Polyunsaturated Fatty Acids, Hyperlipidemia, and Thrombosis

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Conclusions

Polyunsaturated fatty acids in the diet have long been considered essential to the growth and proper nutrition of humans and animals. In recent years, however, these fatty acids have been used to treat and prevent atherosclerosis, and, on an experimental basis, to inhibit thrombosis. It should be emphasized that these additional uses are not at all based upon the premise that atherosclerosis and thrombosis represent essential fatty acid deficiencies, but rather that the polyunsaturated fat may affect these pathological processes through other
mechanisms. The purpose of this review is to explore in depth the actions of the principal polyunsaturated fatty acids upon the plasma lipids and thrombosis and, in particular, to differentiate the effects of the two different families of essential fatty acids: those derived from vegetable oils (chiefly the ω-6 fatty acids) and those derived mainly from fish oils (the ω-3 fatty acids, see table 1). Past studies on the effects of feeding polyunsaturated fats have not appreciated the possible metabolic differences between these two structurally different families of polyunsaturated fatty acids.

The effects of dietary fat upon atherosclerosis may be mediated through two key processes: first, the influx of lipids and lipoproteins, such as low density lipoproteins (LDL), from the plasma into the arterial wall and, second, the formation of platelet thrombi in advanced atherosclerosis.1,2 Dietary fat affects lipid infiltration through its actions to elevate or depress the concentrations of the circulating lipoproteins, especially LDL and very low density lipoproteins (VLDL), which are the major transport moieties for cholesterol and triglyceride. Atheromatous lipid deposits especially occur when the plasma levels of cholesterol and LDL are high.3 Less well understood are the effects of dietary fat upon platelet function and thrombosis. These effects may be mediated, in part, through prostaglandins synthesized from essential fatty acids.4

Since 1952, the plasma cholesterol lowering effects of polyunsaturated fat in the human diet have been demonstrated by many investigators.5,6 In a typical experiment, a polyunsaturated fat has been substituted for a predominantly saturated fat; i.e., corn oil for cocoa butter. After several weeks of such a feeding, there occurs a remarkable lowering of plasma cholesterol levels which can be promptly reversed if saturated fat is reintroduced into the diet. Gram for gram, saturated fat is twice as effective in raising the plasma cholesterol level as polyunsaturated fat is in lowering it.7,8 Although increased fecal excretion of cholesterol and its derivatives usually accompanies the fall in plasma cholesterol levels, the precise mechanisms of action of polyunsaturated fats remain the object of intensive study.

Suggestions that dietary fat might affect thrombosis date back to studies conducted over 20 years ago in which saturated fat was considered to be "thrombogenic" and polyunsaturated fat "nonthrombogenic."9-11 Only recently, with the discovery of platelet and vessel wall prostaglandins, has a more precise mechanism of how dietary fatty acids might affect thrombosis been elucidated.4 Twenty-carbon fatty acids of both the ω-6 and ω-3 families serve as substrates for the synthesis of different prostaglandins having diverse activities. The thromboxanes induce platelet aggregation and vasoconstriction; the prostacyclins inhibit platelet aggregation and produce vasodilation.12 There is currently great interest in the possibility that dietary ω-3 polyunsaturated fatty acids may alter platelet function in an anti-thrombogenic direction.

Table 1. Major Families of Polyunsaturated Fatty Acids

<table>
<thead>
<tr>
<th>Family designation</th>
<th>Parent fatty acid</th>
<th>Major metabolites</th>
<th>Characteristic structure</th>
<th>Principal sources</th>
</tr>
</thead>
<tbody>
<tr>
<td>ω-9</td>
<td>C18:1 ω-9 oleic acid</td>
<td>C20:3 ω-9 eicostrienoic acid</td>
<td>H₃C-C-C-C-C-C-C-C-C-CCCCCC = C-R-RCOOH</td>
<td>Synthesis from acetate; animal and vegetable fats</td>
</tr>
<tr>
<td>ω-6</td>
<td>C18:2 ω-6 linoleic acid</td>
<td>C20:4 ω-6 arachidonic acid</td>
<td>H₃C-C-C-C-C-C-C-C-C = C-R’COOH</td>
<td>Many vegetable oils</td>
</tr>
<tr>
<td>ω-3</td>
<td>C18:3 ω-3 linolenic acid</td>
<td>C20:5 ω-3 eicosapentaenoic acid</td>
<td>H₃C-C-C-C-C-C-C-C-CCCCCC = C-R’COOH</td>
<td>Some vegetable oils, leaves (18:3)</td>
</tr>
<tr>
<td></td>
<td></td>
<td>C22:6 ω-3 docosahexaenoic acid</td>
<td></td>
<td>Marine oils (20:5, 22:6)</td>
</tr>
</tbody>
</table>

A fourth family of polyunsaturated fatty acids (ω-7) can be synthesized from palmitoleate (C16:1 ω-7), but only trace amounts of ω-7 polyunsaturated fatty acids are present in the tissues. The omega (ω) number indicates the location of the first double bond counting from the methyl end of the fatty acid (ω is the last letter of the Greek alphabet). An alternative nomenclature frequently used is the "n" system where "n" replaces the ω (e.g., 18:2 ω-6 = 18:2 n-6).13

*Accumulates only in essential fatty acid deficiency.
Polyunsaturated Fatty Acid Families and Interrelationships

The three major families of unsaturated fatty acids are the oleic acid (ω-9) family, the linoleic acid (ω-6) family, and the linolenic acid (ω-3) family. These fatty acid families, which are not metabolically interconvertible, are described in Table 1.

Polyunsaturated fatty acids are ultimately derived from plants, seeds, leaves, and phytoplankton. Seeds and leaves are the principal sources of linoleic and linolenic acids, whereas phytoplankton synthesize eicosapentaenoic acid and docosahexaenoic acid, the major ω-3 fatty acids. Since phytoplankton are at the bottom of the marine food chain, all other forms of marine life eventually become enriched with these ω-3 fatty acids. Indeed, the ω-3 fatty acids may provide the required degree of unsaturation to allow fish membranes to remain fluid in very cold water. Tables 2 and 3 list some of the common dietary sources of polyunsaturated fatty acids and provide a detailed breakdown of three fats frequently used in the experimental studies alluded to in this review.

Members of the ω-6 and ω-3 families are regarded as essential fatty acids (EFA) since they cannot be synthesized de novo in the body. The principal and best characterized EFA is linoleic acid, C18:2 ω-6. A dietary intake of 1% to 2% of total calories as linoleic acid is sufficient to prevent EFA deficiency, whereas higher levels (2% to 4% of calories) are needed to reverse the condition. Precisely how linoleic acid participates in human nutrition is becoming clearer. Linoleic acid is the obligatory precursor of arachidonic acid, which, in turn, serves as a principal substrate for prostaglandin synthesis. This relationship between linoleic acid, arachidonic acid, and prostaglandins has led to much speculation that EFA deficiency is, in fact, a "prostaglandin deficiency." This contention, however, has not been supported.

Table 2. Common Dietary Sources of Polyunsaturated Fatty Acids

<table>
<thead>
<tr>
<th>Source†</th>
<th>Linoleic</th>
<th>Arachidonic</th>
<th>Linolenic</th>
<th>Eicosapentaenoic</th>
<th>Docosahexaenoic</th>
<th>Saturated</th>
</tr>
</thead>
<tbody>
<tr>
<td>Predominantly ω-6</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Safflower oil</td>
<td>73</td>
<td>—</td>
<td>0.5</td>
<td>—</td>
<td>—</td>
<td>9</td>
</tr>
<tr>
<td>Corn oil</td>
<td>57</td>
<td>—</td>
<td>1.0</td>
<td>—</td>
<td>—</td>
<td>13</td>
</tr>
<tr>
<td>Cottonseed oil</td>
<td>50</td>
<td>—</td>
<td>0.4</td>
<td>—</td>
<td>—</td>
<td>26</td>
</tr>
<tr>
<td>Sunflower seed oil</td>
<td>56</td>
<td>—</td>
<td>0.3</td>
<td>—</td>
<td>—</td>
<td>10</td>
</tr>
<tr>
<td>Peanut oil</td>
<td>29</td>
<td>—</td>
<td>1.0</td>
<td>—</td>
<td>—</td>
<td>19</td>
</tr>
<tr>
<td>Predominantly ω-3</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Linseed oil</td>
<td>15</td>
<td>—</td>
<td>55.0</td>
<td>—</td>
<td>—</td>
<td>13</td>
</tr>
<tr>
<td>Salmon oil</td>
<td>1</td>
<td>—</td>
<td>1.0</td>
<td>5</td>
<td>5</td>
<td>26</td>
</tr>
<tr>
<td>Cod liver oil</td>
<td>2</td>
<td>—</td>
<td>1.0</td>
<td>12</td>
<td>12</td>
<td>19</td>
</tr>
<tr>
<td>Channel catfish oil</td>
<td>6</td>
<td>2.0</td>
<td>0.7</td>
<td>4</td>
<td>9</td>
<td>26</td>
</tr>
<tr>
<td>Mackerel</td>
<td>2</td>
<td>2.0</td>
<td>1.0</td>
<td>10</td>
<td>16</td>
<td>35</td>
</tr>
<tr>
<td>Whale oil</td>
<td>1</td>
<td>4.0</td>
<td>—</td>
<td>3</td>
<td>7</td>
<td>19</td>
</tr>
<tr>
<td>Both ω-6 and ω-3</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Soybean oil</td>
<td>51</td>
<td>—</td>
<td>7.0</td>
<td>—</td>
<td>—</td>
<td>15</td>
</tr>
<tr>
<td>English walnut oil</td>
<td>55</td>
<td>—</td>
<td>11.0</td>
<td>—</td>
<td>—</td>
<td>11</td>
</tr>
<tr>
<td>Low in both ω-6 and ω-3</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Cow milk fat</td>
<td>2</td>
<td>—</td>
<td>1.0</td>
<td>—</td>
<td>—</td>
<td>62</td>
</tr>
<tr>
<td>Human milk fat</td>
<td>7</td>
<td>0.2</td>
<td>0.7</td>
<td>0.6</td>
<td>0.3</td>
<td>50</td>
</tr>
<tr>
<td>Lard</td>
<td>10</td>
<td>—</td>
<td>1.0</td>
<td>—</td>
<td>—</td>
<td>36</td>
</tr>
<tr>
<td>Chicken fat</td>
<td>17</td>
<td>—</td>
<td>1.0</td>
<td>—</td>
<td>—</td>
<td>33</td>
</tr>
<tr>
<td>Beef tallow</td>
<td>4</td>
<td>—</td>
<td>0.5</td>
<td>—</td>
<td>—</td>
<td>48</td>
</tr>
<tr>
<td>Egg yolk</td>
<td>11</td>
<td>6.0</td>
<td>0.2</td>
<td>—</td>
<td>—</td>
<td>53</td>
</tr>
<tr>
<td>Beef liver</td>
<td>10</td>
<td>6.0</td>
<td>0.5</td>
<td>—</td>
<td>—</td>
<td>39</td>
</tr>
<tr>
<td>Coconut oil</td>
<td>2</td>
<td>—</td>
<td>—</td>
<td>—</td>
<td>—</td>
<td>88</td>
</tr>
<tr>
<td>Olive oil</td>
<td>8</td>
<td>—</td>
<td>0.7</td>
<td>—</td>
<td>—</td>
<td>14</td>
</tr>
<tr>
<td>Cocoa butter</td>
<td>3</td>
<td>—</td>
<td>0.2</td>
<td>—</td>
<td>—</td>
<td>60</td>
</tr>
<tr>
<td>Palm oil</td>
<td>9</td>
<td>—</td>
<td>0.3</td>
<td>—</td>
<td>—</td>
<td>48</td>
</tr>
</tbody>
</table>

