

DEPARTMENT OF HEALTH AND HUMAN SERVICES**Food and Drug Administration****21 CFR Part 101**

[Docket No. 91N-0103]

RIN 0905-AB67**Food Labeling: Health Claims and Label Statements: Omega-3 Fatty Acids and Coronary Heart Disease**

AGENCY: Food and Drug Administration, HHS.

ACTION: Final rule.

SUMMARY: The Food and Drug Administration (FDA) is announcing its decision not to authorize the use on the label or labeling of foods of health claims relating to an association between omega-3 fatty acids and coronary heart disease (CHD). The agency has determined, based on: (1) The totality of the publicly available scientific evidence; and (2) the agency's review of comments received in response to its November 27, 1991, proposed rule on omega-3 fatty acids and CHD, including scientific information included in those comments, that there is not significant scientific agreement among experts that such evidence supports a health claim for omega-3 fatty acids and CHD. Further, FDA has determined that the new information does not change the conclusions that the agency reached on the basis of the information reviewed in its proposal. Therefore, FDA has concluded that such a claim is not justified. This action is in response to provisions of the Nutrition Labeling and Education Act of 1990 (the 1990 amendments) that bear on health claims, and is developed in accordance with the final rule on general requirements for health claims, published elsewhere in this issue of the *Federal Register*.

EFFECTIVE DATE: May 8, 1993.**FOR FURTHER INFORMATION CONTACT:** John C. Wallingford, Center for Food Safety and Applied Nutrition (HFS-465) Food and Drug Administration, 200 C St. SW., Washington, DC 20204, 202-205-5461.**SUPPLEMENTARY INFORMATION:****I. Background**

In the *Federal Register* of November 27, 1991 (56 FR 60663), FDA proposed not to authorize a health claim relating diets high in omega-3 fatty acids to reduced risk of heart disease. The proposed rule was issued in response to provisions of the 1990 amendments (Pub. L. 101-535) that bear on health claims and in accordance with the

proposed general requirements for health claims for food (November 27, 1991, 56 FR 60537). As amended in 1990, the Federal Food, Drug, and Cosmetic Act (the act) provides that a food is misbranded if it bears a claim that characterizes the relationship of a nutrient to a disease or health-related condition unless the claim is made in accordance with section 403(r)(3) or (r)(5)(D) of the act (21 U.S.C. 343(r)(3) or (r)(5)(D)).

Congress enacted the health claims provisions of the 1990 amendments to help U.S. consumers maintain good health through appropriate dietary patterns and to protect consumers from unfounded health claims. Section 3(b)(1)(A) of the 1990 amendments specifically requires the agency to determine whether health claims for 10 nutrient-disease relationships meet the requirements of section 403(r)(3) or (r)(5)(D) of the act. The relationship of omega-3 fatty acids and heart disease is one of the claims required to be evaluated. In the *Federal Register* of March 28, 1991 (56 FR 12932), FDA published a notice requesting scientific data and information on the 10 specific topic areas identified in the 1990 amendments. Relevant scientific studies and data received in response to this request were considered as part of the agency's review of the scientific literature on omega-3 fatty acids and CHD and were included in the proposed rule.

In addition, on January 30 and 31, 1992, FDA held public hearings on all aspects of the proposed rules (57 FR 239).

FDA requested in the *Federal Register* of November 27, 1991 (56 FR 60663), written comments in response to its proposed rule, FDA reviewed all of the comments it received, including new data submitted in the comments, and scientific articles referred to in the comments. FDA also reviewed additional scientific articles, reviews, and recommendations published from August 1991 through February 1992.

The Dietary Supplement Act of 1992 (DS Act) established a moratorium on the implementation of the 1990 amendments with respect to dietary supplements. The DS Act says that FDA can grant health claims for food, including dietary supplements, under section 403(r)(3)(B)(i) of the act. However, it may not act on such claims under section 403(r)(5)(D) of the act until it establishes a standard to implement that section of the act, which the DS Act says may not occur until December 1993. Section 3(b)(1)(A)(x) of the 1990 amendments directs the agency to evaluate the omega-3 fatty acids/CHD

claims based on the standard that FDA is establishing for determining the reliability of health claims under section 403(r)(5)(D) of the act. In the November 27, 1991, proposal on general requirements for health claims, FDA proposed to adopt the standard that the 1990 amendments provide for conventional foods, which is set forth in section 403(r)(3)(B)(i) of the act, as the standard for dietary supplements; Given this fact, and the fact that omega-3 fatty acids are found in numerous conventional foods as well as in dietary supplements, FDA broadened its inquiry to a determination as to whether it should grant a health claim on omega-3 fatty acids and CHD for any foods.

Because the DS Act provides that FDA may grant claims using the significant scientific agreement standard specified in section 403(r)(3)(B)(i) of the act, and given the breadth of FDA's November 1991, proposal on omega-3 fatty acids, FDA has decided to move forward to determine whether it can authorize a claim under section 403(r)(3)(B)(i) for omega-3 fatty acids and CHD.

However, this rule does not apply to dietary supplements. While a manufacturer of a dietary supplement can make a claim on omega-3 fatty acids and CHD without rendering its product misbranded under section 403(r)(1)(B) of the act, the manufacturer should assure itself that the making of the claim will not misbrand the product under section 403(a).

II. Summary of Comments and the Agency's Response

FDA received 80 letters, each containing one or more comments, from consumers, health care professionals, universities and research institutes, health profession associations, consumer advocacy organizations, State and local governments, foreign governments, trade organizations, industry, and professional organizations. In addition to these comments, the agency also considered statements made on omega-3 fatty acids and CHD at the January 30 and 31, 1992, public hearings. Some of the comments agreed with one or more of the aspects of the proposed rule, without providing further grounds for support other than those provided by FDA in the preamble to the proposal. Other comments disagreed with one or more aspect of the proposal without providing specific grounds for the disagreement. A few comments addressed issues outside of the scope of this document and will not be discussed in this document. Most of the comments provided specific grounds in support of their positions concerning aspects of this health claim

as proposed. The agency has summarized and addressed the issues raised in the sections of this document that follow.

A. General Comments

1. Definition of omega-3 fatty acids and composition of omega-3 fatty acid supplements

1. One comment criticized the definition of omega-3 fatty acids used in the proposed rule, on the basis that omega-3 fatty acids were not distinguished from other polyunsaturated fatty acids (PUFA's).

In the proposed rule, FDA limited the term omega-3 fatty acids to eicosapentaenoic acid ((EPA), 20 carbons, 5 double bonds) and docosahexaenoic acid ((DHA), 22 carbons, 6 double bonds) (56 FR 60663 at 60664). FDA noted that most of the relevant research has used fish or fish oils rich in these two fatty acids.

FDA acknowledges that its statement Defining omega-3 fatty acids did not explicitly refer to omega-3-fatty acids. The sentence: "Their unique characteristic is the location of the first double bond, which occurs at the third carbon from the methyl (or omega) end of the fatty acid." (56 FR 60663 at 60664) was intended to refer to omega-3 fatty acids. This definition distinguishes omega-3 fatty acids from other PUFA's, which have their first, unsaturation at the sixth or ninth carbon from the omega end of the fatty acid.

2. One comment argued that the definition of omega-3 fatty acids should include land-based (primarily plant) omega-3 fatty acids (i.e., linolenic acid). (For the purposes of this document, the term linolenic acid is used to indicate the omega-3 fatty acid, alpha linolenic acid. In contrast, gamma linolenic acid has its first double bond at the sixth carbon from the omega end of the fatty acid, and is not an omega-3 fatty acid.)

FDA disagrees with this comment. FDA defined omega-3 fatty acids as EPA and DHA, primarily as a functional definition derived from the scientific literature. The hypothesis for a relationship between omega-3 fatty acids and CHD derived from correlations between low rates of CHD and high consumption of fish oils. Similarly, most of the intervention studies have used fish oil or fish as a source of EPA and DHA, not plant oils rich in linolenic acid. The comment provided no evidence that linolenic acid has biochemical effects comparable to EPA or DHA, nor has FDA found any evidence of a relationship between linolenic acid and CHD. Moreover, only a limited amount of linolenic acid is

converted in the body to EPA and DHA (Ref.100). Therefore, FDA believes it has represented the potential nutrient-disease relationship appropriately by limiting its attention to EPA and DHA.

3. Two comments stated that FDA's position on fish as opposed to the omega-3 fatty acids in fish was a tautology, because: "if polyunsaturated fatty acids have beneficial effects on CHD, and if fish oils are a member of this class of fatty acids, it should not be counted against their beneficial effects on CHD."

FDA disagrees with this comment. FDA considers the claim for omega-3 fatty acids to reflect the unique biochemistry of these fatty acids. In particular, the prevailing theory about the mode of action of omega-3 fatty acids is that they compete with omega-6 fatty acids (fatty acids with their first double bond at the sixth carbon from the methyl end, and which comprise the largest amount of dietary PUFA's). Thus, a clear separation of effects of omega-3 fatty acids from effects of other (primarily omega-6) PUFA's is needed to support a claim.

4. One comment stated that FDA did not consider the importance of the ratio of omega-3 fatty acids to arachidonic acid (AA), a 20 carbon omega-6 PUFA with four double bonds, and stated that evidence exists for a relationship between the saturated fat:unsaturated fat ratio in the diet and the omega-6:omega-3 fatty acid ratio in the diet and the risk of CHD.

FDA considers concerns about the ratio of AA to omega-3 fatty acids and the ratio of omega-6 fatty acids to omega-3 fatty acids to be reasonable in view of the competition between these classes of fatty acids in human biochemistry. FDA considered all types of foods and supplements used to provide omega-3 fatty acids in its evaluation of the claim. Although the AA content of the supplement was often reported, studies did not report data for total dietary AA. FDA is aware of only very limited data regarding the ratios of AA to omega-3 fatty acids, and of the omega-6 fatty acid:omega-3 fatty acid ratio in the diet and the risk of CHD. Therefore, it is not possible for FDA to draw any conclusions about these ratios and their possible modifying effects on the omega-3 fatty acids CHD health claim.

However, because the fish oils used contained high concentrations of omega-3 fatty acids, FDA believes that the amounts of fish oils supplemented to the various test diets would have affected the AA:omega-3 fatty acid ratio and the omega-6:omega-3 fatty acid ratio of the diets to some extent. FDA advises

that interested persons may petition FDA under § 101.70 (21 CFR 101.70) to issue a regulation regarding a health claim that relates these ratios to the risk of CHD.

5. Another comment pointed out that supplements used currently have contained various amounts of short- and long-chain omega-3 fatty acids and that many supplements also contain saturated fat. The comment stated that some of the discrepancies in reported findings may be due to the type of supplement used.

FDA agrees that numerous supplements varying in fatty acid composition have been used, and that the variation in the fatty acid composition of supplements may have influenced the outcome. FDA reexamined the studies cited in its proposal and the new data submissions for evidence that the nature of the supplement used was related to the outcome. However, the agency found that the same results are observed regardless of the source of omega-3 fatty acids. For example, in eight well-designed studies cited in the proposal on the total serum cholesterol response among normal subjects (Refs., 6, 9, 14, 49, 54, 73, 156, and 166,) six different sources of omega-3 fatty acids were used: Salmon oil, SuperEPA, MaxEPA, a fish oil triglyceride, Promega, and mackerel paste. None of these supplements produced a change in total serum cholesterol. Similarly, four different sources of omega-3 fatty acids (fresh water fish, salmon oil, purified EPA, MaxEPA) were shown in seven well-designed studies to reduce platelet aggregation in normal subjects (Refs. 2, 6, 24, 54, 96, 143, and 166).

FDA did note that some differences in response have been produced by supplements that vary in ratio of EPA to DHA. For example, one fish oil (pollock oil) with a high EPA:DHA ratio increased low-density lipoprotein (LDL) cholesterol, LDL triglyceride and apoprotein B (apoB) (a protein component of LDL) in comparison to a butter-rich diet, but two fish oils with a low EPA:DHA ratio (tuna oil, salmon oil with added palmitic acid) reduced apoB and LDL cholesterol, and increased LDL triglyceride to a smaller extent than the pollock oil in comparison to the butter-rich diet (Ref. 17). However, the effects of the two major omega-3 fatty acids have not yet been systematically investigated. FDA recognizes that purified EPA and DHA are now available for research; such supplements will enable the study of the individual effects of these fatty acids.

6. One comment stated that conservation of omega-3 fatty acids in

the body calls into question the importance of the amounts of omega-3 fatty acids used in scientific studies. However, the comment did not suggest any alternate method to describe intake.

FDA recognizes that fish is not ordinarily consumed daily. However, the 1990 amendments require that health claims on foods be stated in such a way as to enable the public to understand the relative significance of such information in the context of a total daily diet (section 403(r)(3)(B)(iii) of the act). Thus, a reasonable estimate of daily dietary intake of omega-3 fatty acids is needed when assessing the relationship between omega-3 fatty acids and the risk of CHD. Most of the studies reviewed by the agency used daily supplementation with a known amount of omega-3 fatty acids, but others estimated intake of omega-3 fatty acids from foods consumed in the daily diet. Both types of intake estimates are important. Daily supplementation is useful to relate changes to a carefully controlled amount of omega-3 fatty acids. The average daily intake of omega-3 fatty acids in nonintervention studies provides a basis upon which to determine whether the amounts of omega-3 fatty acids fed in supplementation studies are reasonable in the context of the total daily diet

2. Criteria used in evaluating studies

In the proposed rule, FDA listed some of the criteria used in evaluating epidemiological studies on the relationship of omega-3 fatty acids to CHD: (1) The reliability and accuracy of the methods used in food intake analysis and measurements of disease endpoints, (2) the choice of control subjects, (3) the representativeness of the subjects, (4) the control of confounding factors in data analysis, (5) the potential for misclassification of individuals with regard to dietary exposure or disease endpoints, (6) the presence of bias, and (7) the degree of compliance and how compliance was assessed (56 FR 60667).

However, FDA stated that it considered randomized, double-blind, placebo-controlled trials to be more valuable than other types of human studies because they were less susceptible to bias, and because they allowed inference about the specific effects of omega-3 fatty acids. Studies in which the endpoint was CHD, by definition, provide the most persuasive type of evidence, but studies measuring CHD to date have not provided the specificity to show that the observed effects were due to omega-3 fatty acids.

