Platelet Function, Thromboxane Formation and Blood Pressure Control During Supplementation of the Western Diet with Cod Liver Oil

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SUMMARY Epidemiologic and experimental data suggest an antiatherothrombotic potential of ω-3 polyunsaturated fatty acids. Therefore, the Western diet, which supplies predominantly ω-6 polyunsaturated fatty acids, was supplemented with 40 ml/day of cod liver oil, which provides about 10 g of ω-3 polyunsaturated fatty acids daily, for 25 days in eight volunteers. The ω-3 polyunsaturated fatty acids were incorporated in platelet and erythrocyte membrane phospholipids at the expense of ω-6 polyunsaturated fatty acids. Bleeding time increased (p < 0.01) and platelet count (p < 0.05), platelet aggregation upon ADP and collagen (p < 0.01–0.05), and associated thromboxane B2 formation (p < 0.01) decreased. Blood pressure (p < 0.05) and blood pressure response to norepinephrine (p < 0.01) and angiotensin II (NS) fell, without major changes in plasma catecholamines, renin, urinary aldosterone, kallikrein, prostaglandins E2 and F2α, and red cell cation fluxes. Biochemical and functional changes were reversed 4 weeks after cod liver oil was discontinued. Formation of prostaglandins derived from eicosapentaenoic acid and interference of eicosapentaenoic acid with formation and action of prostaglandins derived from arachidonic acid were evident in vitro. Whatever the mechanism, this moderate supplement of ω-3 polyunsaturated fatty acids markedly changed membrane phospholipids, which was associated with a shift toward less reactive platelets and a blunted circulatory response to pressure hormones.

SEVERAL independent lines of evidence suggest a protective potential of dietary eicosapentaenoic acid (all cis C20:5ω3) against cardiovascular disease. Greenland Eskimos, and to a lesser extent, some Japanese, have a high dietary intake of long-chain ω-3 polyunsaturated fatty acids from seafood and a low incidence of cardiovascular disease, even compared with their Westernized ethnic counterpart. A low prevalence of hypertension in Eskimos, a favorable pattern of serum lipids, and especially a hemorrhagic “defect” evidenced by a bleeding tendency and reduced platelet aggregability, have been invoked as underlying protective mechanisms. Furthermore, ω-3 polyunsaturated fatty acid-enriched diets have been shown to reduce the size and sequelae of cerebral and myocardial infarction in experimental animals.

On a normal Western diet, in which ω-6 polyunsaturated fatty acids predominate, the prostanooids of the two series, derived from membrane-bound arachidonic acid (all cis C20:4ω6) prevail (fig. 1). The balance

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between proaggregatory and vasoconstrictor thromboxane A2 (TXA2) and antiaggregatory and vasodilator prostacyclin (PGI2) has been proposed as one factor that determines platelet reactivity, endothelial thromboresistance, vascular tone and, perhaps, in the long run, the natural history of atherothrombotic vessel disease. One of the ω-3 polyunsaturated fatty acids, eicosapentaenoic acid, the precursor fatty acid of the 3-series prostanoids (fig. 1), interferes at several sites with this balance. Its action could cause a shift toward less reactive platelets and lowered pressure reactivity of the vascular system.

In a previous study, we demonstrated that it is possible to induce in Western Europeans an “Eskimotype” pattern of platelet membrane fatty acids as well as decreased platelet aggregability and thromboxane B2 (TXB2) formation by substituting mackerel as the sole source of their dietary fat. In the present study, a small supplement of cod liver oil added to an otherwise unchanged Western diet was given as a source of long-chain ω-3 polyunsaturated fatty acids, and plasma lipids and membrane fatty acids were measured. Platelet function, red cell cation fluxes, prostanoid formation, blood pressure, urinary and plasma levels and sensitivity to vasoactive hormones were assessed to determine if these factors could be favorably influenced by changes in dietary fatty acids.

