The Effects of Dietary ω3 Fatty Acids on Platelet Composition and Function in Man: A Prospective, Controlled Study

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The rarity of atherosclerotic vascular disease and a mild bruising tendency in Greenland Eskimos has been linked to their ingestion of ω3 fatty acids contained in foods obtained from the sea. Previous studies have shown that feeding salmon oil to normal volunteers resulted in reductions of plasma cholesterol and triglycerides. We wished to learn whether salmon oil feeding would result in the incorporation of ω3 fatty acids into platelets and whether platelet function or platelet-vascular interactions would be altered. Diets containing salmon oils led to the incorporation of eicosapentaenoic acid (C20:5 ω3) into platelets (6.1%) with a reduction in arachidonic acid (C20:4 ω6). The ratio of C20:5/C20:4 increased from 0.0045 on the control diet to 0.3 on the salmon diet. Bleeding times were prolonged (from 6.75 to 10 min, p < 0.005), platelet retention on glass beads was mildly reduced (from 89% to 78%, p < 0.0005), and platelet aggregation in response to dilute concentrations of ADP was inhibited in the subjects ingesting the salmon oil. We conclude that in normal subjects dietary ω3 fatty acids derived from salmon oil are incorporated into platelet phospholipids and that these changes are accompanied by alterations in bleeding time and platelet function.

The relationships of diet, vascular disease, platelets and thrombosis have intrigued clinical investigators for several generations. Recently, Danish workers have focused attention on Eskimos from the West Coast of Greenland who have a low incidence of myocardial infarction and stroke as well as a mild tendency to bruise. The dietary fat is derived largely from whale, fish, and seal. Compared to Danes, the Eskimos had lower levels of plasma cholesterol and triglyceride, increased bleeding times, and increased plasma concentrations of the distinctive ω3 very long chain and highly polyunsaturated fatty acids (i.e., eicosapentaenoic and docosahexaenoic acids) characteristic of fish and other marine animals.

Dietary fatty acids may be classified into families by the location of the first (ω) double bond from the methyl end of the molecule. For example, arachidonic acid (C20:4 ω6) is the familiar ω6 series fatty acid precursor for prostaglandin synthesis in platelets, endothelial cells, and other tissues, whereas the ω3 family is composed of fatty acids such as eicosapentaenoic acid (C20:5 ω3) found principally in nature in fish, shellfish, phytoplankton, and in animals that ingest marine life for food.

In platelets, arachidonic acid is converted by the enzyme cyclooxygenase to cyclic endoperoxides, which are rapidly transformed by thromboxane synthetase to thromboxane A2, an extremely potent vasoconstrictor and platelet aggregating substance. In contrast, the vascular endothelial cell converts arachidonic acid via the same endoperoxides to PGI2 (prostacyclin), which is a vasodilator and inhibits platelet aggregation. To explain the prolonged bleeding times and lack of vascular disease in the Greenland Eskimos, it has been suggested that eicosapentaenoic acid (C20:5 ω3) may compete with arachidonic acid for cyclooxygenase and may thereby alter platelet–vascular interactions.

With this background, we performed a prospective, controlled trial in which normal subjects were fed diets containing large amounts of ω3 fatty acids in the form of Pacific Northwest Chinook salmon and salmon oil. Initial studies indicated that these dietary changes led to the appearance of ω3 fatty acids in the plasma and to a significant reduction of plasma cholesterol and triglyceride. In the present study, we wished to learn whether salmon oil feeding would result in the uptake of ω3 fatty acids into platelets and whether platelet function would be inhibited or bleeding times prolonged. If so, the combination of reduced blood...
lipids and inhibition of platelet–vessel interactions induced by salmon oil feeding might prove to be beneficial in the prevention or therapy of vascular disease in man.

MATERIALS AND METHODS

Subjects

Informed consent was obtained from 6 normal healthy male and 5 female nonsmoking volunteers who were taking no medications that could influence platelet function. The mean age of the study subjects was 39 yr.

Study Design

The subjects were outpatients in a clinical research center (CRC) environment and ate all meals under the supervision of the dietary staff. Each subject was randomly assigned to either the control or salmon diet for 4 wk. After a 3-wk rest period during which the subjects usual home diet was eaten, the alternate diet was eaten for an additional 4 wk. Plasma and platelet lipid analyses, platelet function tests, and bleeding times were performed at least twice during the last 2 wk of each dietary period.

