REVIEW OF EPIDEMIOLOGICAL AND CLINICAL EVIDENCE ON
THE ROLE OF OMEGA-3 FATTY ACIDS IN HEALTH AND DISEASE

June 1986

Quick Response Report #3

Prepared for

CENTER FOR FOOD SAFETY AND APPLIED NUTRITION
FOOD AND DRUG ADMINISTRATION
DEPARTMENT OF HEALTH AND HUMAN SERVICES
WASHINGTON, D.C. 20204

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prepared by
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FOREWORD

The Federation of American Societies for Experimental Biology (FASEB) recognizes that its resources are particularly suited to marshaling scientific expertise for review and assessment of topics in the biological and medical sciences. The Life Sciences Research Office (LSRO) was established by FASEB in 1962 as an operational arm of the Executive Director's staff to provide a means for conducting such scientific reviews and analyses. Reports of LSRO studies are based upon comprehensive literature reviews and the scientific opinions of knowledgeable investigators in specific areas of biology and medicine.

This technical report was prepared by Susan A. Klasing, Ph.D., Scientific Consultant, and Susan M. Pilch, Staff Scientist, LSRO, FASEB. It was prepared as Quick Response #3 for the Center for Food Safety and Applied Nutrition, Food and Drug Administration, in accordance with the provisions of FDA Contract No. 223-84-2059, Task Order #7. This report has been reviewed by the consultants listed in Chapter X and their viewpoints and opinions were incorporated; however, the listing of their names does not imply endorsement of the conclusions of this report.

In accordance with the policies and guidelines developed by the LSRO Advisory Committee, this report has been reviewed and approved for submission by the Chairman of the LSRO Advisory Committee. Reports prepared by LSRO do not necessarily reflect the opinion of the individual members of the FASEB constituent societies. The authors and LSRO are solely responsible for the content of this report.

June 30, 1976

Date

Kenneth D. Fisher, Ph.D.
Director
Life Sciences Research Office
SUMMARY

This report reviews evidence on the role of omega-3 fatty acids, especially those derived from fish or fish products, in human health and disease. It summarizes the results of the major epidemiological studies and clinical trials, reviews the physiological effects of these compounds, provides a discussion of their potential beneficial and deleterious effects, and identifies ongoing investigations.

Epidemiological surveys of populations consuming high amounts of fish have indicated some degree of protection against the development of cardiovascular disease. Most studies have found an inverse relationship between fish consumption and coronary heart disease mortality. The omega-3 fatty acids in fish have been presumed to be responsible for these effects, but whether other components in fish may be involved remains to be determined.

Consumption of omega-3 fatty acids results in a number of metabolic and physiological alterations. Dietary omega-3 fatty acids are incorporated into circulating lipid complexes and into membrane lipids. Most studies show a decrease in plasma triglyceride level, and many also show a decrease in plasma total cholesterol level in response to dietary treatment with fatty fish or fish oils containing omega-3 fatty acids. Some studies have demonstrated a decrease in plasma low-density and very-low-density lipoprotein concentrations. Studies on hemostasis have shown that dietary omega-3 fatty acids promote a shift to a more antiaggregatory state by influencing the balance of prostaglandin I and thromboxane A. These fatty acids also alter the balance of the leukotrienes B and B. Very slight reductions in blood pressure have also been detected following omega-3 fatty acid feeding.

Clinical trials of the use of omega-3 fatty acids to reduce serum lipid levels in patients with various genetic and induced hyperlipidemias have generally been positive. Preliminary trials suggest that these fatty acids may be useful in treatment of peripheral vascular disease, migraine, and possibly some types of hypertension; additional research is needed to confirm these possibilities.

Despite positive results in studies of several-weeks duration, the lack of long-term, systematic studies of omega-3 fatty acids prevents definitive conclusions about their effects on human health at this time. Animal studies indicate that omega-3 fatty acids may have beneficial health effects unrelated to their influence on cardiovascular disorders. Further study is required to determine the relevance of these findings to humans. Although investigators have noted few side-effects resulting from human consumption of omega-3 fatty acids, animal studies indicate the potential for several deleterious effects. Toxicological evaluation of products containing these fatty acids, especially fish oil concentrates and derivatives, is needed.
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</table>
I. INTRODUCTION

Recent epidemiological and clinical reports on potential health benefits of the consumption of omega-3 polyunsaturated fatty acids have stimulated considerable scientific and public interest. Because the Food and Drug Administration (FDA) has responsibility for assessing the safety and nutritional quality of the food supply, a request was made by the Center for Food Safety and Applied Nutrition to the Life Sciences Research Office (LSRO), Federation of American Societies for Experimental Biology (FASEB), to review the major epidemiological and clinical studies which have examined the health effects of omega-3 fatty acids. Specifically, the LSRO was asked to provide the following:

- A brief historical perspective on research interest in omega-3 fatty acids, and the omega-3 fatty acid content of commonly eaten foods.
- A review of epidemiological data on omega-3 fatty acid consumption.
- A review of clinical trials involving omega-3 fatty acid feeding.
- A summary of research studies conducted to examine physiological effects (both beneficial and harmful) of omega-3 fatty acids.
- Identification of ongoing studies.
- Conclusions on the physiological benefits and potentially harmful effects of omega-3 fatty acid consumption.

This report presents a review of relevant information on dietary omega-3 fatty acids, as requested. Names of reviewing consultants for this report are listed in Chapter X.
II. BACKGROUND ON RESEARCH

Prior to the early 1950s, relatively little was known about nutritional aspects of fat, primarily because of difficulty in analysis. Although certain fats had been deemed "essential" for pregnancy, lactation, prevention of certain skin disorders, and resistance to x-ray radiation, it was not until the development of gas-liquid chromatography that the terms omega-6 and omega-3 fatty acids came into use (Reed, 1984). While omega-3 fatty acids enabled animals to grow, only omega-6 fatty acids could prevent dermal symptoms; thus, omega-3 fatty acids were not considered to meet the full requirements for essentiality and were not studied in great detail. The typical structures and characteristics of the major omega-6 (n-6) and omega-3 (n-3) polyunsaturated fatty acids (PUFA)* are given in Table 1.

The richest dietary sources of omega-3 fatty acids are fish oils and the lipids of higher marine animals. The omega-3 fatty acids of marine animals are predominantly 20- and 22-carbon fatty acids, which are synthesized by marine plants, and which vary in marine animals depending upon physiological and environmental conditions as well as diet (Dyerberg, 1986). Terrestrial food chains are dominated by the omega-6 family of PUFA. The fatty acids of land plants are not chain-elongated above the 18-carbon level by animals; thus, the PUFA found in edible plant oils and animal muscle meat are primarily linoleic acid (C18:2ω6), with a small amount of linolenic acid (C18:3ω3) (Dyerberg, 1986). The content of the major omega-3 fatty acids in some selected foods is shown in Table 2. These data on fatty acid composition were compiled by the Nutrient Data Research Branch of the U.S. Department of Agriculture's (USDA) Human Nutrition Information Service. Many of the data were published in a series of 13 articles under the heading "Comprehensive Evaluation of Fatty Acids in Foods" in the Journal of the American Dietetic Association in 1973 to 1975. They have also been entered into the USDA Nutrient Data Bank. Additional sources of data on the fatty acid composition of fish oils include Ackman (1967) and Gruger (1967). As indicated in Table 3, the omega-3 fatty acid content of human milk is relatively low, but can be influenced by dietary intake.

Early research in PUFA nutrition concentrated heavily on its relationship to high blood cholesterol levels and coronary heart disease (CHD). Hypercholesterolemia and hyperlipidemia were once postulated to be the result of essential fatty acid deficiency. This theory led to work examining the effect of fish oil on human serum lipids. Ahrens et al. (1959) fed an iso-caloric liquid diet (described in Ahrens et al., 1954) containing

* See Appendix for list of acronyms.
Table 1. Omega-6 and omega-3 polyunsaturated fatty acids*

<table>
<thead>
<tr>
<th></th>
<th>ω-6 Fatty Acids</th>
<th>ω-3 Fatty Acids</th>
</tr>
</thead>
<tbody>
<tr>
<td>Parent fatty acid</td>
<td>C18:2ω6 linoleic acid</td>
<td>C18:3ω3 linolenic acid</td>
</tr>
<tr>
<td>Major metabolites</td>
<td>C20:4ω6 arachidonic acid</td>
<td>C20:5ω3 eicosapentaenoic acid (EPA)</td>
</tr>
<tr>
<td></td>
<td></td>
<td>C22:6ω3 docosahexaenoic acid (DHA)</td>
</tr>
<tr>
<td>Characteristic** structure</td>
<td>H₃C-C-C-C-C-C=C-RCOOH</td>
<td>H₃C-C-C=C-R′COOH</td>
</tr>
<tr>
<td>Principal sources</td>
<td>Many vegetable oils</td>
<td>Some vegetable oils, leaves (18:3)</td>
</tr>
<tr>
<td></td>
<td></td>
<td>Marine oils (20:5, 22:6)</td>
</tr>
</tbody>
</table>

* Adapted from Goodnight et al. (1982). The correct chemical designations for these two classes of polyunsaturated fatty acids are n-6 and n-3. Because the terms omega-6 and omega-3 are widely used in the nutrition literature, they will be used in this report.

** The classes of fatty acids are named on the basis of the position of the first double bond from the terminal methyl group, as indicated in the abbreviated structures shown.
Table 2. Omega-3 fatty acid content of some selected foods*

<table>
<thead>
<tr>
<th>Food item</th>
<th>Total PUFA</th>
<th>C18:3</th>
<th>C20:5</th>
<th>C22:6</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>g/100 g</td>
<td>g/100 g</td>
<td>g/100 g</td>
<td>g/100 g</td>
</tr>
<tr>
<td><strong>Finfish</strong></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Anchovy, European</td>
<td>1.6</td>
<td>--</td>
<td>0.5</td>
<td>0.9</td>
</tr>
<tr>
<td>Catfish, channel</td>
<td>1.0</td>
<td>Tr</td>
<td>0.1</td>
<td>0.2</td>
</tr>
<tr>
<td>Cod, Atlantic</td>
<td>0.3</td>
<td>Tr</td>
<td>0.1</td>
<td>0.2</td>
</tr>
<tr>
<td>Cod, Pacific</td>
<td>0.2</td>
<td>Tr</td>
<td>0.1</td>
<td>0.1</td>
</tr>
<tr>
<td>Haddock</td>
<td>0.2</td>
<td>Tr</td>
<td>0.1</td>
<td>0.1</td>
</tr>
<tr>
<td>Halibut, Greenland</td>
<td>1.4</td>
<td>Tr</td>
<td>0.5</td>
<td>0.4</td>
</tr>
<tr>
<td>Halibut, Pacific</td>
<td>0.7</td>
<td>Tr</td>
<td>0.1</td>
<td>0.3</td>
</tr>
<tr>
<td>Herring, Atlantic</td>
<td>2.1</td>
<td>0.1</td>
<td>0.7</td>
<td>0.9</td>
</tr>
<tr>
<td>Herring, Pacific</td>
<td>2.4</td>
<td>0.1</td>
<td>1.0</td>
<td>0.7</td>
</tr>
<tr>
<td>Herring, round</td>
<td>1.5</td>
<td>0.1</td>
<td>0.4</td>
<td>0.8</td>
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<tr>
<td>Mackerel, Atlantic</td>
<td>3.7</td>
<td>0.1</td>
<td>0.9</td>
<td>1.6</td>
</tr>
<tr>
<td>Salmon, Atlantic</td>
<td>2.1</td>
<td>0.2</td>
<td>0.3</td>
<td>0.9</td>
</tr>
<tr>
<td>Salmon, chinook</td>
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<td>0.1</td>
<td>0.8</td>
<td>0.6</td>
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<tr>
<td>Sturgeon, Atlantic</td>
<td>2.1</td>
<td>Tr</td>
<td>1.0</td>
<td>0.5</td>
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<tr>
<td>Trout, brook</td>
<td>0.9</td>
<td>0.2</td>
<td>0.2</td>
<td>0.2</td>
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<tr>
<td>Trout, lake</td>
<td>3.4</td>
<td>0.4</td>
<td>0.5</td>
<td>1.1</td>
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<td>Tuna, albacore</td>
<td>1.8</td>
<td>0.2</td>
<td>0.3</td>
<td>1.0</td>
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<tr>
<td>Tuna, unspecified</td>
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<td>--</td>
<td>0.1</td>
<td>0.4</td>
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<td><strong>Crustaceans</strong></td>
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<td></td>
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<tr>
<td>Crab, Alaska king</td>
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<td>Tr</td>
<td>0.2</td>
<td>0.1</td>
</tr>
<tr>
<td>Lobster, northern</td>
<td>0.2</td>
<td>--</td>
<td>0.1</td>
<td>0.1</td>
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<tr>
<td>Shrimp, Atlantic white</td>
<td>0.6</td>
<td>Tr</td>
<td>0.2</td>
<td>0.2</td>
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<tr>
<td><strong>Mollusks</strong></td>
<td></td>
<td></td>
<td></td>
<td></td>
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<tr>
<td>Clam, hardshell</td>
<td>0.1</td>
<td>Tr</td>
<td>Tr</td>
<td>Tr</td>
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<tr>
<td>Conch, unspecified</td>
<td>1.1</td>
<td>Tr</td>
<td>0.6</td>
<td>0.4</td>
</tr>
<tr>
<td>Mussel, blue</td>
<td>0.6</td>
<td>Tr</td>
<td>0.2</td>
<td>0.3</td>
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<tr>
<td>Oyster, eastern</td>
<td>0.7</td>
<td>Tr</td>
<td>0.2</td>
<td>0.2</td>
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<tr>
<td>Scallop, Atlantic deepsea</td>
<td>0.3</td>
<td>Tr</td>
<td>0.1</td>
<td>0.1</td>
</tr>
</tbody>
</table>

