Dietary Eicosapentaenoic Acid and Docosahexaenoic Acid From Fish Oil
Their Incorporation Into Advanced Human Atherosclerotic Plaques

Joseph H. Rapp, William E. Connor, Don S. Lin, and John M. Porter

The incorporation of fatty acids from dietary fish oil was measured in obstructive atherosclerotic plaques removed from 11 patients fed fish oil, rich in ω-3 fatty acids, for 6–120 days before a planned arterial endarterectomy. The fatty acids of plasma and atheroma were analyzed with special reference to docosahexaenoic acid (DHA, 22:6) and eicosapentaenoic acid (EPA, 20:5), the principal ω-3 fatty acids of fish oil. The ω-3 fatty acid content increased greatly in plasma from 0.9% of fatty acids to 14.8% in cholesteryl esters, from 3.8% to 22.1% in phospholipids, and from 1.3% to 21.9% in triglycerides. The ω-3 fatty acid content of the atherosclerotic plaques was also greater when compared with that of plaques removed from 18 non-fish oil-fed controls. The ω-3 fatty acid in cholesteryl esters of the plaques was 4.9% in the experimental group versus 1.4% in control plaque, in phospholipids it was 8.8% versus 1.8%, and in triglycerides it was 4.7% versus 0.7% (p<0.001 for each lipid class). The two major ω-3 fatty acids (DHA and EPA) behaved differently. Compared with their respective plasma levels, relatively more DHA than EPA was deposited into the plaques. Whereas the increase of ω-3 fatty acids in plasma reached a plateau 3 weeks after initiation of fish oil feeding, a linear increase in plaque ω-3 fatty acids continued with time. As a result of the changes in fatty acid composition, the lipid classes of both plasma and plaque had a higher unsaturation index in the fish oil-fed group. Our data demonstrate that dietary ω-3 fatty acids are readily incorporated into advanced human atherosclerotic plaques. Changes in the fatty acids of plasma lipids are reflected in substantial changes in the plaque fatty acid content over a relatively short time. Because ω-3 fatty acids affect so many factors predisposing to both thrombosis and atherosclerosis, these data may have clinical implications. (Arteriosclerosis and Thrombosis 1991;11:903–911)

Several lines of evidence suggest that dietary ω-3 fatty acids may ameliorate the atherosclerotic process. Populations that consume more ω-3 fatty acids from fish and sea mammals have less coronary heart disease,1,2 and coronary artery disease patients who eat fish appear to have both a lower subsequent coronary artery disease rate and a lower total mortality.3 Fish oil–feeding experiments in humans have demonstrated potential antiatherogenic effects, that is, a lowering of plasma lipid and lipoprotein levels and decreased platelet aggregation.4,5 Other factors believed to be involved in the pathogenesis of atherosclerosis also appear to be affected by ω-3 fatty acids. These include the inhibition of intimal hyperplasia in canine autologous vein grafts,6,7 decreased endothelial cell production of a platelet-derived growth factor–like protein,8 increased activity of endothelium-derived relaxing factor,9 and a potential reduction in the intensity of the inflammatory response.10,11 Furthermore, fish oil has prevented the development of experimental atherosclerosis in pigs and rhesus monkeys.12,13 In the pig study, the intima of the coronary arteries was damaged by a balloon catheter at the same time that the animals were fed cholesterol and fat. Considerably less atherosclerosis was found in the pigs fed cod liver oil despite little lowering of plasma lipid levels. This result suggested...
that fish oil had an effect on atherosclerosis unrelated to plasma lipid concentrations. Ingestion of fish oil led to less aortic atherosclerosis in monkeys but with a reduction in total and low density lipoprotein (LDL) cholesterol. In humans a reduced restenosis rate after coronary artery balloon angioplasty has been reported, although other studies have not shown such a positive result.