Diets

Diets for all subjects were prepared daily by CRC dieticians. The control diet approximated the "typical American diet," was iso- and contained 45% of calories as fat, 15% as protein, and 40% as carbohydrate. The fat was largely saturated (36%) and monounsaturated (46%) and was derived from egg yolk, cocoa butter, and peanut oil. Virtually no w3 fatty acids were present. The dietary cholesterol was 500 mg/day. The diet consisted of fruits, vegetables, bread, and other cereal products plus a blended formula that contained the cholesterol and most of the fats. Vitamin E (50 mg/day) was further added to the diet.

The salmon diet was essentially identical in composition, calories, cholesterol, and percent fat, protein and carbohydrate except that almost all of the dietary fat and all of the dietary cholesterol were derived from salmon steaks (about 1 lb/day) and salmon oil (60–90 ml/day). Each subject ingested approximately 10 g of w3 fatty acids, which totaled 15% of fat calories. Since fish oils contain relatively smaller amounts of linoleic acid, the salmon diet contained 4% of fat calories as linoleic acid compared to 18% in the control diet.

Both diets were well tolerated and body weights did not change throughout the study.

Test Methods and Procedures

Platelets were enumerated and sized with electronic counting equipment (Coulter Instruments, Hialeah, Fla.) and results verified by phase microscopy. Wright’s stained blood smears were examined by light microscopy.

Platelet Lipids

Platelet-rich plasma (PRP) was prepared from EDTA-treated blood and the platelet count determined. Platelets were then sedimented at 7000 g for 20 min and the lipids extracted according to Bligh and Dyer. A recovery standard 4-14C-cholesterol was added for the assay of platelet cholesterol. The extracted lipids were separated into individual lipid classes according to previously described methods. After separation, methyl heptadecanoate was added to the phospholipid band as a recovery marker and an internal standard for gas liquid chromatography. Cholesterol was quanti-
the 11 study subjects. Platelet phospholipid fatty acids as measured by gas liquid chromatography changed significantly after the subjects ingested the salmon diet. Both ω3 fatty acids (C20:5, C22:6) rose and ω6 fatty acids C20:4 and C18:2 fell during the experimental period. The most dramatic change was the rise in C20:5 from 0.1% of total fatty acids to 6.1%. Moreover, the ratio of C20:5/C20:4 greatly increased during salmon feeding from 0.0045 to 0.30. There were no significant changes in the levels of other platelet fatty acids.

Although dietary cholesterol intake was the same during the 2 dietary periods, and the plasma cholesterol was lower during the salmon period, the platelet cholesterol rose from a mean of 56 to 68 μg/10⁹ platelets (p < 0.025). Since platelet protein increased from 1.11 ± 0.3 mg/10⁹ platelets on the control diet to 1.24 ± 0.4 mg/10⁹ platelets on the salmon diet, the calculated platelet cholesterol/mg of protein also rose slightly from 45.8 to 48.8 μg platelet cholesterol/mg platelet protein.

**Platelet Counts (Figs. 1 and 2)**

The platelet count fell from 318,000 to 209,000/cu mm in the subjects fed salmon. Although the mean value of the entire group stayed within the normal range, the platelet count in 2 subjects fell below 150,000/cu mm and one subject fell to 90,000/cu mm after 3 wk of the salmon diet.

The platelet count rose rapidly to normal in these subjects once salmon feeding was discontinued (Fig. 2). There were no changes in leukocyte counts or red cell values. Visual evaluation of the stained peripheral blood smear allowed easy identification of increased numbers of larger platelets (megathrombocytes) in the subjects ingesting salmon. This was verified using platelet sizing equipment by a doubling in mean platelet volume in two subjects with thrombocytopenia.

**Platelet Function (Fig. 1)**

Platelet function was assessed by measurements of platelet retention on glass beads, platelet aggregation, ¹⁴C-serotonin release, and an estimate of platelet prostaglandin production, the formation of malondialdehyde. Platelet–vessel wall interactions were studied using the standardized Ivy bleeding time.

In 11 subjects receiving the salmon diet, the bleeding time was prolonged from 6.75 to 10 min (p < 0.005). The bleeding time in the subject with a platelet count of 90,000/cu mm was 9 min 45 sec. Platelet retention on glass beads using freshly drawn nonanticoagulated blood was reduced from 89% to 78% (p < 0.0005) during the salmon period.

Platelet aggregation testing was carried out using a wide variety of aggregating agents including collagen, ADP, epinephrine, thrombin, and arachidonic acid (Table 2). The magnitude of platelet aggregation to ADP decreased significantly when subjects were ingesting the salmon diet. These findings were seen only at low dilutions of ADP (e.g., a final concentration of 1.4 or 2 μM). The concentration of ADP needed to induce 90% or 50% maximal aggregation is greater during the salmon phase, indicating less responsivity of the platelets to the aggregating agent. However, these findings were not significantly different at a p value of <0.05. Using relatively high concentrations of
collagen as an aggregating agent, no significant differences were seen in aggregation or release of $^{14}$C-serotonin. Aggregations to thrombin, arachidonic acid, and epinephrine did not change.