*Monounsaturated fatty acids constitute the remaining fatty acids. They are thought to have neutral effects upon plasma lipid levels.†See refs. 78, 114–118.
Table 3. Detailed Fatty Acid Composition of Dietary Fats Commonly Used in Experimental Studies

<table>
<thead>
<tr>
<th>Fatty acid</th>
<th>Name</th>
<th>Butter fat (saturated)</th>
<th>Corn oil (ω-6)</th>
<th>Salmon oil (ω-3)</th>
</tr>
</thead>
<tbody>
<tr>
<td>4:0-12:0</td>
<td>—</td>
<td>11</td>
<td>—</td>
<td>—</td>
</tr>
<tr>
<td>14:0</td>
<td>Myristic</td>
<td>10</td>
<td>—</td>
<td>4</td>
</tr>
<tr>
<td>16:0</td>
<td>Palmitic</td>
<td>26</td>
<td>11</td>
<td>15</td>
</tr>
<tr>
<td>16:1 ω-7</td>
<td>Palmitoleic</td>
<td>2</td>
<td>trace</td>
<td>6</td>
</tr>
<tr>
<td>18:0</td>
<td>Stearic</td>
<td>12</td>
<td>2</td>
<td>6</td>
</tr>
<tr>
<td>18:1 ω-9</td>
<td>Oleic</td>
<td>25</td>
<td>26</td>
<td>23</td>
</tr>
<tr>
<td>18:2 ω-6</td>
<td>Linoleic</td>
<td>2</td>
<td>60</td>
<td>3</td>
</tr>
<tr>
<td>18:3 ω-3</td>
<td>Linolenic</td>
<td>1</td>
<td>1</td>
<td>1</td>
</tr>
<tr>
<td>20:1 ω-9</td>
<td>Eicosenoic</td>
<td>—</td>
<td>—</td>
<td>10</td>
</tr>
<tr>
<td>20:4 ω-6</td>
<td>Arachidonic</td>
<td>trace</td>
<td>—</td>
<td>trace</td>
</tr>
<tr>
<td>20:5 ω-3</td>
<td>Eicosapentaenoic</td>
<td>—</td>
<td>—</td>
<td>9</td>
</tr>
<tr>
<td>22:1 ω-11</td>
<td>Cetoleic</td>
<td>—</td>
<td>—</td>
<td>9</td>
</tr>
<tr>
<td>22:5 ω-3</td>
<td>Docosapentaenoic</td>
<td>—</td>
<td>—</td>
<td>3</td>
</tr>
<tr>
<td>22:6 ω-3</td>
<td>Docosahexaenoic</td>
<td>—</td>
<td>—</td>
<td>8</td>
</tr>
</tbody>
</table>

Polyunsaturated/saturated 0.05 5.5 1.3
Iodine value 31 123 167

*Trace = < 1%.

by experimental evidence, which has shown that prostaglandins per se do not uniformly alleviate the symptoms of EFA deficiency. Thus, some of the biochemical mechanisms through which linoleic acid functions remain obscure.

The essentiality of the ω-3 family has been questioned because of the finding that some, but not all, of the symptoms of EFA deficiency can be reversed by linolenic acid (e.g., normalization of 20:3/20:4 ratios but not reproductive function). Recent studies, however, have raised the possibility that ω-3 fatty acids may be essential, not for prostaglandin synthesis, but as vital structural components of specialized membranes. For example, docosahexanoic acid (C22:6 ω-3) appears to play a role in the proper functioning of nervous tissues, and impaired maze-running abilities have been found in ω-3 fatty-acid-deficient rats. Remarkably high levels of C22:6 ω-3 are found in brain and retinal cells as well as in spermatozoa. Although further studies will be required to document structural or metabolic defects resulting from dietary ω-3 fatty acid deprivation, the presence of high levels of these unique acids in certain tissues and the known inability of mammalian cells to synthesize these de novo imply a dietary requirement for ω-3 fatty acids.

Omega-6 Fatty Acids and Plasma Lipids

Plasma Cholesterol

The hypocholesterolemic effects of polyunsaturated fatty acids present in vegetable oils has been appreciated for almost 30 years. In 1952, Kinsell et al. suggested that it was not simply the amount of fat in the diet, but the type of fat (animal or vegetable) that affected plasma cholesterol. Later, this point was clearly demonstrated by Ahrens et al., who showed that feeding two vegetable fats (coconut oil and corn oil) led to vastly different plasma cholesterol levels. The iodine number (total unsaturation) of the oil, rather than its source, was found to be strongly correlated with the degree of cholesterol lowering. About the same time, it was shown that the addition of polyunsaturated fat to a fat-free diet resulted in a reduction in plasma cholesterol levels, which was of the same magnitude as the rise observed when saturated fats were added. The possibility that hypercholesterolemia resulted from EFA deficiency was discounted when it was shown that a fish oil high in ω-3 polyunsaturates but low in linoleic acid effectively reduced plasma cholesterol levels.
On the basis of this and other similar studies, Hegsted et al. and Keys et al. developed predictive equations from which the magnitude of change in plasma cholesterol that occurs after substitutions in the diet could be calculated. Both equations predict that, on a gram for gram basis, saturated fatty acids will raise cholesterol levels about twice as much as polyunsaturated fatty acids will lower them. These equations were derived from studies in which linoleic acid was fed; they may not apply to fish oil feeding studies.

Several previous studies have demonstrated that polyunsaturated fatty acids derived from vegetable oils (principally linoleic acid) will reduce plasma cholesterol levels in normal, healthy volunteers. Typically, vegetable oils comprised 40% of the total calories, and the cholesterol intake was about 400 mg/day. The average decline in plasma cholesterol levels in these studies was about 13%, but ranged from 0 to 28%.

The extent of cholesterol lowering was related to at least two factors: 1) the magnitude of the difference between the P/S ratios (polyunsaturated to saturated fatty acids as a percent of total calories) of the saturated and the polyunsaturated diets; and 2) the presence of cholesterol in the diet. For example, in eight of these studies, the P/S ratio of the polyunsaturated diet averaged about 7-fold higher than that of the saturated diet (1.5 vs 0.2), and these studies reported an average decrease in plasma cholesterol levels of only 8%. In six other studies, the P/S ratio of the polyunsaturated diet averaged 27-fold higher than that of the saturated diet (4.6 vs 0.17), and this difference was associated with an average cholesterol decrease of 20%.

In terms of the effects of cholesterol in the diet, only three of these studies employed cholesterol-free diets. In one, a P/S ratio difference of 25-fold produced only an 8% decrease in plasma cholesterol levels, and in another, no change in cholesterol levels was found with a 9-fold P/S ratio range. Connor, et al. repeated the latter experiment using a 94-fold difference in P/S ratios and produced a 20% decrease in cholesterol levels.

Thus, it appears that plasma cholesterol levels are less responsive to changes in the dietary P/S ratio when cholesterol is absent from the diet. The relatively small decrease in plasma cholesterol levels (8%) induced by the more practical shift in P/S ratios (7-fold) may indicate that simply changing the P/S ratio of the dietary fat without regard to other factors (e.g., the total amount of fat in the diet) may not produce physiologically important changes in the plasma cholesterol levels.

In hyperlipidemic patients, the effects of polyunsaturated vegetable oils have been similar to, or in some cases, greater than those reported in normal subjects. The average percent decrease was somewhat higher than that found in normals (22%; range, 13% to 33%). However, even in these hypercholesterolemic patients, those fed diets with the smallest difference in P/S ratios (2.0 vs 0.2) experienced the smallest decreases in plasma cholesterol levels (13%).

All phenotypic forms of hyperlipidemia showed some hypolipidemic response to dietary polyunsaturated fats. In the 17 Type IIa patients reported, the average decline in plasma cholesterol levels was 19%; in 15 Type IIb patients, 21%; in 29 Type IV, 15%; and in seven Type V, 16%. Thus, no particular phenotype was unusually responsive or resistant to changes in the amount of polyunsaturated fat in the diet.

**Plasma Triglyceride**

Investigators who have studied the effects of polyunsaturated vegetable oils upon plasma triglyceride levels have occasionally reached divergent conclusions. In four studies, vegetable oils decreased triglyceride levels somewhat in normal subjects and were no effects. Among those reporting that polyunsaturated vegetable oils were hypotriglyceridemic, one study involved dietary periods of only 10 days' duration. In the study that employed the longest dietary periods (7-9 weeks per period), no effects of vegetable oil upon triglyceride levels could be demonstrated, which suggests that the 10-day study may have been too short to allow for plasma lipids to stabilize. In other studies, plasma triglyceride levels were depressed by only 13% to 15% during vegetable oil feeding. Thus, the overall consensus suggests that the feeding of polyunsaturated vegetable oils has minimal effects upon the plasma triglyceride levels in normolipidemic subjects. Much confusion in this area has been due to the frequent, although not universal, findings in hyperlipidemic subjects that polyunsaturated vegetable oils lower plasma triglyceride levels. It appears that the hypotriglyceridemic response is influenced both by the initial triglyceride levels as well as the genotypic form of hyperlipidemia present. The physiological significance of these changes is not known.

**Plasma Lipoproteins and Apolipoproteins**

There is general agreement that increased dietary intakes of polyunsaturated vegetable oils lower the concentrations of LDL cholesterol and HDL cholesterol in hyperlipidemic patients. Reductions in high density lipoprotein (HDL) cholesterol levels have been found in four studies, but not in four others. The results of both of our recent metabolic studies support the conclusion that no change in plasma HDL levels occurs during polyunsaturated fat feeding. In the studies in which HDL concentrations fell, similar or greater reductions in the concentrations of LDL also occurred, so that the LDL:HD ratio remained fairly constant.
The effects of dietary ω-6 polyunsaturated fatty acids on the concentrations of plasma apolipoproteins are consistent with the lipoprotein changes. Decreases have occurred in the concentrations of apoprotein B35, 42, 45, 53, 54, 59 and either no change46, 56 or a decrease in the concentrations of apoprotein A-I.40, 56, 58, 59 The effects of polyunsaturated fatty acids upon the concentrations of apoprotein E are not known.

In summary, there is universal agreement that a high intake of polyunsaturated vegetable oils will reduce plasma cholesterol and LDL levels. There is probably little effect of ω-6 polyunsaturated fatty acids upon plasma triglyceride and VLDL levels40, 44, 47 although the data in this area are not clear.40, 44, 47, 56 HDL cholesterol levels have been variably affected by diets rich in linoleic acid. Future studies to settle these questions must be carefully conducted to allow adequate time for plasma lipids to stabilize on different dietary regimes; they must control caloric intake to prevent the confounding influences of weight changes upon lipid metabolism; and they should examine the effects upon plasma lipids of reasonable changes in the amount of polyunsaturated fat in the diet.

**Hypollipidemic Mechanisms of Linoleic Acid**

The mechanisms by which polyunsaturated ω-6 fatty acids exert their hypollipidemic effects have been examined extensively, but to date no unifying mechanism has been found to be operative in all situations.29-33 Proposed mechanisms have included: 1) decreased cholesterol absorption; 2) increased excretion of neutral and acidic steroids; 3) decreased cholesterol synthesis; 4) transfer of cholesterol from plasma to tissues; 5) changes in the cholesterol-to-protein ratio in LDL; and 6) changes in the rates of synthesis or catabolism of individual lipoproteins. In addition, the changes in the fatty acid composition of membrane lipids (fluidity) may affect lipoprotein metabolism in the following ways: changes in the lipoprotein particle itself may make it a better substrate for catabolic enzymes, or for binding to cellular receptors; or changes in cellular membranes may affect the local milieu of receptors and membrane-bound enzymes so as to increase their reactivity toward their lipoprotein substrates. All of these possible mechanisms will be considered.