These diverse effects of fish oils have focused attention on the potential of ω-3 fatty acids to alter the biochemistry and pathology of the atherosclerotic process. We reasoned that ω-3 fatty acids must bring about at least some of these beneficial effects by virtue of their incorporation into lipid membranes and other constituents of the atherosclerotic lesion itself. The next question was whether actual experiments would prove this to be the case.

Eicosapentaenoic acid (EPA) and docosahexaenoic acid (DHA) are ordinarily present in only very small quantities in both the plasma and the atherosclerotic plaque of individuals consuming a diet low in marine products. This is the usual American diet. Previously we had found that when fish oil was incorporated into the diet, the plasma concentrations of EPA and DHA increased severalfold within a few weeks. Therefore, in the present study the substantial changes in plasma lipid fatty acid content induced by diet could, over time, alter the fatty acid composition of the lipid classes of advanced human atherosclerotic plaques.

**Methods**

**Subjects**

Eleven patients (nine men and two women) with known obstructive atherosclerosis consumed fish oil for 1–16 weeks (6–120 days) before scheduled peripheral vascular surgery (Table 1). Two patients had multiple operations. Their mean±SD fasting plasma lipid levels were as follows: total cholesterol, 216.0±42.4 mg/dl; high density lipoprotein (HDL) cholesterol, 39.0±6.4 mg/dl; and LDL cholesterol, 152.9±36.1 mg/dl (LDL to HDL ratio=4.0). Mean triglyceride value was 140.1 mg/dl. Seven subjects were hyperlipidemic, with a mixture of phenotypes. There was a history of moderate to heavy tobacco use in all but one subject.

Control values of the ω-3 fatty acid composition of the lipid classes in human atherosclerotic plaques were established by analyzing 18 endarterectomy specimens, 11 from the carotid artery and seven from the femoral artery, from individuals undergoing vascular reconstructions who did not habitually consume marine foods. These are provided in Table 3 and Figure 4.

**Study Design**

Diet histories, complete medical examinations, and plasma lipid and lipoprotein determinations were performed for all subjects on admission to the study. Individuals with a habitual consumption of marine foods were excluded. During the experimental study fish oil (Maxepa oil, Marfleet Refining Company, Hull, England, composition listed in Tables 2 and 4) was substituted for other fats in the diet as much as possible, thus allowing a minimal change in total fat consumption but a considerable increase in ω-3 fatty acid consumption. Subjects were instructed to make no other changes from their customary diets during the study period. The amount of fish oil was calculated so that the ω-3 fatty acid content equaled 6% of total calories. Depending on height, weight, age, and physical activity, fish oil consumption ranged from 48 to 64 g/day (16.0–21.3 g/day of ω-3 fatty acids). Over the entire study period the total ω-3 fatty acid consumption varied from 115...
to 2,256 g, depending on the length of the fish oil-feeding period before surgery. Weekly-interval diet histories were taken. Body weight was checked biweekly to ensure that there was no weight gain during the study period.

All subjects gave informed consent according to the protocols approved by the Human Research Committee of the Oregon Health Sciences University.

**Laboratory Methods**

Blood was drawn twice a week. Plasma lipid and lipoprotein levels were determined by the methods described in the Lipid Research Clinics manual. Fatty acid content of the plasma lipid classes was analyzed by the same methods used for atheroma, which are described subsequently. Platelet counts were determined biweekly, and Ivy bleeding times were measured weekly.

Fifteen endarterectomy specimens were obtained at surgery from 11 patients, and only those portions of the arterial wall grossly involved with the atherosclerotic lesion were analyzed (Table 1). The dissection plane included the media when it was obviously diseased. Specimens were immediately washed thoroughly with cold saline to remove blood and thrombi, blotted dry, minced, weighed, freeze dried, and reweighed. The specimens were finely ground with a mortar and pestle and then extracted five times with CHCl₃/CH₃OH (2:1). Plasma lipids were extracted by the CHCl₃/CH₃OH procedure of Folch et al.