* Adapted from "Provisional Tables on the Content of Omega-3 Fatty Acids and Other Fat Components in Selected Foods" (Hepburn et al., 1986).
Table 2. (continued)

<table>
<thead>
<tr>
<th>Food item</th>
<th>Total PUFA</th>
<th>C18:3</th>
<th>C20:5</th>
<th>C22:6</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>g/100 g</td>
<td>g/100 g</td>
<td>g/100 g</td>
<td>g/100 g</td>
</tr>
<tr>
<td><strong>Fish Oils</strong></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Cod liver oil</td>
<td>25.8</td>
<td>0.7</td>
<td>9.0</td>
<td>9.5</td>
</tr>
<tr>
<td>Herring oil</td>
<td>16.1</td>
<td>0.6</td>
<td>7.1</td>
<td>4.3</td>
</tr>
<tr>
<td>Menhaden oil</td>
<td>29.5</td>
<td>1.1</td>
<td>12.7</td>
<td>7.9</td>
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<tr>
<td>MaxEPA™ concentrated fish body oils</td>
<td>41.1</td>
<td>0</td>
<td>17.8</td>
<td>11.6</td>
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<tr>
<td>Salmon oil</td>
<td>29.9</td>
<td>1.0</td>
<td>8.8</td>
<td>11.1</td>
</tr>
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<td><strong>Legumes</strong></td>
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<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Bean, common, dry</td>
<td>0.9</td>
<td>0.6</td>
<td>--</td>
<td>--</td>
</tr>
<tr>
<td>Lentils, dry</td>
<td>0.5</td>
<td>0.1</td>
<td>--</td>
<td>--</td>
</tr>
<tr>
<td>Soybeans, dry</td>
<td>12.3</td>
<td>1.6</td>
<td>--</td>
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<td><strong>Nuts and Seeds</strong></td>
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<td>Beechnuts, dried</td>
<td>20.1</td>
<td>1.7</td>
<td>--</td>
<td>--</td>
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<tr>
<td>Hickory nuts, dried</td>
<td>21.9</td>
<td>1.0</td>
<td>--</td>
<td>--</td>
</tr>
<tr>
<td>Walnuts, black</td>
<td>37.5</td>
<td>3.3</td>
<td>--</td>
<td>--</td>
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<tr>
<td><strong>Meats</strong></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Beef, ground, regular, raw</td>
<td>1.0</td>
<td>0.2</td>
<td>--</td>
<td>--</td>
</tr>
<tr>
<td>Chicken, dark meat, without skin, raw</td>
<td>1.0</td>
<td>Tr</td>
<td>--</td>
<td>--</td>
</tr>
<tr>
<td>Chicken, light meat, without skin, raw</td>
<td>0.4</td>
<td>Tr</td>
<td>Tr</td>
<td>Tr</td>
</tr>
<tr>
<td>Lamb, leg, raw</td>
<td>1.0</td>
<td>0.3</td>
<td>--</td>
<td>--</td>
</tr>
<tr>
<td>Pork, cured, bacon, raw</td>
<td>6.8</td>
<td>0.8</td>
<td>--</td>
<td>--</td>
</tr>
<tr>
<td>Pork, fresh, ham, raw</td>
<td>2.2</td>
<td>0.2</td>
<td>--</td>
<td>--</td>
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<tr>
<td><strong>Fruits</strong></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Avocados, California, raw</td>
<td>2.0</td>
<td>0.1</td>
<td>--</td>
<td>--</td>
</tr>
<tr>
<td>Raspberries, raw</td>
<td>0.3</td>
<td>0.1</td>
<td>--</td>
<td>--</td>
</tr>
<tr>
<td>Strawberries, raw</td>
<td>0.2</td>
<td>0.1</td>
<td>--</td>
<td>--</td>
</tr>
</tbody>
</table>
Table 2. (continued)

<table>
<thead>
<tr>
<th>Food item</th>
<th>Total PUFA</th>
<th>C18:3</th>
<th>C20:5</th>
<th>C22:6</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>g/100 g</td>
<td>g/100 g</td>
<td>g/100 g</td>
<td>g/100 g</td>
</tr>
<tr>
<td><strong>Vegetables</strong></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Beans, navy, sprouted, cooked</td>
<td>0.5</td>
<td>0.3</td>
<td>--</td>
<td>--</td>
</tr>
<tr>
<td>Broccoli, raw</td>
<td>0.2</td>
<td>0.1</td>
<td>--</td>
<td>--</td>
</tr>
<tr>
<td>Lettuce, butterhead, raw</td>
<td>0.1</td>
<td>0.1</td>
<td>--</td>
<td>--</td>
</tr>
<tr>
<td>Spinach, raw</td>
<td>0.1</td>
<td>0.1</td>
<td>--</td>
<td>--</td>
</tr>
<tr>
<td><strong>Cereal Grains</strong></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Corn, germ</td>
<td>18.0</td>
<td>0.3</td>
<td>--</td>
<td>--</td>
</tr>
<tr>
<td>Oats, germ</td>
<td>12.4</td>
<td>1.4</td>
<td>--</td>
<td>--</td>
</tr>
<tr>
<td>Rice, bran</td>
<td>6.6</td>
<td>0.2</td>
<td>--</td>
<td>--</td>
</tr>
<tr>
<td>Wheat, bran</td>
<td>2.4</td>
<td>0.2</td>
<td>--</td>
<td>--</td>
</tr>
<tr>
<td>Wheat, germ</td>
<td>6.6</td>
<td>0.7</td>
<td>--</td>
<td>--</td>
</tr>
<tr>
<td><strong>Dairy and Egg Products</strong></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Cheese, cheddar</td>
<td>0.9</td>
<td>0.4</td>
<td>--</td>
<td>--</td>
</tr>
<tr>
<td>Milk, whole</td>
<td>0.1</td>
<td>0.1</td>
<td>--</td>
<td>--</td>
</tr>
<tr>
<td>Egg yolk, chicken, raw</td>
<td>4.3</td>
<td>0.1</td>
<td>--</td>
<td>--</td>
</tr>
<tr>
<td><strong>Fats and Oils</strong></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Butter</td>
<td>3.0</td>
<td>1.2</td>
<td>--</td>
<td>--</td>
</tr>
<tr>
<td>Lard</td>
<td>11.2</td>
<td>1.0</td>
<td>--</td>
<td>--</td>
</tr>
<tr>
<td>Linseed oil</td>
<td>66.0</td>
<td>53.3</td>
<td>--</td>
<td>--</td>
</tr>
<tr>
<td>Margarine, hard, soybean</td>
<td>20.9</td>
<td>1.5</td>
<td>--</td>
<td>--</td>
</tr>
<tr>
<td>Margarine, liquid, soybean (hydrog.),</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>soybean and cottonseed</td>
<td>35.8</td>
<td>2.4</td>
<td>--</td>
<td>--</td>
</tr>
<tr>
<td>Margarine, soft, soybean (hydrog.) and palm</td>
<td>34.6</td>
<td>1.9</td>
<td>--</td>
<td>--</td>
</tr>
<tr>
<td>Rapeseed oil (Canola)</td>
<td>33.3</td>
<td>11.1</td>
<td>--</td>
<td>--</td>
</tr>
<tr>
<td>Shortening, household, lard and veg. oil</td>
<td>10.9</td>
<td>1.1</td>
<td>--</td>
<td>--</td>
</tr>
<tr>
<td>Soybean oil</td>
<td>57.9</td>
<td>6.8</td>
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<td>--</td>
</tr>
<tr>
<td>Wheat germ oil</td>
<td>61.7</td>
<td>6.9</td>
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</tr>
</tbody>
</table>
Table 3. Effects of dietary fish oil on the content of omega-3 fatty acids in human milk*

<table>
<thead>
<tr>
<th>Fatty Acids</th>
<th>Dietary Fish Oil (%)</th>
<th>Baseline</th>
<th>Human Milk (%)</th>
<th>4 wk at 5 g/day</th>
<th>2 wk at 10 g/day</th>
<th>8 days at 47 g/day</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>n=8</td>
<td>n=6</td>
<td>n=5</td>
<td>n=1</td>
<td></td>
<td></td>
</tr>
<tr>
<td>C18:3ω3</td>
<td>0.5</td>
<td>0.8 ± 0.5</td>
<td>1.0 ± 0.8</td>
<td>1.0 ± 0.2</td>
<td>0.8</td>
<td></td>
</tr>
<tr>
<td>C20:5ω3</td>
<td>17.1</td>
<td>Tr</td>
<td>0.3 ± 0.15</td>
<td>0.5 ± 0.2</td>
<td>1.8</td>
<td></td>
</tr>
<tr>
<td>C22:5ω3</td>
<td>2.6</td>
<td>Tr</td>
<td>0.2 ± 0.09</td>
<td>0.4 ± 0.1</td>
<td>0.8</td>
<td></td>
</tr>
<tr>
<td>C22:6ω3</td>
<td>10.7</td>
<td>0.1 ± 0.1</td>
<td>0.5 ± 0.09</td>
<td>0.8 ± 0.2</td>
<td>1.9</td>
<td></td>
</tr>
<tr>
<td>Total ω3 &gt;C20</td>
<td>31</td>
<td>0.24 ± 0.06</td>
<td>1.05 ± 0.32</td>
<td>1.7 ± 0.43</td>
<td>4.5</td>
<td></td>
</tr>
</tbody>
</table>

* Adapted from Harris et al. (1984).
either menhaden oil or corn oil as the sole source of dietary fat to two patients, one with hypercholesterolemia and one with hyperlipidemia. The authors hypothesized that menhaden oil, with its low content of essential fatty acids, would raise serum cholesterol levels in these patients, with corn oil having the opposite effect. As expected, the corn oil diet significantly lowered serum cholesterol, phospholipid, and triglyceride levels in both patients when compared to an ad libitum diet. However, when menhaden oil was substituted for corn oil, blood lipid measurements were further decreased in the patient with hyperlipidemia and remained depressed in the patient with hypercholesterolemia. The fatty acid composition of serum phospholipids, cholesterol esters, and triglycerides changed dramatically in response to diet, reflecting fatty acid patterns of the dietary lipid source.

Ahrens and coworkers (1959) supported earlier evidence regarding hypocholesterolemic effects of different fish oils. Bronte-Stewart et al. (1956) maintained volunteers with initial low serum cholesterol levels on a cholesterol-free, low-fat diet (not more than 3% of total calories). Various dietary fats and oils were added to the diet individually to increase the proportion of calories derived from fat to 35, 45, or 60-70%. Supplementation of the diet with marine oils was expected to increase serum cholesterol as suggested by epidemiological data concerning consumption of fats of animal origin. However, pilchard oil and seal oil depressed serum cholesterol levels below those of the low-fat basal diet. Pilchard oil was also more efficacious than sunflower-seed oil in countering the hypercholesterolemic effect of 10 eggs per day.

Malmros and Wigand (1957) argued that the hypocholesterolemic effect of pilchard oil might be the result of its relatively high content of linoleic acid, an essential fatty acid. Thus, experiments using nine healthy volunteers were performed using nonhydrogenated whale oil, essentially devoid of this fatty acid. However, replacement of hydrogenated coconut oil with nonhydrogenated whale oil caused a decrease in total serum cholesterol below levels found during the initial free-choice diet. Effects were postulated to be the result of another essential fatty acid found in whale oil, arachidonic acid.

The theory of Sinclair (1956) that coronary heart disease was absent in Eskimos and rare in Norwegians because of their consumption of fish oils was termed "imaginative" by Keys et al. (1957) who compared corn, sunflower, and fish oil for their cholesterol-lowering effect. Subjects on a metabolic ward were fed a controlled diet differing only in the source of fat. All three oils resulted in lower serum cholesterol values than a butter fat control, with corn oil the most hypocholesterolemic. The investigators concluded that the data did not support the hypothesis of an inverse relationship between essential fatty acid content or degree of unsaturation of the fatty acids in the diet and serum cholesterol levels.
Kingsbury et al. (1961), in studies with small groups of students, demonstrated that 25 g of cod liver oil was more effective in lowering blood cholesterol than an equal amount of corn oil. In subsequent studies (Kingsbury et al., 1962), dietary unsaturated fats from cod liver oil were shown to appear rapidly in plasma lipids. The authors noted that changes in dietary fat were not necessarily reflected by similar alterations in depot fats; however, Hegsted et al. (1962) reported that individuals consuming large amounts of fish had higher than average amounts of C20 fatty acids in adipose tissue.

Noting the plasma lipid-decreasing effect of menhaden oil established by Ahrens et al. (1959), Kinsell et al. (1961) attempted to replicate this response in mildly diabetic patients. Menhaden oil was fractionated to obtain the ethyl esters ("Escambia oil") and these were substituted for butter fat in the diet isocalorically. In doses of 8 to 30 g/day, Escambia oil had greater ability to decrease plasma lipids compared with similar amounts of ethyl linoleate or vegetable fats evaluated in previous experiments.

By 1965, research with marine oils had established several facts: the hypocholesterolemic effect of marine oils was associated with their PUFA content; the structure and nature of these PUFA had been identified; 5- and 6-double bonded fatty acids could accumulate in blood and vascular tissues; and, the PUFA fraction of marine oils promoted a favorable blood cholesterol:phospholipid ratio (Reed, 1984).
III. REVIEW OF EPIDEMIOLOGICAL DATA

In the early 1970s, expeditions were made to western Greenland in an attempt to explain the observed low incidence of heart disease and diabetes mellitus in Greenlandic Eskimos. The diet of this population consisted predominantly of animal meat. The positive correlations among consumption of animal fat, serum cholesterol levels, and atherosclerotic disease were well known at this time; thus, findings from this region seemed inconsistent with the then current scientific theories.