Methods

Subjects and Protocol

Eight healthy male volunteers, 22–42 years old, were studied. Control data were randomly accumulated either before or 4 weeks after the end of the experimental period. Intervention data were assessed after 25 days of supplementation with cod liver oil (Møller a/s), 20 ml twice daily, to an otherwise unaltered Western diet. This fish oil contains about 11% of eicosapentaenoic acid (C20:5ω3) and 16% of docosahexaenoic acid (C22:6ω3), 100 IU/ml of vitamin D3, 1 ng/ml vitamin E and 8 kcal/ml, and provides a daily intake of 4 g of C20:5ω3 and 6 g C22:6ω3, with only minor changes in the ratio of dietary ω-3 and ω-6 polyunsaturated to saturated fatty acids.

At 8 a.m., after an overnight fast, blood for laboratory studies was drawn from an antecubital vein, blood pressure was recorded sphygmomanometrically, urine was voided to terminate a 24-hour collection period and an i.v. cannula was placed for infusion studies. After 1 hour of supine rest, basal blood pressure, the blood pressure response to norepinephrine infusion (5 μg/min for 15 minutes), and, after another 1-hour supine recovery period, to angiotensin II infusion (1 μg/min for 15 minutes) were recorded.

Laboratory Analyses

Complete blood counts, plasma and urinary electrolytes, plasma cholesterol, triglycerides and lipoprotein fractions, uric acid and liver and kidney function tests were determined by routine laboratory methods. Subaqual bleeding time was assessed by a standard “hemostile” earlap prick.

Fatty acid spectra of plasma, washed red cell ghost and platelet membrane phospholipids were quantitatively determined as described previously. Collagen-, ADP- and arachidonic acid–stimulated platelet aggregation was tested in vitro by the method of Born and in platelet-rich plasma as described in detail. Aggregation-associated TXB2 formation and TXB2 in plasma from the first 2 ml of blood drawn through a 19-gauge needle or a 10-inch plastic catheter on EDTA and meclofenamic acid (5 mM and 10 μg/ml, final concentrations) were measured after acidic organic solvent extraction by radioimmunoassay using a specific antibody (provided by L. Levine, Brandeis University) and 3H-TXB2 (100 Ci/mM) (NEN), as described previously. Plasma renin activity, plasma catecholamines, urinary aldosterone, urinary kallikrein using a chromogenic substrate (S-2266, Kabi) and urinary prostaglandins (PG (E)1 and F(α)24) were determined by established techniques. Sodium-potassium cotransport and sodium-lithium countertransport across red cell membranes were measured as described elsewhere.

The 14C-arachidonic/14C-eicosapentaenoic acid (56/50 mCi/mM) (NEN) conversion products on incubation with washed human platelets, human umbilical arteries and rabbit kidney cortex microsomes were separated on thin-layer and reversed-phase, high-performance liquid chromatography, as described elsewhere. According to, 12C-eicosapentaenoic acid incubates of washed human platelets and rabbit renal cortex microsomes were screened for TXB2 and Δ17-6-keto-PGF1α, the stable hydrolysis product of PGI2, by

![Figure 1: Nutritional intake and metabolism (broken arrows) of polyunsaturated fatty acids. The Western diet predominantly supplies ω-6 family polyunsaturated fatty acids, in the first-line linoleic acid. Linoleic acid can be metabolized to arachidonic acid, the precursor fatty acid of the 2-series of prostaglandins. In certain seafoods, long-chain ω-3 polyunsaturated fatty acids prevail, especially eicosapentaenoic acid, the direct precursor fatty acid of the 3-series of prostaglandins. The metabolism of α-linolenic acid contained in certain vegetable oils to eicosapentaenoic acid is controversial. In man, ω-6 and ω-3 fatty acids cannot be interconverted.](image-url)
gas chromatography–mass spectrometry (Varian MAT 44S system; 20-m SE-30 glass capillary column) after extraction, purification and derivatization.14, 26 The derivatives used were the methoxime-methylester-trimethylsilyl ether.26, 28

Data are reported as mean ± sd in the tables and mean ± sem in the figures. The t test for paired samples was used where appropriate.