Portions of the extracts were plated on silica gel G thin-layer chromatography plates after carbon-14-labeled cholesterol and cholesteryl stearate (4-[¹⁴C]cholesterol and cholesteryl-4-[¹⁴C]stearate) were added as internal standards (recovery of 90–95%). The plates were developed in n-hexane/CHCl₃/diethyl ether/acetic acid (80:10:10:1). After the plates were sprayed with rhodamine G, the lipid bands were visualized with ultraviolet light and removed separately.

The free cholesterol and cholesteryl ester bands were removed from the gel by adding 4 ml diethyl ether at room temperature, vortexing for 30 seconds, and then separating the phases by centrifugation. This procedure was repeated five times. The cholesteryl ester was then saponified with 0.5 M KOH in 50% ethanol (50% H₂O), and the cholesterol was extracted with n-hexane. The fatty acids of cholesteryl ester were recovered by acidifying the aqueous phase and reextraction with n-hexane. Cholesterol content was determined by gas chromatography (model 7610A, Hewlett-Packard, Avondale, Pa.) on a 3.8% SE-30 glass column. Cholestane and stigmasterol were used as internal standards according to the method of Miettinen and colleagues. The fatty acids of phospholipids, triglycerides, and cholesteryl esters were methylated with BF₃ in CH₃OH, and relative concentrations were determined by gas-liquid chromatography (model Sigma 3B, Perkin-Elmer, Norwalk, Conn.) by use of a glass capillary column.

![Figure 1](http://atvb.ahajournals.org/)

**Figure 1.** Bar graph showing incorporation (percent of total fatty acids) of eicosapentaenoic acid (EPA, □, left panel), docosahexaenoic acid (DHA, ⊿, middle panel), and total ω-3 fatty acids (other, ▪, right panel) into cholesteryl ester, phospholipid, and triglyceride fractions, respectively, of plasma after fish oil feeding. Each value represents mean of 11 subjects’ plasma ω-3 fatty acids before fish oil feeding and on the final day of feeding (6–120 days). p values before (pre) versus after (post) feeding are as follows: p<0.001 for total ω-3 fatty acids in all lipid classes; p<0.001 for EPA in all lipid classes; p<0.001 for DHA in phospholipids; and p<0.005 for DHA in cholesteryl esters and triglycerides.
TABLE 2. Influence of Dietary ω-3 Fatty Acids From Fish Oil on Fatty Acid Composition of the Major Lipid Classes in Human Plasma