Bang et al. (1971) examined 61 male and 69 female Eskimos from eight settlements in a western, coastal district of Greenland. Data from this study were discussed further in Bang and Dyerberg (1972). All participating individuals were over 30 years of age and were hunters and/or fishermen and their wives. Blood samples were drawn following a 10-hour fast and plasma was separated for subsequent determination of total lipids, cholesterol, and triglycerides. Plasma lipoprotein electrophoresis was performed within 12 hours of blood collection. Data were grouped according to age and sex and compared with appropriate healthy Danish controls. Statistical analyses of the frequency distribution were performed by testing the fitness of the values to the normal distribution by the Kolmogorov-Smirnov one-sample test, before and after logarithmic transformation of the values. Eskimo and Danish control values were compared using Student's t-test after logarithmic transformation.

Overall, plasma levels of total lipids, cholesterol, and triglycerides were lower in Eskimos than in Danes (p <0.001). The only comparisons for age-sex groups that did not reach statistical significance were total lipids and cholesterol in males aged 31 through 40 years. Differences between Eskimos and Danes in all lipid fractions increased with increasing age. Pre-beta-lipoprotein levels were lower (p <0.001) in Eskimos of all age-sex groups than in Danish controls. Beta-lipoprotein levels were also lower in most Eskimos although these differences were not significant in males and females aged 31 through 40 years. Alpha-lipoproteins were higher in Eskimo males versus Danish males (p <0.001), but were similar in both female populations. The authors attributed lower plasma lipid fractions in Eskimos to the high degree of polyunsaturation in the animal meat consumed, although knowledge of exact fatty acid composition of this meat was limited. The authors also acknowledged that, although striking, the relationship between low plasma lipids and low incidence of ischemic heart disease in the Greenlandic Eskimo population was not necessarily causal.

In an attempt to determine whether the cause of decreased blood lipids in Eskimos was genetic or environmental, the blood lipid pattern of 25 female Eskimos living in Denmark was compared with Greenlandic Eskimos and Danish controls (Bang
and Dyerberg, 1972; Bang et al., 1971). Plasma levels of total lipids, cholesterol, triglycerides, beta-lipoproteins, and pre-beta-lipoproteins were higher in Eskimo women living in Denmark than Greenlandic Eskimos (p <0.025 to 0.001) and were similar to Danes. Additionally, Greenlandic Eskimos were divided into "pure" Eskimos and "mixed types", which were typically of both Eskimo and Scandinavian descent. All blood lipid fractions were similar in these two groups with the exception of beta-lipoproteins, which were higher in the "mixed group" (p <0.025). These results were interpreted as favoring an environmental rather than genetic role in the determination of blood lipids in Greenlandic Eskimos.

In a subsequent paper, the plasma fatty acid composition of individuals evaluated in the previous study was reported (Dyerberg et al., 1975). Statistical comparisons between medians were made by the Mann-Whitney rank-sum test. A Gaussian distribution of rank-sums was assumed. The differences in esterified plasma fatty acid composition between Eskimos living in Denmark and Danish controls were relatively small; however, differences were marked when Greenlandic Eskimos were compared with Eskimos and non-Eskimos living in Denmark. Palmitic, palmitoleic, oleic, and eicosapentaenoic acid concentrations in cholesterol esters were higher in Greenlandic Eskimos while linoleic acid levels were lower. Palmitoleic, eicosaenoic, and eicosapentaenoic acid concentrations of triglycerides were higher in Greenlandic Eskimos while triglyceride oleic and linoleic acids were lower in this population. Palmitic and stearic acid levels were slightly higher in phospholipids of Greenland Eskimos while eicosaenoic and eicosapentaenoic acid levels were markedly higher in this group. Linoleic acid content of phospholipids was greatly lower while arachidonic acid was moderately lower in phospholipids of Eskimos residing in Greenland. In contrast to expected plasma lipid composition given the low incidence of atherosclerotic heart disease in Greenland Eskimos, total saturated fatty acid concentration in plasma cholesterol esters and phospholipids was higher in this group while total PUFA concentration was lower compared to Danish controls. The authors speculated that quantitative dietary differences, particularly the high level of eicosapentaenoic acid (EPA), were responsible for differences in plasma lipid and lipoprotein concentrations and in the incidence of coronary atherosclerosis.

A pilot study of food intake data was also conducted on a subset of the previously described population (Bang et al., 1976). Seven individuals, five males and two females, were selected to approximate normal intake in one of the eight settlements studied. These persons also participated in serum lipid analyses. Daily food samples were obtained from each person for all meals using the double-portion technique of Keys and Kimura (1970). Additionally, subjects were interviewed daily regarding food consumption of the previous day. The water, protein, fat, salts (ash), carbohydrate, cholesterol, and fatty acid composition of Eskimo meals was determined and compared to average
Danish food values. Statistical methods were not reported. Dietary intakes of protein, fat, and carbohydrate were 26 vs. 11%, 37 vs. 42%, and 37 vs. 47% for Greenland Eskimos vs. previously determined Danish food values, respectively. Fatty acid content of the diet was reflected in the fatty acid composition of the blood, with palmitoleic, eicosapentaenoic, and docosahexaenoic acids occurring more frequently and linoleic acid less often in Eskimo food and blood lipids than in Danish controls. The substantially lower blood cholesterol levels of Greenland Eskimos (Bang and Dyerberg, 1972) were not predicted by the Keys Formula, used to determine the influence of fatty acid intake on serum cholesterol level. The authors concluded that the longer chain PUFA consumed by Greenlandic Eskimos may be of particular importance in explaining decreased blood cholesterol levels. Additionally, the authors speculated that the even more profound decreases in blood triglycerides and beta-lipoproteins may also be elicited by the longer chain PUFA and that these blood lipid fractions may be related to low incidence of coronary occlusions exhibited by Greenland Eskimos.

The composition of Eskimo food in western Greenland was examined further in 1976 by Bang et al. (1980). Twenty-five females and 25 males from this area, aged 20 to 76 years, participated in a dietary intake study for 3 to 7 consecutive days. The double-portion technique of Keys and Kimura (1970) was used to obtain 178 daily food samples. Food intake data obtained was similar to Bang et al. (1976); protein, carbohydrate, and fat intakes of Eskimos were within a few percentage points of those reported earlier. Daily cholesterol intake was 264 mg/1000 kcal for Eskimos versus 138 mg/1000 kcal for Danish controls. As a percent of total fatty acids, saturated fatty acid intake was markedly lower, and polyunsaturated and monounsaturated fatty acid consumption of Eskimos was higher than average Danish food intake values. Intake of polyunsaturated fatty acids was predominantly of the linolenic (omega-3) class.

Because of the epidemiological data on the dietary habits of the Eskimos and their low incidence of ischemic heart disease, Dyerberg and Bang (1979) evaluated other blood variables which might be related to antithrombotic activity. Preliminary in vitro work indicated that EPA might inhibit platelet aggregation (Dyerberg and Bang, 1978); therefore, in vivo hemostasis of Greenland Eskimos was investigated (Dyerberg and Bang, 1979). Twenty-four adult volunteers were selected from a population of 50 adults who had been determined to have typical Eskimo dietary habits. The study group consisted of 10 females and 14 males, aged 21 to 77 years. Blood values were compared with 21 healthy age- and sex-matched Danish controls. Bleeding time was compared using the Wilcoxon matched-pair test and found to be longer in Eskimos than Danish controls (8.1 vs. 4.8 min., p <0.01).

Eskimos also showed a higher threshold for ADP-induced secondary-phase platelet aggregation (sign test, p <0.01) and lower platelet counts (Wilcoxon matched-pair test, p <0.01). In
10 of the 24 Eskimos, secondary-phase aggregation could not be induced; however, arachidonic acid induced a normal aggregation response in platelet-rich plasma in these individuals. Thus, the authors concluded that the ability to synthesize prosta-
glandins was probably normal. Platelet adhesiveness to glass 
beads, activated partial thromboplastin time, and two-stage 
prothrombin time were similar in both groups. Bleeding time was 
shortened in the four Eskimos who had the longest bleeding time 
when measured 24 hours after ingestion of 1.5 g acetylsalicylic acid. Although fatty acid composition of platelets could not be 
compared statistically, the ratio of omega-3 to omega-6 PUFA was 
1.38 and 0.10 for Eskimos and Danes, respectively. The C20:5ω3 
to C20:4ω6 ratio was 0.94 in Eskimos and 0.02 in Danish controls. 
The authors concluded from these results that increased bleeding 
time resulted from decreased platelet aggregation and that 
differences in hemostatic function could probably be accounted 
for by alterations in prostaglandin production. Because platelet 
counts lower than those experienced by Eskimos are required to 
substantially influence bleeding time, this variable was not 
considered to affect hemostasis in Eskimos. The investigators 
suggested that dietary modification based on these results might 
be as effective as large-scale drug use in the prevention of 
cardiovascular disease.

This recommendation was supported recently by a long-
term study conducted by Kromhout et al. (1985). In 1960, a 
longitudinal investigation of the relationship between diet, 
risk factors, and chronic disease was begun in Zutphen, the 
Netherlands. A random sample of 1088 men was selected from a 
population of 25,000. All study participants had lived in 
Zutphen for at least 5 years and were born between 1900 and 1919. 
A subset of this group consisted of 872 men between the ages of 
40 and 59 years who participated in both the medical evaluation 
and dietary survey of 1960. Typical weekday food consumption was 
estimated by the cross-check dietary history method (Burke, 1947; 
Hartog et al., 1965). Accuracy of this technique was assessed by 
sampling and chemical analysis of the diet of a group of 49 men. 
Although no one was lost to follow-up, individuals with CHD 
(criteria not stated) at the beginning of the study were excluded 
from the analyses. Thus, complete data were available for evalu-
ation of 852 men of which 78 died of CHD during the 20-year 
period. Individuals were divided according to consumption of 
fish in 1960: 0, 1-14, 15-29, 30-44, and 45 or more g/day. Risk 
ratios (plus a 95% confidence interval) were calculated for these 
groups. Analysis of variance was used to determine relationships 
between diet, risk factors, and fish consumption; the Scheffe 
method was used to test significance of contrasts. Whether a 
dose-response relationship existed between fish consumption and 
death from CHD was evaluated by a chi-square test and multiple 
logistic-regression was used to determine the independent 
contribution of fish consumption to CHD deaths. The follow-up 
The average consumption of fish by the Zutphen men was 20 g/day, while 19% of study participants did not consume fish. About two-thirds of the fish consumed consisted of lean fish (such as cod and plaice) and one-third consisted of fatty fish (such as herring and mackerel). Information on the methods of preparation of fish dishes was not supplied. Inverse relationships were found between fish intake and death from CHD in all three follow-up periods. Risk ratios tended to decrease with increasing fish consumption, although this was significant only during the 1960 to 1980 period. Fish consumption was not related to major risk factors for CHD, including age, serum cholesterol, systolic blood pressure, and cigarette smoking, nor was it related to subscapular skinfold thickness, physical activity, occupation, or energy intake. Individuals in the highest group of fish intake had higher consumption of monounsaturated and polyunsaturated fats, cholesterol, animal protein, and alcohol, and lower intake of polysaccharides than men who did not consume fish. After adjustment for risk factors, fish consumption in 1960 remained inversely related to 20-year CHD mortality (p < 0.05). Death rate from CHD was approximately 2.5 times lower in individuals who consumed 30 g or more fish per day.

From these results, the authors concluded the recommendation of one or two fish dishes per week for the prevention of CHD is justifiable. Fish consumption was shown to be an independent factor which reduced risk for CHD; however, because lean fish consumption was also inversely related to CHD, protective effects could not be attributed solely to EPA content of the diet. In agreement with other studies in which only moderate amounts of fish were consumed (Bronsgeest-Schoute et al., 1981; Fehily et al., 1983), no relationship was found between fish consumption and serum cholesterol in the Zutphen men. The authors thus speculated that the effect of fish intake on CHD was not the result of alterations of lipid or lipoprotein metabolism, but no comprehensive biological explanation for this effect could be provided.

Evidence indicating an inverse relationship between CHD and an EPA-rich diet was also collected in Chiba Prefecture, Japan (Hirai et al., 1980, 1982). Rates of hyperlipidemia and atherosclerosis traditionally have been low in Japan, reportedly a consequence of dietary habits (Goto, 1980). Two villages in Chiba Prefecture, consisting predominantly of either fishermen or farmers, were chosen for study because of their conventional way of life and stable population. In Kawazu, the fishing village, 22 females and 20 males were examined in 1980. These villagers normally consumed a high-fish diet. Residents from a farming village were age- and sex-matched to individuals from the fishing village and also examined in 1980. Food intake data were collected and calculated as an average value for 3 days of consumption. Dietary and plasma fatty acid composition, serum lipid, platelet aggregation, and blood viscosity were determined. Statistical methods were not described. Fish consumption (and consequently EPA intake) was higher in the fishing village versus
the farming village \((p < 0.001)\). Fat and protein intakes were both higher in residents of the fishing village than those of the farming village \((p < 0.05 \text{ and } p < 0.01, \text{ respectively})\). Total fatty acid concentrations in plasma lipids were similar in both groups, although plasma arachidonic acid levels and EPA:arachidonic acid ratios were higher in fishing village residents than in those of the farming village \((p < 0.05)\). Serum triglyceride level was also significantly lower in individuals from the fishing village versus those from the farming village. Plasma viscosity was comparable between groups, but ADP-induced platelet aggregation was lower \((p < 0.001)\) in residents of the fishing village. Hirai and coworkers (1980, 1982) interpreted the data as a possible explanation for the relatively low incidence of cardiovascular thrombotic disease in Japan.
IV. PHYSIOLOGICAL EFFECTS

Many alterations in mammalian physiology have been attributed to the effects of omega-3 fatty acids, particularly those associated with fish oils. While dietary linolenic acid (from plant sources) can be converted to EPA and docosahexaenoic acid (DHA) in vivo and may thus exhibit similar physiological effects, this conversion is quite slow and is hampered by high levels of linoleic acid (Harris, 1985). Human and animal studies of the physiological effects of omega-3 fatty acids and their implications have been reviewed recently by Herold and Kinsella (1986) and Dyerberg (1986).