**Decrease in Cholesterol Absorption**

Significant differences in cholesterol absorption have not been found in normal48 or in hyperlipidemic29 subjects fed either saturated or polyunsaturated fats. Studies from our laboratory have also failed to reveal any differences in cholesterol absorption in normolipidemic subjects fed diets rich in saturated, monounsaturated, or polyunsaturated fatty acids of either the ω-3 or the ω-6 families.32, 44

**Increase in Fecal Steroid Excretion**

Several groups have explored the possibility that greater amounts of fecal steroids are excreted during polyunsaturated fat feeding. In normal subjects this was found to be the case in six studies31, 40, 41, 44, 46, 49 but not in two others.39, 48 Recent studies from our laboratory reconfirmed these findings and showed a 56% increase in total sterol excretion during 4 weeks of polyunsaturated fat feeding as compared to a comparable period on a saturated fat diet.44 Increased fecal steroid excretion has also been reported in hypertriglyceridemic subjects26 but not in those with hypercholesterolemia.29, 55 In a more prolonged study (40 days) in normal subjects, fecal steroid excretion increased when polyunsaturated fat was substituted for saturated fat.46 After adaptation during the later phases of the experimental diets, however, the fecal steroid excretions were similar. These findings imply that, after a change to a polyunsaturated fat diet, fecal steroid excretion rises as the plasma cholesterol level declines, but then returns to normal levels once a new steady state is reached. The enhanced fecal steroid excretion more than accounts for the loss of cholesterol from the plasma compartment as the plasma level falls.31 The increased excretion of fecal sterols after ω-6 polyunsaturated fat diets may not be essential to their hypollipidemic effect, particularly in familial hypercholesterolemic subjects since they do not always have increased fecal steroid excretion. In fact, much of the current controversy concerning the effects of vegetable oil feeding upon fecal steroid excretion has been due to the failure to recognize that subjects with genetically distinct hyperlipidemias may respond differently. The reasons for these differences are unclear.

**Reduced Cholesterol Synthesis and the Transfer of Cholesterol to the Tissues**

The possibility that cholesterol synthesis is reduced by polyunsaturated fatty acids has been largely ruled out.23, 61 The lack of change in the steady-state excretion of steroids observed by Nestel et al.46 further supports this view. Recent studies have shown similar rates of cholesterol synthesis in freshly isolated mononuclear cells from subjects fed saturated vs polyunsaturated fat diets.51 Since mononuclear cells are an easily accessible 'peripheral tissue' possessing LDL receptors,63 these findings also imply that cholesterol does not shift into the peripheral tissues during polyunsaturated fat feeding. Such a shift would be expected to lower the rate of cholesterol synthesis in the cell by feedback inhibition.63 Further evidence that polyunsaturated fatty acids do not enhance tissue uptake of cholesterol came from an analysis of liver tissue from subjects consuming diets rich in polyunsaturated and saturated fatty acids. No increase in hepatic cholesterol levels after feeding polyunsaturated fat was found.62 Direct evidence that polyunsaturated
fatty acids increase tissue cholesterol concentra-
tions in humans is lacking; they may even, on a theo-
retical basis, decrease tissue concentrations. 31

Decrease in the Cholesterol Content of
Lipoproteins

In 1969, Spritz and Mishkel 56 proposed that as
dietary polyunsaturated fatty acids became incorpo-
rated into plasma lipoproteins, the tortuous geometry
of the linoleate molecule would reduce the amount of
space available for cholesterol transport, thus lower-
ing the proportions of cholesterol in each LDL part-
cle. The hyperlipidemic patients in this study had
decreased cholesterol-to-protein ratios in LDL. How-
ever, this decrease was not found in two other stud-
ies. 43, 44 Some 52, 58 but not all 53 of the studies in
normolipidemic subjects have reported similar de-
creases in the cholesterol content of LDL.

Changes in the Rates of Synthesis and Catabolism
of Lipoproteins

Comparative studies of apolipoprotein metabolism
in both normal and hyperlipidemic patients fed diets
high in saturated fatty acids or rich in ω-6 polyunsatu-
rases have been undertaken. Although most stud-
ies have examined parameters of LDL metabolism,
reports of the influence of dietary polyunsaturated
fatty acids on the synthesis and catabolism of VLDL
and HDL have also been published. The kinetics of
VLDL apoprotein B (apo B) have been compared in
subjects fed diets rich in polyunsaturated vs satu-
rated fatty acids. 42 The synthesis of apo B after the
polyunsaturated-fat-rich diet was significantly reduced in
all studies, but the fractional catabolic rate did not
change appreciably. Substitution of polyunsaturated
fatty acids for saturated decreased the synthesis of
apo A-I, the major apoprotein in HDL, by 26%. 59 No
change in the fractional catabolic rate of apo A-I was
noted.

The influence of polyunsaturated fatty acids on
LDL apo B metabolism has been studied in both
normal and hypercholesterolemic subjects. The frac-
tional catabolic rate of LDL has consistently been
increased after the substitution of polyunsaturates
for saturated fats in the diet. In some 43, 60 but not all 38, 41 studies, these changes were paralleled by
significant decreases in the absolute synthetic rates
for LDL apo B. In all studies, however, the trends
have been similar (decreased synthetic rate and in-
creased fractional catabolic rate with polyunsatu-
rated fat), but in some cases the difference has not
reached statistical significance. A recent study in
which saturated or ω-6 polyunsaturated fatty acids
were isocalorically substituted for monounsaturated
fatty acids has provided additional insights into the
individual effects of saturated vs polyunsaturated
fats on LDL metabolism. 41 The fractional catabolic
rate of LDL was significantly higher from the polyun-
saturated diet than from either the monounsaturated
or saturated regimens. This suggests that increased
catabolism of LDL results primarily from the addition
of polyunsaturated fat to the diet rather than from the
removal of saturated fatty acids.

Membrane Fatty Acid Composition and
Lipoprotein Metabolism

Polyunsaturated fat feeding alters the fatty acid
composition of cellular membranes and plasma lipo-
proteins 56, 59, 64 with a resultant increase in fluidity. 54
A report that lipoprotein lipase is more reactive to-
w ard the more fluid lipoprotein particles 65 implies
that linoleate-enriched lipoproteins may be cleared
more rapidly. In addition, the fatty acid composition
of cellular membrane phospholipids can influence the
activity of membrane-bound enzymes. 56 An in-
crease in the unsaturation of membrane phospho-
lipids in cultured human fibroblasts led to a more
rapid removal of LDL particles but no change in the
number of LDL receptors. 57 Thus, the activity of re-
ceptors or membrane-bound enzymes toward lipo-
proteins appears to be enhanced by higher levels of
linoleic acid in the diet.

In summary, the literature attests to the fact that no
single mechanism of action of ω-6 polyunsaturated
fatty acids has yet been proven. Multiple pathways
have been described in both normal and hyperlipide-
mic subjects, and these pathways are greatly af-
ected by the type of lipid disorder present. Many of
the observed effects of polyunsaturated fatty acids
may be interrelated. For example, increases in fecal
steroid excretion could be secondary to an increased
rate of catabolism of LDL. This may be caused by
changes in lipoprotein or membrane fluidity leading
to changes in the activity of lipolytic enzymes or
membrane receptors. Based on available evidence,
we believe that the most promising mechanisms to
explain the hypolipidemic effects of polyunsaturated
fats relate to changes in the fluidity of lipoproteins
and/or cellular plasma membranes. These physio-
chemical changes (which follow increased con-
sumption of polyunsaturated fatty acids) may affect
lipid and lipoprotein metabolism leading to reduced
steady state plasma lipid levels.

Omega-3 Fatty Acids and Plasma Lipids

Epidemiologic Studies

Recent studies by Bang and Dyerberg 68 have
stimulated interest in the metabolism of ω-3 fatty
acids and have suggested a link between the habit-
ual ingestion of these fatty acids in the diet and the
low death rates from atherosclerotic disease ob-
served in Greenland Eskimos. Although little reliable
data on cardiovascular morbidity and mortality is
available among this particular group of Eskimos,
Bang and Dyerberg 68 noted that during the years
1953–1967 only two cases of atherosclerotic heart
disease and no cases of diabetes mellitus were registered in the population of 1300 people. In 1970, Arthrand estimated deaths from cardiovascular disease to be only 16% of all deaths in Alaskan coastal Eskimos (aged 41–70 years) over the years 1959–1968. Bang and Dyerberg have provided evidence that the high levels of ω-3 fatty acids consumed in the Eskimo diets of seal, whale, and fish (5.8 g/day) may be responsible for the lower plasma lipid levels in this population compared to levels in Danes (0.8 g/day). In addition, ω-3 fatty acids may also be responsible for the prolonged bleeding times that are common in this group of Eskimos.

Animal Studies

Because of the large body of data available on the effects of dietary ω-6 polyunsaturated fatty acids in humans, we have not reviewed the ω-6 studies carried out in experimental animals. However, because dietary ω-3 polyunsaturated fatty acids have not been as extensively studied in humans, a brief review of the ω-3 fatty acid feeding studies in animals is desirable.

Cholesterol metabolism in animals fed various fish oils has been reviewed in depth by Stansby. Rats have been fed sardine oil, cuttlefish liver oil, cod liver oil, and tuna and menhaden oil. In the latter studies, the highly unsaturated ω-3 fatty acids found in the fish oils were compared to the ethyl esters of linolenic, linoleic, palmitic, and oleic acids in hypercholesterolemic rats. Despite the differences in unsaturation, both of the fish oils and linolenic acid produced equivalent cholesterol lowering. Linoleic acid was the next most effective, followed by oleate and palmitate.

In contrast to these findings in rats, studies in hypercholesterolemic chickens have shown that fish oils were more hypocholesterolemic than either linolenic or linoleic acids. Thus, in some species, fatty acids of the ω-3 family (from fish oils and linolenic acid) may be equally hypocholesterolemic. In other animals, the fatty acids with the greatest unsaturation (eicosapentaenoic and docosahexaenoic from fish oils) are more hypocholesterolemic than other ω-3 fatty acids that contain less unsaturation (linoleic acid).

The hypolipidemic effects of a diet containing mackerel oil (which is rich in ω-3 fatty acids) vs one supplemented with olive oil (rich in monounsaturates) has also been compared in piglets. Although plasma cholesterol levels were not affected by the mackerel oil feeding, the concentrations of plasma triglyceride were significantly depressed. Failure to balance the high cholesterol intake during the fish oil feeding (400 mg/day) by adding similar amounts of cholesterol to the olive oil diet may explain why plasma cholesterol levels were not reduced by the fish oil.

Human Studies

Between 1956 and 1963, eight studies of fish-oil feeding were carried out in humans. Although they varied in the kinds of subjects studied, in the degree of dietary control, in the sources of fish oil fatty acids, and in duration, there has been good agreement that fish oils were at least as hypocholesterolemic as polyunsaturated vegetable oils. The principal intent of these studies was to demonstrate that polyunsaturated fats from any source (fish or vegetable) were hypocholesterolemic. There was no indication from these studies that fish oils had any unique properties not shared by other polyunsaturated oils from vegetable sources.

Two interesting features of these studies were perhaps not appreciated at the time. First, since fish oils contain large amounts of cholesterol (300 to 500 mg/dl) and vegetable oils contain none, the vast majority of these investigators fed higher levels of cholesterol during the fish oil phase than during the vegetable oil phase. Thus, the finding of similar hypocholesterolemic effects of these two types of fat implied that the fish oils might have been even more hypocholesterolemic if they had not contained dietary cholesterol.