<table>
<thead>
<tr>
<th>Fatty acids</th>
<th>Dietary fish oil (%)</th>
<th>Cholesteryl ester (%)</th>
<th>Phospholipid (%)</th>
<th>Triglycerides (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Before feeding</td>
<td>After feeding</td>
<td>Before feeding</td>
<td>After feeding</td>
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<tr>
<td>Saturates</td>
<td>28.4</td>
<td>14.0±2.6</td>
<td>44.7±4.3</td>
<td>37.5±8.8</td>
</tr>
<tr>
<td></td>
<td></td>
<td>16.1±2.8</td>
<td>37.5±8.8</td>
<td>28.3±5.4</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td></td>
<td>25.2±7.9</td>
</tr>
<tr>
<td>Monounsaturates</td>
<td>21.1</td>
<td>19.8±2.3</td>
<td>12.8±1.6</td>
<td>46.1±5.5</td>
</tr>
<tr>
<td></td>
<td></td>
<td>21.0±3.5</td>
<td>12.3±3.4</td>
<td>32.3±6.0*</td>
</tr>
<tr>
<td>Total polyunsaturates</td>
<td>39.9</td>
<td>65.0</td>
<td>39.6</td>
<td>23.7</td>
</tr>
<tr>
<td>Total ω-3</td>
<td>35.1</td>
<td>0.9±0.5</td>
<td>3.8±1.2</td>
<td>1.3±1.7</td>
</tr>
<tr>
<td>18:3 ω-3</td>
<td>0.5</td>
<td>0.4±0.1</td>
<td>0.1±0.1</td>
<td>0.7±0.4</td>
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<tr>
<td>20:5 ω-3</td>
<td>17.1</td>
<td>12.0±2.8*</td>
<td>10.2±4.9*</td>
<td>0.1±0.1</td>
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<tr>
<td>22:5 ω-3</td>
<td>4.5</td>
<td>0.2±0.4</td>
<td>0.7±0.2</td>
<td>0.2±0.1</td>
</tr>
<tr>
<td>22:6 ω-3</td>
<td>10.7</td>
<td>0.2±0.3</td>
<td>2.0±1.7</td>
<td>0.2±0.2</td>
</tr>
<tr>
<td>Total ω-6</td>
<td>4.4</td>
<td>64.1±4.1</td>
<td>35.8±4.1</td>
<td>22.4±4.2</td>
</tr>
<tr>
<td>18:2 ω-6</td>
<td>1.2</td>
<td>56.5±3.7</td>
<td>22.4±4.0</td>
<td>20.6±3.9</td>
</tr>
<tr>
<td>20:4 ω-6</td>
<td>0.8</td>
<td>5.5±2.1</td>
<td>9.9±2.1</td>
<td>1.2±0.4</td>
</tr>
<tr>
<td>Unsaturation index</td>
<td>200.4</td>
<td>158.9</td>
<td>119.1</td>
<td>96.9</td>
</tr>
<tr>
<td></td>
<td></td>
<td>199.0</td>
<td>184.0</td>
<td>175.6</td>
</tr>
</tbody>
</table>

Values are percent of total fatty acids. mean±SD.

p values: Experimental group versus baseline, *p<0.001, †p<0.005, ‡p<0.01, §p<0.025. Plasma values shown are mean concentrations of ω-3 fatty acids in plasma taken the day before surgery from 11 patients.

(Supelco SP 2330, 30 m, Bellefonte, Pa.).16 Fatty acid standards were run daily.

The unsaturation index of each lipid class of both plasma and plaque was calculated by multiplying the number of double bonds by the percentage composition of fatty acids in the class and then summing the value.51

Statistical Methods

Comparisons of ω-3 fatty acid incorporation were done with Student’s t or paired t tests as appropriate. Bonferroni corrections were added for multiple comparisons. An “uptake function,” B[1-exp(Bt)], was fitted to the sets of data for the incorporation of EPA and DHA into the cholesteryl ester fractions of plasma by nonlinear least-squares method by use of a modified Gauss-Newton procedure. Tests of linearity of the data for the incorporation of EPA and DHA into plaque also were done by a least-squares method, and linearity was proven as the best expression of the data trend. The intercept, proportionality coefficients (slope), and standard errors are listed with each figure.

Results

Plasma ω-3 Fatty Acid Content

After initiation of fish oil feeding, there was a substantial increase in the total ω-3 fatty acid content of plasma cholesteryl esters and other lipid classes (Figure 1 and Table 2). This amounted to a rise of more than 15-fold for both cholesteryl esters and triglycerides and a rise of more than fivefold for phospholipids at the termination of fish oil feeding.

The incorporation of EPA and DHA into the cholesteryl ester fraction was examined in detail. The increase in both EPA and DHA was exponential and then reached a steady state in approximately 3 weeks for EPA and slightly later for DHA (Figure 2). EPA had the higher level of incorporation in this fraction, accounting for more than 80% of the ω-3 cholesteryl ester fatty acids (Table 2). With the increase in ω-3 fatty acids in the cholesteryl ester fraction there was a reciprocal decrease in ω-6 polyunsaturates, particularly linoleic acid (18:2; p<0.001). Total polyunsaturates were not significantly changed.