A. BLOOD AND PLATELET FATTY ACIDS

The most frequently documented physiological change resulting from consumption of omega-3 fatty acids is the dramatic alteration in the fatty acid composition of plasma and membrane lipids. Numerous epidemiological surveys have noted this characteristic in populations consuming high levels of fish (see Chapter III).

von Lossonczy et al. (1978) studied 19 monks in a Dutch monastery and 23 nuns in a Belgian convent in a cross-over design to test the effect of a mackerel diet on serum lipid composition. All subjects were lacto-ovo-vegetarians and maintained diaries regarding adherence to the experimental diet. A 24-hour recall questionnaire was used twice during the trial to determine energy and fat intake. During a preliminary period, 2 weeks prior to initiation of the experiment, margarine low in linoleic acid was used to replace traditional high-linoleic acid soft margarine. Subjects were placed in either the control or experimental group for 3 weeks and then switched for the remaining 3 weeks of the 6-week experiment. Two fasting blood samples, 2 days apart, were taken at the beginning of the experiment, after 3 weeks when diets were changed, and just prior to the end of the experiment. The experimental diet consisted of 200 g mackerel per day incorporated into the habitual diet, providing a daily intake of approximately 8 g of omega-3 fatty acids. The control diet substituted 150 g of Gouda cheese per day in place of fish. All subjects received 100 mg of vitamin E on alternate days. Because of the cross-over design, each subject served as his or her own control; a simple two-tailed rank-sum test was used to determine significance and a Student's t-test was used to compare results between groups.

The fatty acid pattern of serum lipid fractions was changed by the fish diet: C20:5ω3 increased in all fractions and C22:6ω3 increased in triglycerides and phospholipids, but not sterol esters. Concomitant decreases occurred in C18:2ω6, C18:1ω9, C20:1, and C22:1.
Siess et al. (1980) studied changes in platelet and plasma fatty acids in seven healthy white men consuming an almost exclusively mackerel diet for 1 week. The diet consisted of 500-800 g mackerel per day which provided 7-11 g EPA. Pilot studies showed changes in plasma lipid profiles 3-6 days after starting the diet; therefore, blood samples were taken 1 and 2 days before and 3 and 6 days after beginning the experiment. Statistical analyses were performed using a Student's t-test. The mackerel diet induced changes in the fatty acid composition of plasma and platelets and plasma phospholipids consonant with the lipid composition of mackerel. The fatty acid pattern in platelet membranes was similar to that found in Greenland Eskimos (Dyerberg and Bang, 1979). Plasma and platelet EPA levels were increased (p < 0.01) and arachidonic acid levels were decreased (p < 0.05) leading to an elevated C20:5 to C20:4 ratio, also found in Eskimos.

Ten normolipidemic volunteers (five males and five females, aged 20 to 52 years) were randomly assigned to either a control diet or a salmon diet, similar except for composition of fatty acids (Harris and Connor, 1980). Individuals were fed each diet for 4 weeks as outpatients in a clinical research center, allowing at least 3 weeks between diets. Additionally, three subjects with Type IIb hyperlipoproteinemia were admitted as inpatients and fed the salmon diet alone. Statistical analyses were performed using a Student's t-test. Omega-3 fatty acids C20:5 and C22:6 were rapidly incorporated into plasma lipid fractions during the salmon diet. Goodnight et al. (1980) analyzed the platelet composition of these subjects. The C20:5ω3 fatty acid level of platelets rose from 0.1 to 6.06% (p < 0.025) and the C22:6ω3 level increased from 0.6 to 3.6% during the salmon diet. Levels of linoleic and arachidonic acids in platelet phospholipids decreased concomitantly. Initial platelet studies were repeated with 11 different subjects and the same experimental design (Goodnight et al., 1981). Similar changes in platelet composition were found with an overall increase in omega-3 fatty acids in the phospholipid fraction.

The effect of fish oil on blood lipid composition was also examined by Sanders et al. (1981). Twelve healthy male subjects (aged 19 to 31 years) took a 20-ml cod liver oil supplement daily for 6 weeks which provided 1.8 g EPA and 2.2 g DHA per day. Two sets of baseline measurements, 1 week apart, were made for each subject. Statistical analyses were performed using analysis of variance and a paired-sample t-test. Levels of EPA and DHA were increased (p < 0.01) in platelet and erythrocyte phosphoglycerides. Both C20:4ω6 and C22:4ω6 decreased in these fractions (p < 0.01). Platelet phosphoglyceride fatty acid composition returned to normal 5 weeks after withdrawal of supplement, but erythrocyte phosphoglycerides did not. This would be expected because of the more rapid turnover of platelets versus erythrocytes.
Thorngren and Gustafson (1981) conducted a longer study than previous investigators on the effect of a diet rich in EPA. Ten healthy male volunteers, aged 28 to 35 years, partially replaced their normal diet with fish for 11 weeks; EPA intake was increased by 2-3 g/day. Blood samples were taken on two occasions, 2 weeks apart, to establish baseline values. The fatty acid composition of phosphatidylcholine was altered by the EPA-rich diet. Levels of C20:5ω3 and C22:6ω3 rose after 3 weeks (p < 0.001) as did C22:5ω3 after 6 weeks (p < 0.05); however, the proportion of C20:4ω6 remained essentially unchanged throughout the experiment. The ratio of C20:5ω3 to C20:4ω6 rose threefold. Phosphatidylcholine fatty acid patterns returned to normal at 6 and 11 weeks after cessation of the fish diet.

Comparative effects of corn oil and cod liver oil on blood lipid composition were studied by Brox et al. (1981). Ten healthy male subjects, aged 20 to 30 years, were fed a 25-ml corn oil or cod liver oil supplement daily for 6 weeks in a blind cross-over design study. Subjects were selected on the basis of answers to a dietary questionnaire; only individuals whose diet consisted of approximately 40% fats with a P/S ratio of 0.5, 15% protein, and 45% carbohydrates were included. The two dietary periods were separated by 3 weeks when no supplement was given. Subjects remained on test for 16 weeks after supplementation ceased. Measurements were taken prior to the trial, after each dietary period, and again at the end of the trial. Statistical analyses were performed using a Student's t-test. Plasma levels of palmitic and oleic acids were slightly lower during the corn oil supplement (p < 0.02), but changes in other fatty acids did not reach significance. Cod liver oil supplementation induced a reduction in palmitic and oleic acid concentrations (p < 0.05) and an increase in EPA and DHA levels (p < 0.01).

Additional studies on cod liver oil supplementation and resultant physiological changes were conducted by Lorenz et al. (1983) [see also Fischer and Weber (1983)] to supplement their earlier work (Siess et al., 1980). Eight healthy male volunteers, aged 22 to 42 years, were fed 40 ml cod liver oil daily for 25 days to provide 4 g EPA and 6 g DHA per day. The normal "western" diet was not altered. Blood samples were drawn after overnight fast and 24-hour urine collections were made at the end of the supplement period. Control data were accumulated randomly either before or 4 weeks after the experimental period. Fatty acid patterns of plasma, red cell membrane, and platelet microsomal phospholipids were altered to reflect the omega-3 fatty acid content of cod liver oil, but these changes were rapidly reversed after supplementation ended.

B. PLASMA TRIGLYCERIDES

Studies in hyperlipidemic subjects have indicated a substantial decrease in plasma triglyceride levels after consumption of fish and fish oils (see Chapter V). Experiments
in normal individuals have also indicated a possible hypotriglyceridemic effect resulting from dietary fish products. The mechanism of this effect was discussed by Harris et al. (1984).

von Lossonczy et al. (1978) (see Section IV-A) found serum triglyceride levels lower (p < 0.01) in both men and women after fish consumption. The magnitude of decrease in serum triglyceride level was strongly correlated with initial level. In another study, normolipidemic subjects showed a 40% decline in plasma triglyceride level (p < 0.01) when consuming a salmon diet versus controls (Harris and Connor, 1980) (see Section IV-A). Plasma triglyceride levels in hyperlipidemic patients on this diet were 67% lower on the salmon diet compared with their habitual hypolipidemic diet. The authors hypothesized that the alteration in plasma membrane lipids (see Section IV-A) might provide a better substrate for lipoprotein lipase, the enzyme responsible for clearance of triglyceride-rich lipoproteins. This theory of salmon-induced accelerated clearance of triglycerides was supported by the much lower postprandial rise in plasma triglyceride levels following the salmon meal versus the control meal.

A fish oil diet was found to be hypotriglyceridemic in a study by Connor et al. (1981) of 12 normal subjects. Subjects were fed diets containing either saturated fatty acids (P/S=0.5), omega-3 fatty acids (P/S=0.9), or omega-6 fatty acids (P/S=3.4). Fat sources were incorporated into the diet in a random order for 4 weeks each. Omega-3 fatty acids were supplied by salmon and omega-6 fatty acids were supplied by safflower oil. Plasma triglyceride levels were reduced by the omega-3 fatty acid diet versus either the omega-6 fatty acid diet or the control (p < 0.005 and p < 0.025, respectively).

Bronsgeest-Schoute et al. (1981) extended their earlier work (von Lossonczy et al., 1978) by studying effects of an omega-3 fatty acid concentrate derived from cod liver oil on 52 selected volunteers for 4 weeks. The study population consisted of healthy men and women between the ages of 18 and 60 years, with serum cholesterol level not greater than 250 mg/dl, serum triglyceride level less than 200 mg/dl, and consumption of fish products not more than four times per month. Subjects were randomly allotted to five groups of 10 individuals per group. The two remaining subjects were placed in the group with the highest consumption of omega-3 fatty acids. The concentrate was provided in capsule form and provided either 0, 1.37, 2.27, 4.09, or 8.19 g omega-3 fatty acids daily. In accord with previous observations, serum triglyceride levels decreased from 90 mg/dl at the start of the experiment to 55 mg/dl at the end (p < 0.01) in the group consuming the highest amount of omega-3 fatty acids (8.19 g/day). This effect was not found in other groups, leading the researchers to speculate that only large amounts of omega-3 fatty acids in the diet lead to a decrease in blood triglyceride levels. Plasma triglyceride levels were also depressed (p < 0.02) in 12 healthy male subjects consuming cod liver oil for 6 weeks (Sanders et al., 1981) (see
Section IV-A). Values returned to initial levels after withdrawal of the supplement.

A few studies have shown no alterations in blood triglyceride levels following consumption of diets high in omega-3 fatty acids. Brox et al. (1981) (see Section IV-A) found that plasma triglycerides did not change during 6 weeks of supplementation of cod liver oil. Lorenz et al. (1983) (see Section IV-A) also found no changes in plasma triglyceride levels following cod liver oil supplementation for 25 days.

C. PLASMA CHOLESTEROL

Most well-controlled studies of normal subjects and patients with hypertriglyceridemia have shown decreases in blood cholesterol levels in response to dietary omega-3 fatty acids.

von Lossonczy et al. (1978) (see Section IV-A) reported a decline in serum cholesterol of 14.1 mg/dl in subjects consuming the control diet. After treatments were switched, individuals consuming the control diet showed a 14.3 mg/dl increase in serum cholesterol while cholesterol levels dropped 18.0 mg/dl in those on the fish diet. The response of serum cholesterol to fish consumption was greater for women than for men. In subsequent work by the same authors (Bronsgeest-Schoute et al., 1981) (see Section IV-B), serum cholesterol level was not altered in response to fish oil concentrate. The authors theorized that responses of blood cholesterol levels to omega-3 fatty acids in the diet may be "occasional".

In a study by Harris and Connor (1980) (see Section IV-A), consumption of a salmon diet in normal subjects for 4 weeks depressed plasma cholesterol level 17% (p < 0.01) when compared to the control diet. In those individuals who were hyperlipidemic initially, plasma cholesterol level declined 20% compared with their habitual hypolipidemic diet. Sanders et al. (1981) (see Section IV-A) reported no change in plasma total cholesterol level in subjects following 6 weeks of cod liver oil supplementation. This finding agreed with results of Brox et al. (1981) and Lorenz et al. (1983) (see Section IV-A) regarding responses to cod liver oil supplementation.

Harris et al. (1983) noted reductions in plasma cholesterol levels in subjects resulting from 4 weeks on a diet rich in omega-3 fatty acids supplied by salmon (p < 0.01) and a diet high in omega-6 fatty acids supplied by safflower oil (p < 0.05). Both omega-3 and omega-6 fatty acids increased excretion of neutral steroids and bile acids. Omega-3 fatty acids appeared to be more potent, gram for gram, in hypocholesterolemic effects than omega-6 fatty acids.

Nestel (1986) studied six healthy normolipidemic volunteers, aged 21-35 years, during three dietary periods: habitual
diet for 1 week, fish oil-enriched diet (40 g/day MaxEPA) for 3 weeks, and fish oil, cholesterol-supplemented diet (750 mg/day cholesterol from egg yolk) for 3 weeks. Changing from the habitual to the fish oil diet significantly lowered cholesterol levels in plasma, very-low-density lipoprotein (VLDL), low-density lipoprotein (LDL), and high-density lipoprotein (HDL) (p < 0.001, p < 0.05, p < 0.01, and p < 0.001, respectively, by the paired t-test). The addition of cholesterol to the fish oil diet failed to raise lipoprotein cholesterol levels significantly, although the total plasma cholesterol level rose from 149 ± 40 to 157 ± 37 mg/dl (p < 0.05). Thus, in this study, omega-3 fatty acids were found to reduce plasma and lipoprotein cholesterol levels even when dietary cholesterol intake was high.