Results

This 25-day supplementation of cod liver oil to normal Western diet resulted in a marked change in the fatty acid composition of plasma, red cell membrane and platelet microsomal phospholipids. The ω-3 polyunsaturated fatty acids (C20:5ω-3, C22:6ω-3) significantly increased (p < 0.01). In contrast, the ω-6 polyunsaturated fatty acids (C18:2ω-6, C20:4ω-6) decreased (p < 0.05–0.01) — except C20:4ω-6 in red cell membranes — with little change in saturated and monounsaturated fatty acids (fig. 2). This pattern was found in all subjects and, as seen in the control measurements made 4 weeks after cessation of the cod liver oil supplementation, reversed rapidly, with, at most, a small residual C22:6ω-3 increase and a C20:4ω-6 decrease (fig. 3).

Despite an increase in total fat intake of about 300 kcal/day, plasma cholesterol, triglycerides and high- and low-density lipoprotein (HDL and LDL) subfractions were unaltered. There was also no change in hemoglobin, hematocrit, calcium, sodium and potassium in serum (table 1), white cell count, uric acid and screening tests for kidney and liver function.

Bleeding time increased significantly (p < 0.01), platelet count fell (p < 0.05) and TxB2 in plasma obtained by fresh venipuncture tended to fall during the cod liver oil supplement period. Plasma TxB2 levels in blood drawn through a freshly placed plastic catheter were much higher under control conditions, but were found significantly (p < 0.05) reduced with cod liver oil supplementation (table 2). Accordingly, a significant reduction of ex vivo platelet aggregation on ADP (p < 0.05) and the lower doses of collagen (p < 0.05–0.01), as well as a decreased TxB2 formation (p < 0.01) on all concentrations of collagen were found. Aggregation and TxB2 formation in platelet-rich plasma on exogenous arachidonic acid remained virtually unchanged (fig. 4).

Radiochromatographic analysis by thin-layer chromatography and high-performance liquid chromatography of products obtained by parallel incubation of 14C-arachidonic and 14C-eicosapentaenoic acid showed comparable conversion of both fatty acids by washed human platelets to compounds co-chromatographing with authentic TxB2 in two systems. Direct proof of
TABLE 1. Serum Lipids, Electrolytes, Hemoglobin, Hematocrit, and Red Cell Count

<table>
<thead>
<tr>
<th></th>
<th>Cholesterol</th>
<th>Potassium</th>
<th>Calcium</th>
<th>Hemoglobin</th>
<th>Hematocrit</th>
<th>Red cell count</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>(mg/dl)</td>
<td>(mg/dl)</td>
<td>(mmol/l)</td>
<td>(mmol/l)</td>
<td>(%)</td>
<td>(10^12/l)</td>
</tr>
<tr>
<td>Control diet</td>
<td>85±45</td>
<td>209±38</td>
<td>54±9</td>
<td>143±1.8</td>
<td>4.5±0.3</td>
<td>4.8±0.2</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td></td>
<td>14.9±0.7</td>
<td>42.8±2.9</td>
<td>4.7±0.2</td>
</tr>
<tr>
<td>Cod liver oil</td>
<td>68±15</td>
<td>201±37</td>
<td>52±6</td>
<td>143±2.7</td>
<td>4.35±0.3</td>
<td>4.8±0.2</td>
</tr>
<tr>
<td>supplementation</td>
<td></td>
<td></td>
<td></td>
<td>15.3±1.2</td>
<td>43.8±3.7</td>
<td>4.8±0.3</td>
</tr>
</tbody>
</table>

Values are mean ± sd; n = 8.
Abbreviation: HDL = high-density lipoprotein.