Platelet production of malondialdehyde was assessed following stimulation of the platelets with the sulfhydryl blocking agent n-ethylmaleimide. No reduction, and in fact, an increase of malondialdehyde, occurred in platelets from subjects consuming the salmon diet.

**DISCUSSION**

We prospectively studied platelet lipid composition and function in 11 normal individuals ingesting a diet rich in salmon oil under carefully defined conditions and compared the findings to those obtained during a control (typical American) diet. Distinctive $\omega$3 fatty acids (e.g., C20:5, C22:6) increased greatly in the plasma and in the platelets of the study subjects during the salmon phase. In recent reports, these fatty acids were also found in the plasma and platelets of Eskimos eating a seal and whale diet and in German subjects fed large quantities of mackerel for 6 days.

The platelet content of cholesterol was measured since plasma cholesterol in our subjects decreased by 17% and alterations in platelet reactivity have been
linked to changes in platelet cholesterol. In our study, platelet cholesterol did not decrease, and therefore, reduced platelet reactivity induced by lowered membrane cholesterol levels cannot explain the prolongation of bleeding times observed in the test subjects.

A mild to moderate fall in the platelet count (but not WBC or RBC) occurred in all but one of the subjects, and platelet numbers fell to less than 150,000/cu mm in three subjects. In these instances the platelet count rose to normal in about 1 wk after salmon feeding was discontinued. Whether the diminished circulating platelet count was due to decreased or altered marrow production, splenic sequestration or accelerated consumption is not known, but the presence of megathrombocytes may indicate shortened platelet survival and consequent increased marrow production of platelets or perhaps a direct effect of the lipid changes on the megakaryocyte. The reductions in platelet count cannot explain the prolonged bleeding times since levels of less than 100,000/cu mm are necessary to significantly prolong the bleeding time in normal individuals. No subject in our study had any bleeding phenomenon. The falls in platelet count are consonant with the lower levels of platelets seen in Eskimos compared to Danish controls (171,000/cu mm versus 232,000/cu mm).

The increase in Ivy bleeding times from the salmon diet was similar to the longer bleeding times found in Greenland Eskimos when compared to a Danish control population. Studies of platelet aggregation in response to a variety of aggregating agents showed no dramatic alterations but did show a significant diminution of aggregation to dilute concentrations of ADP. These findings are similar to those reported by Dyberg and Bang in the Eskimo population. At a time when our study was largely complete, Siess et al. reported that subjects ingesting a mackerel diet had decreased platelet aggregation to very low concentrations of collagen. We used higher concentrations of collagen and found no difference in aggregation, although 14C-serotonin release was reduced by 20%.

We did observe a small but significant reduction in platelet retention to glass beads when freshly drawn, nonanticoagulated, and immediately processed blood was used for the assay. Plasma factors known to influence platelet adhesiveness such as fibrinogen or von Willebrands factor (VIII:VWF) did not change during the dietary periods. Whether another plasma factor such as PGI2 or PGI1 generated during venipuncture may have had an effect is not known.

The ω3 fatty acid, eicosapentaenoic acid (C20:5) from fish oil has been shown to participate in prostaglandin metabolism in vitro. Moncada postulated that C20:5 would displace C20:4 (arachidonic acid) in the prostaglandin pathway and produce thromboxane A2, which was originally thought to be inactive, rather than the active thromboxane A2. In contrast, PGI1 generated from endothelial cells inhibited platelet aggregation. More recently, however, Needleman et al. have suggested that C20:5 competitively inhibits platelet cyclooxygenase and is itself a poor substrate for thromboxane synthetase leading to diminished production of both thromboxane A2 and thromboxane A1.

Since assays for thromboxane B2 were not available to us at the time of our study, the platelet production of malondialdehyde was chosen as an estimate of prostaglandin synthesis in an effort to determine if ω3 fatty acids from fish oil might inhibit prostaglandin synthesis. For example, aspirin in low doses will virtually eliminate malondialdehyde production by platelets in humans through inhibition of cyclooxygenase and has been used to estimate thromboxane synthesis in clinical studies. In 10 of 11 subjects, the production of malondialdehyde by platelets failed to decrease and, in fact, increased during the salmon phase of the study. The explanation for this finding is, as yet, unknown and differs from the "in vitro" work previously cited and the short-term mackerel feed-


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