D. LIPOPROTEINS

Changes in levels of lipoproteins have been noted occasionally in human studies. Levels of VLDL decreased substantially in male and female subjects consuming a fish diet (von Loosnecz et al., 1978) (see Section IV-A), but HDL and LDL levels were increased in males only (statistics not reported). A significant rise in HDL-cholesterol level (p < 0.02) after fish consumption was seen only in men who consumed the control diet first. In another study, plasma VLDL and LDL levels declined following a salmon diet (p < 0.005 and p < 0.05, respectively), but HDL level remained unchanged (Harris and Connor, 1980) (see Section IV-A).

In studies comparing omega-3 and omega-6 fatty acids, LDL-cholesterol level was reduced by both high PUFA diets when compared with the control diet (p < 0.01 and p < 0.05, respectively) (Connor et al., 1981) (see Section IV-B). Plasma VLDL levels were also lower following the omega-3 fatty acid diet versus either the omega-6 fatty acid diet or the control (p < 0.025). The level of HDL-cholesterol did not change in response to diet.

Nestel et al. (1984) compared lipoprotein production in response to short-term (2 to 3.5 weeks) feeding of diets containing either safflower oil or fish oil (MaxEPA) in a small number of normal and hyperlipidemic subjects. Statistical methods were not reported. The investigators noted that, compared with the safflower oil diet, the fish oil diet lowered VLDL-lipid and apoprotein B concentrations. The concentrations of HDL lipids and apoprotein A, were also lowered, but the effect on LDL concentrations was inconsistent. In kinetic studies, the daily production of VLDL-apoprotein B was substantially less in six subjects after 3 weeks of the fish oil diet, compared to the safflower oil diet. Effects on the irreversible fractional removal rate were inconsistent. The daily production of VLDL-triglyceride also showed a reduction. The suppression of VLDL-apoprotein B and triglyceride production was found not to be due to diminished plasma total free fatty acid or EPA flux.
Illingworth et al. (1984) studied LDL kinetics in seven healthy adults who consumed a control diet containing a blend of peanut oil or cocoa butter and a fish oil diet containing either salmon oil or EPA for 4-week periods. The fish oil diet provided 24 ± 8 g/day of omega-3 fatty acids, whereas the control diet provided none. Cholesterol content was balanced in the control diet by the addition of egg yolk. Statistical analyses were performed by paired t-test. Plasma total and LDL-cholesterol levels fell significantly (p < 0.005 and p < 0.025) 23 and 20%, respectively, on the fish oil diet. The cholesterol:protein ratio of LDL was similar on both diets, indicating that the decreased concentration of LDL cholesterol was due to a decrease in LDL particles in plasma. Kinetic studies with $^{125}$I-LDL indicated a significantly lower rate of synthesis of LDL-apo-protein B on the fish oil diet as compared to the control diet; in contrast, the fractional catabolic rate was similar on both diets. The investigators concluded that dietary omega-3 fatty acids lower plasma LDL levels in normal subjects by reducing the rate of synthesis of apoprotein B.

The acute effects of a fatty meal containing either cream or cod liver oil on plasma lipoproteins were examined by Aviram et al. (1986). Blood was collected from six healthy male subjects, aged 20-38 years, after a 12-hour fast and 3 hours after consuming 900 kcal of either cream or cod liver oil. Statistical tests were not reported. The HDL-cholesterol level remained the same after cod liver oil, but decreased after cream, whereas the HDL-protein concentration was increased after both meals. After cream, the saturated fatty acid percentage was increased in VLDL, decreased in LDL, and was unchanged in HDL, whereas the omega-3 PUFA percentage decreased in all lipoprotein fractions. After cod liver oil, the saturated fatty acid relative concentration was unchanged in VLDL, decreased in LDL, and increased in HDL, and the omega-3 fatty acid percentage increased in VLDL and HDL but was unchanged in LDL. The fatty acid composition in HDL suggested that dietary fatty acids were transferred to HDL from the chylomicrons and VLDL after the cod liver oil, but not after cream.

E. HEMOSTASIS

Because of the low incidence of cardiovascular disease and increased bleeding tendency in Greenland Eskimos (Dyerberg and Bang, 1979), many researchers have studied changes in platelet counts and function, thromboxane production, anti-thrombin-III levels, and plasma clotting factors.

Herold and Kinsella (1986) have reviewed possible mechanisms to explain changes in hemostasis following omega-3 fatty acid ingestion noted in epidemiological surveys and clinical trials (Figure 1). Arachidonic acid is the precursor of the prostaglandins [thromboxane (TXA$_2$), produced in platelets and prostacyclin (PGI$_2$), produced in vessel walls] and is converted
Figure 1. Prostaglandin synthetic pathways in blood vessels and platelets. The scheme shows the key sites in the conversion of dietary linoleic acid to arachidonic acid and then to prostanoids and leukotrienes. The key enzymatic steps likely to be affected by dietary omega-3 fatty acids are Δ6 desaturase and cyclooxygenase. Adapted from Herold and Kinsella (1986).
to these compounds by the action of cyclooxygenase. TXA\textsubscript{2} is considered proaggregatory and PGI\textsubscript{2} antiaggregatory; their balance regulates initial steps of blood clotting. Dietary omega-3 fatty acids are believed to compete with arachidonic acid for cyclooxygenase, thus altering formation of pathway endproducts (Herold and Kinsella, 1986), possibly in favor of trienoic prostanoids (e.g., TXA\textsubscript{2} and PGI\textsubscript{3}) (Begent and Born, 1984). This alteration may be responsible for a reduction in platelet aggregation and increased bleeding time noted in individuals with high consumption of omega-3 fatty acids (Herold and Kinsella, 1986).

Siess et al. (1980) examined platelet aggregation and thromboxane formation in seven healthy white men (see Section IV-A). TXB\textsubscript{2}, the stable hydrolysis product of TXA\textsubscript{2}, was measured by radioimmunoassay. High-dose collagen-induced TXB\textsubscript{2} synthesis and platelet aggregation were both reduced (p < 0.02 and p < 0.01, respectively) by the mackerel diet which the authors theorized might be the result of the increased C20:5 to C20:4 ratio. Arachidonic acid stimulates platelet aggregation by formation of proaggregatory TXA\textsubscript{2}, and EPA has been shown to inhibit platelet aggregation in vitro (Needleman et al., 1979). High-dose collagen-induced TXB\textsubscript{2} synthesis was not affected, however.

Goodnight et al. (1980) found platelet counts dropped from 350,600 to 212,000/cu mm (p < 0.05) in individuals consuming a salmon diet for 1 month (see Section IV-A); however, these values were still considered within the normal range. Standardized Ivy bleeding times increased from 7.45 min to 10.13 min (p < 0.05) following this diet. In subsequent experiments (Goodnight et al., 1981) (see Section IV-A), platelet counts dropped from a mean of 318,000 to 209,000/cu mm during the salmon diet. Although mean platelet counts remained in the normal range, several subjects developed thrombocytopenia which rapidly returned to normal when the salmon diet was discontinued. Megathrombocytes were present while the subjects were on the salmon diet which the authors theorized may have indicated decreased platelet survival. The standardized Ivy bleeding time was used to determine platelet-vessel wall interactions and increased from 6.75 to 10.0 min (p < 0.005) during consumption of the salmon diet. Decreased platelet counts cannot explain increased bleeding times in these subjects because counts less than 100,000/cu mm are necessary to prolong bleeding time in normal individuals (Harker and Slichter, 1972). Platelet aggregation induced by low concentrations of ADP was decreased during the salmon diet (p < 0.05), but did not change in response to collagen induction.

The effect of fish oil on hemostasis was also examined by Sanders et al. (1981) (see Section IV-A). Bleeding time was increased (p < 0.02) after 3 weeks of supplementation and remained elevated throughout the experiment, approaching initial values 5 weeks after withdrawal of the supplement. Leukocyte and erythrocyte counts; dilute clot-lysis time; plasma fibrinogen; and factors II, VII, VIII, and X did not change in response to
cod liver oil, although the estimated maximum response to platelet aggregation induced by ADP increased (p < 0.01). Antithrombin III—levels decreased (p < 0.01) after 6 weeks of supplementation and remained depressed 5 weeks after cessation of the supplement. Although patients with inherited antithrombin-III deficiency have a high risk of thrombosis (Mackie et al., 1978), the authors speculated that low levels may reflect a reduced requirement for this protective mechanism. These results contrast with reports that immunoreactive antithrombin-III concentration and antithrombin-III activity are significantly higher in Greenland Eskimos than Caucasian Danes (Stoffersen et al., 1982). Additionally, Greenland Eskimos who have emigrated to Denmark have intermediate antithrombin-III levels leading the authors to hypothesize that high dietary PUFA may play a role in these differences.

Thorngren and Gustafson (1981) used acetylsalicylic acid (ASA) to further test effects of a diet rich in EPA (see Section IV-A). The ASA was given at doses of 3.5 mg/kg and 10 mg/kg 2 weeks apart during the baseline period. These same doses of ASA were given 2 hours apart after 11 weeks on the diet. Bleeding times were determined before and 2 hours after administration of ASA. Pre-ASA bleeding times were increased throughout the experiment: 31% (p < 0.01) at 3 weeks, 42% (p < 0.01) at 6 weeks, and 33% (p < 0.05) at 11 weeks, but returned to baseline values in the post-dietary period. Blood platelet and fibrinogen concentration, prothrombin time, and activated partial thromboplastin were not altered in response to treatment. Collagen-induced platelet aggregation was depressed (p < 0.05 or p < 0.01) at all levels of collagen after 3 weeks of the fish diet. The ADP-threshold (the smallest amount of ADP required to induce the second phase of aggregation) was increased (p < 0.01) after 6 weeks on the dietary regimen. The increase was still evident 11 weeks after the end of the diet (p < 0.01). Therefore, dietary effects on platelet aggregation were sustained longer than alterations in platelet membrane phospholipids and in vivo bleeding time. Low-dose and high-dose ASA increased bleeding time 29% (p < 0.05) and 41% (p < 0.01), respectively, during the baseline period. During the dietary period, bleeding time was increased 55% (p < 0.01) by low-dose ASA and 44% (p < 0.05) by high-dose ASA. ASA during the diet increased bleeding time by more than the sum of EPA or ASA alone, but the synergistic effect was not significantly more than additive. Because ASA is thought to inhibit aggregation by acetylation of cyclooxygenase, preventing synthesis of TXA₂ (Roth et al., 1975; Smith and Willis, 1971), the authors theorized that EPA does not inhibit platelet and vessel wall interactions exclusively by altering prostaglandin synthesis. However, neither enzyme activities nor product concentrations were measured in this experiment, thus, evidence for this hypothesis is, at best, circumstantial.

Collagen-induced aggregation was decreased by corn oil and cod liver oil supplementation in a study by Brox et al. (1981) (see Section IV-A); however, cod liver oil reduced
low-dose collagen-induced aggregation to a greater extent than did corn oil (p <0.05). Platelet aggregation returned to normal 16 weeks after supplementation. Low- and medium-dose collagen-induced TXA₂ production was reduced by both corn oil and cod liver oil supplementation (p <0.05). Only cod liver oil supplementation reduced high-dose collagen-induced TXA₂ production (p <0.01). Arachidonic acid-induced TXA₂ production and collagen-induced malondialdehyde production were not changed by diet. Clotting time of recalcified whole blood was also not affected by dietary treatment. Differences in blood variables including platelet counts, hematocrit, and primary bleeding time were not observed and excretion of prostaglandin metabolites (E and F series) did not change as a result of treatment. Data were insufficient to determine if prostacyclin production by the vessel wall was altered by dietary supplement.

Kobayashi et al. (1981) presented a brief report of the use of concentrated EPA from sardine oil to decrease blood viscosity in Japanese volunteers. Capsules containing 21.4% EPA, 7.4% DHA, 0.7% arachidonic acid, and 300 IU tocopherol were given to provide 1.4 g EPA per day. This dose approximately equaled the difference in consumption patterns between a Japanese farming community and a fishing village where blood viscosity had been shown to be notably different (see Chapter III, Hirai et al., 1980). The EPA supplementation significantly decreased blood viscosity after 4 weeks; subjects with initial higher viscosity showed a larger decline. Platelet aggregability was not affected by the supplement.

Kobayashi and coworkers extended this work using a highly purified ethylester of EPA (EPA-E) derived from sardine oil (Terano et al., 1983). Eight healthy males, aged 29 to 42 years, consumed 12 capsules per day (four with each meal) for 4 weeks in addition to their ordinary Japanese diet containing approximately 100 g fish (0.9 g EPA/day). Each 400 mg capsule comprised ethylesters containing 75% EPA, 6.2% DHA, 6.3% eicosatetraenoic acid, 3.8% octadecatetraenoic acid, and 0.2% alphatocopherol. Fasting blood samples were obtained prior to the experiment, at 2 weeks, and at 4 weeks after initiation of the study. Collagen-, epinephrine-, and ADP-induced maximum platelet aggregation decreased following consumption of EPA-E for 4 weeks (p <0.001, p <0.05, and p <0.01, respectively). Arachidonic acid-induced aggregation was not altered by treatment. Plasma retention was decreased (p <0.05) after 4 weeks of supplementation as was whole blood viscosity (p <0.01); plasma viscosity was not changed. Red cell deformability was evaluated in 4 subjects during the experiment and was significantly increased after 4 weeks (p <0.05). A positive correlation was noted between red cell deformability and EPA content in erythrocyte membrane phospholipids. This suggests that elasticity in the erythrocyte membrane is enhanced by increased erythrocyte EPA content. Certain cerebro- and cardiovascular diseases are known to exhibit impaired red cell deformability and increased whole blood viscosity. Production of TXB₂ from thrombin-induced arachidonic
acid-labelled platelets was reduced (p < 0.05) after supplementation; however, TXB₂ production from EPA-labelled platelets could not be detected.