TxB₃ formation from exogenous ¹²C-eicosapentaenoic acid was provided by the detection of characteristic fragments (m/e 612, 299) by gas chromatography-mass spectrometry (fig. 5). In contrast to arachidonic acid, however, eicosapentaenoic acid in the same concentration did not induce platelet aggregation in platelet-rich plasma or platelet suspension. Conversion of ¹⁴C-eicosapentaenoic acid by human umbilical arteries to a compound co-chromatographing with authentic 6-keto-PGF₁α and by rabbit kidney cortex microsomes to compounds comigrating with 6-keto-PGF₁α, PGF₂α, and PGE₃ was somewhat less effective than conversion of ¹⁴C-arachidonic acid. Further direct proof of PG₁₂ formation from exogenous eicosapentaenoic acid was provided by detection of a fragment (m/e 612) characteristic for Δ17-6-keto-PGF₁α by gas chromatography-mass spectrometry.

Upright blood pressure and blood pressure response to a 15-minute norepinephrine infusion (5 μg/min) were significantly reduced (p < 0.05–0.01) during cod liver oil supplementation, and blood pressure after 1 hour of supine rest and the pressor response to a 15-minute angiotensin II infusion (1 μg/min) tended to decrease (NS) (fig. 6). Plasma norepinephrine and epinephrine levels remained unchanged and plasma renin activity and the 24-hour excretion of aldosterone, kalilrein, prostaglandin E₂ and F₂α, tended to fall during cod liver oil supplementation (table 3). Sodium-potassium cotransport and sodium-lithium countertransport across red cell membranes were also unaltered.

At the end of the supplementation period, we observed, quite unexpectedly, a slight but significant increase in 24-hour sodium excretion (p < 0.01) and a slightly reduced potassium excretion (NS). Furthermore, sodium-potassium cotransport (r = −0.63; p < 0.01) and sodium-lithium countertransport (r = −0.71; p < 0.01) correlated inversely with arachidonic acid content in red cell membrane phospholipids, both before and during cod liver oil supplementation.

**Discussion**

The low cardiovascular morbidity in Greenland Eskimos has been linked to their unique diet of seafood rich in ω-3 polyunsaturated fatty acids, hypothetically because these compounds may alter the participation of platelet aggregation and platelet-vessel wall interaction in the natural history of atherothrombosis in Greenland Eskimos.

**TABLE 2. Bleeding Time, Platelet Count and Plasma Levels of Immunoreactive Thromboxane B₂ from Blood Samples by Fresh Venipuncture (19-gauge Needle or 10-inch Catheter)**

<table>
<thead>
<tr>
<th></th>
<th>Bleeding time (sec)</th>
<th>Platelet count (× 10^12/μl)</th>
<th>Plasma i.r. TxB₂ (19-g needle) (pg/ml)</th>
<th>Plasma i.r. TxB₂ (10-inch catheter) (pg/ml)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Control diet</td>
<td>104±34</td>
<td>217±27</td>
<td>113±47</td>
<td>323±210</td>
</tr>
<tr>
<td>Cod liver oil</td>
<td>145±52†</td>
<td>193±28*</td>
<td>89±39</td>
<td>159±40*</td>
</tr>
</tbody>
</table>

Mean ± sd; n = 8.
*<p < 0.05 vs control diet.
†<p < 0.01 vs control diet.
Figure 4. Platelet aggregation in platelet-rich plasma (PRP) was stimulated with increasing concentrations of ADP and collagen (Coll) and with arachidonic acid (C20:4). Aggregation response measured as light transmission (left ordinate) is given as circles and associated thromboxane B2 formation by vertical bars (right ordinate). The range of reversible (1st wave) aggregation on ADP is given by the small parts of the horizontal bars, and the range of irreversible (2nd wave) aggregation on ADP by the dotted parts of the horizontal bars. Open symbols represent control data and closed symbols represent data after 25 days of cod liver oil supplement. Data are mean ± SEM; n = 8. *p < 0.05; **p < 0.01.