7. Some comments expressed the concern that it was unlikely that

additional clinical trials will be done due to their expense, and that, therefore, FDA should rely more heavily on epidemiologic studies, animal studies, and biochemical and physiological interventions that suggest an effect of omega-3 fatty acids on risk of CHD.

FDA has no basis upon which to agree or disagree with the comments' assertion that further clinical trials are unlikely. FDA disagrees that its emphasis on clinical trials was misplaced. Because the 1990 amendments addressed nutrient-disease relationships, FDA considered human studies that used CHD as the endpoint to be the most directly relevant studies, although these studies do not demonstrate that the effects are specifically due to omega-3 fatty acids. Human studies in which a surrogate marker for CHD risk was measured as the endpoint of the treatment were also considered carefully. The advantage of these studies is that they are able to demonstrate specificity of the effects due to omega-3 fatty acids. However, the relationships between many of these surrogate markers and risk of CHD are not well established, making it difficult to relate changes in these endpoints brought about by omega-3 fatty acids to the risk of disease. Biologic markers can serve as markers of a developing disease, but the relevance of such evidence depends directly on the strength of the association between the marker and the disease (Ref. 115).

FDA agrees that there are considerable additional data in animal studies, in vitro studies, and biochemical and physiological interventions regarding the effects of omega-3 fatty acids. However, it is not clear that the results of such studies are relevant to the risk of human disease. Thus, FDA believes that these other types of data are of secondary importance compared to clinical data that measure either CHD per se or established surrogate markers for CHD.

However, in response to the comments, FDA has provided a more thorough description of animal and in vitro studies that suggest a role for omega-3 fatty acids in reducing the risk of CHD, particularly with respect to the effects of omega-3 fatty acids on the development of atherosclerosis and with respect to the responsiveness of blood vessels to ischemia (see comments 38 and 49 and section II.C.3.a. of this document).

8. Many comments stated that the agency's position on omega-3 fatty acid and CHD was inconsistent with its position on other health claims, and argued that for each of the four claims proposed to be allowed by FDA, the

data were no stronger than the data supporting the link between omega-3 fatty acids and CHD. The comments asserted that, by basing its decision on the relationship between the nutrient and a surrogate marker for the disease, or for a susceptible subpopulation, FDA held other claims to a less restrictive standard. One comment stated: "The FDA statement is internally consistent in denying health claims for omega-3 fatty acids, but this is only in the context of holding these food components to essentially impossible standards not required for other, allowable, claims."

Specific comparisons were made to the proposed claims on fat and CHD, fat and cancer, calcium and osteoporosis, and sodium and hypertension. Other comments indicated that qualified claims, such as that for calcium and osteoporosis, were appropriate models for the claim relating omega-3 fatty acids to CHD.

FDA disagrees with these comments. FDA believes that for these other claims there is significant scientific agreement among qualified experts regarding the relationship between the nutrient and the disease, whereas there is not such agreement regarding the relationship between omega-3 fatty acids and CHD, or between omega-3 fatty acids and agreed surrogate markers for risk of CHD. For example, based on the totality of the publicly available scientific evidence, FDA determined that there is significant scientific agreement about the role of calcium in maintaining bone mineral density (the relationship of the nutrient to the intermediate marker for the disease), and about the relationship between peak (maximal) bone mass and the risk of developing osteoporosis and related bone fractures later in life (the relationship between the intermediate marker and the disease itself) (see 56 FR 60689; see also the final rule on calcium and osteoporosis published elsewhere in this issue of the *Federal Register*). Similarly, FDA relied on a long history of Federal Government and other consensus statements to conclude that there is significant scientific agreement about the role of sodium as a causal factor in hypertension for a segment of the population. (See 56 FR 60825; see also the final rule on sodium and hypertension, published elsewhere in this issue of the *Federal Register*.) FDA also recognized the history of significant scientific agreement about the relationships between fat and cancer and between fat and CHD evidenced by statements in reports issued by Federal Government and other authoritative bodies. (See 56 FR 60764, 56 FR 60726; see also final rules on fat and cancer and

fat and CHD, published elsewhere in this issue of the Federal Register.).

Thus, these other nutrient-disease relationships have a history of being recognized in Federal Government and authoritative reports, indicating significant scientific agreement, whereas the relationship between omega-3 fatty acids and CHD has not been so recognized. For two of these other nutrient-disease relationships, the data relate to the disease itself, rather than to markers for the disease. In the other two, calcium and osteoporosis and fat and CHD, there is significant scientific agreement that the dietary factors are related to surrogate markers for the diseases, and that the surrogate markers are related to the diseases.

There is significant scientific agreement that serum cholesterol and blood pressure are risk factors for CHD, as indicated by the emphasis on these factors in Federal Government and other authoritative documents (Refs. 34 through 36, 100, 115, and 169). Data regarding the effects of omega-3 fatty acids on these endpoints have been carefully reviewed. However, the other endpoints measured in studies of the effects of omega-3 fatty acids, e.g., in vitro platelet aggregation, various growth factors, fibrinogen, have not achieved the same extent of scientific agreement.

Where authorized health claims include qualifications, the qualifications are intended to assure that the wording of allowed claims reflects those particular aspects of the substance-disease relationship for which there is significant scientific agreement, not to qualify the extent of agreement.

9. Some comments stated that FDA relied heavily on material published in the National Academy of Sciences 1989 report, "Diet and Health: Implications for Reducing Chronic Disease Risk" (Ref. 115) and the Surgeon General's 1988 report (Ref. 34), and did not place enough emphasis on information published since that time.

FDA acknowledges that the two reports in question were important to its assessment of the scientific evidence. However, the agency does not agree that it failed to give appropriate weight to subsequently published research. The 1990 amendments required the agency to consider the totality of publicly available scientific evidence in assessing nutrient-disease relationships. Given the time constraints imposed by the 1990 amendments for developing and publishing proposed regulations, FDA depended on Federal Government reports and reports of authoritative bodies (e.g. the National Academy of Sciences) for assessment of the scientific

evidence published before 1988. The reports were also used as a way of determining whether there was significant scientific agreement among qualified experts that the evidence supports a relationship between omega-3 fatty acids and CHD. The agency's reliance on these reports is consistent with the 1990 amendments, which require the agency to consider reports from authoritative scientific bodies of the United States in assessing health claim petitions and to justify any decision rejecting the conclusions of such reports (section 403(r)(4)(C) of the act).

Recognizing, however, that considerable research had been published since these reports, and that these reports had not been updated, FDA also reviewed the available studies on humans published since 1988. FDA relied on its own review of individual studies rather than review articles, because review articles generally reflect the bias of the author and may not consider the totality of the evidence. FDA focused its independent review on primary papers published between January 1988 and August 1991. Surveys and cross-sectional or prospective studies that were published before 1988 and used to generate the hypothesis of a relationship between omega-3 fatty acids and CHD were also reexamined. Thus, by utilizing the two reports in question, supplemented with an independent review of the subsequently published research, FDA was able to assess the totality of the scientific evidence on omega-3 fatty acids and CHD in compliance with the statutory standard.

10. One comment suggested that FDA was inconsistent with the conclusions of the major reviews of this area, published after the Federal Government and other comprehensive reports. They stated that of the nine major reviews (excluding Kinsella, and Connor and Connor), eight concluded that omega-3 fatty acids played a beneficial role with factors affecting heart disease.

Although FDA did not rely on review articles to assess the strength of association between omega-3 fatty acids and CHD, each review was read, and the agency interprets these reviews as supporting the hypothesis in concept. However, each review contained reservations about the extent to which the relationship between omega-3 fatty acids and CHD was established. The cautionary statements suggest general agreement that the area of omega-3 fatty acids and CHD holds promise for further research along a number of lines, but that, at present, there are not sufficient data to have certainty about the

relationship between omega-3 fatty acids and CHD. Placed in chronological order, the concluding sections from the cited review articles exemplify the lack of certainty as to the relationship between omega-3 fatty acids and risk of CHD.

The review of the relationship between omega-3 fatty acids and CHD by Leaf and Weber (Ref. 91) was considered in the National Academy of Sciences' "Diet and Health: Implications for Reducing Chronic Disease Risk" report (Ref. 115). FDA elected to include the Leaf and Weber review in its citations because it covered, in the most comprehensive manner of all available reviews, the state of scientific knowledge about omega-3 fatty acids in CHD at the time the Federal Government and other comprehensive reviews were published. Leaf and Weber wrote: "Despite claims that n-3 fatty acids can help prevent atherosclerosis, recommendations to the public on diet have been conservative; people have been advised to increase their consumption of fish by replacing two or three meals a week containing red meat with meals containing fish." Their concluding sentence was: "If prospective double-blind, placebo-controlled clinical trials were to show that n-3 fatty acids helped to prevent atherosclerosis, these agents apparently would represent one of the most benign interventions in our pharmacopeia." (Emphasis added.)

Bonaa (Ref. 10) wrote in his conclusion that the data on blood pressure:

* * * provide some support for the hypothesis that dietary marine lipids influence blood pressure in man. Supplementation of n-3 PUFA [polyunsaturated fatty acids] to Western diets consistently lowered systolic blood pressure, while results for diastolic blood pressure were conflicting * * *. There is no evidence of any substantial hypotensive response to marine lipids and further studies should be designed to detect small effects.

Lands (Ref. 89) did not review the relationship between omega-3 fatty acids and any specific disease, but presented the hypothesis that the balance of omega-3 and omega-6 fatty acids in the diet may be related to diseases associated with overproduction of eicosanoids from AA. He indicates in the introduction that, "We are now in an uncertain time of evaluating the benefits and risks of dietary n-6 and n-6 polyunsaturated fats."

Weber (Ref. 161) concluded:

The promise of n-3 fatty acids deduced from biochemical and functional effects will have to be evaluated in ongoing and future carefully designed and conducted studies. So

far, published data of controlled clinical trials incorporating clinical endpoints after n-3 PUFAs are available only in abstract form. Therefore, the gap between biochemical and functional effects of dietary fatty acids assumed to be of clinical benefit in the prevention of atherothrombotic and allergic/inflammatory disorders is only beginning to be closed. (Emphasis added.)

Connor and Connor (Ref. 21) wrote in their summary:

The exact place of omega-3 fatty acids from fish and fish oil remains to be defined. However, this much seems certain. Fish provides an excellent substitute for meat in the diet. Fish is lower in fat, especially saturated fat, and contains the omega-3 fatty acids. Fish oil may have promise as a therapeutic agent in certain hyperlipidemic states, especially the chylomicronemia of type V hypolipidemia. Fish oil has logical and well-defined antithrombotic and antiatherosclerotic activities since it depresses thromboxane A2 production and inhibits cellular proliferation responsible for the progression of atherosclerosis. As the years pass and more experiments are reported, it seems reasonable to place the omega-3 fatty acids from fish oil in a prominent position for specific hypolipidemic, antithrombotic and antiatherosclerotic activity.

Kinsella et al. (Ref. 82) wrote:

The cumulative findings concerning fish oils suggest that further amelioration of coronary heart disease may be feasible by dietary manipulation and by optimizing the intake of n-6 and n-3 PUFAs, not only to reduce plasma lipids but to ensure balanced eicosanoid metabolism—a prospect that deserves more research ***. Overall, in view of the prevalence of coronary heart disease, consumption of n-3 PUFA oils should be considered as a useful complementary option for the amelioration of coronary vascular diseases.

Knapp (Ref. 84) introduced his paper stating: "The role of dietary polyunsaturated fats in the prevention of human vascular disease has not been defined, but population and intervention studies have suggested that w-3 fatty acids (FAs) from marine lipids may have a number of potentially beneficial effects." (Emphasis added.) And in conclusion he wrote: "The proof of our hypotheses must be derived from increasingly ambitious clinical trials, which assess the potential benefits of dietary polyunsaturates in particular clinical settings, the recent demonstration that three helpings of oily fish per week prolongs survival after MI (Ref. 16) is an example of this." (Emphasis added.)

Nestel (Ref. 111) concluded: "More basic understanding of the actions of fish oils is necessary before fish oils can be recommended widely to the public."

Nordoy and Goodnight (Ref. 112) cautioned that until additional data

become available, "clinicians should be advised to follow the dietary recommendations of the National Cholesterol Education Program's expert panel," which is silent on omega-3 fatty acids and limits the total: polyunsaturated fat to 10 percent of calories. These reviewers added their own recommendation that omega-6/omega-3 ratio of the PUFA's be approximately 3/1, with the omega-3 fatty acids from marine sources.

Weber and Leaf (Ref. 162) stated:

Despite all the laboratory, human, animal, and epidemiologic studies suggesting an anti-atheromatous action of w-3 fatty acids, we have been lacking adequate clinical trials which will determine in prospective, placebo-controlled, randomized studies, whether all the above experimental and epidemiologic evidence adds up to a demonstrable effect of fish oils to prevent atherosclerosis, e.g., coronary heart disease in humans at high risk for heart attacks.

The Burr paper (Ref. 16) was described in Weber and Leaf's review, and thus was considered in the above summary statement.

In summary, these reviews indicate that what is agreed is that there is a plausible biochemical basis for a relationship between omega-3 fatty acids and CHD, and that there are some data supporting some of the hypothesized mechanisms by which omega-3 fatty acids might be related to CHD. What is not agreed, as indicated by the cautious tones of these concluding statements, is that such a relationship already has been established by the evidence.

11. A concern raised by many comments was that FDA's conclusions were different from the conclusions reached in the report from the Life Sciences Research Office (LSRO) of the Federation of American Societies for Experimental Biology (Ref. 100), the most recent comprehensive review, and that FDA did not explain why it reached a different conclusion from that reached in the LSRO report.

The LSRO report was contracted for by FDA as an independent review of the scientific evidence about the relationship between omega-3 fatty acids and CHD. A draft of the tentative final report was received immediately prior to the publication of the proposed rule. Thus, there was insufficient time for the agency to prepare a detailed discussion of the report. The final report was submitted to FDA as a comment to the proposal. The LSRO report's conclusions on hypertension, thrombosis, the development of atherosclerotic plaque and intimal hyperplasia, plasma lipids and lipoproteins, diabetic and prediabetic

patients, and epidemiologic observations are grouped with other comments on these topics and discussed in this document.

12. One comment considered FDA's caution against extrapolation of results from studies conducted in at-risk populations to the general population to be questionable, and possibly biased against hypertensives. The comment stated that the health claim should be allowed, based on data showing that omega-3 fatty acids reduce blood pressure among hypertensives.