Mortensen et al. (1983) examined hemostasis in a double-blind, cross-over study of 20 healthy males aged 25 to 40 years. Subjects supplemented their usual diets with approximately 4 g/day of omega-3 (from MaxEPA) or omega-6 (from corn oil and olive oil) PUFA for 4 weeks separated by 4 weeks without dietary addition. Bleeding time was significantly prolonged in response to the fish oil, but no change was seen after vegetable oil. A small but insignificant decrease in platelet count was noted after fish oil; neither oil affected mean platelet volume. Collagen- and ADP-induced aggregation were not altered significantly, although the threshold concentration for collagen increased 26% after fish oil. Immunoreactive antithrombin-III concentration and activity increased after vegetable oil; only immunoreactive antithrombin-III concentration increased after fish oil.

Lorenz et al. (1983) reported that bleeding time increased (p < 0.01) while platelet count fell (p < 0.05) during supplementation of cod liver oil (see Section IV-A). Plasma TXB₂ tended to be lower during cod liver oil supplementation when obtained from fresh venipuncture, but this change did not reach significance. When blood samples were drawn through an i.v. catheter, TXB₂ was lower (p < 0.05) with supplementation. Low-dose collagen² and ADP-induced platelet aggregation decreased (p < 0.05) as did collagen-induced TXB₂ production (p < 0.01). Arachidonic acid-induced platelet aggregation and TXB₂ formation did not change with dietary supplementation. Gas chromatography-mass spectrometry (GC-MS) analysis detected in vitro conversion of ¹⁴C-arachidonic and ¹⁴C-EPA to TXB₂ and TXB₃, respectively. Conversion of in vitro EPA to antiaggregatory PGI₂ was also confirmed by GC-MS. Obtaining control measurements 4 weeks after the supplemental period was a potentially serious flaw in experimental design. Sanders et al. (1981) and Thorngren and Gustafson (1981) showed antithrombin-III values, systolic and diastolic blood pressure, plasma fatty acid composition, and some platelet function had still not returned to normal values 5 and 11 weeks post-supplementation. Some results of this experiment, therefore, must be interpreted with caution.

Formation of trienoic prostanoids has been implicated in the functional effects observed after consumption of diets high in omega-3 fatty acids. This theory was investigated by Fischer and Weber (1983). Eight healthy males, aged 22 to 42 years, consumed 20 ml cod liver oil twice daily for 25 days (Lorenz et al., 1983). Platelet-rich plasma was obtained from subjects prior to the experiment and after 25 days of cod liver oil supplementation and examined for ex vivo generation of TXB₃, the stable metabolite of nonaggregatory TXA₃. Stimulation of platelet-rich plasma with a high concentration of collagen led
to generation of TXB$_3$, whereas low-dose collagen-stimulation reduced platelet aggregation and formation of immunoreactive TXB$_2$ after 25 days of cod liver oil supplementation. TXB$_3$ was not formed after stimulation of platelet-rich plasma during the control period. The authors concluded that formation of inactive TXA$_3$ and a decrease in proaggregatory TXA$_2$ production may contribute the changes in hemostasis following dietary EPA. Fischer and Weber (1984) also demonstrated the in vivo production of the trienoic prostanoid PGI$_3$ in subjects who had ingested either cod liver oil (approximately 4 g/day EPA) or mackerel (approximately 10-15 g/day EPA).

A more recent study by the same group (von Schacky et al., 1985) examined the responses of six healthy male volunteers who supplemented their diets with cod liver oil (10-40 ml/day) for 40 weeks. In this long-term trial, TXA$_3$ (measured in serum from whole clotted blood) was formed in small amounts, whereas TXA$_2$ formation was decreased to 50% of control values. As determined from its major urinary metabolite, PGI$_3$ was formed from EPA at rates up to 50% of unaltered PGI$_2$ formation. This study confirmed that long-chain omega-3 fatty acids can produce a dose-related and sustained shift in prostaglandin I$_2$/thromboxane A balance to a more antiaggregatory and vasodilatory state.

F. BLOOD PRESSURE

Blood pressure was examined following consumption of cod liver oil in 12 healthy males (Sanders et al., 1981) (see Section IV-A). Systolic and diastolic blood pressures decreased ($p < 0.01$) after 6 weeks of supplementation and remained depressed for 5 subsequent weeks. A double-blind cross-over study would be needed to verify blood pressure changes in this trial as repeated measurements are known to lead to lower values. Lorenz et al. (1983) noted that cod liver oil supplementation decreased upright systolic blood pressure and blood pressure response to a 15-minute infusion of 5 µg/min norepinephrine ($p < 0.05 - p < 0.01$) in eight healthy males (see Section IV-A). Supine resting systolic blood pressure and the pressor response to angiotensin II infusion (1 µg/ml) tended to decrease with cod liver oil supplementation, but results were not significant. As classic humoral and neuronal systems that control blood pressure remained unchanged, the authors could not determine possible mechanisms for depressed systolic blood pressure. Potential flaws in experimental design of this study have already been discussed (see Section IV-E). Mortensen et al. (1983) (see Section IV-E) found that both recumbent and upright systolic blood pressures were slightly but significantly decreased by fish oil supplementation compared to vegetable oil supplementation. Diastolic blood pressure was not altered, nor were the urinary excretion of sodium and potassium or plasma renin concentration.
LEUKOCYTE FUNCTION

Leukotrienes are produced in neutrophils (polymorphonuclear leukocytes) from arachidonate via the lipoxygenase pathway (Figure 2). In comparison to arachidonic acid, EPA appears to be a preferred substrate for lipoxygenase in subcellular human and guinea pig neutrophils (Lee et al., 1985). Leukotriene B₅ has been implicated in both the inflammatory response and vascular damage resulting from a variety of diseases (Ford-Hutchinson et al., 1980; Klickstein et al., 1980; Palmblad et al., 1981; Sacks et al., 1978).

Strasser et al. (1985) studied leukotriene formation in neutrophils from six healthy men, aged 26-37 years, who supplemented their otherwise unchanged diet with 40 ml/day cod liver oil (approximately 4 g/day EPA) for 4 weeks. The investigators found that leukotriene B₅ was not detectable before supplementation, but that it increased significantly (to 70.2 ± 18.7 pmol/10⁷ polymorphonuclear leukocytes) after cod liver oil treatment. The synthesis of leukotriene B₄ was not significantly altered. The ratio of leukotriene B₄:leukotriene B₅ corresponded to the ratio of arachidonic acid:EPA in polymorphonuclear leukocyte phospholipids. These findings provided evidence that leukotriene B₅ was formed from cellular EPA in the neutrophils of subjects taking supplements of a fish oil with a high EPA content.

Prescott et al. (1985) studied four healthy human subjects consuming an EPA-rich diet (increased fish consumption with additional supplemental MaxEPA) for 3 weeks. The diet was designed to provide 8-10 g/day EPA. Neutrophils were isolated from blood before and after dietary treatment. Statistical analyses were performed using a t-test. The EPA and DHA content of neutrophils increased from nondetectable levels to 7.4 and 3.9 mole %, respectively (p < 0.01), following the EPA-rich diet. Whereas leukotriene B₄ and its stereoisomers were the only products observed after stimulation of neutrophils in the pre-dietary phase, leukotriene B₅ and its stereoisomers were identified from stimulated neutrophils after dietary supplementation. Decreases in leukotriene B₄ production following supplementation (p < 0.05) were offset by increases in leukotriene B₅ production (p < 0.05). Although leukotriene B₅ is considered 20 times less potent in stimulating neutrophils than is leukotriene B₄ (Prescott, 1984), functional studies performed on neutrophils indicated no alteration in aggregatory response or adherence to nylon fibers after dietary treatment.

Different results were found by Lee et al. (1985) who studied seven healthy males, aged 22 to 53 years, for 6 weeks with a lower dose of omega-3 fatty acids. Subjects supplemented their usual diet with 18 capsules of MaxEPA/day to provide 3.2 g EPA/day and 2.2 g DHA/day. Blood samples were taken prior to the experiment, after 3 weeks, and at the end of the study. In four subjects, blood was also sampled 6 weeks after supplementation.
Figure 2. Oxidative metabolism of arachidonic acid, eicosapentaenoic acid, and docosahexaenoic acid by the 5-lipoxygenase pathway. Adapted from Lee et al. (1985). 5-HPETA denotes 5-hydroperoxyeicosatetraenoic acid, 5-HPEPA 5-hydroperoxyeicosapentaenoic acid, 7-HPDHA 7-hydroperoxydocosahexaenoic acid, 4-HPDHA 4-hydroperoxydocosahexaenoic acid, 5-META 5-hydroxyeicosatetraenoic acid, 5-HEPA 5-hydroxyeicosapentaenoic acid, 7-HDHA 7-hydroxydocosahexaenoic acid, 4-HDHA 4-hydroxydocosahexaenoic acid, and LT leukotriene.
Changes did not occur in leukotriene B₄ production from neutrophils after 3 weeks on the experiment; however, leukotriene B₄, related diastereoisomers, and 5-HETA produced by activation with calcium ionophore were decreased approximately 48% following 6 weeks of MaxEPA supplementation (p < 0.001 for all variables, by analysis of variance). Whereas leukotriene B₄ and 5-HEPA were not generated prior to supplementation, detected values were 3.1 ng/million cells and 12.6 ng/million cells, respectively, at 3 weeks and 2.6 ng/million cells and 2.8 ng/million cells, respectively, at 6 weeks. Summed values for leukotrienes and monohydroxy fatty acids at 3 weeks were not significantly different from control values; however, at 6 weeks, total leukotrienes decreased 42% (p < 0.05) and monohydroxy fatty acids decreased 55% (p < 0.05). Neutrophil function was altered by dietary treatment in this experiment; after 6 weeks neutrophil adherence to bovine endothelial-cell monolayers was suppressed (p < 0.05) and neutrophil chemotaxis in response to leukotriene B₄ was decreased 70% (p < 0.001). The authors suggested that by inhibiting leukotriene B₄-mediated functions of neutrophils, diets high in omega-3 fatty acids may have anti-inflammatory effects.
V. REVIEW OF CLINICAL TRIALS

Few clinical trials evaluating the ability of dietary fish, or omega-3 fatty acids, to affect health were performed prior to epidemiological reports showing an inverse relationship between fish consumption and incidence of heart disease. A 19-year study of Nelson (1972) examined the effect of dietary modification, including increased fish consumption, on subsequent mortality in 80 patients with coronary artery disease. Critical experimental and reporting errors preclude interpretation of results, however, and data from this study will not be included in this report.

Several investigators have examined the potential therapeutic benefit of omega-3 fatty acids for treatment of heart disease. Saynor et al. (1984) studied 107 individuals, 92 with heart disease or hypertriglyceridemia, for periods up to 2 years. Subjects included 88 males and 19 females, aged 31 to 75 years. Each subject consumed 10 ml of MaxEPA (19% EPA) with food twice per day; the normal diet was otherwise unaltered. Blood samples were obtained twice at 7-day intervals before supplementation and at 1, 3, 6, 12, and 24 months after supplementation. Statistical analyses were performed using a paired t-test or a Wilcoxon two-sample test.

Serum triglyceride levels in all subjects decreased by a mean of 37% after 1 month of supplementation (p < 0.001), then remained constant throughout 24 months of observation. Subjects with initial hypertriglyceridemia showed a mean decrease in serum triglyceride levels of 42% after 1 month and 47% after 24 months of MaxEPA. Total cholesterol concentration was also depressed, although this reduction occurred much more slowly than the change in serum triglyceride level and did not reach statistical significance until 24 months (p < 0.001). The HDL-cholesterol concentration was increased (p < 0.05 to p < 0.001) at 1, 3, 6, and 24 months; values decreased slightly at 9 and 12 months, but did not return to pre-treatment levels. Platelet counts were lower at 1, 3, and 6 months (p < 0.01 to p < 0.005), but then returned to normal levels. Bleeding time was compared in eight subjects taking 10 ml MaxEPA daily versus 11 subjects consuming 20 ml MaxEPA per day. Measurements were made before supplementation and after 12 months of dietary treatment. Bleeding time increased (p < 0.001) in individuals consuming 20 ml MaxEPA per day compared with those consuming 10 ml/day. In 12 patients taking glyceryl trinitrate for relief of angina pain, the number of tablets consumed weekly was dramatically decreased after 9 months of MaxEPA supplementation. Patients also reported fewer angina attacks and increased exercise tolerance. The VLDL and LDL fractions decreased (p < 0.001 and p < 0.05, respectively) and the HDL fraction increased (p < 0.01) after 6 months of MaxEPA consumption. The authors concluded that use of omega-3 fatty acid supplements may decrease the incidence of arterial
thrombosis and the development of atheroma. However, little information on the dose-response was given.

Dietary fish oil also had hypolipidemic effects in a metabolic study of patients with Type IIb and Type V hypertriglyceridemia (Phillipson et al., 1985). Twenty subjects, 8 males and 12 females (aged 36 to 69) with long-standing moderate to severe hypertriglyceridemia, were placed on one of three metabolically controlled diets. Ten of the patients were classified as having Type IIb hypertriglyceridemia and 10 were classified as having Type V hypertriglyceridemia.