Figure 5. In vitro, several test systems were incubated in parallel either with arachidonic acid (AA) (left side) or with eicosapentaenoic acid (EPA) (right side). (first panel) Washed human test platelet suspensions (PS) were incubated with 14C-AA or 14C-EPA and the conversion products identified by radiot chromatography on thin-layer chromatography (TLC scan). The start is indicated by arrows. Spots of authentic standard prostanoids are indicated by numbers: 1 = 6-keto-PGF1α; 2 = PGF2α; 3 = TXB2; 4 = PGE2; 5 = PGD2; and 6 = AA. Comparable conversion of AA and EPA to TXB2 and TXB3 and hydroxy fatty acids (main peak between standards 5 and 6 is evident). (second panel) Platelet suspensions (PS, a) and platelet-rich plasma (PRP, b) were stimulated with unlabeled fatty acids (14C-FAs). A response appears upon arachidonic acid challenge only, but not with eicosapentaenoic acid. Platelet suspension incubates were then evaluated for formation of thromboxanes by gas chromatography–mass spectrometry (GC-MS SCAN). Characteristic ion pairs of TXB2 (m/z 612, 299) were detected in eicosapentaenoic incubates of platelets (in accordance with conversion of labeled EPA; see first panel). Characteristic ion pairs of TXB2 (m/z 614, 301) were detected in arachidonic incubates and, to a lesser extent, in eicosapentenoic acid incubates of platelets revealing concomitant conversion of endogenous platelet membrane arachidonic acid. (third panel) Rings of human umbilical artery comparably converted 14C-AA and 14C-EPA to a major product co-chromatographing with 6-keto-PGF1α. For identification, see legend to first panel. (fourth panel) Rabbit renal cortex microsomes converted 14C-AA and 14C-EPA to a comparable spectrum of 2-series and 3-series prostanoids. For identification see legend to first panel. In identical incubates with unlabeled AA and EPA peaks (1) were further evaluated by GC-MS. A characteristic fragment of Δ17-6-keto-PGF1α (m/z 612) was detected in EPA incubates (in accordance with conversion of labeled EPA). A characteristic fragment of 6-keto-PGF1α (m/z 614) and common fragments (m/z 558, 468) in AA- and to a lesser extent in EPA-incubates reveal concomitant conversion of endogenous membrane arachidonic acid.
equilibrate continuously with their current lipid environment. The consistency and reversibility of these changes is demonstrated by their uniform shift in all subjects (fig. 3).

Concomitantly with the changes in platelet membrane fatty acids, a prolonged bleeding time and reduced TxB₂ formation and platelet aggregation on ADP and collagen (two stimuli of possible significance for in vivo clot formation) were observed. These findings parallel epidemiologic observations in Eskimos and findings after a seafood diet or aspirin administration. After aspirin, which irreversibly blocks cyclooxygenase by acetylation at the active site, an increased platelet count has been found. In contrast, in our study, a reduced platelet, but not red cell, count during cod liver oil supplementation was observed that fits well with previous, as of yet unexplained, experimental and epidemiologic findings.

The inhibition of thromboxane formation and platelet aggregation during the cod liver oil supplementation was most marked at the lower concentrations of ADP and collagen. Platelet aggregation induced by low concentrations of ADP and collagen is also most sensitive to aspirin and probably dependent on thromboxane formation. However, in contrast to aspirin pretreatment, thromboxane formation and thromboxane-dependent aggregation were unaltered in this study when tested with exogenous arachidonic acid. These findings suggest a reduced supply of endogenous prostaglandin precursor fatty acids or impaired handling of these fatty acids when eicosapentaenoic acid-loaded and arachidonic acid-depleted platelets are challenged with ADP or low-dose collagen, which acts by precursor fatty acid release and thromboxane formation.