FDA disagrees with this comment. FDA stated that, although it considered studies in the healthy population to be the most relevant, it also considered studies in a subpopulation with CHD or risk factors for CHD, in part because high risk populations may be more sensitive to showing a nutrient-disease relationship than the general population (56 FR 60663 at 60667). FDA stated that it extrapolated positive results from at-risk populations cautiously, and that comparable findings in the general population were needed to support a health claim.

13. Two comments discussed FDA's criteria for weighing various types of data. One comment stated that epidemiologic data are the "most significant class of evidence," and that FDA should give priority to various types of data in the same order that various types of data were reviewed in the proposal. One comment stated that FDA should not have considered epidemiological studies separately from clinical trials.

FDA considered the totality of publicly available scientific evidence in its assessment of the relationship of omega-3 fatty acids to CHD. However, some types of evidence were weighted more heavily than others because they were more useful in establishing whether or not the scientific basis of the claim was valid. In particular, the agency was concerned that both the substance (omega-3 fatty acids) and the disease (CHD) be carefully characterized. FDA also considered it important that the amount of omega-3 fatty acids tested was reasonably related to normal dietary intake, and that the findings apply to the general population. FDA agrees that epidemiologic studies in which the endpoint was CHD provide persuasive evidence for a relationship between fish consumption and CHD, but these studies did not provide the specificity to show that the observed effects were due to omega-3 fatty acids. Intervention trials using fish oil supplements often showed that the effects were specific to omega-3 fatty acids (by controlling with

other types of fatty acids) but typically did not measure the primary endpoint, CHD. Thus, these different types of data complement each other and must be considered together in assessing the totality of the scientific evidence.

14. One comment offered the services of the International Society for the Study of Fatty Acids and Lipids for the evaluation of the relationship between omega-3 fatty acids and CHD.

FDA appreciates this offer. In the final rule on general requirements for health claims published elsewhere in this issue of the *Federal Register*, FDA advises that it welcomes the input of any professional organization that can provide expertise in reviewing data and in developing a thoughtful and well-organized petition for a health claim on a particular topic. In fact, FDA has added to § 101.70(b) the provision that information submitted with petitions may include any findings, along with the basis of the findings, of an outside panel with expertise in the subject area at issue. FDA, however, retains the authority to review such petitions and, through rulemaking, to decide whether or not to authorize the claim.

15. Two comments stated that it was contradictory for the U.S. Government to contract for research on the omega-3 fatty acids through the biologic test materials program but not to allow a health claim. Another comment pointed out that the Food and Agriculture Organization and the World Health Organization support research on omega-3 fatty acids. Other comments slated that the tone of FDA's proposed rule was unduly negative and that, by taking such a position, FDA may retard further research.

FDA disagrees that Federal Government sponsorship of a program to provide test materials for research on the effects of omega-3 fatty acids and the denial of the omega-3 fatty acid-CHD health claim are contradictory actions. The purpose of the biologic test materials program is to develop, and standardize a source of omega-3 fatty acids and enable carefully controlled research on the effects of particular omega-3 fatty acids. In the proposed rule, FDA's intent was to examine the total available scientific evidence, some of which was generated using omega-3 fatty acids from the biologic materials test program, and to state its conclusions about the relationship between omega-3 fatty acids and CHD.

In its proposal and in this final rule, FDA has identified a number of areas where agreement is lacking that an observed effect of omega-3 fatty acids is related to the risk of CHD, or where there are ambiguities in the data that

may be resolved by further research. Thus, FDA's analysis should provide guidance for additional research rather than inhibit it.

B. Relationship Between Omega-3 Fatty Acids and CHD

In the proposed rule, FDA tentatively concluded that the totality of the scientific evidence does not provide a basis upon which to authorize a claim that omega-3 fatty acids are associated with the risk of CHD (56 FR 60663). FDA noted that:

the epidemiological research on this topic revealed that the available studies applied only to the consumption of fish, which contain omega-3 fatty acids, and * * * it was not possible to ascribe any effects specifically to the omega-3 fatty acids. Examination of data from clinical studies revealed that the effects on blood lipids of fish oils containing omega-3 fatty acids were primarily a reduction of blood triglycerides, a blood lipid variable not considered to be an independent risk factor for CHD, but they had no effect on serum cholesterol, low-density lipoprotein (LDL) cholesterol, or high density lipoprotein (HDL) cholesterol, the blood lipid variables most closely associated with risk of CHD. The scientific data are ambiguous on the effects of omega-3 fatty acids on blood pressure and other risk factors for CHD. (56 FR 60663.)

A number of comments supported the agency's position on this health claim, but without any specific reasons for that support. One comment agreed with the agency's position in principle, but contested the agency's interpretation of the scientific information in some areas. Other comments disagreed with the agency's review of the scientific information and its conclusion regarding the strength of the evidence supporting the proposed health claim. Specific comments are summarized below.

1. Epidemiologic evidence

In the proposed rule, FDA reviewed correlational and cross-sectional studies, prospective studies, and intervention studies available since 1988. (See 56 FR 60663 at 60667 through 60668.) Except for the intervention studies (which were typically clinical trials) these studies used fish as a source of omega-3 fatty acids. FDA concluded that those studies that used fish as the source of omega-3 fatty acids were: "ambiguous, because they are not capable of distinguishing the effects that are specific to omega-3 fatty acids from those that are related to fish consumption." (56 FR 60663 at 60668.)

16. A number of comments considered the evidence from epidemiologic studies that relates the

consumption of fish inversely to CHD to be sufficient to support a health claim, but did not supply any new information or arguments to support their position.

FDA disagrees with the comments FDA found that:

Only a few studies found an association between, fish intake and CHD, while others have found no association. Thus there was not consistency of findings. None of the studies that reported a relationship distinguished fish consumption from other factors associated with fish consumption, and therefore they did not demonstrate specificity. Even in those studies reporting a relationship between fish consumption and CHD, it was not clear that the effects were because of the omega-3 fatty acids in fish. Also, the omega-3 fatty acid content of the fish diet associated with reduced CHD was so low that the importance of omega-3 fatty acids is questionable * * *

(56 FR 60663 at G0672.)

17. One comment described the results of the Dolecek and Grandits analysis of multiple risk factor intervention trial (MKFIT) data (Ref. 38) as indicating a greater protective effect against CHD due to consumption of 0.6 gram (g) omega-3 fatty acids than all other conventional efforts combined (reducing saturated fat, cholesterol, cigarette smoking, and hypertension).

FDA agrees with this comment that the association between omega-3 fatty acid consumption and CHD mortality reported in this study has the potential to make a very important public health impact. Notably, the results were obtained on data adjusted for age, race, smoking at entry to the study, diastolic blood pressure, and high-density lipoprotein (HDL) and LDL concentrations. Furthermore, the omega-3 fatty acids were obtained in the normal diet, providing evidence that the amount of omega-3 fatty acids consumed in a normal dietary intake is sufficient for the effect.

The researchers' adjustments for lipoprotein measurements should control for some other dietary variables that have been associated with CHD through their effects on these lipoproteins, e.g., saturated fat, but other dietary variables associated with CHD were not controlled, e.g., alcohol. The association between omega-3 fatty acid consumption and CHD mortality described in this study is among the most provocative findings to date in this area, and merits additional study using a design that will document that the active dietary component is or is not the omega-3 fatty acids (i.e., specificity of the effect).

18. One comment pointed out that the Burr paper (Ref. 16) deserved close consideration, because, in contrast to

trials on lipid lowering drugs, it showed that consumption of fish containing omega-3 fatty acids or dietary supplements of omega-3 fatty acids may reduce the risk of heart disease. One comment stated that it considered the Burr paper to be a positive finding, but gave no reason for this conclusion. The LSRO final report, submitted as a comment, also recognized the Burr paper as a very important trial. LSRO pointed out that, although separate results were not shown for those consuming fatty fish and those consuming supplemental fish oil, the results were dramatic, especially since all-cause mortality was reduced, in contrast to results from trials of plasma lipid-lowering drugs. LSRO concluded that "future research will be needed to define the amount and duration of w-3 fatty acid supplementation required to produce the beneficial effects."

FDA agrees that the Burr paper provides valuable evidence consistent with the hypothesized relationship between omega-3 fatty acids and CHD. However, FDA noted in its proposal (56 FR 60663 at 60668) that there are two specific shortcomings in this paper: the absence of separate data for subjects who consumed fish and those who consumed fish oil capsules, and the absence of dose-response data. These data would have provided evidence for a specific effect of omega-3 fatty acids. Ideally, other data regarding the subjects diet would also show that there was no difference in consumption of other dietary factors related to CHD. The study design specifically included two such dietary factors, dietary fat and dietary fiber, and the lack of significant effects of these components argues against dietary factors other than omega-3 fatty acids as responsible for the association.

FDA does not consider the Burr paper to have established a beneficial effect of omega-3 fatty acids, although its results are consistent with such an action. The LSRO conclusion indicates that neither the amount of omega-3 fatty acids necessary for beneficial effects nor the duration of their intake has been established. The specificity of the substance responsible for the beneficial effects, the quantitative amount needed to produce the effect and the duration of intake needed to produce the effect need to be established before FDA can authorize a claim linking omega-3 fatty acids to reduction of risk of CHD.

19. Some comments stated that the amount of fish in the Zutphen and Burr studies was so low that the association between fish consumption and reduced CHD mortality could not be explained

by the displacement by fish of other atherogenic foods from the diet.

FDA is not persuaded by these comments. The limitation in these studies is that they did not control for dietary factors associated with CHD, not that fish consumption displaced other atherogenic foods. FDA noted in its proposal that the Zutphen study found significant correlations between fish consumption and other dietary factors (i.e., alcohol, polyunsaturated fats) related to CHD. Comparable correlations were not addressed in the Burr paper because dietary intake data were not reported. Also, the design of the Burr paper was to encourage consumption of fish, which would likely have resulted in a reduction in the consumption of red meat (and, therefore, saturated fat).

20. Two comments discounted the Curb et al. study (Ref. 25), which showed no association between fish consumption and CHD mortality among subjects in Hawaii. The comments stated that the dietary source of fish was likely tropical fish, and since tropical fish feed on coral they have a high content of AA, which would counteract the effect of omega-3 fatty acids.

FDA disagrees with the comments. No data regarding the AA content of the diet in this study, or in other correlational studies, have been reported. Indeed, most epidemiologic correlation studies have not quantified the intakes of omega-3 fatty acids, a fundamental measurement to establish an association between omega-3 fatty acids and CHD. Finally, there is an abstract reporting that the omega-3 fatty acid to omega-6 fatty acid ratio of tropical fish is comparable to or greater than that of fish in higher latitudes (Ref. 237). Thus, the comments' explanation for a negative finding must be considered theoretical.

21. One comment argued that the lack of an association between fish consumption and CHD in two populations in Canada, a prairie province and a coastal province (Ref. 74), was because the prairie population consumed more alcohol and the coastal population smoked more. This comment criticized FDA for not pointing out the cautions raised by the authors about potential confounders like the difference in alcohol consumption.

FDA believes it presented the results of this paper fairly. While the authors reported small differences in smoking (more in the coastal population) and alcohol consumption (more in the prairie population), they stated, "It seems unlikely that these differences are sufficiently large to offset any strong effect of fish consumption." FDA is keenly aware that dietary and

behavioral factors (e.g., smoking, alcohol) must be controlled before meaningful conclusions may be drawn about the effects of omega-3 fatty acids. FDA notes that alcohol consumption was also a confounding factor in a study that reported an association between fish consumption and CHD (Ref. 87).

22. A few comments stated that many of the reported effects come from studies on fish consumption, but that all measured biochemical changes related to CHD that are produced by fish have also been produced with fish oil concentrates.

FDA agrees in part with this comment. The fact that the same biochemical results have been obtained using fish oils rather than fish provides strong evidence that particular biochemical markers are affected specifically by omega-3 fatty acids. Also, since most studies have used fish oils, these results add consistency to the effects reported for studies that used fish. However, FDA disagrees that the comparable findings in studies that used fish oils and fish are sufficient to support the health claim that omega-3 fatty acids reduce the risk of CHD, because the particular biochemical markers affected by both fish and fish oils are not recognized with significant scientific agreement as useful surrogate risk factors for CHD in the general population.

23. One comment argued that the fact that Greenland Eskimos ate diets with half the saturated fat and more polyunsaturated fat than Danes and had much less CHD than Danes strengthens the case for fish oil-derived omega-3 fatty acids.

FDA agrees with the comment that diets lower in saturated fat are consistent with reduced CHD mortality (see the final rule on "Dietary Lipids and Coronary Vascular Disease" published elsewhere in this issue of the *Federal Register*). The differences in saturated fat intake, however, do not strengthen the case for omega-3 fatty acids, because they do not distinguish omega-3 fatty acids from polyunsaturated fats. Rather, the differences in dietary fat intakes strengthens the argument that saturated fat is associated with CHD mortality. The numerous dietary differences between the Greenland Eskimos and Danes make it difficult to ascribe to any single dietary factor the differences in CHD.

24. One comment pointed out that, of the ten prospective studies cited in the proposal (including three in Table 1 of the proposal), six support an inverse relationship between fish consumption and CHD. The comment noted that one

study only showed a relationship in men under 45, and argued that this result is promising because one might expect to find positive effects of long-term fish consumption on CHD in younger, relatively healthy men rather than in older men.

FDA agrees that some but not all reports find an inverse relationship between fish consumption and CHD. FDA does not agree, however that this effect would more likely be noticed among younger, relatively healthy men. Older men would have had a greater duration of intake of omega-3 fatty acids and a greater incidence of CHD than younger men. Both these factors would favor finding an effect in older men rather than in younger men.

25. One comment noted that only two studies found a positive dose-response. Another comment stated that the studies that show no effect are those where the base group already consumed fish, whereas in studies that showed an effect, the base group did not consume fish. A third comment stated that there were data that described an inverse dose-response relationship between serum EPA and CHD deaths among Japanese, but did not identify a particular study.