Diets for Type IIb patients were a low-fat (20-30% of calories), low-cholesterol (150 mg/day) control diet, followed by a 4-week fish oil diet, followed in some patients by a 4-week high-vegetable oil diet. The fish oil and vegetable oil diets provided 325 mg cholesterol/day and 30% of calories as fat. Type V patients consumed a low-fat (5%), no-cholesterol control diet, followed by a high-fish oil diet and then a high-vegetable oil diet. The fish and vegetable diets contained 350 mg cholesterol and 20-30% of calories as fat. Vegetable oil contained 50% stripped corn oil and 50% safflower oil; fish oil was either salmon oil or MaxEPA, providing 20 or 30 g omega-3 fatty acids per day, respectively. Blood samples were collected after an overnight fast and statistical analyses were performed using a paired t-test or a one-way analysis of variance followed by the Newman-Keuls procedure for post hoc multiple comparisons.

Patients with Type IIb hypertriglyceridemia had a 64% lower plasma triglyceride level when consuming fish oil compared to the control diet (p < 0.01). The high vegetable oil diet also decreased plasma triglyceride levels (p < 0.05), but these values were higher than those resulting from the fish oil diet (p < 0.01). Total cholesterol levels were similarly affected by diet although to a lesser degree (p < 0.01). The level of VLDL-cholesterol was decreased by both oil supplements with fish oil exhibiting the greatest response (p < 0.01); VLDL-triglyceride level showed alterations similar to those in VLDL-cholesterol (p < 0.01). The LDL-cholesterol level was only decreased by vegetable oil (p < 0.05), while LDL-triglyceride level was not affected by diet. The level of HDL-cholesterol was increased in response to the vegetable oil diet (p < 0.05). Plasma apolipoprotein B and C-3 levels were decreased by the fish oil diet (p < 0.01 and p < 0.05, respectively). Apolipoprotein levels were not measured following the vegetable oil diet.

Responses to the fish oil diet were even more dramatic in Type V patients. The extremely low-fat control diet depressed plasma lipids in these subjects although values were still greatly elevated with evidence of chylomicronemia remaining in five patients. Chylomicronemia disappeared following the fish oil diet. Additionally, total cholesterol level decreased from 377 mg/dl on the control diet to 195 mg/dl following the fish oil diet (p < 0.01). The total cholesterol level was also depressed.
following the vegetable diet, although to a lesser degree. Total triglyceride levels were lower following the fish oil diet than either the control (p < 0.01) or vegetable oil diet (p < 0.05). The VLDL-cholesterol level was lower after fish oil supplementation (p < 0.01). The VLDL-triglyceride level was also lowered by the vegetable oil diet. The LDL-cholesterol level was increased by fish oil (p < 0.05), whereas LDL-triglyceride and HDL-cholesterol levels were unchanged in response to diet. Apo-lipoprotein B, C-3, and E levels were decreased in response to fish oil supplementation (p < 0.05), p < 0.025, and p < 0.05, respectively). The authors suggested that high omega-3 fatty acid diets may be an important technique for control of hypertriglyceridemia.

Singer et al. (1985a) examined the influence of mackerel and herring diets on eight patients with Type IV and Type V primary hyperlipoproteinemia. Six males and two females (mean age 46.8 years) were placed on the mackerel and herring diets for 2 weeks each in a cross-over design study. The diets were isocaloric, with the mackerel diet supplying 2.2 g/day EPA and 2.8 g/day DHA, and the herring diet supplying 1.0 g/day EPA and 2.8 g/day DHA. The two experimental periods were separated by 3 months of normal food; control values were obtained before and 3 months after each dietary period. Fasting blood samples were obtained from an antecubital vein 20 minutes after insertion of an indwelling catheter. Statistical analyses were performed using a matched-pair t-test.

Serum triglyceride levels fell markedly following both dietary regimens, but the decrease only reached significance after the mackerel diet (p < 0.05) because of the high standard deviation. Decreases in triglyceride levels were more pronounced with higher initial serum triglyceride levels. Values had returned to basal levels 3 months after treatment. Total cholesterol values reacted similarly to serum triglyceride values which were significantly lower (p < 0.05) at the end of the mackerel period. Data indicated that omega-3 fatty acids might have desirable effects in patients with hyperlipoproteinemia. Long-term studies are currently in progress to determine the efficacy of such a dietary regimen.

More evidence of the benefit of omega-3 fatty acids in the treatment of hyperlipidemic patients was provided by Simons et al. (1985) in a double-blind, placebo-controlled, 6-month cross-over design study of hyperlipidemic patients. Of the 25 subjects enrolled in the study, nine had the Type IIa phenotype, eight had Type IIb, seven had Type IV, and one had Type V. Subjects were given either 6 (n=13) or 16 (n=12) capsules/day of MaxEPA (providing 1.1 g/day EPA or 2.9 g/day EPA, respectively) or an identical placebo capsule of olive oil for 3 months and then switched to the alternate treatment for 3 months. In addition, a single-blind, placebo-controlled study was conducted in 3 patients with Type V hyperlipoproteinemia. These patients initiated the experiment with 2 months on placebo followed by
16 capsules/day MaxEPA for 3 months. Subjects from both groups had indications of primary hyperlipidemia and consisted of 22 males and 6 females, aged 32 to 77 years. Twelve-hour fasting blood samples were obtained at monthly intervals. Data were analyzed statistically using the Statistical Package for the Social Sciences. Single measurements were compared using the Student's t-test and interrelationships between measurements were examined using the Pearson correlation coefficient. The MaxEPA capsules were well-tolerated during the study with no evidence of toxicity or changes in body weight or blood pressure. In the double-blind study, patients with Type IIa hyperlipidemia did not show changes in mean plasma cholesterol values in response to MaxEPA when compared to placebo; however, plasma triglyceride levels fell an average of 22%. In Type IIb patients, plasma cholesterol levels fell 3% after MaxEPA treatment and plasma triglyceride levels fell 28%. Levels of plasma cholesterol decreased 6% and plasma triglycerides fell 41% after MaxEPA consumption in patients with Type IV hyperlipidemia. In one Type V patient, plasma cholesterol level decreased 26%, while plasma triglyceride level dropped 63%. Analysis of the data indicated that results were independent of treatment sequence. Alterations in plasma lipids were dose-related; the 45% mean decrease in plasma triglyceride level represented a 33% fall in patients taking six capsules/day and a 58% fall in patients taking 16 capsules/day. Much of this decrease was the result of a significant, dose-related decrease in VLDL level. The HDL levels rose 4.9% following six capsules MaxEPA/day and 7.3% after 16 capsules/day. In contrast to other studies (Dyerberg et al., 1978; Lorenz et al., 1983), bleeding time, platelet count, plasma fibrinogen, and blood or plasma viscosity did not change in response to treatment.

Although the single-blind study was not analyzed statistically, results were consistent and identified a decline of 49-70% in plasma triglyceride levels after consumption of 16 capsules/day MaxEPA versus placebo. Triglyceride-rich lipoprotein levels were also reduced, whereas, HDL levels increased from 0.26 to 0.56 mmol/l following MaxEPA therapy. The LDL levels were unchanged. In both studies, therapeutic effects of MaxEPA were obtained within 1 month of treatment. Sanders et al. (1985) obtained similar results to those of Simons et al. (1985) when comparing 15 g/day of MaxEPA supplementation for 4 weeks versus 15 g of a corn oil-olive oil mixture in hypertriglyceridemic patients.

The beneficial effects of MaxEPA supplementation have also been studied in patients on hemodialysis who have an exceptionally high incidence of cardiovascular disease and hypertriglyceridemia (Rylance et al., 1984). Six male and three female hemodialysis patients, aged 26 to 64 years, participated in an 8-week study involving consumption of 20 ml/day MaxEPA. Pre- and post-treatment blood samples were taken following a 12-hour fast during the interdialysis period. Platelet aggregation studies were also performed. Results were compared using a
paired Student's t-test. Plasma triglyceride levels (initially 1.8 mmol/l) decreased 51% (to 0.89 mmol/l) (p < 0.005) following MaxEPA treatment. Although plasma total cholesterol (initially 5.68 mmol/l) remained essentially unchanged as a result of therapy, HDL-cholesterol levels (initially 1.42 mmol/l) increased 25% (to 1.78 mmol/l) (p < 0.005), primarily in the form of HDL₃-cholesterol (p < 0.02). Collagen- and ADP-induced in vitro platelet aggregation was reduced following MaxEPA treatment. A 13-week study of the effects of fish oil on hemodialysis patients was conducted by Hamazaki et al. (1984). No side effects of treatment were observed. Levels of serum triglycerides, total cholesterol, and phospholipids were all decreased (p < 0.001, p < 0.025, p < 0.005, respectively) after 4 and 13 weeks of supplementation of 9.0 g fish oil/day providing 1.6 g EPA and 1.0 g DHA per day. The platelet count decreased from 156 ± 49 to 126 ± 41 (x10³/cu mm) after 13 weeks (p < 0.005). However, blood viscosity was not altered by treatment in these patients. The authors recommended long-term studies to evaluate the potential therapeutic use of MaxEPA to help reduce the incidence of cardiac and vascular complications in hemodialysis patients.

Additional therapeutic trials of omega-3 fatty acids have been conducted in patients with other vascular disorders such as ischemic heart disease, peripheral vascular disease, stroke, and hypertension. Subjects with confirmed ischemic heart disease participated in a 5-week study to test the effects of 20 ml/day MaxEPA (providing 3.5 g/day EPA) on platelet kinetics (Hay et al., 1982). Nine males and four females, aged 33 to 70 years participated in the study. Blood samples were obtained after a 12-hour fast. A Student's paired t-test was used. All subjects showed an increase in platelet survival, with a mean increase of 10% (p < 0.0005). Plasma-beta-thromboglobulin fell 31% (p < 0.025) as a result of supplementation and platelet counts were reportedly 16% lower (p < 0.005). Plasma platelet factor 4 decreased 25% compared to pre-treatment levels. White-cell counts fell slightly (p < 0.05) during treatment, but hemoglobin level and other red-cell variables did not change. Plasma HDL level increased 18% (p < 0.025) after MaxEPA supplementation; however, contrary to other studies, serum cholesterol level was not altered and the drop in serum triglyceride level did not reach significance. The authors indicated that many subjects had consumed the fish oil during the fasting period prior to blood sampling which may have significantly altered plasma triglyceride levels. While Hay et al. (1982) suggested that data justified initiation of long-term studies to explore toxicity and inhibition of atheroma from dietary fish oil, Jones and Davies (1982) were critical of this interpretation. In their opinion, reduced platelet numbers and assay variability may have been responsible for reported changes in platelet kinetics. They suggested further studies on platelet reactivity prior to initiation of long-term clinical trials on patients with ischemic heart disease.
Woodcock et al. (1984) conducted a double-blind, randomized trial of omega-3 fatty acids in 19 patients with intermittent claudication. Patients received supplements of either fish oil (MaxEPA to provide 1.8 g/day EPA) or an identical capsule containing a mixture of corn and olive oil for 7 weeks. Statistical analyses were by Student's t-tests. A statistically significant (p < 0.05) reduction in whole blood viscosity was observed in patients after the fish oil, but not after the vegetable oil supplement. Plasma viscosity, hemoglobin concentration, packed cell volume, and platelet count were not changed. The preliminary results suggested a potential therapeutic role for EPA in arterial disease.

MaxEPA was used unsuccessfully for treatment of stroke patients (Green et al., 1985). Seven males and four females, aged 53 to 76 years, completed a double-blind, placebo-controlled, cross-over design study for 12 weeks. Occlusive cerebrovascular disease was confirmed by clinical examination, computed tomography, and angiography. Patients were randomly assigned to either 10 capsules MaxEPA per day to provide 1.8 g EPA or an identical placebo capsule containing olive oil. After 6 weeks of supplementation, treatments were switched. Of the 18 original subjects, four were unable to swallow 10 capsules per day and were removed from the study. Laboratory analyses were performed at baseline and at 3-week intervals during the study and values were compared using paired t-tests.

Serum total cholesterol and HDL-cholesterol levels did not differ between treatments; however, serum triglyceride level was lower in individuals consuming MaxEPA after 6 weeks (p < 0.038). Bleeding time was increased slightly (p < 0.023) after 3 weeks MaxEPA supplementation compared to placebo, but this difference was no longer significant after 6 weeks. Platelet count and agonist-induced platelet aggregation were lower after 6 weeks, but values did not reach significance. Factor VIII-related antigen was not affected by treatment. Possible explanations for this lack of effect of fish oil in stroke patients were enumerated: dose of MaxEPA may have been insufficient to produce desired effects, duration of the test may have been inadequate, MaxEPA may be less potent than unprocessed fish oil, and other trials with less rigorous experimental design may have had uncontrolled factors influencing results (Green et al., 1985). In addition, the statistical power of this study may not have been great enough to detect differences, and elderly patients with atherothrombotic disease may be more resistant to dietary change than the younger subjects examined in most studies.

Most studies relating the feeding of omega-3 fatty acids to changes in blood pressure have been done in healthy individuals. However, Singer et al. (1985a), in a paper discussed earlier in this chapter, found systolic blood pressure in recumbent and upright positions lower (p < 0.05) in hyperlipoproteinemic patients following a mackerel, but not a herring,
diet. Diastolic blood pressure was unchanged in response to both diets. Basal blood pressure had been 144/95 mm Hg.