Second, the daily intake of ω-6 fatty acids was invariably greater than the intake of ω-3 fatty acids, and yet similar or greater reductions in plasma cholesterol levels occurred from ω-3 fatty acids. Thus, gram for gram, the ω-3 fatty acids were considerably more hypocholesterolemic than linoleic acid. Others have shown that tetra-, penta-, and hexaenes produced two to three times greater cholesterol lowering than did the di- or trienes.

More recently, dramatic reductions in the concentrations of plasma triglycerides and VLDL have been observed in both normolipidemic and hyperlipidemic subjects fed diets supplemented with fish oils. In one study, normal subjects were fed diets enriched with salmon oil, polyunsaturated vegetable oils, and a relatively saturated control diet for periods of 4 weeks each. Plasma cholesterol levels were reduced similarly with both the salmon and vegetable oil rich diets. In contrast, however, plasma triglyceride levels fell 33% as a result of the salmon oil diet but were unchanged after the vegetable oil diet (figure 1). This direct comparison provided the first clear demonstration that ω-3 fatty acids present in salmon oil possess unique hypotriglyceridemic properties not found in the polyunsaturated ω-6 fatty acids of vegetable oils. In these studies it was further demonstrated that dietary ω-3 fatty acids significantly decreased plasma VLDL (50%) and LDL (16%) levels but did not change the concentrations of HDL (figure 2). Possible mechanisms for this effect will be discussed subsequently. The fact that HDL levels were not depressed has been confirmed by others, and, in some studies, HDL cholesterol levels have actually increased on the ω-3 fatty acid rich diets.

The effects of fish oils in hypercholesterolemic and hypertriglyceridemic patients have been reported in...
only two studies. Ahrens et al.,38 examined two sub-
jects (probably Type II-b) given isocaloric amounts of
either corn oil or menhaden oil at 40% of calories.
Plasma cholesterol and triglyceride levels were both
reduced more by the fish oil than the vegetable oil.
Phillipson et al.,5'-M recently reported the effects of
feeding diets rich in salmon oil, polyunsaturated
vegetable oils, and saturated fats to hyperlipidemic
subjects. In patients with Type II-b hyperlipidemia,
plasma cholesterol levels (mg/dl) decreased from
294 to 239 (ω-6 diet) and 200 (ω-3 diet). Plasma tri-
glyceride levels were 397 (control), 247 (ω-6 diet),
and 135 (ω-3 diet). Thus, the fish oil diet was signifi-
cantly more hypolipidemic than the polyunsaturated
vegetable oil diet.

The effects of dietary fish oil upon the plasma
lipids of patients with severe hypertriglyceridemia
(Type V phenotype) were first reported in 1981.94
These patients, in whom hypertriglyceridemia is nor-
mally exacerbated by even moderate intakes of di-
etary fat, were able to consume up to 30% of their
calories as fish oil, with a concomitant lowering of
their plasma lipid levels. The ω-3 fatty acid-rich diet
resulted in a 46% decline in plasma cholesterol lev-
els and a 77% drop in triglyceride levels when com-
pared to a control diet having only 5% fat (figure 3).
Comparative diets containing 30% of calories as ω-6
fatty acids caused rapid increases in the concentra-
tions of both plasma cholesterol and triglycerides so
that it became necessary to stop these diets after 14
days because of the increasing risk of abdominal
pains, hepatomegaly, and acute pancreatitis. The ω-
3 fatty acids had distinctly different effects upon
plasma lipid levels than ω-6 fatty acids and may be of
substantial therapeutic benefit in hypertriglyceride-
mic patients.

A few attempts have been made to determine the
minimum intake of ω-3 fatty acids necessary to pro-
duce significant changes in plasma lipid levels. Although dose responses have not been fully ascertained, it has been shown that approximately 4 g/day of ω-3 fatty acids from 20 ml of cod liver oil produced a greater cholesterol lowering than did 16 g of linoleic acid. In a second study, however, the addition of up to 8 g of ω-3 fatty acids (from cod liver oil) did not change the plasma cholesterol levels but did result in a significant reduction in plasma triglycerides. A further study showed that 4 g of ω-3 fatty acids per day were able to reduce the levels of plasma triglycerides but not of cholesterol.

A major problem with many of these studies is that the fish oils were simply given as supplements and were not incorporated into a metabolic diet as a replacement for a portion of the customary dietary fat. In addition, the cholesterol intake was usually higher during the fish oil phase. For these reasons it seems likely that more significant hypocholesterolemic effects might have occurred if the dietary periods had been more rigorously matched in terms of both fat and cholesterol content.

In summary, although relatively few studies have examined the effects of ω-3 polyunsaturated fatty acids upon plasma lipids and lipoproteins, there has been an impressive consistency in the findings, not only in metabolic studies but also with free-living human beings consuming high levels of ω-3 fatty acids. Fish oils have always been at least as effective as vegetable oils (if not more so) in lowering plasma cholesterol levels. These reductions occurred when ω-3 fatty acids constituted from 1% to 8% of total calories. In light of the fact that linoleic acid intakes of 15% to 20% of calories were needed to achieve similar depressions in plasma cholesterol levels, the dietary ω-3 fatty acids were roughly 2 to 5 times more potent than the ω-6 acids.

The most striking effects of fish oil feeding have been the rapid and drastic decreases in the levels of plasma triglyceride and VLDL. Since vegetable oils produce little or no depression in plasma triglyceride concentrations, it is evident that ω-3 fatty acids have unique effects not shared with the ω-6 fatty acids. Studies in hyperlipidemic subjects have demonstrated that small quantities of ω-3 fatty acids are much more hypolipidemic than large amounts of linoleic acid. Thus, dietary ω-3 fatty acids may prove to be very effective in the treatment of hyperlipidemia.
Hypolipidemic Mechanisms of Omega-3 Fatty Acids

Plasma Cholesterol

The mechanism of the ω-3 induced hypocholesterolemia has not been studied as extensively as that resulting from ω-6 fatty acid ingestion. Nevertheless, there is data to suggest that the following mechanisms may be involved: 1) increased fecal steroid excretion; 2) changes in the fatty acid composition (fluidity) of lipoproteins; and 3) changes in the rates of synthesis and/or catabolism of VLDL and LDL.

In studies recently reported from our laboratory, both polyunsaturated fat diets (ω-6 or ω-3) led to increased fecal steroid excretion. Net fecal excretion of cholesterol and its metabolites was 673 mg/day during the saturated control diet, 926 mg/day with the vegetable oil diet, and 975 mg/day after the salmon oil diet. The slightly greater excretion with ω-3 fatty acids as compared to the ω-6 fatty acids occurred primarily from an increase in the excretion of bile acids. Thus, increased steroid excretion was a common result of feeding both ω-6 and ω-3 polyunsaturated fatty acids.

In terms of lipoprotein composition, we found that the cholesterol-to-protein ratio was not different after saturated and ω-3 dietary periods despite a decrease in LDL levels (Harris, unpublished observations). Hence, changes in this ratio would not explain the hypocholesterolemic effect of ω-3 fatty acids.

Although no data on the fatty acid composition of individual lipid classes from the major lipoprotein fractions have been reported as yet, the fatty acids of each plasma lipid class have been studied during ω-3 diets. The percent of total fatty acids occurring as ω-3 in each class of plasma lipids (triglyceride, phospholipid, and cholesteryl esters) was 1% to 3% on the control diet and increased to 31%, 33%, and 26%, respectively, after the ω-3 diet. Since these ω-3 fatty acids are highly unsaturated (5 to 6 double bonds), the fluidity of the lipoprotein particles would probably be affected and the interaction between lipoproteins and lipolytic enzymes may be enhanced.

Omega-3 fatty acids have affected plasma triglycerides and VLDL cholesterol levels more extensively than total and LDL cholesterol levels. This suggests that ω-3 fatty acids may primarily affect the synthesis or clearance of triglycerides and/or VLDL. Because of the well-established precursor-product relationship between VLDL and LDL, reductions in the synthesis of VLDL may in turn lead to lower concentrations of LDL. Chait et al. and Grundy have provided evidence that ω-6 fatty acids may reduce plasma cholesterol levels in this manner in hyperlipidemic subjects. Recent studies in our laboratory have shown that feeding ω-3 fatty acids to normal subjects reduced the synthetic rate of LDL apo B by 23% as compared to a saturated-fat control diet. The fractional catabolic rate did not differ during the two diets (Illingworth, unpublished observations). These findings indicate that a decrease in VLDL synthesis could be responsible for lower LDL levels. Comparative studies of VLDL kinetics during the feeding of diets rich in ω-3, ω-6, and saturated fatty acids will be needed to clarify this issue.

Plasma Triglyceride

Reductions in the concentrations of plasma triglycerides and VLDL may occur from lower rates of synthesis of the triglyceride or apo B moieties of VLDL or may result from an increased rate of clearance of VLDL from the plasma. In light of the first possibility, Iritani et al. showed in rats that eicosapentaenoic acid (20:5 ω-3) reduced the activity of the lipogenic enzyme acetyl CoA carboxylase when compared to linoleic acid. Similar findings have been reported by others. Thus, ω-3 fatty acids may reduce hepatic lipogenesis with a concomitant reduction in the supply of fatty acids for triglyceride synthesis.

The most important source of fatty acids for triglyceride synthesis is the adipose tissue. A reduction in adipose tissue lipolysis could lower fasting free fatty acid levels and reduce triglyceride synthesis. Such a mechanism has been proposed for the hypolipidemic effects of nicotinic acid. However, Chait et al. found no difference in plasma free fatty acid levels in subjects fed saturated and ω-6 polyunsaturated fat diets. This finding does not preclude the possibility that ω-3 fatty acids could affect triglyceride levels via a reduction in free fatty acid release, especially in view of the dramatic and consistent triglyceride lowering that accompanies ω-3 fatty acid feeding. One possible way in which ω-3 fatty acids could influence adipose tissue lipolysis is via their participation in prostaglandin metabolism. In vitro, prostaglandin E2 has been shown to inhibit lipolysis of adipose tissue triglyceride. However, other investigators have failed to show any effects of prostaglandins on lipolysis in vivo and the possible role of these compounds in the regulation of free fatty acid levels remains unsettled. Nevertheless, the possibility remains that prostaglandins derived from ω-3 fatty acids may have anti-lipolytic properties which may contribute to their overall hypotriglyceridemic effects.

A second mechanism by which dietary ω-3 fatty acids could reduce plasma triglyceride levels is the acceleration of VLDL clearance. Since chylomicrons and VLDL share a common clearance mechanism, we assessed the effects of ω-3 fatty acids on the rate of chylomicron clearance after a standardized test meal. We consistently found that the postprandial rise in plasma triglyceride levels after a salmon oil test meal was markedly reduced as compared to the rise that followed a saturated fat test meal. After verifying that the salmon oil was not malabsorbed, we concluded that dietary salmon oil may increase the rate of clearance of chylomicrons. Likewise, the reduced levels of fasting triglycerides may be due to more rapid removal of VLDL from the circulation.
Should ω-3 fatty acids be shown to accelerate triglyceride clearance rates, at least two possible mechanisms should be considered: an increase in plasma lipolytic activity (lipoprotein lipase or hepatic triglyceride lipase) or the lipoprotein particle itself may be changed in such a way that it becomes a better substrate for lipolytic enzymes. Support for increased enzyme activity is sparse. In preliminary studies from our laboratory, we have found no significant difference in post-heparin lipolytic activity in normal subjects fed ω-3 and saturated fat diets (Illingworth, unpublished observations). As noted earlier, the microenvironment of membrane-bound lipolytic enzymes may be affected by dietary fatty acids, thereby altering either the affinity or activity of receptors and enzymes. Such phenomena have not been examined during ω-3 fatty acid feeding.