FDA disagrees that only two studies found a dose response correlation. Each study that response a relationship between fish consumption (or, in one study, the calculated intake of omega-3 fatty acids) and CHD found a dose-response relationship. FDA agrees that most of the dose-response relationships reported suggest that the primary difference in rate of CHD is between those who consume no fish at all and those who consume a small amount of fish, and that there appears to be little additional benefit from consumption of large amounts of fish (Ref. 88). An alternate way of describing these data is that those who consume no fish have an increased risk of CHD. These data merit followup, because a showing that the relationship is due to the omega-3 fatty acids may provide evidence that the long-chain omega-3 fatty acids are essential in the diet.

In its proposal, FDA reviewed a study that described on inverse dose-response relationship between serum EPA and CHD mortality among two groups of Japanese (Ref. 70). FDA concluded that these cross-sectional, correlational data were useful in generating a hypothesis. Other notable dietary factors (including a difference in salt intake of 50 percent) and risk factors for CHD (prevalence of hypertension) also differed between the two groups, so it is not possible to conclude that differences in CHD

mortality were due to differences in dietary omega-3 fatty acids.

26. Two comments stated that FDA had erred in stating that no biochemical data were reported in the Burr paper (Ref. 16).

FDA agrees with this comment, and stands corrected. Bun et al. (Ref. 16) did report that the geometric mean percentages of EPA were 0.59 percent and 0.40 percent in men given advice to consume more fish and those not so advised, respectively, a highly significant difference ($p < 0.01$). The fact that a geometric mean rather than an arithmetic mean was reported implies that there was substantial skewing of the data.

It is not clear from the article whether these differences were for the 6-month time into the trial, or for the end of the trial. The authors did not correlate plasma EPA concentrations directly with myocardial infarction (MI) or CHD deaths.

27. One comment argued that it was highly misleading to state in Table 1 that Kromhout et al. (Ref. 67) reported that, "lean fish, low in omega-3 fatty acids, had some protective effect against CHD." because Kromhout did not distinguish between the effects of lean and fatty fish.

FDA disagrees with this comment. The authors made two statements about lean fish that imply that additional data analyses were conducted, although (as the comment correctly notes) results of these analyses were not included in the paper. The authors wrote, "Lean fish was also inversely related to mortality from coronary heart disease," and "Thus, the inverse relation between lean fish and coronary heart disease cannot be explained by eicosapentaenoic acid."

FDA interprets these comments as a caution to the reader against assuming that EPA was the active component responsible for the observed reduction in CHD among fish-consuming subjects.

28. LSRO included in its report two studies that correlated plasma omega-3 fatty acids with dietary intake of these fatty acids (Refs. 213 and 225). Two other papers reviewed by LSRO but not included in the FDA proposal were correlation studies of mortality from different diseases among Greenlanders and Danes (Ref. 170) and diet-disease correlations in Japan (Ref. 284).

FDA agrees with LSRO's descriptions of these studies. FDA notes that the authors of the studies that correlated intake and plasma levels of omega-3 fatty acids did not relate their data to CHD. The correlation studies of Mortality did not provide any specific data regarding omega-3 fatty acids.

29. One comment provided new dose-response data from additional analyses of data of the Dart study, previously reported in part by Burr et al. (Ref. 16), that related the dietary intake of EPA at 6 months into the trial to the risk of CHD events (heart attacks, or MI's) or CHD mortality. The 947 subjects for whom dietary data were obtained were grouped according to EPA intake; 114 consumed less than 1 g per week (1 g/week), 373 consumed 1 to 2 g/week, and 460 consumed 2 or more g/week. The percentage of subjects that experienced either a nonlethal heart attack or died from a heart attack decreased as dietary EPA increased. For heart attacks the rates were 7.9 percent, 7.0 percent and 6.7 percent, for the less than 1 g/week, 1 to 2 g/week and 2 or more g/week groups, respectively. The percentages in each group who died were 6.1 percent, 5.1 percent, and 4.1 percent, respectively. There were no statistical analyses of these data reported.

FDA notes some limitations in these data as reported that caution against strong conclusions. Most notably, the analysis excluded the events and deaths during the first 6 months of the trial, when about half of all events and deaths occurred. This clearly diminishes the sensitivity of the analysis, and may result in an underestimation of the true effect, since the difference in survival between the group advised to eat more fish and the group not advised to eat more fish was most pronounced, during the first 6 months. Alternatively, if the healthiest subjects were also the most compliant subjects, the reduced death rate in the highest EPA-consumption subjects may reflect the underlying health of those subjects, and the importance of dietary EPA may be overestimated.

Also, the unequal group sizes for this analysis places a greater weight on each subject in the smallest group (less than 1 g/week) than in the other groups. This may be particularly important because the smallest group includes those who consume no fish, and who may differ from fish consumers in other dietary or behavioral factors associated with CHD risk. The sensitivity of the results to small changes in outcomes is shown by example: one fewer death (6/114 rather than the reported number, 7/114) makes the CHD death rate of the less than 1 g/week group equal to the rate in the 1 to 2 g/week group.

Finally, although the dietary intake data at 6 months are useful, this study also assayed plasma fatty acids. Use of plasma EPA (or EPA plus DHA) in the dose-response analysis would have been a more powerful analysis, because it eliminates errors in the diet record data

corrects for losses during food preparation and individual differences in bioavailability of the fatty acids, and integrates intake of omega-3 fatty acids over a longer period than the diet record data.

Therefore, FDA finds these dose-response data to be consistent with the hypothesis that omega-3 fatty acids reduce the risk of CHD, but the shortcomings discussed above limit their usefulness in establishing a relationship between omega-3 fatty acids and risk of CHD.

2. Evidence relating omega-3 fatty acids to intermediate or surrogate markers of CHD

In the proposed rule (56 FR 60663 at 60668), FDA stated that most information about the effects of omega-3 fatty acids on CHD has been derived from clinical trials using concentrated fish oils enriched in EPA and DHA, and in some cases purified methyl or ethyl esters of EPA and DHA. FDA concluded that:

* * * there are a few established effects of omega-3 fatty acids from fish oils on thrombosis and hemostasis. Standardized bleeding times are increased, and platelet aggregation and function are reduced. However, direct relationships between the changes in bleeding times or platelet function and risk of CHD have not been established. While there is an established relationship between blood pressure and CHD, it has not been shown that omega-3 fatty acids specifically affect blood pressure in normal subjects in a way that would provide a protective benefit toward the risk of CHD. Effects of omega-3 fatty acids on other markers linked with CHD, e.g., fibrinogen or lipoprotein (a) have not been established. (56 FR 60663 at 60671).

a. Atherosclerosis

i. Blood lipids

30. Numerous comments criticized FDA's focus on blood cholesterol as a surrogate marker for risk of CHD, although one comment noted that such an emphasis would be expected, given the importance of cholesterol in CHD. Another stated that the focus on cholesterol ignores other factors that determine blood cholesterol such as heredity, exercise, and stress, etc.

FDA disagrees with the comments that it overemphasized the importance of studies in which cholesterol was measured as a surrogate marker for CHD. The considerable attention given to blood cholesterol measurements (and measurements of other blood lipids and the protein components of blood lipoproteins) was the consequence of two factors: (1) There was a large number of studies on the effects of fish

oils or fish containing omega-3 fatty acids on these blood lipid measures; and (2) there is general agreement that certain blood lipids are strongly associated with the risk of CHD.

FDA undertook to evaluate findings in these studies whether or not fish or fish oils were used as the source of omega-3 fatty acids and whether or not the outcome measures were generally recognized as predictive of CHD. This approach allowed the identification of biologic activities of omega-3 fatty acids that may be related at some point to risk of CHD, and identified areas where additional research is needed. FDA included in the summary of its proposed rule (56 FR 60663) only measures of generally recognized risk factors. FDA did not intend to imply that data on alternate markers were not considered in its decision.

FDA agrees that other factors contribute to blood lipid measures, but believes that randomization should control for these factors. In nonrandomized studies, these sources of potential bias limit the conclusions that can be inferred from the data. This is an important reason that data from correlation studies do not conclusively establish a relationship between omega-3 fatty acids and risk of CHD.

31. The LSRO report paid considerable attention to changes in blood lipids after increased consumption of omega-3 fatty acids, and reached some conclusions about effects of omega-3 fatty acids on blood lipids that differed from those reached by FDA. LSRO abstracted three studies from before the period covered by FDA review (Refs. 284a, 267, and 2.57). These studies were considered, in Federal Government and other comprehensive reports reviewed in the proposed, rule and not discussed further by FDA. LSRO also included three studies not reviewed by FDA in its proposal (Refs. 168, 226, and 301). Agren et al. (Ref. 168) studied healthy students randomly assigned to their normal diet (one fish meal per 2 weeks), a fish diet, or a fish diet low in saturated fat for 15 weeks. There was no change in total serum cholesterol on the control diet or fish diet, but the low-fat fish diet produced a reduction in total cholesterol. Jensen et al. (Ref. 226) studied 18 healthy subjects supplemented sequentially for 4-week periods with 4-week washouts between, with fish oils containing 1, 3, and 6 g EPA plus DHA, and found no change in total or LDL cholesterol. Wolmarans studied healthy subjects fed a meat diet or fish diet containing 6.1 g EPA plus DHA for 6 weeks each in a crossover design, and found reduced total cholesterol and LDL cholesterol

during the fish diet phase. There was no difference in total fat of the two diets. However, there was significantly less saturated fat in the fish diet, so it is not certain that the omega-3 fatty acids were responsible for the decrease in the cholesterol measures.

FDA is concerned that the studies abstracted by LSRO do not accurately represent the totality of publicly available scientific evidence. For example, in its proposal, FDA included five studies among normal subjects (Refs. 2, 6, 24, 73, and 143) and three studies among subjects with preexisting lipid or lipoprotein abnormalities (Refs. 18, 73, and 93) not included in the LSRO report that had data for effects of omega-3 fatty acids on plasma lipids or lipoproteins. FDA determined that seven studies that reported changes in total cholesterol had the most rigorous designs and the largest numbers of normal subjects. None of these seven studies (Refs. 6, 9, 14, 49, 54, 73, and 166) in normal subjects found a significant change in total cholesterol after fish oil supplementation. FDA found similar results with regard to hyperlipidemic subjects.

Only two of these seven strongest studies in normal subjects were abstracted in the LSRO text, and two others were not cited at all by this report. LSRO did not distinguish between normal and hyperlipidemic subjects in its summary or conclusions. LSRO summarized the evidence on total cholesterol by stating, "Decreases in total cholesterol * * * have also been reported," (emphasis added), without mentioning that the predominant finding is that there is no effect on total cholesterol.

Similarly, FDA stated that the strongest studies among normal subjects (Refs. 6, 9, 14, 49, 54, 73, and 166) found no change in LDL cholesterol, and one reported an increase in LDL cholesterol (Ref. 54). Indeed, most studies on hypertriglyceridemic or hypercholesterolemic subjects reported an increase in LDL cholesterol, following fish oil supplementation (56 FR 60663 et. 60669). Consequently, FDA disagrees strongly with the summary statements in the LSRO report:

Effects of fish oil upon LDL have been variable, in part because of different doses. In normolipidemic individuals, LDL has generally declined significantly. In some patients with primary hypercholesterolemia, consumption of fish (sic) has not resulted in altered plasma cholesterol levels; other studies have shown decreased cholesterol and LDL levels. (Emphasis added.)

32. Two comments stated that FDA had not considered all relevant data on HDL₂ cholesterol, and cited additional

studies that reported increased HDL₂ cholesterol after fish oil supplementation. One comment stated that overall HDL cholesterol tends to rise, and cited a review paper by Harm (Ref 62). The LSRO report also concluded that HDL was increased by fish oil supplementation.

FDA disagrees with the comment regarding the overall HDL cholesterol change after fish oil supplementation. The agency considered HDL changes separately for normal, healthy subjects and for hyperlipidemic subjects (56 FR 60663 at 60669). Nearly all studies on normal subjects found no significant change in HDL cholesterol level. Some investigators reported increased HDL₂, but the data on HDL₂ were equivocal.

FDA also disagrees with the conclusions of the LSRO report regarding HDL cholesterol, because it does not represent the totality of publicly available scientific evidence. The LSRO summary states, "In some studies HDL concentrations have actually increased with consumption of fish oil" (emphasis added), not acknowledging that the balance of available scientific evidence on HDL indicates no change. In the review by Harris cited in the comment, the changes in HDL cholesterol in each study were weighted according to the number of subjects in the study, giving a per-subject change. This method of pooling data from different studies does not account for the variation of the response of subjects in each study, the amount of omega-3 fatty acids fed, the duration of feeding, or the source of the omega-3 fatty acids. Therefore, it must be considered only an estimate of the effects of omega-3 fatty acids on HDL cholesterol. Harris calculated the average HDL cholesterol change for normal subjects to be an increase of approximately 3 percent, a net change smaller than the usual variability in the test used to measure HDL.

The agency agrees with the comment that not all HDLs data were considered in the proposed rule, although FDA noted (56 FR 60663 at 60669) that some studies among normal subjects found increases in the HDLs fraction of HDL cholesterol, and that these reports were the most promising changes in blood lipids. Of the six references cited by the comments as not included among studies showing increased HDL cholesterol after omega-3 fatty acids, two were published after the time period covered in the proposed rule (Refs. 235 and 252). One other paper not cited by FDA in its proposal, although it was published during 1988 (Ref. 291), dealt with insulin-dependent diabetics. The other three papers were cited by

FDA in other contexts, but data from these papers regarding HDL₂ cholesterol levels were not discussed (Refs. 1, 32, and 148).

FDA reexamined those papers that it cited but from which it did not present data regarding HDL₂, together with the newer papers. When fractions of HDL cholesterol have been reported, an increase has generally been found in the HDL₂ fraction (Refs. 1, 32, 148, 235, 251, and 291), with a comparable decrease in the HDL₃ fraction (Refs. 1, 235, and 251). This represents a shift within the HDL fractions toward a lipid-rich lipoprotein, and away from a protein-rich lipoprotein, similar to that reported for LDL, below. This shift has been reported when there is (Refs. 32, 148, 235, and 291) or is not (Refs. 1 and 251) a change in total HDL cholesterol. This raises the possibility that a shift occurred in other studies where total HDL was reported as not changed.