Singer et al. (1985b) performed a similar experiment in patients with mild essential hypertension. Fourteen male patients, aged 23 to 43 years, with diastolic blood pressure of 90 to 104 mm Hg were placed on diets described in a previous experiment (Singer et al., 1985a). All patients were normolipidemic; mild essential hypertension was diagnosed by physical examination, electrocardiogram, x-ray, and laboratory studies. Protocol was as described previously. The mackerel diet decreased serum total cholesterol level by 9%, triglyceride level by 28%, LDL-cholesterol level by 14%, LDL:HDL cholesterol ratio by 20%, and lecithin cholesterol acyl transferase activity by 14% (p <0.05 to p <0.01). The HDL-cholesterol level was increased 12% (p <0.05) by the mackerel diet. Values returned to basal levels within 3 months of cessation of mackerel with the exception of HDL and LDL/HDL-cholesterol ratio. Blood lipid measurements were slightly affected by the herring diet but not to a significant degree. Serum uric acid was increased 24 and 20% (p <0.05) by mackerel and herring diets, respectively. Serum sodium concentration decreased 2% (p <0.05) following the mackerel diet, although the mackerel diet was slightly higher in sodium. Casual recumbent, but not upright, systolic blood pressure was lowered 8% (p <0.01) by the mackerel diet; the herring diet had no effect on casual blood pressure. Both systolic and diastolic blood pressures decreased (p <0.01) before and during the psychophysiological stress test after consumption of the mackerel diet. After 3 months, values had returned to basal levels. Systolic and diastolic blood pressures were only slightly depressed during the psychophysiological stress test during the herring diet. The authors suggested alterations in blood pressure might be the result of changes in lipid composition of cell membranes at receptive sites of vasoactive hormones or direct effects of prostaglandins of the 3-series on vessel wall or transmitter release. Recommendations were made to conduct long-term studies to evaluate the contribution of omega-3 fatty acids to the treatment of mild hypertension.

Hamazaki et al. (1984), in experiments with hemodialysis patients, also found a significant (p <0.05) decrease in diastolic blood pressure after 12 weeks of administration of omega-3 fatty acid-rich fish oil supplementation. However, basal blood pressure values were within normal limits.

Finally, McCarren et al. (1985) reported preliminary results of a double-blind, placebo-controlled (vegetable oil) trial of MaxEPA (1 g/10 lb body weight/day for 6 weeks) in patients with severe migraine. Mean migraine intensity and headache frequency were both decreased during supplementation with fish oil. This effect on migraine might be mediated through changes in prostaglandin synthesis and/or reduction in platelet serotonin release, with an overall decrease in cerebral vasospasm.
VI. ONGOING STUDIES

Table 4 identifies various clinical and experimental studies on omega-3 fatty acids that are currently underway. Many of the studies are supported by the National Institutes of Health which issued a program announcement "Biological Mechanisms of Omega-3 Fatty Acids in Health and Disease States" in December 1985 (Wyngaarden, 1986). Currently three institutes have plans for initiatives in the forms of program announcements. The National Heart, Lung and Blood Institute (NHLBI) also has published an RFA on "Studies of Omega-3 Polyunsaturated Fatty Acids in Thrombosis and Cardiovascular Disease" (RFA-86-HL-24; NIH Guide, Vol. 15, No. 8, page 2, June 20, 1986).

Continuing research on omega-3 fatty acids is being encouraged through the U.S./Japan Cooperative Medical Science Program. The National Institutes of Health has also proposed that studies of omega-3 fatty acids be included as a research priority in the Arctic Health Research Plan developed by the Interagency Arctic Research Policy Committee (Wyngaarden, 1986).

In addition, the National Marine Fisheries Service of the National Oceanic and Atmospheric Administration, in conjunction with the National Institutes of Health, is developing standards for the manufacture and quality control of omega-3 fatty acid products (including specific deuterated fatty acids) for use in research studies.
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<td>Biosynthesis, biology, and metabolism of the leukotrienes</td>
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Table 4. (continued)

NIH support relevant to fish oil research as of April 1986*

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<td>MO1 RR 00749</td>
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Other Support Relevant to Fish Oil Research**

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<td>Sea Grant</td>
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* Source: Human Nutrition Research Information Management System.
** Source: Childs (1986)
VII. CONCLUSIONS AND ADDITIONAL CONSIDERATIONS

A. CONCLUSIONS

Considerable scientific and public interest has been generated by recent epidemiological data indicating decreased cardiovascular mortality and morbidity associated with consumption of fish-oil-derived fatty acids, particularly the omega-3 polyunsaturated fatty acids. Most epidemiological studies have found a low incidence of CHD mortality in populations which consume high amounts of fatty fish. Some studies have detected an inverse dose-response relationship between CHD mortality and fish consumption, but this has not been a universal finding (Curb and Reed, 1985; Vollset et al., 1985).

Human experimental studies generally have shown effects of omega-3 fatty acid consumption, such as decreased plasma triglyceride level, reduced LDL- and VLDL-cholesterol concentrations, and changes in platelet function, which are consistent with reduced development of cardiovascular diseases. Clinical trials with omega-3 fatty acids have produced beneficial changes in the plasma lipid composition of patients with various hyperlipidemias. However, the number of human studies has been limited and some have been subject to design flaws which restrict interpretation (Herold and Kinsella, 1986). For example, in some studies a control group was lacking, subjects were not randomly assigned to treatments, usual intake of fish products was not assessed, compliance with treatment was not monitored, and treatments were not blind to subjects and/or investigators. In addition, widely differing doses have been used and most studies on omega-3 fatty acids have tested intake levels much higher than realistically could be consumed by the American public. A number of dietary variables differ among studies and could influence both results and their interpretation. These include the level of cholesterol in the fish oil supplements, dietary cholesterol balance in control and treatment groups, level and degree of saturation of fat in the control diet, total fat composition in diets in which a supplement is given, and relative quantities of the major omega-3 fatty acids. Because much experimentation has been conducted with intact fish or heterogeneous fish products, there has also been speculation that observed beneficial effects may not be the result of EPA, DHA, or omega-3 fatty acids in general.

The lack of long-term, systematic studies of omega-3 PUFA is a major shortcoming which prevents drawing definitive conclusions about their health effects at this time. Nonetheless, animal and human data are accumulating to suggest that omega-3 fatty acid consumption provides some degree of protection from cardiovascular disease.
B. OTHER CONSIDERATIONS

1. Potential beneficial effects

Animal studies have indicated some other potential beneficial effects in cardiovascular and cerebrovascular disease. For example, Black et al. (1979) found that dietary menhaden oil reduced the neurological deficit and volume of brain infarction after experimentally-induced focal cerebral ischemia in cats. The same group (Culp et al., 1980) also reported that dietary menhaden oil reduced the myocardial damage associated with experimental coronary thrombosis in dogs.

In addition, animal studies have indicated that omega-3 fatty acids may have other beneficial effects unrelated to cardiovascular disorders. Studies with mice have shown that omega-3 fatty acids may protect against deleterious effects of autoimmune diseases such as rheumatoid arthritis and lupus erythematosus (Kelley et al., 1985; Prickett et al., 1983). However, Prickett et al. (1984) found that dietary menhaden oil augmented the development of arthritis in rats immunized with type II collagen. Prickett et al. (1981) demonstrated that a diet high in EPA protected mice of a susceptible strain from the development of glomerulonephritis. Fish oil has also been shown to decrease the development of L-azaserine-induced preneoplastic lesions in the rat pancreas (O'Connor et al., 1985).

Visual acuity was impaired in rhesus monkeys whose dams were fed a diet deficient in omega-3 fatty acids during gestation (Neuringer et al., 1984). This finding, plus the fact that large amounts of DHA have been found in the brain, retina, and sperm (O'Brien and Sampson, 1965), has led some researchers to speculate on the essentiality of omega-3 fatty acids (Harris, 1985). Linolenic acid, EPA, and DHA have also been found in human milk; their concentrations increase during dietary supplementation of fish oil (Harris et al., 1984). The need for these fatty acids during infant nutrition is presently unknown.

Further research is needed to determine the relevance of these findings in animals to humans.

2. Potential deleterious effects

There is little information regarding potential toxicity from long-term or high-level consumption of omega-3 fatty acids, limiting the ability to make sound recommendations on fish consumption (Herold and Kinsella, 1986). Although most researchers noted few side-effects resulting from dietary treatment with fish or fish oils, experiments were generally short-term and not designed to test for toxicity. Goodnight et al. (1982) and others have discussed several possible side-effects resulting from omega-3 fatty acid consumption. Some fish oils
contain high levels of cetoleic acid, an erucic acid isomer. Erucic acid has been shown to cause transient myocardial lipodosis and fibrosis in several animal species (Beare-Rogers, 1977). High intakes of cetoleic acid have not been shown to cause deleterious health effects in Eskimo populations; however, some researchers theorize that Eskimos may have developed protective catabolic mechanisms which may not be in place in people who do not regularly consume high-fish diets (Budowski, 1981).

"Yellow fat disease" has been noted in pigs fed mackerel or whale oil (Garton et al., 1952; Ruiter et al., 1978), as well as certain wild animals that consume large amounts of polyenoic fatty acids (Dormandy, 1978), and may be associated with vitamin E deficiency. This disease has never been observed in human beings (Dyerberg, 1986) and does not appear to have adverse effects other than discoloration of adipose tissue (Goodnight et al., 1982). Fish oils are easily peroxidized; antioxidant requirements are increased during high intakes of omega-3 fatty acids and vitamin E deficiency may be precipitated. This problem has been avoided in most experiments by adequate vitamin E supplementation.

Bleeding tendency has been observed in populations with high fish consumption (Bang and Dyerberg, 1980). Eskimos have been reported to suffer from increased mortality due to stroke (Kromann and Green, 1980). Goodnight et al. (1980) noted that several subjects developed thrombocytopenia during consumption of a salmon diet.

Because EPA is a competitive inhibitor of cyclooxygenase and the biosynthesis of dienoid prostaglandins, it has been speculated that omega-3 fatty acids may induce kidney side-effects similar to those caused by the nonsteroidal anti-inflammatory drugs. These drugs (which also inhibit cyclooxygenase) precipitate reversible acute renal failure in patients with a reduction in real or effective blood volume associated with conditions such as volume depletion, congestive heart failure, cirrhosis (especially with ascites), and post-operative fluid sequestration (Dunn and Zambraski, 1980). No such effects have been observed in animal studies or human trials, but should probably be sought in future and ongoing trials, especially in patients with compromised renal function.

With the exception of possible contamination by heavy metals, pesticides, or PCBs, increased intake of intact fish should pose little hazard for the normal population (Dyerberg, 1986) and may, in fact, provide some physiological benefits. Fish oil concentrates and fish oil derivatives, however, require more caution. Many concentrated fish products contain high levels of vitamins A and D which could result in vitamin toxicity if these products are consumed in excess or in association with other vitamin supplements.
VIII. LITERATURE CITED


IX. LITERATURE NOT CITED


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APPENDIX

LIST OF ACRONYMS

<table>
<thead>
<tr>
<th>Acronym</th>
<th>Definition</th>
</tr>
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<tbody>
<tr>
<td>ADP</td>
<td>Adenosine diphosphate</td>
</tr>
<tr>
<td>ASA</td>
<td>Acetylsalicylic acid</td>
</tr>
<tr>
<td>CHD</td>
<td>Coronary heart disease</td>
</tr>
<tr>
<td>DHA</td>
<td>Docosahexaenoic acid</td>
</tr>
<tr>
<td>DRR</td>
<td>Division of Research Resources</td>
</tr>
<tr>
<td>EPA</td>
<td>Eicosapentaenoic acid</td>
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<tr>
<td>EPA-E</td>
<td>Ethylester of eicosapentaenoic acid</td>
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<tr>
<td>FASEB</td>
<td>Federation of American Societies of Experimental Biology</td>
</tr>
<tr>
<td>FDA</td>
<td>Food and Drug Administration</td>
</tr>
<tr>
<td>GC-MS</td>
<td>Gas chromatography-mass spectrometry</td>
</tr>
<tr>
<td>4-HDHA</td>
<td>4-hydroxydocosahexaenoic acid</td>
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<tr>
<td>7-HDHA</td>
<td>7-hydroxydocosahexaenoic acid</td>
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<tr>
<td>HDL</td>
<td>High-density lipoprotein</td>
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<tr>
<td>5-HEPA</td>
<td>5-hydroxyeicosapentaenoic acid</td>
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<tr>
<td>5-HETA</td>
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<td>4-HPDHA</td>
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<tr>
<td>5-HPETA</td>
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<tr>
<td>LDL</td>
<td>Low-density lipoprotein</td>
</tr>
<tr>
<td>LSRO</td>
<td>Life Sciences Research Office</td>
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<tr>
<td>LT</td>
<td>Leukotriene</td>
</tr>
<tr>
<td>MaxEPA</td>
<td>A commercial concentrated fish oil product containing approximately 18% EPA and 12% DHA</td>
</tr>
</tbody>
</table>
NHLBI  National Heart, Lung and Blood Institute
NIAID  National Institute of Allergy and Infectious Diseases
NIADDK National Institute of Arthritis, Diabetes, Digestive and Kidney Diseases
NIH   National Institutes of Health
NOAA  National Oceanic and Atmospheric Administration
PCBs  Polychlorinated biphenyls
PGE$_2$ Prostaglandin E$_2$
PGE$_2$ Prostaglandin E$_2$
PGG$_2$ Prostaglandin G$_2$
PGH$_2$ Prostaglandin H$_2$
PGI$_2$ Prostacyclin, prostaglandin I$_2$
PGI$_3$ Prostaglandin I$_3$
P/S Polyunsaturated/saturated
PUFA Polyunsaturated fatty acids
RFA   Request for an Application
TXA$_2$ Thromboxane A$_2$
TXA$_3$ Thromboxane A$_3$
TXB$_2$ Thromboxane B$_2$
TXB$_3$ Thromboxane B$_3$
USDA  U.S. Department of Agriculture
VLDL  Very-low-density lipoprotein