In vitro, both ¹⁴C-arachidonic acid and ¹⁴C-eicosapentaenoic acid were converted by human platelets to TxB₁ or TxB₂, respectively. However, eicosapentaenoic acid did not induce aggregation in platelet suspensions or platelet-rich plasma (fig. 5). These data fit well with previous observations showing that TxA₄ is not proaggregatory, and that eicosapentaenoic acid-derived cyclooxygenase products elevate cAMP levels in platelets. A reduced formation of TxB₂ by compe-

**TABLE 3.** Plasma Levels of Hormones, 24-hour Urinary Volume and Excretion of Hormones, Electrolytes and Prostaglandins

<table>
<thead>
<tr>
<th></th>
<th>Norepinephrine (pg/ml)</th>
<th>Epi-epinephrine (pg/ml)</th>
<th>PRA (ng ATU/ml-hour)</th>
<th>Na⁺/K⁺-transport (µmol/ml cells-hour)</th>
<th>Na⁺/Li-exchange (µmol/ml cells-hour)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Control diet</td>
<td>267 ± 77</td>
<td>53 ± 20</td>
<td>2.1 ± 0.8</td>
<td>0.460 ± 0.127</td>
<td>0.058 ± 0.021</td>
</tr>
<tr>
<td>Cod liver oil</td>
<td>237 ± 83</td>
<td>56 ± 39</td>
<td>1.6 ± 0.6</td>
<td>0.424 ± 0.133</td>
<td>0.057 ± 0.022</td>
</tr>
<tr>
<td>supplementation</td>
<td></td>
<td></td>
<td></td>
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<td></td>
</tr>
<tr>
<td>UV (ml/24 hours)</td>
<td>1625 ± 588</td>
<td>159 ± 54</td>
<td>93 ± 42</td>
<td>11.75 ± 3.5</td>
<td>67.2 ± 34</td>
</tr>
<tr>
<td>Sodium (mmol/24 hours)</td>
<td>188 ± 58*</td>
<td>80 ± 19</td>
<td>9.2 ± 5.2</td>
<td>56.4 ± 39</td>
<td>115 ± 58</td>
</tr>
<tr>
<td>Potassium (mmol/24 hours)</td>
<td>1802 ± 646</td>
<td>188 ± 58</td>
<td>80 ± 19</td>
<td>9.2 ± 5.2</td>
<td>56.4 ± 39</td>
</tr>
<tr>
<td>Aldosterone (µg/24 hours)</td>
<td>189 ± 87</td>
<td>1003 ± 348</td>
<td>1003 ± 349</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Kallikrein (IU/24 hours)</td>
<td>189 ± 87</td>
<td>1003 ± 349</td>
<td>1003 ± 349</td>
<td></td>
<td></td>
</tr>
<tr>
<td>PGE₂ (ng/24 hours)</td>
<td>189 ± 87</td>
<td>1003 ± 349</td>
<td>1003 ± 349</td>
<td></td>
<td></td>
</tr>
<tr>
<td>PGF₂α (ng/24 hours)</td>
<td>189 ± 87</td>
<td>1003 ± 349</td>
<td>1003 ± 349</td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

Values are mean ± SD; n = 8.
*p < 0.01 vs control diet.
Abbreviations: UV = urinary volume; PRA = plasma renin activity.
tition of eicosapentaenoic acid and arachidonic acid at the cyclooxygenase step and blockade of the postulated TXA2 receptor by eicosapentaenoic acid or derived products may also explain reduced platelet aggregability on low concentrations of ADP and collagen, and an increased threshold dose for irreversible aggregation on ADP. In eicosapentaenoic acid-enriched platelets. In carefully sampled plasma, TxB2 levels were not significantly reduced during cod liver oil supplementation. In catheter samples in which ex vivo activation of platelets could not be excluded as effectively, a significant reduction of TxB2 levels from a high control level was measured during the period of cod liver oil supplementation. Cyclooxygenation of eicosapentaenoic acid in human umbilical arteries and rabbit renal cortex microsomes to the corresponding prostanooids was somewhat less effective than that of arachidonic acid in these preparations, and was also lower than the conversion to prostanooids in platelets (fig. 5). However, the detection of Δ17-6-keto-prostaglandin F1α by gas chromatography–mass spectrometry shows the capability of vascular tissue to convert eicosapentaenoic acid to PGI2, which may be as potent an inhibitor of platelet aggregation as PGI2.