Since the activity of postheparin lipoprotein lipase does not appear to be greatly affected by dietary ω-3 fatty acids, a second possibility needs to be considered, namely, that ω-3 fatty acids may change the nature of the lipoprotein substrate and thereby enhance triglyceride clearance. Clearly, total unsaturation of plasma lipoproteins is dramatically affected by a diet rich in ω-3 fatty acids. Although lipoprotein lipase has no known fatty acid specificity, the size of the lipoprotein particle being cleared does influence its clearance rate in that larger particles are cleared most rapidly. It is not known whether chylomicrons or VLDL enriched with the long-chain highly unsaturated ω-3 fatty acids are larger than those carrying the more usual fatty acids. When relatively small amounts of fish oils were fed to subjects on otherwise uncontrolled diets, there appeared to be a relationship between decreased plasma triglyceride levels and the in vitro lipolysis of lipoprotein triglycerides by lipoprotein lipase. Since post-heparin lipoprotein lipase activity was derived from one individual in all experiments, the differences in the observed lipolytic rates probably resulted from the diet-induced differences in the lipoproteins themselves. Thus, the nature of the lipoprotein substrate may help determine its rate of clearance.

Polyunsaturated Fatty Acids and Thrombosis

A persistent interest in the relationships of polyunsaturated fatty acids to the role of platelets in atherosclerosis and thrombosis has spanned several decades and has now dramatically accelerated with the appreciation of the links between dietary fatty acids and prostaglandins in platelets and vascular tissue. The following sections will focus first on the early work linking free fatty acids and dietary saturated fats to vascular thrombosis and will then proceed to a detailed examination of the changes in platelet/ves-

ewel phospholipid composition, cellular function, and prostaglandin metabolism induced by ω-6 and ω-3 polyunsaturated fatty acids in the diet. It is hoped that this information will further stimulate inquiry into the potential antithrombotic effects and clinical usefulness of dietary polyunsaturated fatty acids in humans.

Free Fatty Acids, Platelets, and Thrombosis

The first step taken in the study of fatty acids as potential thrombogenic substances was to add free fatty acids directly to whole blood and to administer them intravenously to experimental animals. Initially, the sodium salts of various fatty acids were added to blood and the mixtures injected into a loop of plastic tubing which was rotated semivertically on a wheel (Chandler apparatus) to induce a thrombus. In these experiments, long-chain saturated fatty acids such as stearic acid (C18:0) greatly shortened the "thrombus formation time," while polyunsaturated fatty acids were essentially inactive. Similarly, intravenous infusions of saturated fatty acids into dogs led to thromboses in the heart and great vessels whereas long-chain polyunsaturated fatty acids such as linoleic acid were much less thrombogenic.

These experiments demonstrated the thrombogenic potential of saturated free fatty acids but it was not yet clear whether they acted via the generation of fibrin (perhaps through activation of factor 12) or whether they also possessed the ability to directly aggregate platelets. Accordingly, experiments were performed with platelets and fatty acids in the absence of plasma coagulation factors. The addition of long-chain saturated fatty acids to washed platelets led to the release reaction and irreversible platelet aggregation, whereas polyunsaturated fatty acids had little or no effect. However, the activity of arachidonic acid was not tested in this system. When the added fatty acids were bound by albumin or plasma proteins in solution, their platelet aggregating ability was generally reduced although stearic and oleic acids continued to be active. Thus, saturated free fatty acids, especially when in excess of the tight albumin binding sites, could lead to platelet aggregation in the absence of blood coagulation and the formation of fibrin.

Additional studies examined the platelet aggregating effects of endogenously generated free fatty acids in animals or humans. Acute elevations of plasma free fatty acids following subcutaneous ACTH given to rabbits led to thrombosis in isolated jugular venous segments and the appearance of "circulating platelet aggregates" in arterial blood. Similar increases in total and saturated free fatty acids induced either by fasting in normal subjects or secondary to acute coronary insuffi-
ciency in patients could also be correlated with the appearance of circulating platelet aggregates and elevated platelet factor 4 plasma concentrations in venous blood.\(^{116,117}\)

In sum, under appropriate (but rather artificial) experimental conditions, saturated free fatty acids may lead to thromboses that appear to be mediated, at least in part, by increased platelet reactivity. These effects are not seen with unsaturated fatty acids. Whether acute elevations of free fatty acids from endogenous sources may lead to overt vascular occlusion in susceptible individuals such as those with severe coronary vascular disease requires further clarification.

Dietary Fat, Platelets, and a Predisposition to Thrombosis

Instead of using free fatty acids to initiate thrombosis, an additional series of experiments were carried out in which certain dietary fats were fed to experimental animals for weeks or months and the predisposition of the animals to thrombosis produced by biologic or mechanical stimuli determined. For example, rats fed butter or a diet augmented with specific saturated fatty acids (e.g., stearic acid) developed larger hepatic thrombi than control animals after jugular vein infusions of S. typhosa endotoxin.\(^{116,119}\) As in the experiments using injections of free fatty acids, it was not clear whether the endotoxin infusion (classically associated with disseminated intravascular coagulation) produced the larger hepatic thromboses via fibrin generation or through a diet-induced augmentation of platelet reactivity. Subsequent investigation indicated that the platelets from rats fed a high saturated fat diet showed increased sensitivity to thrombin-induced platelet aggregation and were able to release platelet factor 3 more readily than controls.\(^{120-122}\) Unfortunately, these studies failed to provide more direct evidence of platelet involvement in the endotoxin-induced hepatic thromboses.

Further studies have examined whether different dietary fatty acids may influence the number of platelet-derived pulmonary thrombi produced by an infusion of adenosine diphosphate into the inferior vena cava of rats.\(^{123}\) After the saturated fat diet, the adenosine diphosphate infusion led to the immediate formation of thrombus in 90% of the animals, whereas the incorporation of polyunsaturated fatty acids into the diet (replacing 20% of the saturated fat) significantly reduced, but failed to eliminate, pulmonary thrombi.

Lastly, following surgical insertion of a small polyethylene cannula into the abdominal aorta of male rats, the number of hours required for occlusion of the cannula by a platelet thrombus was recorded.\(^{124-126}\) There was a direct relationship between the dietary linoleic acid content and prolongation of the aortic occlusion time. Conversely, the ingestion of saturated fatty acids of increasing chain length was associated with a progressive shortening of the occlusion time. The platelet count was higher and the platelets more sensitive to aggregation by adenosine diphosphate in the animals fed the saturated fat diet.

These and other experiments indicate that the chronic feeding of diets rich in saturated fat to experimental animals predisposed them to thrombi initiated by endotoxin, adenosine diphosphate, or the insertion of a plastic cannula into the aorta. Although the dietary manipulations resulted in increased platelet reactivity by various in vitro measurements, it remains to be proven that the increased propensity to thrombosis was actually caused by dietary-induced alterations in platelet reactivity.

Prostaglandins Derived from Polyunsaturated Fatty Acids in Platelets and Blood Vessels

Our understanding of the relationships of polyunsaturated fatty acids and their role in platelet and vascular function has been greatly aided by the recent identification and characterization of platelet and endothelial cell prostaglandins (figure 4).\(^4, 12, 127\) Since these potent substances, thromboxane A2 and prostaglandin I2 (PGI2),\(^*\) are ultimately derived from dietary fatty acids, it is possible that manipulation of dietary fat content might alter prostaglandin synthesis and subsequent platelet/vessel interactions.

Arachidonic acid (C20:4 o-6) may be transported in the circulation either in the free fatty acid form bound to albumin or esterified in lipoprotein phospholipids and cholesterol esters.\(^127\) Direct uptake of free arachidonic acid by platelets or the vessel wall leads to its incorporation into membrane phospholipids;\(^128, 129\) the latter also exchange with plasma phospholipids. Following activation of one or more phospholipases, arachidonic acid becomes "available" to the enzyme cyclooxygenase and is rapidly converted to the labile cyclic endoperoxides, PGG2 and PGH2.\(^130\) Enzymes such as thromboxane synthetase in the platelet or prostacyclin synthetase in the endothelial cell convert the endoperoxides to biologically active thromboxane A2 or PGI2 (prostacyclin).\(^131-133\) The potent vasoconstricting and platelet aggregating effects of thromboxane A2 and the vasodilating and platelet inhibitory effects of prostacyclin are now well known and have been extensively studied.\(^4\)

\(^*\)The prostaglandins have been named in the order of their discovery beginning with A and proceeding to I; e.g., PGI2. The numerical subscript refers to the number of double bonds in the prostaglandin side chain.
Other polyunsaturated fatty acids besides arachidonic acid may serve as substrates for prostaglandin synthesis (figure 5). For example, dihomo-gamma-linolenic acid (DHLA) (C20:3 ω-6) acts as a substrate for prostaglandins of the "1" series such as the classical prostaglandin, PGE1 (thromboxane A1 or PG11, if formed at all, appear to be inactive). Eicosapentaenoic acid (C20:5 ω-3) is the substrate for prostaglandins of the "3" series and, under certain conditions, leads to the production of thromboxane A3 and PGI3.

The biochemical relationships and metabolic pools of the polyunsaturated fatty acids in the human require further study. For example, the essential fatty acid, linoleic acid, is converted to C20:3 ω-6 and ultimately to arachidonic acid in the liver. Although the feeding of diets rich in linoleic acid regularly leads to its accumulation in platelet membrane phospholipids, arachidonate levels are found to be unchanged or may even be decreased in platelets. Similarly, feeding of linolenic acid (C18:3 ω-3) does not appear to lead to significant increases of eicosapentaenoic acid in adult human plasma. The feeding of marine foods rich in eicosapentaenoic acid, however, leads to rapid incorporation of this fatty acid into both platelet and endothelial cell membranes.

Recent studies have shown that arachidonic acid can also be converted by the lipoxygenase pathway to a new class of compounds called leukotrienes.

Figure 4. Prostaglandin metabolism in platelets and blood vessels. Conversion of arachidonic acid to PGI2, thromboxane A2, and leukotrienes.

Figure 5. Prostaglandin synthesis from polyunsaturated fatty acids. Conversion of ω-6 and ω-3 fatty acids to various prostaglandins. The dotted lines indicate that the depicted elongation and desaturation steps occur in some but not all tissues.
Since leukotrienes have been shown to have bronchoconstrictive properties (i.e., slow reacting substance of anaphylaxis, SRS-A), chemotactic activity, and possible influences on thromboxane and prostacyclin synthesis, they will provide a fertile area for future biochemical and clinical research. The rapidly increasing knowledge of prostaglandin and leukotriene metabolism will allow reinterpretation of older studies of the effect of dietary fatty acids on platelet function. More important, it will enable future investigators to construct more rational attempts to manipulate cellular prostaglandin synthesis by dietary or other therapeutic means.

Effects of Omega-6 Fatty Acids upon Platelet and Vascular Composition and Function

Linoleic Acid

Early studies of fatty acids and thrombosis generally supported the hypothesis that replacement of saturated by polyunsaturated fatty acids in the diet might be protective against thromboembolic events, and that this protective effect might be mediated through an inhibition of platelet function. Consequently, during the last decade, a number of investigators have examined the effects of diets containing large amounts of polyunsaturated fatty acids (especially linoleic acid) on platelet lipid composition and hemostatic function. Evaluation of these studies has been somewhat difficult because of marked variations in experimental design, dietary control, lipid analyses, and tests of platelet function. However, some important generalizations can be made in spite of these drawbacks.

Platelet and Vessel Lipid Composition

Although linoleic acid is rapidly converted to arachidonic acid in the liver and in cells such as fibroblasts, similar elongation and desaturation does not occur in platelet membranes. Indeed, studies in which linoleic acid was fed to rabbits in the form of corn oil led to a reproducible increase in the percent concentration of linoleic acid in platelet membrane phospholipids compared to a butter fat diet (table 4). However, platelet arachidonic acid was decreased in the corn-oil-fed animals compared to the butter fat group. Similar findings were observed in rats fed diets containing combinations of corn oil and hydrogenated coconut oil. The arachidonic acid content of aortic phospholipids was also found to be increased in butter-fed rabbits.