The ω-3 fatty acid incorporation into plasma phospholipids also was substantial (Table 2). At baseline, the plasma phospholipids contained more ω-3 fatty acids (3.8%) than did plasma cholesteryl esters (0.9%). After fish oil feeding, both EPA and DHA rose strikingly in phospholipids, to 10.2% and 8.2% of total fatty acids, respectively. There was a small but significant increase in docosapentaenoic acid (DPA, 22:5) from 0.7% to 1.7%. As in the cholesteryl ester fraction there was a decrease in linoleic acid, from 22.4% to 14.1% (p<0.001), with the increase in ω-3 fatty acids.

Significant amounts of ω-3 fatty acids were also incorporated into plasma triglycerides (Table 2). The relative incorporation of EPA, DHA, and DPA was similar to that of phospholipids, with the percentages of EPA and DHA being virtually equal. As the ω-3 fatty acids were incorporated into this fraction, the concentration of ω-6 fatty acids was unchanged. However, there was a significant decrease in the ω-9 fatty acid, oleic acid, which declined from 41.5% to 27.5% (p<0.001).

The unsaturation index was greater for all three plasma lipid classes after fish oil feeding.

ω-3 Fatty Acid Content of Atherosclerotic Plaque

Dietary supplementation with fish oil resulted in the incorporation of substantial quantities of ω-3 fatty acids into these advanced atherosclerotic lesions (Table 3). The mean total ω-3 fatty acid in the cholesteryl ester fraction of control plaques was 1.4±0.8% compared...
with a mean of 4.9±3.1% in plaques from fish oil-fed patients, a 350% difference (p<0.001).

As expected, the duration of feeding had a dominant effect on the accumulation of \( \omega-3 \) fatty acids. Total \( \omega-3 \) fatty acid accumulation in the cholesteryl ester fraction was 2.8% among the seven subjects fed fish oil for less than 58 days (eight plaque samples) and 7.3% for the four subjects fed fish oil for 58 days or longer (seven plaque samples) (Figure 3), with a range of 1.8% of total fatty acids in the subject fed fish oil for 6 days to 12.7% in a subject fed fish oil for 120 days. Together, EPA and DHA accounted for more than 80% of the \( \omega-3 \) fatty acid accumulation in the cholesteryl ester fraction. Unlike plasma in which the initial increase was exponential, the incorporation of EPA and DHA into plaque appeared to be linear over the period studied (Figure 4). The calculated slope for each had an intercept of zero, as the baseline plaque cholesteryl ester \( \omega-3 \) fatty acid content was not significantly different from this value. The increase in total polyunsaturated fatty acids over control values was correlated with a difference in the calculated unsaturation index, from 142.4 to 164.5.

Unlike plasma, there was no change in the percentage of \( \omega-6 \) fatty acids in plaque cholesteryl esters, but there were fewer total saturated fatty acids in the plaques from fish oil-fed subjects, 13.0% compared with 17.2% (p<0.005).

The mean \( \omega-3 \) fatty acid incorporation into plaque phospholipids was 8.8% of total fatty acids compared with a control level of 1.8%, a 490% difference. Both DHA and DPA were present in concentrations higher than those found in the cholesteryl ester fraction (Table 3). The accumulation of \( \omega-3 \) fatty acids in phospholipids also increased with the length of the feeding period (Figure 3). The amount of incorporation ranged from 2.3% in the subject fed fish oil for 6 days to 18.6% in the subject fed fish oil for 120 days. This latter amount represented approximately 85% of the total plasma phospholipid \( \omega-3 \) fatty acid concentration. Total polyunsaturates were 27.4% in controls compared with 34.2% in the fish oil-fed group. The unsaturation indexes were 93.7 and 117.8, respectively. Whereas plasma phospholipids had a reduction in \( \omega-6 \) fatty acids with fish oil feeding, in plaque the only significant change was in total monounsaturates, from 20.3% to 17.0% (p<0.001).