However, the importance of the shift in subfractions of HDL is not clear. FDA noted in its proposal (56 FR 60663 at 60869) that there is evidence that the HDLs fraction is the one most closely linked to risk of CHD. However, the agency was unable to find evidence that there was significant scientific agreement that HDL₂ was the fraction of HDL most closely associated with CHD. The National Institutes of Health's National Heart, Lung, and Blood Institute (NHLBI) consensus development conference on Triglyceride, High Density Lipoprotein and Coronary Heart Disease (Ref. 255), anticipated in the proposal (56 FR 60663 at 60664), concluded that, "It is not known to what extent these alterations of HDL contribute to atherosclerosis."

Therefore, data on changes in HDL subfractions after increased consumption of omega-3 fatty acids do not provide a sufficient basis for a health claim, because there is not significant scientific agreement that HDL₂ is directly related to risk of CHD. If the risk of CHD becomes linked with HDL₂, these findings in normal subjects may be of great importance.

33. Many comments indicated that high triglycerides are causally related to decreased HDL, that triglycerides are an independent risk factor for CHD, or that statistical manipulations of data and imprecise measurements of triglycerides obscure the importance of triglycerides as a risk factor for CHD. One comment provided additional citations regarding the relationship between triglycerides and HDL, but these did not bear on risk of CHD. One comment stated that it was generally agreed that triglycerides were not independently associated with CHD.

FDA disagrees with all but the last comment. FDA is aware that there has been, and still is, substantial interest in the potential role of triglycerides in the etiology of CHD (e.g., Ref. 208). Because of the continued interest, the relationship between triglycerides and CHD was the topic of a consensus development conference sponsored by NHLBI on February 26 through 28, 1992. NHLBI had previously addressed this topic in 1983 and concluded at that time that the relationship was controversial. The recent conference (Ref. 255) concluded, "For triglyceride, the data are mixed; although strong associations are found in some studies, the evidence on a causal relation is still incomplete."

FDA agrees that the statistical methods previously used to study the relationship between triglycerides and CHD have lessened the likelihood that triglycerides would be found to be a significant, independent predictor of CHD. Furthermore, the agency believes that study design and analytic measurement methods have contributed to variation in triglycerides that may have resulted in reducing the statistical association between triglycerides and CHD. FDA believes that these sources of variation in triglycerides can be reduced by careful study design and standardized analytical measurement techniques, and also that clinical studies designed to lower triglycerides could provide a basis upon which to reconsider the importance of triglycerides in CHD.

34. Some comments stated that some very recent evidence from the Helsinki Heart Study supports a protective effect of lowering triglycerides, at least for a selected subpopulation of people with a high ratio of LDL cholesterol/HDL cholesterol and very high triglycerides.

FDA agrees that fish oils reduce plasma triglycerides. In its proposal FDA wrote, "The predominant blood lipid effects of fish oils *** are decreased plasma triglycerides and VLDL." (56 FR 60663 at 60669.) In this regard FDA and LSRO were in agreement. The LSRO summary states, "The most striking effect is lowering of plasma triglyceride and VLDL concentrations."

FDA disagrees, however, that triglycerides have been established as an independent risk factor for CHD. The recent results from the Helsinki Heart Study (Ref. 242) were discussed at length at the NHLBI consensus development conference (Ref. 255). While the reduction in CHD mortality following drug intervention was dramatic (i.e., approximately 7-fold) for a particular subgroup with both elevated

triglycerides and elevated LDL to HDL ratio, this result was obtained by a post hoc analysis of earlier results. Because the combination of factors used to connote the high-risk group (i.e., high LDL cholesterol to HDL cholesterol ratio and high triglycerides) was determined after the data were collected, those results are not the results of the testing of a hypothesis, but are the origins of a new hypothesis. The authors indicate that the cut-off points for the ratio of LDL to HDL and triglycerides chosen were to some extent arbitrary. The actual number of cardiac events in the study was small (e.g., 18 events among 138 subjects in the highest risk subgroup), and the reduction in all-cause mortality due to the lipid-lowering drug, gemfibrozil, was not significant. Finally, independent of LDL to HDL ratio, increased triglycerides alone were not associated with an increased risk of heart attack.

The dramatic reduction of triglycerides by omega-3 fatty acids has resulted in their use in the treatment of a rare genetic hypertriglyceridemia (type V) to prevent noncardiovascular effects of high triglycerides (i.e., pancreatitis), but the usefulness of lowering triglycerides as a general strategy in prevention of CHD is not generally agreed. Therefore, FDA believes that the triglyceride-lowering effect of fish oils for some at-risk persons does not provide a basis for a health claim at this time.

35. Numerous comments indicated that postprandial triglyceridemia is a mechanism of action in the development of atherosclerosis. Some comments indicated that the relationship of elevated triglycerides to risk of CHD would be discussed at the NHLBI consensus development conference (Ref. 255). Others pointed out that LSRO had concluded that elevated very low density lipoproteins (VLDL) and triglycerides were atherogenic. LSRO stated that the reduction of postprandial hyperlipidemia is a "most important anti-atherogenic action." LSRO wrote in the summary that, "Since postprandial lipemia has been identified as an atherogenic risk factor, its prevention by w-3 fatty acids would be a most desirable effect" (emphasis added), and in its conclusions LSRO wrote:

Fish oil has a generally accepted hypolipidemic effect without depressing HDL. This applies most to VLDL and triglyceride, lipids now believed to be atherogenic. There is little doubt that there is a reduction of postprandial hyperlipidemia following the ingestion of dietary fat if the background diet contains relatively small

quantities of w-3 fatty acids. This may be a most important anti-atherogenic action.

FDA agrees that fish oils do not generally lower HDL. FDA also agrees that major blood lipid effects of omega-3 fatty acids are reductions of triglyceride and VLDL. The role of omega-3 fatty acids in the reduction of postprandial triglycerides was described in three papers abstracted by LSRO (Refs. 15, 59, and 163). While the first two papers used high levels of omega-3 fatty acids (30 and 9 g of EPA plus DHA/day, respectively), the recent paper used only 5 g of fish oil, containing 1.7 g EPA plus DHA. These studies showed that the concentration of plasma chylomicrons after a high-fat test meal was significantly less if the subjects had been consuming a fish oil diet than if they had been consuming a saturated fat or olive oil supplemented diet. Thus, FDA agrees that fish oils reduce postprandial lipemia.

However, FDA disagrees that there is significant scientific agreement that VLDL and triglycerides are atherogenic, or that the reduction in postprandial hyperlipidemia is a most important anti-atherogenic action. Neither the Federal Government nor other authoritative reports have included these blood lipid measures among those they consider to be independent risk factors associated with CHD (Refs. 34 through 36, and 115). Furthermore, postprandial lipemia was discussed at the February 1992 NHLBI consensus development conference. The summary of that conference stated, "Postprandial triglyceride may be more important than the fasting triglyceride levels [to CHD], but little is known about this at the present time." (Ref. 255).

FDA notes that the only paper in the LSRO report cited in support of this hypothesized mechanism of action of omega-3 fatty acids in the prevention of CHD was a review paper published in 1979 (Ref. 305). Therefore, FDA believes that there is not significant scientific agreement at this time that postprandial triglycerides are related to the risk of CHD.

ii. Vessel wall effects

36. One comment indicated that two new studies support the use of omega-3 fatty acids to prevent restenosis, the closing of a mechanically opened blood vessel (Refs. 172 and 259). This comment suggested that FDA discounted the findings of the Dehmer study (Ref. 30) on the basis that it employed simultaneous treatment with drugs and fish oils.

FDA considered the use of omega-3 fatty acids to prevent restenosis to be a drug usage (56 FR 60663 at 60670), and

notes that patients in these studies are under a physician's care. FDA's description of the Dehmer study points out a limitation of the data that is common in other reports of no effect of omega-3 fatty acids in restenosis (Refs. 56, 106, and 121), that the studies have not controlled for generalized effects of PUFA's that are not specific to omega-3 fatty acids. A better balanced experimental design would be comparison of drugs plus omega-3 fatty acids to drugs plus alternate PUFA's (e.g., corn oil).

FDA agrees that the new studies provide some support for the role of omega-3 fatty acids in prevention of restenosis, although neither was designed to distinguish effects of omega-3 fatty acids from effects of omega-6 PUFA's.

Nye et al. (Ref. 259) studied 79 men and 29 women who were referred for angina and underwent coronary percutaneous transluminal angioplasty (PCTA), i.e., a mechanical opening of a closed heart blood vessel. The subjects were randomly assigned to one of three treatments: (1) A combination of aspirin plus dipyridamole (an anti-platelet combination of drugs), (2) olive oil placebo, or (3) 12 milliliters (mL) fish oil containing 3.2 g EPA plus DHA/day. Subjects were restudied 1 year later or before if symptoms recurred, and 93 percent of all subjects were followed for the year. Although there was no significant difference in angina among the groups, the rate of restenosis, defined in this study as a loss of 50 percent or more of the luminal diameter increased by PCTA, was significantly less in the fish oil group (11 percent) than in the placebo group (33 percent).

The use of olive oil as the placebo did not control for effects due to PUFA's (omega-6). Also, it is notable that the restenosis rate in the aspirin group was somewhat higher (17 percent) than in the fish oil group, because aspirin is a much more potent inhibitor of platelet function than EPA in fish oil. Nonetheless, these results are consistent with an effect of omega-3 fatty acids in reducing restenosis.

The full "Quebec study" was published after the receipt of the comment, but because it was cited in the comment it will be discussed here. In this study, Bairati et al. (Ref. 172) conducted a double-blind, randomized intervention with either fish oil containing 4.5 g EPA plus DHA/day, or olive oil placebo in 205 patients undergoing first PCTA. The treatments were started 3 weeks before the procedure, and continued for 6 months after. Restenosis was assessed angiographically, using a quantitative

computer analysis program. Restenosis was reduced in the fish oil group compared to the olive oil group according to 3 of 4 definitions of restenosis. It was not reduced according to the clinical definition used by Nye et al. (Ref. 259), above, of a loss of 50 percent or more of the luminal diameter increased by PCTA.

This study also collected dietary data. The third of the subjects with the highest consumption of omega-3 fatty acids (0.15 g/day) and the third of the subjects with intermediate consumption of omega-3 fatty acids (0.033 to 0.15 g/day) had significantly lower rates of restenosis than the third consuming the least amount of omega-3 fatty acids. In fact, dietary omega-3 fatty acids (other than the supplement) were associated with a greater reduction in chance of restenosis than was the supplement. This result was somewhat surprising, since the supplement contained 30 times the amount of omega-3 fatty acids in the diet. No differences in rate of restenosis were found according to intake of total fat, polyunsaturated fat, monounsaturated fat, saturated fat, cholesterol, or total seafood consumption. These results suggest that chronic consumption of low amounts of omega-3 fatty acids may be as useful in preventing restenosis as much larger amounts consumed for a few weeks prior to and after PCTA.

In general, the results of Bairati et al. (Ref. 172) and Nye et al. (Ref. 259) are consistent, even though they obtained different results according to one identical definition of restenosis. The Bairati et al. study, like Nye et al. 1990, used olive oil as the control. If the mechanism of action of omega-3 fatty acids in restenosis is through competition with AA, this control is suitable, and an omega-6 fatty acid oil would have made the difference due to omega-3 fatty acids even more pronounced. If, however, the mechanism of action is through nonspecific effects of highly unsaturated fatty acids, then a control of a PUFA (e.g., corn oil) might have reduced the apparent effect of omega-3 fatty acids. It is notable that the only study of restenosis that has used a polyunsaturated fat control (an olive oil-corn oil mix) did not find an effect (Ref. 56).

37. Five studies in humans relevant to the action of omega-3 fatty acids on the vessel wall were referenced in comments (Refs. 200, 213, 259, 268, and 277), including two published since the time period covered by FDA's review in its proposed rule (Refs. 200 and 268). Hamakazi et al. (Ref. 213) found a slower aortic pulse wave velocity (an

electro-physiologic measurement) in persons from a Japanese fishing village compared to those from a farming village. Other data showed the populations differed in their intake of omega-3 fatty acids. Rapp et al. (Ref. 268) measured the amount of omega-3 fatty acids in the atherosclerotic lesion after consumption of omega-3 fatty acids at a high level (6 percent of calories, 16 to 21 g EPA plus DHA/day) for 6 to 120 days prior to planned surgical intervention, and found that the amount of omega-3 fatty acids in the lesion continued to increase throughout the time of ingestion. Force et al. (Ref. 200) studied the effects of fish oils and aspirin on the production of urinary metabolites of AA and EPA. Fish oil feeding resulted in a slight decrease in the amount of thromboxane A₂ made in the platelet, a decrease in the amount of AA-derived prostacyclin made in the endothelial cell, and an increase in the amount of EPA-derived prostacyclin made in the endothelial cell. Schmidt et al. (Ref. 277) described decreased Bionocyte chemotaxis among hypertensive patients after fish oil feeding. The Nye et al. study is discussed in comment 36 of this document.

FDA considers these studies to be observational, not clearly associating omega-3 fatty acids with risk of CHD. The correlation data of Hamakazi et al. do not indicate a specific role for omega-3 fatty acids. The Rapp et al. data verify that it is possible to incorporate omega-3 fatty acids into preexisting atherosclerotic plaque, but the relevance of incorporated omega-3 fatty acids has not been established. The studies of Force et al. and Schmidt et al. relate to a potential mechanism of action of omega-3 fatty acids, but the importance of these actions in reducing risk of CHD has not been established.

38. Many comments stated that the biochemical and physiological actions of omega-3 fatty acids are anti-atherogenic because they favor vasodilatation and inhibit vasoconstriction. One comment by a manufacturer of omega-3 fatty acids considered these actions have potential for future significance. Two comments cited a list of effects of omega-3 fatty acids, suggesting that each of the effects in the list was anti-atherogenic, and other comments referred to one or more of the components in the list. The listed changes were:

- decreased thromboxane;
- increased prostacyclin and leukotriene (LTB₄);
- decreased fibrinogen,
- decreased platelet activating factor (PAF);

decreased platelet-derived growth factor (PDGF);

- decreased superoxide;
- decreased interleukin-1(TNF);
- increased endothelium-derived relaxation factor (EDRF);
- decreased lipoprotein (a) (Lp(a));
- reduced inflammatory response; and
- increased fibrinolytic activity.

The LSRO report stated that other mechanisms, such as cellular growth factors, interleukin-1 and cytokines, and EDRF may be important in the development of atherosclerosis, and be affected by omega-3 fatty acids. However, except for a single *in vitro* study on PDGF, no data are described in the report regarding these factors, nor is their relevance to human CHD discussed.