The decrease in the total amount of arachidonate present in platelet phospholipids may be even greater than has been appreciated since it has been reported recently that total platelet fatty acid concentration may be decreased in animals fed large amounts of linoleic acid. For example, total platelet fatty acids declined by half in rats fed a 40% fat diet in which the P/S ratio was increased from 0.4 to 5.5. A recent study conducted in human volunteers showed that feeding supplemental linoleic acid in the form of corn oil (25 ml/day) for 6 weeks led to an increase in the linoleate content of the phosphatidylcholine component of platelet phospholipids, but not the phosphatidyethanolamine or phosphatidylinositol fractions. Total platelet arachidonate levels were unchanged.

Some additional experimental data clarifying this question have recently become available using cultured endothelial cells or platelets incubated with various fatty acids for 1–72 hours. When the incubation medium was enriched with linoleic acid, the phospholipids in endothelial cells and platelets increased their linoleic acid content from 5% to 25%, but arachidonic acid levels fell by 25% to 40%. This reduction may be due in part to a Δ6 desaturase deficiency in endothelial cells that prevents conversion of C18:2 to C20:4 by interfering with the desaturation of C18:2 to C18:3. In addition, high concent-

Table 4. Platelet Fatty Acid Composition Following Dietary Enrichment with Polyunsaturated Fatty Acids in Humans

<table>
<thead>
<tr>
<th>Platelet fatty acid</th>
<th>Linoleic acid (in vegetable oil)</th>
<th>Dihomo-gamma-linolenic acid (as purified supplement)</th>
<th>Eicosapentaenoic acid (in fish oil)</th>
</tr>
</thead>
<tbody>
<tr>
<td>C18:2 ω-6 (linoleic acid)</td>
<td>Increased</td>
<td>Decreased</td>
<td>Decreased</td>
</tr>
<tr>
<td>C20:3 ω-6 (dihomo-gamma-linolenic acid)</td>
<td>No change</td>
<td>Increased</td>
<td>No change</td>
</tr>
<tr>
<td>C20:4 ω-6 (arachidonic acid)</td>
<td>Decreased</td>
<td>Decreased</td>
<td>Decreased</td>
</tr>
<tr>
<td>C20:5 ω-3 (eicosapentaenoic acid)</td>
<td>Nil</td>
<td>Nil</td>
<td>Increased</td>
</tr>
</tbody>
</table>
trations of linoleic acid may simply reduce arachidonic acid levels by competition for esterification sites in phospholipids.

Therefore, in at least some experimental situations, feeding a diet very rich in linoleic acid may lead to a selective decrease in arachidonic acid, whereas diets containing saturated fatty acids may lead to the opposite effect. Whether these changes in arachidonic acid content will regularly translate into equivalent changes in thromboxane or prostacyclin release remains to be determined. 148

Platelet and Vessel Wall Function

Changes in platelet function have been studied after the chronic feeding of diets rich in polyunsaturated vegetable oils. When bleeding times were performed in human subjects ingesting a diet rich in \( \omega-6 \) polyunsaturated fatty acids and compared to a control dietary period, no significant differences were found in our own studies (unpublished observations) or in those of others (table 5). 140, 149, 150 Despite this lack of prolongation in the bleeding times, some tests of platelet aggregation have been affected by dietary linoleic acid.

In rabbits fed corn oil, the sensitivity for arachidonic acid and collagen-induced platelet aggregation was lowered when compared to aggregation in butter-fed animals. 151 In humans, Renaud et al. 152 reported that platelet aggregation in response to thrombin and adenosine diphosphate (but not collagen or epinephrine) was increased in a group of French farmers with a high saturated fat intake, compared to a second group eating a diet containing less saturated fat. 152 Similarly, platelet aggregation induced by low concentrations of collagen was decreased in a group of Norwegian subjects given a corn oil supplement. 149 In contrast to these studies, a group of normal American men given dietary supplements of margarine and vegetable oils failed to show differences in platelet aggregation but did show a significant reduction in platelet retention on glass bead columns. 153

In sum, it appears that supplementing or replacing saturated fat with oils rich in linoleic acid leads to mild and rather variable effects on platelet function. Although the bleeding time is not significantly prolonged, platelet aggregation to low doses of aggregating agents may be inhibited, platelet retention on glass beads reduced, and the time to platelet aggregation in the filtragometer prolonged. Platelets also appear to be less reactive as evidenced by lengthening of the heparin thrombin clotting time and a reduction in circulating platelet aggregates.

Mechanisms

The mechanisms underlying the changes in platelet function and proposed antithrombotic effects of dietary polyunsaturated fatty acids are not completely understood. However, recent studies examining prostaglandin synthesis and other biochemical pathways of platelets and vessel walls provide some insight and future directions for study.

<table>
<thead>
<tr>
<th>Dietary fatty acid</th>
<th>Bleeding time</th>
<th>Platelet aggregation</th>
<th>Platelet thromboxane B2</th>
<th>Vessel PG12</th>
</tr>
</thead>
<tbody>
<tr>
<td>Linoleic acid (in vegetable oil)</td>
<td>Normal</td>
<td>Inhibited</td>
<td>Decreased</td>
<td>Decreased*</td>
</tr>
<tr>
<td>Dihomo-gamma-linolenic acid (as purified supplement)</td>
<td>Normal</td>
<td>Inhibited</td>
<td>Decreased*</td>
<td>Decreased</td>
</tr>
<tr>
<td>Linolenic acid (in linseed oil)</td>
<td>Normal</td>
<td>Unknown</td>
<td>Decreased*</td>
<td>Decreased</td>
</tr>
<tr>
<td>Eicosapentaenoic acid (in fish oil)</td>
<td>Prolonged</td>
<td>Inhibited</td>
<td>Decreased</td>
<td>Decreased*</td>
</tr>
</tbody>
</table>

*Data are suggestive but not definitive (see text and references).
In one experiment, rabbits were fed a diet containing 8% of their calories as corn oil for 3 weeks and the conversion of exogenous radiolabelled arachidonic acid to labelled thromboxane B2 in platelet-rich plasma was examined. At low substrate concentrations of arachidonic acid, platelet thromboxane production was significantly lowered in the corn-oil-fed animals. The decrease in thromboxane B2 production was associated with less platelet aggregation following stimulation by arachidonic acid or low doses of collagen.

In a second study from the same laboratory, rabbits were fed diets enriched or depleted in linoleic acid for 3 months. The linoleate-rich diet led to an increase in linoleic acid and a decrease in arachidonic acid in platelet phospholipids. This change was paralleled by a reduced production of thromboxane B2 from exogenous radiolabelled arachidonic acid but collagen-induced thromboxane production from endogenous arachidonic acid was not changed. Recently, a preliminary report indicated that arachidonic acid content of rat platelets was directly correlated with changes in thromboxane B2 production after stimulation of the platelets by collagen.

After human volunteers were fed relatively low amounts (25 ml/day) of a corn oil supplement, collagen-induced thromboxane B2 production significantly decreased (60 to 20 ng/ml) even though the arachidonic acid content of platelet phospholipids was unchanged. Therefore, after corn oil feeding, it would seem that platelet thromboxane production is decreased. This occurs when arachidonic acid is added to the prostaglandin pathway from exogenous sources or when arachidonate is released from endogenous pools of phospholipid after stimulation with low concentrations of collagen. It is currently unclear whether the decreased prostaglandin synthesis is a result of decreased precursor fatty acids, alterations in the activity of platelet enzyme systems such as phospholipase, cyclooxygenase, or thromboxane synthetase, or related to other more remote factors such as a possible effect on thromboxane production by dietary cholesterol.

Dietary fatty acids could influence platelet reactivity in ways other than acting directly on thromboxane synthesis. One study examined the half-life of thromboxane A2 in a buffer test system containing albumin. The addition of a mixture of free fatty acids extracted from human plasma to the test system led to accelerated destruction of thromboxane A2, presumably by interfering with the thromboxane stabilizing effects of albumin. Another report suggested that the feeding of linoleic acid resulted in increased levels of platelet cyclic AMP which in turn could lead to decreased platelet reactivity. Lastly, a recent study from Japan found that polyunsaturated fatty acids (especially linoleic and arachidonic acids) markedly inhibited platelet phosphodiesterase in vitro which could prolong the effectiveness of cyclic AMP.

Platelet reactivity could also be altered by variations in vascular prostaglandin synthesis (i.e., prostacyclin). Only a few studies are currently available to examine this important question, and unfortunately variations in methodology and experimental design have made a general interpretation of the results difficult. In 1979, the investigative group from Unilever in Holland examined the PG12-like activity generated from perfused segments of rat aorta taken from animals that had been fed a diet high in sunflower seed oil. Aortas from several animals fed this vegetable oil produced somewhat less PG12-like activity than those from animals ingesting 35% of their calories as hydrogenated coconut oil, but the differences were not statistically significant. A study from Italy using pieces of aorta taken from butter or corn oil fed rabbits also documented decreased production of PG12-like activity in the animals fed the polyunsaturated fat diet. The increased production of PG12 in the saturated fat group correlated well with the increase in the arachidonic acid found in the phosphatidylinositol fraction of the aortic phospholipids. In contrast to the two studies cited above, a second study by the Dutch workers showed a slightly increased aortic production of PG12 in linoleic acid fed rats; this change correlated with the increased C18:2 ω-6 in the diet.

A series of experiments have been carried out using human umbilical endothelial cells cultured in vitro. Enrichment of these cells with linoleic acid led to a significant decrease in spontaneous or stimulated PG12 production. This effect may be due to a competitive inhibition of endothelial cell cyclooxygenase by linoleic acid or to a linoleic acid-induced reduction in phospholipid arachidonic acid content.

In attempting to fit most of the experimental data about mechanisms into a coherent picture, it appears that feeding diets high in linoleic acid to animals or humans leads to increased concentrations of linoleic acid in the platelet membrane and decreased concentrations of arachidonic acid. Thromboxane production is decreased from platelets after they are stimulated with low doses of collagen. PG12 production from blood vessels may also decrease because of the reduced arachidonic acid content of the vascular phospholipids or the inhibition of endothelial cell cyclooxygenase.

**Dihomo-gamma-linolenic Acid (C20:3 ω-6)**

The effects of DHLA on platelets and blood vessels have been of interest because this 20-carbon fatty acid is the precursor to the "1" series of prostaglandins such as PGE1 (figure 5; tables 4 and 5). The latter has an inhibitory effect on platelet function. Since naturally occurring fats and oils contain only minute amounts of DHLA, purified DHLA (usually as the ethyl or methyl ester) must be given to experimental subjects in order to increase its concentration in plasma or tissues. If radiolabelled DHLA is given to
an experimental animal, it is largely converted to arachidonic acid (its natural end product) by the liver and other tissues although it is possible to "load" plasma, platelets, and most likely endothelial cells by DHLA feeding. Endothelial cells do contain the Δ5-desaturase necessary to convert C20:3 ω-6 to C20:4 ω-6. However, the increased concentration of DHLA in platelets and endothelial cells (from feeding purified DHLA) is associated with a decrease in the content of linoleic acid and a significant reduction in arachidonic acid.

Feeding purified DHLA to humans or animals has led to conflicting results in respect to its effects on platelet function. One study showed decreased platelet aggregation in response to collagen or ADP but a second study showed little effect. One study showed that incorporation of DHLA into platelets does not lead to platelet aggregation and, in fact, may be inhibitory.