Thus, a consistent pattern of altered cyclooxygenase-derived compounds due to changes in precursor fatty acid stores, as supported by our in vitro data, could probably explain the effects on platelet function during cod liver oil supplementation. Likewise, interference of eicosapentaenoic acid with the lipooxygenase pathways of arachidonic acid to hydroperoxy fatty acids (which inhibit PGI2 formation), and to leukotrienes (which may be important for detrimental edema formation in critically ischemic tissue) have been found at low conversion of eicosapentaenoic acid itself to be expected under a possibly low in vivo hydroperoxide tone.

Systolic blood pressure tended to decrease during cod liver oil supplementation. This decrease was significant in the upright position and during norepinephrine infusion (fig. 6). At the same time, the activity of several classic humoral and neuronal systems that contribute to blood pressure regulation was largely unaltered (table 3). Therefore, we can only speculate about the underlying mechanism. The decrease in blood pressure might be a result of alterations in the cell membrane lipids at receptor sites of vasoactive hormones or neural transmitters, thereby changing, for example, membrane fluidity and receptor-effector coupling. In addition, direct effects of prostaglandins of the three series such as PGI2 and PGD3 on vessel walls and function could well be operative.

Since membrane polyunsaturated fatty acids have been found to influence certain transmembrane ion transport systems and since transmembrane cation fluxes in erythrocytes may be altered in essential hypertension, sodium-potassium cotransport and sodium-lithium countertransport were studied. Despite incorporation of ω-3 polyunsaturated fatty acids into erythrocyte membranes, no changes in ion fluxes (and arachidonic acid content) were seen. The unexpected increase of 24-hour sodium excretion after 25 days of cod liver oil supplementation may be due either to an unrecognized increase of sodium ingestion or to increased sodium loss, or both. The pattern of decreased plasma renin activity and urinary aldosterone would be consistent with increased sodium intake, but also with reduced renal prostaglandin formation. Indeed, eicosapentaenoic acid, in contrast to arachidonic acid, does not stimulate renin secretion and may even inhibit renal conversion of arachidonic acid to prostaglandins as supported by reduced urinary PGE2 and PGF2α excretion on cod liver oil. A check of sodium, potassium and calcium excretion performed on day 10 of the cod liver oil supplement did not show any change from control. Therefore, a slow onset of either mechanism must be assumed. In any case, reduced blood pressure due to inadvertent volume contraction by sodium loss during cod liver oil supplementation seems unlikely because hemoglobin, hematocrit and heart rate remained constant.

Cod liver oil supplementation in the small dose we studied produced no increase in body weight or clinical side effects. The only change in an extensive routine laboratory check was an anticipated increase in the plasma vitamin D3 level. Calcium homeostasis was not influenced.

In conclusion, dietary intervention by supplementation with ω-3 polyunsaturated fatty acids in a manner compatible with established Western dietary habits rapidly induced biochemical and functional changes in platelets and, to a less extent, in blood pressure control mechanisms. The findings paralleled observations in native Eskimos, who have a unique nutrition and low morbidity from atherothrombotic disease. The specific actions of eicosapentaenoic acid and its products may offer a new rationale for approaching the prevention of atherothrombotic vascular disease that merits comparison with other approaches, such as antiplatelet drugs and cholesterol and ω-6 polyunsaturated fatty acid-centered intervention.

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