FDA addresses fibrinogen, Lp(a), and fibrinolytic activity in comment 46 and in section II.C.2. of this document. FDA does not agree that omega-3 fatty acids produce changes in all of the listed parameters. FDA has determined that for some of these endpoints the changes have not been shown to be specific to omega-3 fatty acids, but may be due to polyunsaturated fats instead. FDA disagrees that the changes brought about by omega-3 fatty acids will prevent atherosclerosis. Most of the data regarding changes in these endpoints brought about by omega-3 fatty acids have been derived from tissue culture or animal experiments, and the relevance to human atherosclerosis has not been demonstrated.

Thromboxanes and prostacyclins are compounds derived from omega-3 fatty acids and omega-6 fatty acids that affect the relaxed state of the blood vessels. Thromboxanes are produced primarily in platelets, and prostacyclins are produced primarily in the endothelial cells of the blood vessels. The thromboxane made from an omega-6 fatty acid called AA, thromboxane A₂ is a potent vasoconstrictor. EPA competes with AA for the enzyme that makes thromboxane A₂, and thereby diminishes the rate of production of thromboxane A₂; the thromboxane made from EPA is a much less potent vasoconstrictor. The prostacyclins made from AA or EPA in the endothelial cells are vasodilators. Thus, the relative amounts of AA and EPA in platelets and endothelial cells play a role in determining the form and amounts of the prostaglandins and thromboxanes that affect the tension of the vessel wall. Excessive constriction may lead to an occlusion, resulting in a heart attack. While there is general recognition that these vasoactive compounds may play a role in the formation of clots and thereby in heart attacks, there is no

agreement about the extent of changes needed in the concentrations of the vasoactive compounds in order to have an effect on heart disease. Changes in the amounts of these vasoactive compounds, produced by consumption of fish oil, are only useful as marker for CHD only insofar as there is significant scientific agreement that the magnitude of the changes is related to CHD. FDA is not aware of any such agreement, nor did the comments provide any evidence of agreement that particular changes in the levels of these vasoactive compounds were related to a reduction in risk of disease. Furthermore, the amount of omega-3 fatty acids needed to produce these changes in humans is not known.

For PDGF the evidence is confined to animal studies (Ref. 201), and the relevance to human disease has only been suggested, not demonstrated. The animal studies on PDGF also did not show that the effect was specific to omega-3 fatty acids. For example, the PDGF effect was observed also after polyunsaturated fats, and was abolished by anti-oxidants, suggesting that any highly unsaturated fatty acids prone to oxidation would have the effect. The experiments on EDRF (Ref. 181) also did not show that the effects were specific to omega-3 fatty acids, since the experiments were carried out in the presence of indomethacin, which, blocks the eicosanoid effects of EPA. In fact, the authors consider changes in membrane fluidity to be a reasonable explanation for the effects. In yet other cases, e.g., TNF, there are conflicting results depending on the species (Refs. 41 and 236), and the findings must be considered preliminary.

FDA considered the effect of omega-3 fatty acids on chemotaxis, one aspect of inflammatory response (56 FR 60663 at 60670). A complete discussion of the role of fish oils in inhibition of the inflammatory process is outside of the scope of this rulemaking, but the relationship between omega-3 fatty acids and inflammatory response could be the subject of a petition for a health claim that includes the necessary information about this relationship.

FDA agrees that the biochemistry of the products formed from the omega-3 fatty acids *in vivo* (i.e., eicosanoids) have been shown under experimental conditions, usually *in vitro*, to have pronounced effects on the vessel wall. However, demonstration of isolated biochemical effects is not a sufficient basis upon which to make a claim regarding the outcome of a multifactorial process. Intermediate markers of CHD are useful only insofar as there is significant scientific

agreement that changes in these markers produced by omega-3 fatty acids are causally related to CHD.

b. Thrombosis and hemostasis

39. A few comments stated that the mode of action of omega-3 fatty acids may be through stabilization of arrhythmia, and noted the reduced rate of death after heart attacks (MI's) in the Dart study (Ref. 16). This comment also stated that certain animal data were consistent with this hypothesis. The comments stated that the fibrillation mechanism suggested by DART was compelling, because 60 percent of sudden deaths are caused by ventricular fibrillation following reperfusion. Many commented that data from nonhuman primate models show that omega-3 fatty acids abolish arrhythmias, whereas polyunsaturated fat (safflower oil) had a lesser effect.

FDA disagrees with these comments. FDA's review of the literature regarding the usefulness of omega-3 fatty acids in arrhythmia and ventricular fibrillation found only one study on arrhythmias in humans, and it reported no significant effect of omega-3 fatty acids (Ref. 58). A review in 1989 also concluded that, even among the animal studies, there was no significant difference between omega-3 fatty acids and other polyunsaturated fats on arrhythmias (Ref. 269).

The data from the studies in Nonhuman primates (i.e., the marmoset monkey) were published only as a Non-peer-reviewed paper in a book (Ref. 188). Two papers by the same author on the same topic were cited in 1990 as in press in a peer-reviewed journal, but have not yet been published. Therefore, FDA regards the data on nonhuman primates as preliminary only. Furthermore, the data for the marmoset monkey were obtained after prolonged feeding for 12 or 24 months with a supplement of DHA-rich fish oil at a level of 8 percent of the diet by weight. FDA calculates that this would provide 2.5 g of omega-3 fatty acids from fish oil/kilogram (kg), over 50 times the usual rate of supplementation in human studies (10 g fish oil or 3 g omega-3 fatty acids/day for a 70 kg subject), and over 300 times the amount of omega-3 fatty acids associated with reduced risk of CHD in the epidemiologic literature (Refs. 16, 38, and 87 report 300 to 660 milligrams (mg)/day). Thus, the relevance of these studies to omega-3 fatty acids in the human diet is questionable.

FDA is aware of *in vitro* data that show a specific protective effect of EPA against toxicity of heart muscle cells in culture. These results provide a

biochemical basis for the hypothesized stabilization of cardiac arrhythmias by omega-3 fatty acids. Although, this study (Ref. 212) was performed, *in vitro* on heart cells from rats, it showed that the protective effect was specific to omega-3 fatty acids (EPA) because a similar effect was not obtained when a highly unsaturated omega-6 fatty acid (AA) was used instead.

FDA also regards the evidence the Burr study of reduced death following a heart attack among men, advised to increase fish consumption as consistent with a stabilization of arrhythmias (Ref. 16). FDA agrees that this postulated mechanism of action is of great potential public health significance. However, the agency finds the clinical data available at this time are not in agreement with animal and *in vitro* data. Because the clinical data are not in agreement with these other types of data and because of the limitations in the animal studies, FDA concludes that there is not sufficient basis for protective effect specific to omega-3 fatty acids on arrhythmias, and, therefore, CHD in humans.

40. One comment criticized the 6-week clinical study by Hardarson et al. that found no effect of omega-3 fatty acids on arrhythmias (Ref. 58), arguing that the time for incorporation of omega-3 fatty acids into heart phospholipids was too short for an effect to be observed.

FDA agrees in part and disagrees in part with this comment. Generally, the time needed for incorporation of omega-3 fatty acids into heart phospholipids is short; studies in animals show such incorporation, in a period of weeks (Ref. 249). In the Hardarson study (Ref. 58), a substantial amount of cod liver oil was fed (20 mL/day) and a 230 percent increase in plasma phospholipid EFA was found. There was no trend toward reduced arrhythmias. Other data, however, show that although plasma phospholipids increase the omega-3 fatty acid content during the first few weeks of supplementation, the incorporation of omega-3 fatty acids in human atherosclerotic plaque continues to increase through 120 days (Ref. 268). Therefore, FDA agrees with the comment the supplementation period in the Hardarson study (Ref. 58) may have been too short to find an effect of fish oils on occurrence of arrhythmias. Also, the agency notes that the absence of a difference in CHD mortality during the first 6 weeks of the Burr study (Ref. 16) is consistent with the hypothesis that prolonged intake of omega-3 fatty acids (longer than 6 weeks) is needed to observe an effect on arrhythmias or

other mechanisms that reduce CHD mortality. FDA agrees that effects of long-term consumption of omega-3 fatty acids on arrhythmias, other platelet or vessel wall functions, and even some blood lipid measures have not been sufficiently studied.

i. Bleeding times

41. Two comments stated that there is no evidence of increased bleeding even among patients who had invested 6 to 8 g of EPA plus DHA/day and underwent emergency surgery, coronary artery bypass surgery or angioplasty. The comments argued that increased bleeding has not a safety concern.

FDA agrees that there are few reports of excessive bleeding after ingestion of omega-3 fatty acids. However, FDA notes that the cited reports are for subjects with CHD, and evidence of the lack of excessive bleeding complications in this population is not sufficient to assure safety of omega-3 fatty acids in the general population. FDA believes that changes in bleeding due to consumption of omega-3 fatty acids remains a valid safety concern (see comment 52 of this document).

ii. Platelet aggregation

In the proposal, FDA stated:

The relationship between platelet aggregation and the risk of heart attacks or CHD death in the general population is an important line of evidence that would support drug claims and perhaps health claims for omega-3 fatty acids. Although there is some evidence that changes in platelet aggregation may help prevent second heart attacks * * * it has not been shown that changes in platelet aggregation in the general population will reduce the risk of CHD. (56 FR 60663 at 60670.)

The agency added: "What has not been established, however is that platelet aggregation is a bona fide surrogate risk factor for CHD in the general population," (56 FR 60663 at 60672.)

42. Many comments argued that platelet aggregation is completely substantiated as a marker for risk of CHD, based on the results of the Physicians' Health Study (Ref. 66). One comment qualified this conclusion stating that the primary effect of omega-3 fatty acids *in vivo* was to reduce platelet deposition at sites of aortic lesions.

FDA acknowledges that aspirin studies provide evidence that platelet aggregation is a risk factor for CHD. The effect of aspirin in inhibiting platelet function has been shown. Among persons who have already had an MI, aspirin is effective in preventing a second infarction. FDA has proposed that aspirin be used to reduce the risk

of death and/or nonfatal heart attack in patients with previous infarction or unstable angina pectoris as a professional labeling indication (provided to health professionals, but not to the general public), in the tentative final monograph for over-the-counter internal analgesic, antipyretic, and antirheumatic drug products (November 16, 1988, 53 FR 46204 at 46259). However, FDA does not consider the effects of aspirin in the Physicians' Health Study sufficient to establish that dietary omega-3 fatty acids would have the same effect in the general population. The Physicians' Health Study did not evaluate omega-3 fatty acids. The study population was highly selected; the rate of heart attacks was approximately 10-fold lower than in the general population, and cardiovascular mortality was only 15 percent of that expected for the general population of white men of the same age. Also, the results of the Physician's Health Study are not as straightforward as presented in the comments. The chairman of the Physicians' Health Study reported that there was a reduced risk of MI in the aspirin group, predominantly in nonfatal MI, but that there was no significant effect on overall cardiovascular mortality (a 2 percent reduction, not statistically significant) (Ref. 66). In addition, the aspirin group in this study had a greater number of sudden deaths (Ref. 282).

In the other primary prevention trial (Ref. 265), aspirin did not have any significant effect on heart attacks, on stroke, or on total vascular mortality. There was a significant increase in disabling stroke in the group taking aspirin.

On the basis of these studies there has not been an endorsement of the use of aspirin as a prophylactic measure against CHD by the general population by the American Heart Association or by the Canadian Medical Association (Ref. 187). Notably, "1992 Heart and Stroke Facts" published by the American Heart Association (Ref. 169) makes no reference to platelet aggregation as a risk factor for heart attacks (although sticky platelets are mentioned to be a consequence of cigarette smoking in the section on stroke), nor is aspirin discussed as an option for CHD prophylaxis, even though other drug and surgical treatments are discussed.

Therefore, FDA concludes that there is not significant scientific agreement at this time that platelet aggregation is a surrogate marker for CHD in the general population.

43. The LSRO report, submitted as a comment, contained abstracts of 19 studies in humans that contained data

regarding changes in platelet function following omega-3 fatty acid consumption. LSRO concluded that omega-3 fatty acids prevented platelet aggregation.

In its proposal, FDA stated: "Platelet aggregation is generally considered to be decreased by fish oil consumption." (56 FR 60663 at 60670.) The agency also stated: " * * * platelet aggregation and function are reduced: However, direct relationships between the changes in * * * platelet function and risk or CHD have not been established." (56 FR 60633 at 60671.) Thus, FDA agrees with the conclusions of LSRO about effects of omega-3 fatty acids on platelet aggregation.

Two of the studies described by LSRO were not considered by FDA in its review, because they were published before 1988, and had been considered by Federal Government and other authoritative reports. One study (Ref. 227) used a large amount of fish oil (50 mL/day) not reasonably related to normal dietary intake. The other study (Ref. 211) involved 13 insulin-dependent diabetics, and therefore is of questionable relevance for the general population.

In its proposed rule, FDA considered 13 of the other 17 studies that were abstracted by LSRO. One of the four studies not addressed by FDA was a study on the effects of added vitamin E to fish oil on fibrinogen and fibrinolysis (Ref. 210). Two papers (Refs. 234 and 244) were published after the time period covered by FDA review. Marckmann et al. (Ref. 244) compared the effects of a fish diet and a lean meat diet on plasminogen activator (t-PA), plasminogen activator inhibitor (PAI-1) and the activity of the inhibitor (PAI-1 activity). Li and Sternier (Ref. 234) described changes in *in vitro* platelet adhesion after fish oil supplementation. The fourth paper was an uncontrolled observation study that found a high frequency of nosebleeds in adolescents supplemented with fish oils (Ref. 189).

Six other papers on thrombosis were not described in the LSRO text, but were included in the table (Refs. 203, 204, 209, 226, 245, and 254). Of these six, one was not relevant to the nutrient-disease relationship (Ref. 245) because it did not study EPA and DHA. Jensen et al. (Ref. 226) found no significant change in bleeding times in normal subjects after 1, 3, or 6 g EPA plus DHA/day in healthy subjects. Green et al. (Ref. 209) found no change in platelet aggregation or platelet count in 27 hyperlipidemic subjects in a randomized double-blind placebo controlled crossover trial. The treatments were 15 g/day fish oil

containing 4.3 g EPA plus DHA and a 50:50 mix of corn:olive oil, with each treatment lasting 8 weeks, and a 4-week washout between. Blood viscosity was decreased by fish oil, Gazso et al. (Ref. 204) found decreased platelet aggregation in healthy subjects after consumption of EFAmol-marine compared to olive oil in a double-blind randomized crossover study. These results of studies confirm others cited by FDA. The other studies (Refs. 203 and 254) pertained to regulators of bleeding and are discussed below.