Although the concentration of \( \omega-3 \) fatty acids in the plasma triglycerides was comparable to that of plasma phospholipids (21.9% versus 22.1%, Table 2), their mean concentration in plaque triglycerides was lower (4.7%) than for phospholipids (8.8%) (Table 2). Nonetheless, this amounted to a 670% change from control levels. The range was from 1.3% of total fatty acids in the subject fed fish oil for 6 days to 12.3% in the subject fed fish oil for 120 days. Total polyunsaturates were 19.4% in control plaques and 27.0% in plaques from the fish oil-fed subjects, with unsaturation indexes of 85.2 and 114.4, respectively.

Relative Concentrations of Eicosapentaenoic Acid, Docosapentaenoic Acid, and Docosahexaenoic Acid

To examine the relation of the three major \( \omega-3 \) fatty acid constituents of the diet, EPA, DPA, and DHA, we calculated their ratios in the diet, plasma, and plaque. The ratio of DHA to EPA was 0.63 in the diet (Table 4). In the plasma cholesteryl ester fraction the ratio was 0.17; in the phospholipid fraction, 0.80; and in the triglyceride fraction, 0.99. The lower ratio in plasma cholesteryl ester in relation to that in plasma triglycerides was comparable to that of plasma phospholipids (21.9% versus 22.1%, Table 2), their mean concentration in plaque triglycerides was lower (4.7%) than for phospholipids (8.8%) (Table 2). Nonetheless, this amounted to a 670% change from control levels. The range was from 1.3% of total fatty acids in the subject fed fish oil for 6 days to 12.3% in the subject fed fish oil for 120 days. Total polyunsaturates were 19.4% in control plaques and 27.0% in plaques from the fish oil-fed subjects, with unsaturation indexes of 85.2 and 114.4, respectively.
The diet indicated a relatively poor incorporation of DHA into this lipid class. In the plaque the ratios were 0.54 for cholesteryl esters, 1.52 for phospholipids, and 1.33 for triglycerides.

The ratio of DPA to EPA in the diet was 0.26. The DPA to EPA ratio was much lower in plasma cholesteryl esters (0.02) and consistently increased in the respective plaque lipid classes. The ratio of DHA to EPA was increased in all plasma lipid classes over the ratio in the diet (2.38) but was reduced to substantially below the dietary ratio in plaque (Table 4).

Plasma Lipids and Platelet Parameters

At the termination of ω-3 fatty acid feeding, the mean plasma cholesterol concentration was reduced a small but significant amount from that at baseline for these 11 subjects, from 216±42 to 194±28 mg/dl (p<0.025). Mean plasma triglycerides were essentially unchanged (140±56 to 133±78 mg/dl). There were no changes in mean platelet counts (280±59 to 269±45×10^9) or bleeding times (7.3±1.8 to 7.3±1.4 minutes) due to ω-3 fatty acid feeding.

Discussion

In this study of human atherosclerotic plaques, dietary ω-3 fatty acids were shown for the first time to be rapidly incorporated into the lipid classes of advanced atherosclerotic lesions. Dietary ω-3 fatty acids have been previously demonstrated to associate themselves rapidly with the cholesteryl ester, phospholipid, and triglyceride moieties of the plasma lipoproteins, (Reference 22 and W.E. Connor et al, unpublished observations), and this feature was again documented in our study. It is known that the isotopically labeled cholesterol of lipoproteins will also exchange with the cholesterol of atherosclerotic plaques.23,24 The information from the present study indicated that ω-3 fatty acids also participated in such a phenomenon, and their concentrations in the plaque increased linearly over the length of time of ω-3 fatty acid feeding. These data suggest that the atherosclerotic plaques of these patients steadily accumulated ω-3 fatty acids from the plasma. It is known that there is an influx of plasma lipoproteins into the atherosclerotic plaque,25,26 and they would presumably then transport the ω-3 fatty acids into this tissue. It seems less likely that the accumulation of ω-3 fatty acids would have resulted from the exchange with other fatty acids present within the arterial wall.