The mechanism for the putative decrease in platelet reactivity to aggregating agents could reflect decreased thromboxane A2 production from arachidonic acid by the platelets. Needelman et al. have shown that incorporation of DHLA into platelets does not lead to the formation of PGE2; rather, DHLA is converted by thromboxane synthetase to hydroxy-heptadecadienoic acid, which is a functionally inactive compound. Thromboxane B2, PGE1, or PGD1 are not formed in appreciable amounts.

The effects of free or albumin-bound DHLA added to endothelial cell cultures has been recently studied by Nordøy et al. who showed that endothelial cell enrichment with C20:3 led to a marked reduction of synthesized PGI2-like material. In contrast, the addition of arachidonic acid led to the endothelial cell production of a factor (presumably prostacyclin) that strikingly inhibited ADP-induced platelet aggregation.

In summary, feeding purified methyl or ethyl DHLA to humans or animals leads to the incorporation of this fatty acid into platelets and vascular cells where it is associated with decreased amounts of arachidonic acid in membrane phospholipids. Consequently, "2" series prostaglandin synthesis (such as thromboxane A2) may be diminished due to a decrease in arachidonate availability or possible competitive inhibition of thromboxane synthetase. Whether the prostaglandin inhibitory effects of DHLA are relatively more pronounced in platelets or in vascular endothelium is not known.

**Effects of Omega-3 Fatty Acids on Platelet and Vascular Composition and Function**

**Linolenic Acid (C18:3  ω-3)**

In most tissues (except, for example, platelets and red blood cells) or in vitro systems, linolenic acid may be desaturated and elongated to eicosapentaenoic acid. The feeding of oils rich in linolenic acid (e.g., linseed oil, which contains 53% C18:3 ω-3) to animals or humans might theoretically lead to the accumulation of eicosapentaenoic acid in tissues such as platelets or endothelial cells. One such study showed that feeding rats up to 4% of the calories as purified methyl linolate led to a decrease in arachidonic acid (23% to 11%) and an increase in eicosapentaenoic acid from 0.1% to 3%-4% in liver and serum lipids. The production of thromboxane B2 from platelets, as measured by radioimmunoassay in serum, substantially declined (130 to 27 ng/ml) as the linolenate content of the diet was increased. In contrast, Dyerberg et al. fed a normal volunteer 45 ml/day of linseed oil and failed to detect any increase in eicosapentaenoic acid in serum lipids. Platelet or vascular concentrations of C20:5 ω-3 were not measured.

Of the few studies that have examined the effects of feeding C18:3 ω-3 upon platelet or endothelial cell function, Borchgrevink et al. found no changes in platelet adhesiveness or bleeding times in humans after dietary supplements of linseed oil. Recently, ten Hoor and coworkers fed a diet containing 25% C18:3 ω-3 to rats and noted that collagen-induced platelet malondialdehyde production and aortic vascular production of PGI2-like material were both lower than in a comparable group of rats fed a diet high in linoleic acid (table 5).

**Eicosapentaenoic Acid (C20:5  ω-3)**

As alluded to earlier, the fascinating series of scientific visits to the West Coast of Greenland by Bang and Dyerberg generated some provocative questions about the relationships of an "Eskimo diet" to platelet and vascular function. Previous work had established that the Eskimo diet contained large amounts of ω-3 polyunsaturated fatty acids such as eicosapentaenoic acid and that these fatty acids were present in plasma and platelets of the Eskimos. A bruising or bleeding tendency had been described among the Greenland Eskimos and was also noted in the 1930s by a French explorer who visited northern Canadian Eskimos eating a similar diet. Pursuing these leads, the Danish investigators studied the bleeding times of their Eskimo subjects and found them to be prolonged in comparison to a control population living in Denmark. These and other observations have stimulated a growing interest in the effects of ω-3 fatty acids derived from marine oils on the composition and function of platelets and on cellular prostaglandin metabolism.

**Platelet Lipid Composition**

Changes in platelet phospholipid composition brought about by feeding dietary supplements of fish oils to animals or humans are now quite well documented. The concentrations of both linoleic acid and...
arachidonic acid were decreased in the Eskimo platelet phospholipids. Similar observations have been noted in volunteers fed mackerel oil, cod liver oil, or salmon oil, and in animals given menhaden or cod liver oils (table 4).74, 92, 136, 137, 139 This reduction in linoleic acid could be a reflection of the low linoleate intake (most fish oils contain only 1% to 2% linoleic acid) or may relate to the displacement of linoleate by other fatty acids.124 The mechanism responsible for the reduction in the arachidonic acid content of platelets is not known, but it is possible that the decreased dietary linoleic acid may lead to reduced arachidonate levels in plasma and hence, platelet membrane phospholipids. Alternatively, arachidonic acid may be simply displaced by other fatty acids.

As might be expected, the consumption of ω-3 rich fish oils led to a marked incorporation of eicosapentaenoic acid and even longer chain polyunsaturated fatty acids (table 5). With one exception,140 bleeding times have invariably been prolonged by 30% to 40% in humans ingesting fish oils for several weeks or longer although a clinical bleeding tendency has not been observed.74, 92, 137, 139 Brox, et al.140 failed to show a lengthening of the bleeding time, but this may have been due to the lesser quantity (25 ml/day) of fish oil that was administered.

Platelet aggregation in response to ADP has been impaired in the Eskimos and in the residents of a fishing village in Japan, as well as in human subjects fed salmon and cod liver oils.74, 92, 139, 172 In other studies, aggregation induced by low doses of collagen was also found to be diminished.136, 139, 140 Platelet retention on glass beads (which may reflect platelet aggregation as well as platelet adhesion) was found to be significantly decreased in subjects receiving a diet containing salmon oil supplements.136 Lastly, the occlusion time of vascular prostheses inserted into the aorta of rats was prolonged when the animals were fed cod liver oil.138

Decreased platelet counts have been observed in subjects ingesting large amounts of ω-3 fatty acids. These include the early Eskimo observations as well as a recent study of the effects of salmon oil feeding in volunteers.74, 139 In general, the reduction in platelet count, while statistically significant, remained within the normal range and was not low enough to affect the duration of the bleeding times (i.e., less than 100,000/mm³). In several individuals, however, there was a more marked fall in the platelet count.139

After the salmon oil feeding was discontinued, the platelet count rose rapidly to normal levels. In one of these subjects, a repeat study feeding salmon oil led to a reduction in platelet count a second time although platelet survival, using 51Chromium plateaued before and during fish oil feeding was unchanged (Goodnight, unpublished observations). At present, it is not clear whether the thrombocytopenia is related to the ω-3 fatty acids in the fish oil, other natural components, or to contaminants.

In sum, feeding ω-3 fatty-acid-rich fish oils to humans leads to a reproducible prolongation of the Ivy bleeding time, inhibition of platelet aggregation by ADP and collagen, as well as a decrease in platelet retention on glass beads. In some settings, there may also be a reduction in platelet count. The mechanisms for these functional alterations may be explained in part by changes in platelet and endothelial cell prostaglandin synthesis induced by alterations in dietary fatty acid composition.

Mechanisms of Fatty Acid Effects

At least three studies in which fish oils have been fed to humans have clearly shown that thromboxane B₂ production (the stable metabolite of thromboxane A₂) was decreased following stimulation of platelets by collagen or adenosine diphosphate (Goodnight, unpublished observations).136, 140 In a rat study, no appreciable production of thromboxane A₃ from platelets was observed after feeding cod liver oil.138 Thus, it seems more likely that the reduced platelet reactivity found with fish oil feeding is a result of reduced thromboxane A₂ levels rather than an increased production of the inactive thromboxane A₃.

Several investigators have also examined the platelet production of malondialdehyde which, in the past, has been assumed to be a measure of thromboxane synthetase. Two studies in humans have shown normal or increased levels of platelet malondialdehyde production associated with decreased thromboxane B₂ production.132, 140 In another study, rat platelets produced less malondialdehyde after fish oil compared to sunflower seed oil feeding.134 Recent work has indicated that thiobarbituric-acid-reactive substances (similar to malondialdehyde) may also be generated via the platelet lipoxygenase pathway during the formation of leukotrienes.173 Since this pathway remains active in spite of eicosapentaenoic-acid-mediated cyclooxygenase inhibition, the increased concentrations of malondialdehyde-like material during fish oil feeding may be derived from this source.

The production of PGF₂α-like activity from vessel walls after fish oil feeding has also been examined.134 Incubated segments of rat aorta produced less platelet inhibitory activity (presumed to be PGF₂α) in animals ingesting cod liver oil compared to sunflower seed oil. In addition, the production of prostaglandin 6-keto-F₁₂o (the stable, inactive breakdown product of PGF₂α) was decreased in the fish-oil-
treated animals as was \( \Delta^{17-6}\)-keto-F\(_{10}\) (the breakdown product of PG\(\mathrm{I}_2\)). It seems clear that feeding of fish oils containing \( \omega-3 \) fatty acids leads to a reduction in thromboxane \( \mathrm{A}_2 \) production by platelets, and there may also be a reduction in prostacyclin-like activity from the walls of blood vessels.

Biochemical explanations for these findings have been sought by a number of in vitro experiments using purified fatty acids and specific assays for prostaglandin metabolites. For example, it has been shown that eicosapentaenoic acid will not induce platelet aggregation when added to platelet-rich plasma and will inhibit the second wave of platelet aggregation that follows the addition of adenosine diphosphate.\(^7\)

One explanation for the reduced formation of thromboxane \( \mathrm{B}_2 \) from platelets enriched in eicosapentaenoic acid may involve competitive inhibition of cyclooxygenase by eicosapentaenoic acid which leads to reduced thromboxane \( \mathrm{A}_2 \) synthesis from arachidonic acid (figure 6).\(^{174, 175}\) In addition, eicosapentaenoic acid has been shown to be a relatively poor substrate for cyclooxygenase, and consequently only very small amounts of thromboxane \( \mathrm{A}_3 \) (a weak platelet aggregating substance) are produced.\(^{174, 176}\) It is also possible that prostaglandins of the D series (e.g., PGD\(_3\)) may be formed from eicosapentaenoic acid and that these may also inhibit platelet aggregation.\(^{177}\)

Several other hypotheses for the decreased platelet responsiveness that follows \( \omega-3 \) fatty acid ingestion are possible. These include the blockade of thromboxane receptors on platelets\(^{178}\) by eicosapentaenoic acid, replacement of arachidonic acid by eicosapentaenoic acid in platelet phospholipids, with a concomitant reduction in the amount of arachidonate available for prostaglandin synthesis, or an inhibitory effect of eicosapentaenoic acid on phospholipase \( \mathrm{A}_2 \) leading to a reduced release of arachidonic acid from platelet phospholipids.

The effects of eicosapentaenoic acid on prostaglandin production by endothelial cells has received less attention. Preliminary evidence indicates that both PG\(\mathrm{I}_2\) and PG\(\mathrm{I}_3\) are decreased, although some platelet inhibitory activity may still be present.\(^{179}\) If it should be conclusively shown that vascular PG\(\mathrm{I}_2\) as well as platelet thromboxane production is diminished, then further studies on the magnitude of these decreases will be required. Since the bleeding time is prolonged in humans fed fish oil, it may be that there is a relatively greater decrease in thromboxane generation than in prostacyclin production.

In conclusion, ingestion of dietary fish oils containing the \( \omega-3 \) fatty acid eicosapentaenoic acid may have rather profound effects on platelet or vessel composition and function. Cellular phospholipid concentrations of arachidonic acid are decreased, bleeding times are prolonged, and various in vitro tests of platelet function are inhibited. One explanation for the platelet inhibition may be the significant reduction in platelet thromboxane synthesis, which has been repeatedly demonstrated. More data are

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**Figure 6.** Effects of eicosapentaenoic acid (EPA) on platelet prostaglandin synthesis. Possible sites of action upon the conversion of arachidonic acid to thromboxane \( \mathrm{A}_2 \).
needed on the vascular production of prostacyclin and particularly on the balance between thromboxane and PGI₂ under various experimental or clinical conditions in vivo.

