2 biosynthesis achieved by fish oil would be necessary to influence these end points. Consistent with this hypothesis, the combination of TXA2 and serotonin antagonists40 and the 7E3 antibody directed against glycoprotein IIb/IIIa16 prevent the development of reocclusion.

Experiments in vitro have suggested that biological properties of fish oils, unrelated to eicosanoid forma-
tion, may contribute to an antithrombotic effect of these compounds. These include modulation of the vascular expression of growth factors44 and the possible regulation of plasminogen activator inhibitor formation.45 Despite these possibilities, the modest interaction of fish oil with rt-PA is explicable in terms of an effect on platelets that is consistent with the alterations in eicosanoid biosynthesis that we observed. We found that the capacity of platelets to form TXA2 was reduced. This is most commonly assessed by measurement of immunoreactive serum TB2. However, in the present study, we demonstrated that our antibody cross-reacted with TB3 and confirmed the contribution of TB3 to the radioimmunoassay by direct measurements with GC-MS. It has been proposed that TXA3 is biologically inert10; however, the possibility that it retains the biological properties of TXA2 has not been completely excluded. Whether it is biologically active or not, the residual capacity of platelets to form TXA3, as reflected in the reduced amounts in serum from animals fed fish oils, would be consistent with the limited effects of this intervention on platelet function. Thus, the relations between inhibition of the capacity of platelets to form TXA2 and both inhibition of actual TXA2 biosynthesis14 and inhibition of TXA2-dependent platelet activation16 are nonlinear. For example, studies of the recovery of platelet TXA2 formation after administration of aspirin suggest that TXA2-dependent platelet activation ex vivo is largely maintained by roughly 10% of synthetic capacity.47

This study is the first to report the effects of fish oil supplementation on eicosanoid biosynthesis during thrombosis and thrombolysis. The functional effects that we observed were also consistent with the effects of fish oils on actual in vivo synthesis of TXA2, which were assessed by measurement of 2,3-dinor-TXB2, a major urinary metabolite of TXB2, and of TXA2.
Thus, in the animals receiving fish oil, TxA2-M excretion was depressed under basal and stimulated conditions. Although TxA2 biosynthesis increased, it did not compensate for the reduction in TxA2. This may be because the precursor endoperoxide, PGH3, is a relatively poor substrate for Tx synthase. Although biosynthesis of total Tx was reduced by fish oil, it was not completely inhibited, which is consistent with the incomplete inhibition of serum TxB2. These results would imply an incomplete inhibition of Tx-dependent platelet activation in vivo.

The limited interaction of fish oil with rt-PA may also be partially due to concomitant inhibition of PGI biosynthesis. Both PGI2 and its trienoic analogue, PGI3, are platelet inhibitory vasodilators in vitro. PGI2 biosynthesis is increased in the setting of platelet activation, possibly as a homeostatic reaction to accelerated platelet–vessel wall interactions. In the present study, fish oil supplementation increased the formation of PGI2 under basal conditions, consistent with observations in healthy volunteers. However, the increase in biosynthesis of PGI2, coincident with thrombosis and thrombolysis, was suppressed. This is consistent with our previous observation that, in contrast to the lack of effect of fish oil on PGI2 in healthy volunteers, the elevated PGI2 formation observed in patients with platelet activation is suppressed when they are administered fish oil. In the present study, there was no increase in PGI2 formation to compensate for this effect, perhaps reflective of a reduced affinity of PGI synthase for PGH2 similar to that observed in vitro, which is only evident in vivo in the face of stimulated production of this eicosanoid. Given the biological properties of these eicosanoids, the suppressive effects on prostacyclins under these conditions may serve further to constrain the antithrombotic potential of fish oils in vivo.

In summary, the present study provides functional evidence for a modest, but significant, amplification of the efficacy of a thrombolytic agent by fish oils in vivo. This was mediated by effects on TxA formation. Incomplete suppression of TxA and limitation of the homeostatic increase in PGI formation may have served to constrain the inhibitory effects of fish oil on TxA-dependent platelet activation in vivo. Long-term administration of very large doses of fish oils was used to achieve this effect.

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G A Braden, H R Knapp, D J Fitzgerald and G A FitzGerald

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