The plaques became more polyunsaturated after fish oil feeding. Fatty acids of five (EPA and DPA) and six (DHA) carbon–carbon double bonds replaced saturated fatty acids in the cholesteryl ester and triglyceride fractions and replaced monounsaturated fatty acids in the phospholipid fraction. Consequently, the unsaturation index of the different lipid classes of arterial plaques increased greatly after fish oil feeding. The precise effect of the change in unsaturation on the atherosclerotic process is unknown at the present time. However, the degree of unsaturation of the fatty acid of phospholipids has been reported to have a significant effect on membrane fluidity and the activity of membrane-bound enzymes.27,28 LDL from monkeys fed fish oil has a lower transition temperature than that of LDL from control animals.29 Analyzing arterial plaques by physical and chemical techniques, Small and colleagues30 stated that the lipid composition of the lesion determines the physical state of plaque lipid, that is, liquid–crystalline, crystalline, or oil droplet. It is possible that fish oil feeding would alter the physical state of plaque lipids, as well as increase the fluidity of plaque cellular components and infiltrating lipoproteins.27,28

To examine the interrelation of the three major long-chain polyunsaturated ω-3 fatty acids, EPA, DPA, and DHA, we have compared the ratios of

### Table 3. Influence of Dietary ω-3 Fatty Acids From Fish Oil on Fatty Acid Composition of the Major Lipid Classes in Human Atherosclerotic Plaques

<table>
<thead>
<tr>
<th>Fatty acids</th>
<th>Cholesteryl ester (%)</th>
<th>Phospholipid (%)</th>
<th>Triglycerides (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Controls (n=18)</td>
<td>After ω-3 feeding (n=15)</td>
<td>Controls (n=18)</td>
</tr>
<tr>
<td>Saturates</td>
<td>17.2±3.6</td>
<td>13.0±2.3†</td>
<td>47.7±5.1</td>
</tr>
<tr>
<td>Monounsaturates</td>
<td>29.7±5.0</td>
<td>28.4±3.5</td>
<td>20.3±3.1</td>
</tr>
<tr>
<td>Polyunsaturates</td>
<td>Total polyunsaturates</td>
<td>50.8</td>
<td>55.9</td>
</tr>
<tr>
<td></td>
<td>Total ω-3</td>
<td>1.4±0.8</td>
<td>4.9±3.1*</td>
</tr>
<tr>
<td></td>
<td>18:3 ω-3</td>
<td>0.3±0.3</td>
<td>0.4±0.1</td>
</tr>
<tr>
<td></td>
<td>20:5 ω-3</td>
<td>0.5±0.4</td>
<td>2.4±1.6*</td>
</tr>
<tr>
<td></td>
<td>22:5 ω-3</td>
<td>0.1±0.1</td>
<td>0.7±0.7*</td>
</tr>
<tr>
<td></td>
<td>22:6 ω-3</td>
<td>0.4±0.2</td>
<td>1.3±1.0*</td>
</tr>
<tr>
<td></td>
<td>Total ω-6</td>
<td>49.1±6.9</td>
<td>51.0±6.2</td>
</tr>
<tr>
<td></td>
<td>18:2 ω-6</td>
<td>38.8±7.3</td>
<td>40.0±5.2</td>
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<tr>
<td></td>
<td>20:4 ω-6</td>
<td>7.2±2.4</td>
<td>7.9±2.5</td>
</tr>
<tr>
<td>Unsaturation index</td>
<td>142.4</td>
<td>164.5</td>
<td>93.7</td>
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</table>

Values are percent of total fatty acids, mean±SD. *p<0.001, †p<0.005, ‡p<0.025.
FIGURE 3. Bar graphs showing comparison of incorporation of the ω-3 fatty acids into lipids (cholesteryl esters, upper panel; phospholipids, middle panel; and triglycerides, lower panel) of atherosclerotic plaques from 18 control and 11 fish oil-fed subjects. Fish oil-fed group is divided into seven subjects fed fish oil for less than 58 days (<58d; eight plaque samples) and four subjects fed fish oil longer than 58 days (>58d; seven plaque samples). p values are as follows: * control versus plaques from subjects fed <58 days; † plaques from subjects fed <58 days versus those from subjects fed >58 days. Mean plasma ω-3 fatty acid levels (m) of the fish oil-fed group the day before their respective operations are included for comparison.