Eight papers on platelet fraction were reviewed by FDA but not by LSRO (Refs. 2, 6, 18, 24, 73, 93, 131, and 143). Three studies were uncontrolled (Refs. 18, 93, and 143), while two were randomized (Refs. 2 and 131). Three were randomized, double-blind, placebo-controlled trials that used saturated vegetable oil (Ref. 6), vitamin E (Ref. 24) or wheat germ oil (Ref. 73) as the placebos. The two studies that used vegetable oil or vitamin E as controls found a reduction in platelet aggregation after omega-3 fatty acids, where no difference was reported in the trial that used a wheat germ oil placebo, although the data were not provided in this paper.

FDA and LSRO reached the same conclusions with regard to the effects of omega-3 fatty acids on platelet function. LSRO also concluded that platelet survival is also enhanced, but the only two studies published since 1987 that reported increased platelet survival (Refs. 94 and 144) were both uncontrolled, so the effect cannot be attributed specifically to omega-3 fatty acids.

44. One comment agreed in principle with the agency's assessment of the effects of omega-3 fatty acids on bleeding times, platelet aggregation, regulators of bleeding and blood pressure. The comment pointed out the extent of inhibition of platelet adhesion is as much, as 60 percent, a marked reduction in adhesion.

FDA agrees that the reported extent of reduction of platelet adhesion omega-3 fatty acid intake is remarkable (Ref. 234). The agency notes that this effect appears specific to omega-3 fatty acids at reasonable intake levels. FDA notes that animal studies (Refs. 230 through 233) published since the proposed rule provide evidence of reduced platelet adhesion to blood vessel endothelium *in vivo* in response to agents that provoke such adhesion. Because of the magnitude of the effect of omega-3 fatty acids on platelet adhesion, FDA considers this action of omega-3 fatty acids on blood platelet function to have great potential with

regard to the development of atherosclerosis and the risk of CHD. However, as for platelet aggregation, FDA does not believe that there currently is significant scientific agreement that platelet adhesion is an accepted risk factor for CHD in the general population.

iii. Regulators of bleeding

In its proposal (56 FR 60663 at 60670 through 60671), FDA reviewed data on the effects of omega-3 fatty acids on other factors that are involved in the regulation of bleeding—fibrinogen, fibrinolytic activity and Lp(a)—and that have been associated with CHD.

45. Some comments, including the LSRO report, stated that omega-3 fatty acids increase fibrinolysis.

FDA disagrees with these comments. FDA found that there was no clear relationship between omega-3 fatty acids and factors involved in dissolving blood clots (56 FR 60663 at 60671). FDA noted that the data did not establish that omega-3 fatty acids reduced fibrinogen, because most studies did not control for other factors that might have reduced fibrinogen, e.g., other PUFA's.

FDA has reviewed the relevant studies again, as well as studies brought to its attention in the LSRO report. LSRO cited three papers on fibrinogen or fibrinolysis not cited by FDA. One placebo (vitamin E) controlled study found no change in fibrinolytic activity (Ref. 210). Mullertz et al. (Ref. 254) supplemented seven healthy adults with 0.55 g ERA plus DBA/day for 21 days and found increased levels of PAI-1, but no change in t-PA, suggesting that fish oil decreased fibrinolytic capability. Cans et al. (Ref. 203) reported no change in fibrinogen concentration after EFAmol-marine compared to corn oil, which is rich in polyunsaturated fat. These studies do not support the conclusion that omega-3 fatty acids reduce fibrinogen, or increase fibrinolysis.

The selection of studies abstracted by LSRO may not have represented the publicly available scientific evidence. For example, five papers abstracted found either a decrease in fibrinogen or an increase in fibrinolytic activity (Refs. 57, 71, 98, 104, and 117). In contrast, two studies found no change in fibrinolytic activity (Refs. 150 and 166), and only one found increased fibrinogen (Ref. 144), leaving the impression that omega-3 fatty acids usually have been reported to enhance fibrinolysis.

However, three other studies not abstracted by LSRO but included in their tables reported no effect of omega-3 fatty acids on fibrinogen compared to corn oil (Refs. 10, 118, and 203). One

found a decrease compared to olive oil (Ref. 49) and one found a decrease compared to soybean oil only when 30 mL of fish oil were consumed, but not when 15 mL were consumed (Ref. 57). Additional well-designed studies not cited by LSRO, but considered in the FDA proposal, reported no change (Ref. 24) and an increase (Ref. 131) in fibrinolytic activity.

Therefore, FDA stands by its earlier conclusion that the publicly available scientific evidence does not support a relationship between omega-3 fatty acids and decreases in fibrinogen or increases in fibrinolysis. This conclusion is supported by findings that consumption of other PUFA's have effects comparable to those produced by consumption of omega-3 fatty acids.

46. Two comments cited unpublished data by Kostner and Herrmann, reporting reduced Lp(a) after fish oil consumption.

FDA was unable to find the full paper by these authors showing the decrease in Lp(a). FDA did find a paper by these researchers published in 1991 (Ref. 241) that reported no effect of fish oils on Lp(a) and did not cite conflicting work from their laboratory.

iv. Blood pressure

In its proposal, FDA considered the relationship between omega-3 fatty acids and blood pressure, one of the recognized risk factors for CHD. FDA stated:

These results for effects of omega-3 fatty acids on blood pressure of normal subjects are ambiguous. Some studies found a reduction in systolic blood pressure after consumption of fish oils containing omega-3 fatty acids, whereas others did not. None of the studies found a significant reduction in diastolic blood pressure. Therefore, it also remains to be established that the normal, healthy population will reduce their risk of CHD via a reduction in blood pressure following consumption of omega-3 fatty acids.

(56 FR 60663 at 60671.)

FDA also stated that it was not known whether or not the magnitude and duration of the effect would persist after longer term supplementation. FDA recognized that studies among hypertensives found an effect more consistently than studies among normal subjects, although sometimes large amounts of fish oils were used.

47. Some comments considered the effects of omega-3 fatty acids on hypertension as evidence, of a reduction in CHD risk. Other comments called for FDA to reassess the studies on blood pressure. One of these comments that the results of studies on blood pressure are not "completely

ambiguous. One comment agreed in principle with the agency's assessment of the blood pressure studies. One comment considered a number of animal models to be relevant for hypertension. The LSRO report also considered the evidence relating omega-3 fatty acids to blood pressure to be important in relation to CHD. The LSRO report concluded that, "Fish oil probably has a mild hypotensive effect, especially in high doses."

FDA disagrees that the publicly available scientific evidence supports a relationship between omega-3 fatty acids and hypertension. At best, as stated in the proposal, the data are ambiguous. Qualifiers are needed to indicate that the reductions in blood pressure have not generally been shown to be specific to omega-3 fatty acids. Also, many valid studies have reported no effect.

LSRO reported a total of 13 studies on hypertension. Four were published before 1988, and were not reviewed by FDA in the proposed rule. Three of these studies used fish as the source of omega-3 fatty acids and therefore did not show the effect specifically to be due to omega-3 fatty acids. In fact, in one study (Ref. 292), the control diet of meat produced a decrease in blood pressure comparable to that of the fish diet. The study that used fish oils (Ref. 271) used an olive oil control, rather than an oil high in PUFA'S. This study is the only study to show an effect of omega-3 fatty acids on diastolic blood pressure in normal subjects.

Of the other 10 studies on hypertension described in the LSRO report, 6 were also reviewed by FDA (Refs. 11, 57, 80, 85, 95, and 101). The LSRO and FDA interpretations of the results from these papers did not differ in any significant regard, except that FDA specifically noted that two of these studies (Refs. 85 and 95) used very high amounts (50 mL) of fish oil to show the effect. In fact, FDA singled out the Bonaa et al. (Ref. 11) and Kestin et al. (Ref. 80) studies as well-designed studies that showed an effect specific to omega-3 fatty acids in hypertensive and normal subjects, respectively (56 FR 60663 at 60671).

The LSRO report reviewed four papers not originally reviewed by FDA (Refs. 190, 285, and 299), including one study on linolenic acid outside of the scope of the definition of omega-3 fatty acid, as used in this regulation (Ref. 262). Two other papers that appeared in the LSRO table but not in the text (Refs. 203 and 247) were also not reviewed by FDA in its proposal.

FDA agrees with the LSRO interpretation of the Wing et al. study

(Ref. 299), where subjects remained on blood pressure lowering medications and no effects of added fish oils were observed.

FDA disagrees with the LSRO descriptions of the Singer and Cobiac studies. The placebo in the Singer study (Ref. 285) was olive oil, but this was not pointed out in the LSRO text. The reduction of blood pressure observed after fish oil, therefore, may have been due to a general unsaturated fatty acid effect not specific to omega-3 fatty acids. In the description of the Cobiac et al. study (Ref. 190), LSRO did not note that fish oil treatment alone (without simultaneous reduction of salt) had no effect on blood pressure.

Two other studies were cited in the LSRO tables but not in the text and were not included in the FDA review. Neither of these found an effect on blood pressure. Gans et al. (Ref. 203) used a randomized double-blind, placebo-controlled design and found a reduction in diastolic blood pressure for both fish oil and corn oil (placebo). Meland et al. (Ref. 247) carried out a randomized, double-blind multicenter trial among 40 mildly hypertensive subjects, using 6.8 g EPA plus DHA/day, but found no difference in blood pressure compared to a 50:50 olive:corn oil control.

Three other large and appropriately controlled studies not in the text of the LSRO report but included in its table were also reviewed by FDA. Two randomized studies on normal subjects (Refs. 9 and 49) and one controlled study among mildly hypertensive subjects (Ref. 20) reported no differences in blood pressure attributable to omega-3 fatty acids.

FDA reviewed in its proposal three other randomized, double-blind, placebo-controlled studies among healthy subjects that were not included in the LSRO review. Two of these studies were on normal, healthy subjects (Refs. 6 and 24) and found a decrease in systolic blood pressure compared to a saturated vegetable oil or vitamin E, respectively. The third study (Ref. 73) found that omega-3 fatty acids did not affect blood pressure in hypertensives or normal men compared to wheat germ oil.

Therefore, FDA concludes that the evidence of an effect of omega-3 fatty acids on blood pressure in normal subjects is ambiguous, because some studies reported a blood pressure lowering effect, whereas other equally well-designed studies found no specific effect. Studies among hypertensives found an effect more consistently than studies among normal subjects, although sometimes large amounts of fish oils were used, and many studies

did not show that omega-3 fatty acids were more effective than other polyunsaturated fats.

48. Comments stated that other lines of evidence were not discussed in the proposal. Examples given were changes in plasma viscosity, increased vascular compliance, and reduced white blood cell (WBC) count.

FDA disagrees with the comment with respect to plasma viscosity and vascular compliance. In its proposed rule, FDA acknowledged that plasma viscosity was decreased and red cell deformability was increased by omega-3 fatty acids, but that the importance of these effects on the risk of CHD had not been established (56 FR 60663 at 60670).

The agency agrees that it did not systematically consider WBC count among the effects produced by omega-3 fatty acids. WBC count was not included among the actions of omega-3 fatty acids considered in major reviews. FDA notes that WBC count has only recently been identified as associated with risk of CHD by the Caerphilly Collaborative Heart Disease Study (Ref. 301a). Only two papers among literature from 1988 to present have reported a reduction of WBC count after fish oil supplementation (Refs. 183 and 253).

3. Other relevant information

a. Animal studies

49. Numerous comments asserted that animal studies did not receive an appropriate amount of discussion. One of these same comments stated that animal studies are not sufficient to support the claim, and that clinical trials on effects of omega-3 fatty acids directly on CHD are needed. One comment criticized FDA's review of animal studies because the negative findings have been in inappropriate models and should not have been discussed. Another comment stated that they did not believe that there is an appropriate animal model for human cardiovascular and CHD. The LSRO report considered animal studies to provide important evidence for an anti-atherogenic effect of fish oils, stating, "Omega-3 fatty acids have been shown to retard the development of the atherosclerotic plaque in experimental animals including the pig and rhesus monkey."

FDA agrees that the evidence from studies in animals warrants additional discussion. FDA has reviewed here those animal studies that were cited in its proposed rule and those that were cited in the LSRO report that were relevant to the development of atherosclerosis. Other animal studies relevant to the development of

atherosclerosis, and animal studies on aspects of CHD other than atherosclerosis are reviewed under section II.C.3.a. or this document.

In its proposal, FDA cited eight animal studies and one abstract on the development of atherosclerosis that were not included in the LSRO review (Refs. 19, 51, 65, 81, 97, 123, 126, and 151); seven of which reported either no beneficial effect or an adverse effect in fish oil supplemented animals. Only one animal study on the effects of omega-3 fatty acids on restenosis was abstracted by LSRO, although the others cited by FDA were described in the LSRO tables.

Three studies in nonhuman primates have been reported (Refs. 27, 47, and 116). In the Davis et al. (Ref. 27) and Parks et al. (Ref. 116) studies, the polyunsaturated fat intake was higher in the fish oil groups, and polyunsaturated fat is known to lower total plasma cholesterol. Also, the control diet of the Davis et al. study had more saturated fat than the fish oil diet. Thus, the effects on atherosclerosis may not have been specific for omega-3 fatty acids.

In these studies, the total cholesterol values for the fish oil groups were substantially lower than for the control group, which may explain the observed differences in atherosclerosis. In support of this interpretation, Parks et al. (Ref. 116) noted that one of monkeys fed the fish oil diet responded differently than the other 11 monkeys fed fish oil. This one monkey had a plasma cholesterol level comparable to that of the lard-fed control monkeys, and also had atherosclerosis comparable to the lard-fed monkeys.

Changes in total cholesterol levels were noted by authors of another study in pigs that showed a reduction in atherosclerosis concomitant with a reduction in time-weighted total cholesterol (Ref. 81). Since cholesterol concentrations are not changed by fish oils in humans, animal studies where fish oil treatment lowered total cholesterol levels are of questionable relevance to the role of omega-3 fatty acids in the development of human atherosclerosis.