**Possible Risks from Increased Amounts of Polyunsaturated Fat in the Diet**

While the evidence is reasonably good that a reduction of risk factors for atherosclerotic disease might occur if the dietary intake of polyunsaturated fats was increased, we must consider also the potential risks that could be associated with this dietary alteration. The discussion that follows will examine the possible untoward effects of both the ω-6 and ω-3 polyunsaturated fatty acids.

**ω-6 Fatty Acids: Safety and Side Effects**

There is no historical precedent that attests to the safety of a diet high in linoleic acid. For most of humankind, prior to the advent of 20th century technology, the total fat content of the diet has ranged from 8% to 12% of the total calories and linoleic acid constituted from 4% to 8% of the total calories, which has been the typical intake of most Americans.¹⁸⁰ These historical observations indicate that there is no evidence available concerning the long-term beneficial or harmful effects of consuming diets that might contain up to 15%–25% of the total calories from linoleic acid. In addition, studies in present-day populations consuming both high and low fat diets have revealed that the linoleic acid content of adipose tissue is very similar worldwide.¹⁸¹,¹⁸² Since linoleic acid in adipose tissue can only be derived from the diet, this finding implies that the typical intake in America is usual around the world.

Possible harmful effects of dietary ω-6 fatty acids include enhanced formation of cholesterol gallstones, a stimulus to carcinogenesis, increased vitamin E requirements, promotion of obesity, increased uptake of plant sterols, and increased cholestrol absorption. In a long-term clinical trial, the incidence of gallstones in autopsied individuals whose long-term diet had contained a large amount of polyunsaturated fat was significantly increased over a control group.¹⁸³ However, this finding was not confirmed in another study.¹⁸⁴ An indication that polyunsaturated fat might produce more lithogenic bile (or at least a change in bile composition) has been provided in a metabolic study in humans.⁵² Furthermore, in humans the increased excretion of bile acids and neutral steroids in the stool after polyunsaturated fat feeding might also imply a change in bile composition.

An increased incidence of malignant neoplasias in individuals who consumed a diet high in polyunsaturated fat has been reported in one study but not in others¹⁸⁵–¹⁸⁷ (see review by Enig, et al.¹⁸⁸). Nevertheless, the potential threat of enhanced carcinogenesis must still be considered. Theoretically, such an effect could be promoted by an increased accumulation of peroxides from linoleic acid, which may enhance the production of carcinogens.

With regard to weight control, use of large quantities of polyunsaturated vegetable oils in the diet poses a serious problem because fat is the most calorically dense food. A high-fat diet thus becomes a major contributor to the excessive calories ingested by the obese. Weight control implies the reduction of fat intake. Such a reduction involves little nutritional loss since fat is a foodstuff that provides few nutrients other than calories. The artificial addition of tablespoons of polyunsaturated oils into many recipes or taking it "straight" cannot be justified in weight-control diets. In fact, a most successful weight-reduction diet is the Kempner rice-fruit diet, which is extremely low in fat, high in bulk, and hypolipidemic.

**ω-3 Fatty Acids: Safety and Side Effects**

There are several aspects of dietary fish oils that may be of possible concern. Some fish oils contain high levels (about 10%) of cetoleic acid (C₂₂:₁ ω-11), which is an isomer of erucic acid (C₂₂:₁ ω-9). Erucic acid is found in rapeseed oil and other *Brassica*-derived oils, and, when fed in high levels, is known to cause transient myocardial lipidosis and fibrosis in several species of experimental animals (see FAO review¹⁸⁹). However, erucic acid (not to mention cetoleic acid) has never been shown to have detrimental effects in humans. Eskimo populations have consumed diets high in cetoleic acid for centuries, and no myocardial damage has been reported. In human subjects fed fish oils experimentally, this particular fatty acid has not even been detected in the plasma. This would suggest that in humans cetoleic acid is either very poorly absorbed or is rapidly metabolized.¹⁸³ Therefore, there is little evidence to suggest that the cetoleic acid found in fish oils has any detrimental effects in humans.

Another possible side effect of feeding high levels of fish oils has been reported in pigs fed mackerel oil⁸³ or whale oil.¹⁹¹ These animals developed a disorder known as "yellow fat disease," which has been associated with vitamin E deficiency. No symptoms other than discoloration of the adipose tissue were noted, and growth was normal. This condition has not been reported in Eskimos nor in any other species of experimental animals. Because of the highly unsaturated nature of the ω-3 fatty acids, vitamin E requirements may indeed be increased during the consumption of fish oils as they are with polyunsaturated vegetable oils. This potential problem could be easily prevented by providing adequate amounts of vitamin E in the diet, as is commonly done in the experimental feeding of fish oils in humans.

Two effects of fish oil feeding upon platelet function may be of concern: the increased bleeding times...
and thrombocytopenia. Although in our studies the mean bleeding time increased from 6 minutes (control) to 10 minutes during the salmon oil diet, some patients with subclinical platelet defects had more dramatic prolongation. For example, one normal volunteer who had a prolonged bleeding time during the control diet (13 minutes), had a bleeding time of over 30 minutes during the salmon oil phase (Goodnight, unpublished observations). However, no clinical bleeding of any significance has been observed in any of these subjects.

A mild thrombocytopenia has been reported in most fish-oil feeding studies in humans. This has not been a cause for concern except in two subjects who transiently developed platelet counts below 100,000 (83,000 and 87,000) after 3 weeks of a diet containing 40% of the calories as salmon oil. A few days after the salmon oil diet was discontinued, the platelet counts returned to normal levels; no bleeding tendencies occurred. Such thrombocytopenia has not been observed in subjects receiving lesser amounts of salmon oil.

In summary, the expected benefits of modest increases in dietary polyunsaturated fat would far outweigh the possible risks outlined above. While linoleic acid intakes of up to 10% of the total calories would not be considered dangerous or even deleterious, only slight effects upon plasma lipid levels have been observed in subjects receiving lesser amounts of fatty acids. Platelet function may be expected. If such an increase in linoleic acid intake is accompanied by a reduction in the intakes of saturated fat and dietary cholesterol, then maximum lowering of plasma lipids might be expected.

On the other hand, the intake of ω-3 fatty acids is currently very low. Thus, the inclusion of as much as 4 to 6 g per day (1% to 2% of calories) may have substantial effects upon the plasma lipid levels, platelet functions, and bleeding times without incurring significant risk. In addition, past recommendations to limit the intake of fish high in fat content may now be inappropriate. The consumption of these foods might well be encouraged. Certainly little harm would be anticipated, and the likelihood of benefit would be great. Large amounts of fish are currently consumed by some coastal populations.

Clearly, use of fish oil supplements to substantially increase the intake of ω-3 fatty acids (particularly eicosapentaenoic acid) would not be indicated at the present time except under experimental conditions. The uncontrolled consumption of large amounts (15–50 ml/day) of fish liver oils (such as cod liver oil) should certainly be discouraged because of the high levels of vitamins A and D they contain. For example, even a relatively small amount of cod liver oil (20 ml) would provide about four times the Recommended Dietary Allowance (RDA) for each vitamin. Toxic symptoms have been reported with as little as five times the RDA of vitamin D.

On the other hand, the daily consumption of a half a pound of salmon would provide approximately 30 g of fish oil (4 to 6 g of ω-3 fatty acids) and very small amounts of the fat soluble vitamins.

Summary

The substitution of polyunsaturated fat in the diet for saturated fat has a definite hypolipidemic effect which has been demonstrated in a variety of experiments in both animals and humans. Both the ω-6 and ω-3 families of fatty acids (as present in vegetable oil and in fish oil) are hypocholesterolemic and sharply reduce the plasma concentrations of LDL. However, only the ω-3 fatty acids from fish oils have a pronounced hypotriglyceridemic effect in both normal and hypertriglyceridemic subjects. In some subjects, ω-6 fatty acids may have a mild hypotriglyceridemic action, whereas in others no demonstrable effect upon plasma triglyceride concentrations can be shown. In patients with severe hypertriglyceridemia (Type V hyperlipidemia), dietary ω-6 fatty acids may elevate the concentrations of plasma triglyceride (both chylomicrons and VLDL) and cause a recurrence of symptoms in patients with hepatosplenomegaly. On the other hand, in all trials described to date, ω-3 fatty acids in the diet from salmon oil and other fish oils have had a profound hypotriglyceridemic effect in both normal subjects and in patients with the Types V and IIb phenotypes. Whether linoleic acid would have similar hypolipidemic effects has not been adequately tested.

The hypolipidemic effect of dietary polyunsaturated fatty acids presumably operates through a change in the fatty acid composition of cell membranes and lipoproteins, particularly in the phospholipid and cholesteryl ester moieties. This membrane change is frequently associated with an increased loss of cholesterol and bile acids from the body in the form of increased fecal steroid excretion. Coincident with, or perhaps even preceding, the increased fecal steroid excretion, the rate of removal of plasma LDL increases (increased fractional catabolic rate). This is matched by a decrease in LDL synthesis in some instances.

The profound hypotriglyceridemic action of ω-3 fatty acids from fish oils remains as yet unexplained. Perhaps the synthesis of VLDL and LDL may be decreased or their clearances enhanced. A not unreasonable hypothesis is that the flux of free fatty acids from adipose tissue to liver in fish oil-fed humans may be reduced, perhaps mediated through the prostaglandin system. Omega-3 fatty acids certainly have the capacity to modify prostaglandin synthesis. Another possibility would be reduced synthesis of triglyceride in the liver.

Polyunsaturated fat has an additional antithrombotic action in the body when it is substituted for saturated fat in the diet. The antithrombotic effects may result from either the simple reduction of more thrombogenic saturated fat by substitution with polyunsaturated fat in the diet, or, alternatively, from an independent modification of platelet or vessel wall membrane function via perturbations of prostaglandin metabolism. While experimental feeding of both ω-6 and ω-3 fatty acids leads to clear inhibition of platelet aggregation in vitro, only fish oil feeding rela-
ably leads to a significant prolongation of the Ivy bleeding time in humans. These effects may reflect the changes in the fatty acids composition that occur with polyunsaturated fat feeding (vegetable or fish oils). High levels of either linoleic acid or fish oil ω-3 fatty acids appear to reduce the levels of arachidonic (oils). High levels of either linoleic acid or fish oil ω-3 fatty acids prolong bleeding times, the balance between these two compounds appears to be more favorable with fish oils than with vegetable oils. There remains a great need for studies to characterize the in vivo production of platelet thromboxane and vessel wall prostacyclin during fish oil and vegetable oil feeding.

Recommended dietary changes to fit the experimental evidence as presented in this review could be stated as follows: saturated fat in the diet, because of its thrombogenic and hyperlipidemic effects, should certainly be reduced from the current American intake of well over 20% of the total calories to less than 8% of the total calories. If dietary linoleic acid is at the level of 4% to 8% of the total calories (the present intake in the U.S. diet), there is little indication that increasing it further would result in significant health benefits. On the other hand, there are indications that benefit would be obtained and no harm produced by the ingestion of moderate amounts (4 to 8 g/day) of ω-3 fatty acids derived from fish oils. The effects of dietary polyunsaturated fatty acids of both the ω-6 and the ω-3 families will certainly continue to be the object of intensive research. The antithrombotic and hypolipidemic properties of fish oils need to be further characterized and translated into specific nutritional interventions, which may be helpful in the prevention and treatment of atherosclerotic diseases.

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Index Terms: unsaturated fatty acids • dietary fats • fish (liver) oil • atherosclerosis • hyperlipidemia • thrombosis • platelets • prostaglandins • thromboxane • prostacyclin