TABLE 4. DHA to DPA, DPA to EPA, and DHA to DPA Ratios in Various Lipid Classes of Plasma and Plaque of Patients Fed Fish Oil

<table>
<thead>
<tr>
<th>Location</th>
<th>DHA/EPA</th>
<th>DPA/EPA</th>
<th>DHA/DPA</th>
</tr>
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<tr>
<td></td>
<td>CE</td>
<td>PL</td>
<td>TG</td>
</tr>
<tr>
<td>Plasma</td>
<td>0.17</td>
<td>0.80</td>
<td>0.99</td>
</tr>
<tr>
<td>Plaque</td>
<td>0.54</td>
<td>1.52</td>
<td>1.33</td>
</tr>
</tbody>
</table>

In dietary fish oil, the DHA to EPA ratio was 0.63, the DPA to EPA ratio was 0.26, and the DHA to DPA ratio was 2.38. Plasma values shown are the mean of the ω-3 fatty acid concentrations for 11 patients taken the day before surgery.

DHA, docosahexaenoic acid (22:6 ω-3); EPA, eicosapentaenoic acid (20:5 ω-3); DPA, docosapentaenoic acid (22:5 ω-3); CE, cholesteryl ester; PL, phospholipid; TG, triglycerides.
plasma phospholipids. However, the incorporation of both DPA and DHA into cholesteryl esters relative to that of phospholipids was dramatically reduced.

A general comparison of the ratios of EPA, DPA, and DHA between plasma and plaque suggests that DHA and DPA were deposited in the plaque much more readily than EPA, and DPA was deposited more readily than DHA. These differences could be explained by three possibilities. First, there may have been preferential uptake of different fatty acids from plasma to plaque (DPA>DHA>EPA). Second, there may have been preferential oxidation of these fatty acids (EPA>DHA>DPA). Third, elongation and/or desaturation may have occurred in the arterial wall with relatively more elongation (EPA to DPA) than desaturation (DPA to DHA). Howard has demonstrated the de novo synthesis and elongation of fatty acids by subcellular fractions of monkey aorta. Although the exact mechanism is unknown at the present, the data in the present study suggest that the incorporation of these fatty acids into arterial plaque was not via a simple uptake phenomenon and may involve more complex mechanisms that remain to be delineated.

It is possible that these findings have clinical significance. ω-3 fatty acids have discreet yet powerful biologic effects. They may inhibit the production of thromboxane A₂, stimulate the production of endothelium-derived relaxing factor, inhibit intimal hyperplasia in vein grafts, decrease the endothelial cell production of a platelet-derived growth factor-like protein, and potentially reduce the inflammatory response. In addition, there are two well-documented studies showing inhibition of atherosclerosis by ω-3 fatty acids in monkeys and pigs. This inhibition occurred even though the plasma cholesterol levels remained high and HDL was fairly low, suggesting that the mechanism(s) were other than simply an alteration of plasma lipid levels.

While the influx of ω-3 fatty acids from the plasma into the atherosclerotic arterial wall as shown in this study could have an ameliorating effect on the atherosclerotic process, as yet there are no data on the effect of fish oil feeding on preexisting atherosclerotic lesions, even in experimental animals. Indeed, because lipid hydroperoxidation has been shown to be an important stimulus for macrophage uptake of lipoproteins, loading the plaque with ω-3 fatty acids theoretically could promote atherogenesis. Further studies will hopefully clarify these issues and establish a more mechanistic basis for possible therapeutic effects of fish oil on the atherosclerotic process.

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