A recent study (Ref. 47) in nonhuman primates (vervet) compared the effects of fish oil supplementation to sunflower oil supplementation in either an atherogenic diet (high fat, low polyunsaturated fat to saturated fat ratio, high cholesterol) or, following the atherogenic diet, in a therapeutic diet (low fat, high polyunsaturated fat to saturated fat ratio and low cholesterol). Animals in each diet group were matched for serum cholesterol. Sixteen separate measures of atherosclerosis

were scored, including various measures of the extent of plaque, loss of endothelium, intimal thickening, and inflammation. Overall there was no benefit of fish oil; in some cases, the atherosclerotic measure indicated more disease in the fish-oil fed animals.

The LSRO report considered the amount of fish oil in the diets in this experiment (1.3 to 1.8 percent of calories) too low to observe an effect. In fact, FDA calculates a lower percent of calories from fish oil (1.0 to 1.5 percent) than calculated by LSRO. This level is about half the amount used in short-term human studies (i.e., 10 mL/day), and FDA agrees that the low level makes it less likely that an effect would be observed than if a higher amount had been used. However, diets were supplemented for a prolonged period of time (20 months) and in the therapeutic diet other dietary factors were also changed that might have made the effects of omega-3 fatty acids more noticeable (e.g., ratio of polyunsaturated to saturated fatty acids). Finally, the fact that there were differences between the fish oil-supplemented group and the polyunsaturated fat group while on either the atherogenic or therapeutic regimens suggests that there was sufficient sensitivity in the experimental design to detect protective effects of omega-3 fatty acids.

In some animal studies that showed a protective effect of fish oils, an invasive procedure was used to accelerate atherosclerosis, either mechanical injury (Ref. 122) or vein grafts (Refs. 90 and 186). These studies may be most relevant to the late stages of atherosclerosis, and to CHD in humans following invasive procedures. All of the animal studies cited in the LSRO report except Kim et al. (Ref. 81) and Fincham et al. (Ref. 47) did not control for PUFA's, so the effects observed have not been shown to be specific to omega-3 fatty acids. Additionally, the level of use of fish oils has been high, e.g., 22 percent of calories in Parks et al. (Ref. 116) and 25 percent of the diet in Davis et al. (Ref. 27), which limits the extrapolation of findings in these studies to levels that might be reasonably consumed by humans.

FDA disagrees with the summary of the literature in animals, as expressed in the LSRO-report, because that report fails to mention important limitations in the data. FDA notes that most studies did not have an adequate design to show specificity of effects as due to omega-3 fatty acids. Furthermore, reductions in total cholesterol in the fish oil fed animals may explain the reported reductions in atherosclerosis. Since reasonable amounts of fish oils in

human diets do not alter serum cholesterol concentrations, the results from these animal experiments are of questionable importance regarding human atherosclerosis. Finally, LSRO did not review numerous animal studies that found no effect or an adverse effect of supplementation with fish oils, and therefore the LSRO conclusion does not represent the totality of publicly available scientific information.

With these qualifications in mind, FDA notes that some of the reported effects of the dietary interventions with fish oils on the development of atherosclerosis have been dramatic. Also, FDA recognized that animal studies are of great importance for study of long-term effects on chronic diseases of consumption of amounts of omega-3 fatty acids, particularly in amounts that might be obtained in a reasonable diet. Therefore, FDA encourages further research in this area using rigorous study designs and amounts of omega-3 fatty acids reasonably available in a normal diet to elucidate any effects specific to these fatty acids.

After closer scrutiny of the animal studies cited in FDA's proposal and in the LSRO report, the agency has reached the same conclusion that it reached in its proposed rule: there are some data in studies from animals which suggest the possibility of a beneficial effect of omega-3 fatty acids on CHD; however, the data are equivocal. (56 FR 60663 at 60671.)

b. Safety considerations

50. One comment stated that the increases in LDL cholesterol observed were a chance occurrence, and another stated that increased LDL should not be considered an adverse finding in light of the results of the Burr study.

FDA disagrees with this comment. FDA found that increased LDL cholesterol was ordinarily found when hyperlipidemics or diabetics were given fish oil supplements. This may be due in part to the fact that fairly large amounts of omega-3 fatty acids (i.e., 5 g EPA plus DHA/day or more) were used in these studies. Increased LDL is not ordinarily seen in the studies on normal subjects.

FDA does not consider the Burr study (Ref. 16) to have established that omega-3 fatty acids reduce the risk of CHD, and therefore remains concerned that increases in LDL cholesterol could be adverse for some subjects. FDA notes that concern about increased LDL cholesterol was expressed in the report of the NHLBI concensus development conference (Ref. 255).

51. One comment stated that it was inappropriate to consider adverse effects

in subpopulations without describing the advantages of omega-3 fatty acids in those same populations.

FDA disagrees with this comment. As noted in the final rule on general requirements for health claims, published elsewhere in this issue of the *Federal Register*, it would be a violation of the agency's responsibility under the act to authorize a health claim about a substance without being satisfied that the use of the substance was safe. The agency attempted to examine all available scientific evidence regarding the effects of omega-3 fatty acids. FDA separated out the potential adverse effects discovered during its review, because it wanted to draw attention to these issues as impediments to a health claim for omega-3 fatty acids and CHD. Such potential adverse effects must be resolved, and may be important in setting the conditions under which FDA would allow a health claim to appear on the label and labeling of foods and food supplements.

52. Two comments stated that the safety issues raised in the Mitre Corp. report (Ref. 72) were outdated but did not indicate which issues, or suggest why they were outdated.

FDA recognizes that there has been considerable debate regarding the clinical importance of bleeding times since the publication of the Mitre Corp. report (Ref. 72). However, the agency believes that the issues raised in that report have been restated in subsequent literature, and that all issues of safety are important in deciding whether or not to authorize a health claim.

53. A few comments recommended that FDA balance the benefits of reduced risk of CHD against the risk of reduced glycemic control among diabetics when deciding whether or not to authorize a health claim. One comment stated that physicians could adjust the dose of insulin if omega-3 fatty acids reduced their glycemic control, but another comment stated that glycemic control must be considered a real adverse effect.

FDA agrees that limitations on the use of a substance by a subpopulation (e.g., diabetics) do not necessarily exclude a substance from bearing a health claim for the general population, because the claim may be appropriately restricted. However, FDA agrees that the loss of glycemic control is a potentially serious adverse effect that must be fully addressed before a health claim could be authorized.

54. Another comment stated that a major concern about omega-3 fatty acids not mentioned in the proposed rule is that they may be oxidized and, as

oxidized products, may have adverse effects.

FDA agrees that oxidation of omega-3 fatty acids is a concern. In fact, there are many studies that have been reported since the publication of the proposed rule, or that were not included in FDA's literature review, that indicate such a concern (Refs. 184, 210, 217, 229, 240, 248, 263, 283, and 290).

Antioxidants have been successfully added to supplements and may be adequate to protect the omega-3 fatty acids in foods. It may be necessary to establish conditions that protect against oxidation of omega-3 fatty acids and incorporate those conditions into any future regulation authorizing health claims for omega-3 fatty acids.

55. A related comment indicated that the majority of the fish oil preparations that have been used are severely oxidized, including National Institutes of Health Fish Oil Test Materials. However, no data regarding the extent of oxidation, the nature of the oxidation products, or the physiologic action of these products was provided.

FDA agrees with this comment. Many of the biologically active products of omega-3 fatty acids are oxidation products. Oxidation of test materials may explain some contradictory findings in the literature.

56. One comment pointed out that increased prothrombin times and possibility of increased stroke were not discussed.

FDA agrees with this comment. FDA did not specifically review data on prothrombin times, although data on bleeding times as a measure of hemostasis were discussed for both normal subjects and for subjects with risk factors for CHD. The importance of the increase in bleeding time brought about by supplemental fish oils or increased fish consumption is not clear. FDA noted in the proposal that most reports suggest that serious bleeding is not an issue in patients supplemented with omega-3 fatty acids, and also that standardized bleeding times do not closely correlate with clinically significant bleeding. However, concerns about untoward bleeding after supplemental fish oils have been raised in the literature (Refs. 106, 120, and 189).

FDA did not discuss the possibility of increased occurrence of stroke as a consequence of increased consumption of omega-3 fatty acids. The papers that reported a correlation between high consumption of omega-3 fatty acids from fish and other marine animals and low rate of CHD mortality also noted an increase rate of stroke, particularly hemorrhagic stroke (Refs. 8 and 84).

Also, the possibility of increased rates of stroke are raised by the data from studies on aspirin (Ref. 66).

Thus, FDA considers these potential adverse reactions to be legitimate concerns, primarily in the context of very high intakes of omega-3 fatty acids.

57. One comment stated that even if adverse effects were only suspected in a medical disorder, pronounced warnings or contradictions would be required.

As noted above, the agency must be satisfied that the use of a substance is safe before it will authorize a health claim about the substance. Thus, suspicions about potential adverse effects would need to be resolved prior to the authorization of a claim. Certain health claims may require appropriate qualifications as a way of minimizing potential safety concerns.

C. New Scientific Data

To determine whether or not new scientific data published since the proposed rule provided a basis for modifying FDA's conclusions regarding the relationship between omega-3 fatty acids and risk of CHD, FDA conducted a search of the scientific literature for relevant studies. Reviews published since the period covered in the literature review in FDA's proposed rule were used to identify recently published studies.

1. Epidemiologic studies

a. Cross-sectional studies and surveys (Table 1)

Bulliyya et al. (Ref. 185) found lower total serum cholesterol and higher HDL cholesterol in a fish-consuming coastal village population than in a nonfish-consuming population from the interior of India. These correlational data are consistent with a beneficial effect of omega-3 fatty acids on blood lipids, but many possible confounding variables prevent strong conclusions regarding a specific role for omega-3 fatty acids.

In a retrospective study, Popeski et al. (Ref. 266) found that women from communities with higher marine oil consumption had significantly lower diastolic pressure in the last 6 hours of pregnancy than women from communities with low fish oil consumption. Pregnancy associated hypertension was 2.6 times more common in communities with low fish consumption. These correlational data are consistent with an effect of omega-3 fatty acids on blood pressure in this particular situation. Again, many possible confounding variables prevent strong conclusions regarding a specific role for omega-3 fatty acids.

b. Prospective studies (other than intervention studies) (Table 1)

Bjerregaard and Dyerberg (Ref. 176) reported age-standardized mortality rates per 10,000 person-years for CHD in men in Greenland settlements (5.3) as half of that reported for men in Denmark (10.0). There was an increasing rate of CHD from settlements to towns in Greenland. The difference in rates of CHD in women were less apparent, with lower rates in Denmark than in towns in Greenland. These studies do not have sufficient specificity to identify omega-3 fatty acids as causal in reducing CHD, but are consistent with the hypothesis that they are.

Van Houwelingen et al. (Ref. 294) found that, while men from a high fish consumption group had higher concentrations of plasma phospholipid EPA and DHA than men from a low fish consumption group, there was no significant difference in collagen-induced platelet aggregation, cutaneous bleeding time, ATP-release in whole blood, or platelet number between the two groups. This study suggests that the outcome measures found commonly to be affected in clinical studies may not be related to consumption of omega-3 fatty acids in the free-living population.

c. Intervention studies

There were no new prospective intervention studies measuring occurrence of heart attacks or CHD mortality.

2. Evidence relating omega-3 fatty acids to intermediate or surrogate markers of CHD (Table 2)

a. Atherosclerosis

i. Blood lipids

Through its own literature review, FDA has found another 34 studies not reviewed in the proposal that report data for serum cholesterol after consumption of fish containing omega-3 fatty acids or fish oil concentrated in omega-3 fatty acids. Among these, 25 found no change in blood cholesterol levels, 3 found an increase, and 6 found a decrease.

Studies among normal healthy subjects generally reported no change in total cholesterol (Refs. 168, 196, 202, 210, 217, 220, 235, 241, 253, 254, and 277), although none of these studies was controlled for nonspecific effects of the omega-3 fatty acids as polyunsaturated fats.

One study among normal subjects found that feeding a high fish diet did not change total cholesterol, unless combined with a low total fat and low saturated fat diet (Ref. 168). Another study (Ref. 301) reported decreased total

cholesterol after switching from a meat diet to a fish diet, but the fish diet had significantly less saturated fat than the meat diet. One study (Ref. 283) found a slight increase after 5.4 g EPA plus DHA/day from MaxEPA (with 30 percent saturated fatty acids), and one study (Ref. 224) found a slight reduction in total cholesterol after 2.7 g purified EPA/day, but neither study was placebo controlled for effects of polyunsaturated and saturated fat contained in the supplements.

Similarly, nearly all of the 17 studies on subjects in at-risk subpopulations, including all of the studies that controlled for PUFA's (Refs. 203, 209, 247, and 258), found no effect of supplemental omega-3 fatty acids on total cholesterol (except for a post hoc analysis of a subgroup in one study; (Ref. 209)). One study in diabetics (Ref. 252) found an increase in serum cholesterol, but the statistical significance of the result may have been due in part to a change in the opposite direction in the control group. One study among hyperlipidemics (Ref. 191) found decreased cholesterol after relatively high doses (4.6 to 6 g EPA plus DHA/day) but not after 3.6 g EPA plus DHA/day, and did not control for PUFA effects of the supplements. The other study that reported decreased cholesterol after supplemental omega-3 fatty acids (Ref. 268), similarly, found the effect after a high level (6 percent of calories, 16 to 21 g EPA plus DHA/day) and did not control for the polyunsaturated fat effects of the supplement.

These studies support the conclusion reached in the proposed rule, that among normal, healthy subjects there is no significant effect of omega-3 fatty acids from fish or fish oils on total serum cholesterol.

FDA concluded in the proposed rule that the best studies among normal subjects found no effect of fish oils on LDL cholesterol. All of the additional studies among normal healthy subjects obtained in FDA's updated literature search have reported no change in LDL cholesterol (Refs. 220, 253, 253, and 277).

One study (Ref. 224) reported that purified EPA produced a significant decrease in a subtraction of large, light LDL cholesterol (LDL₁), and a significant increase in small, dense LDL cholesterol (LDL₂), but FDA calculates no change for the sum of these two fractions of LDL cholesterol. Some clinical studies reviewed in the proposed rule (Refs. 1, 43, 53, and 129) described changes in the composition of LDL particle after consumption of fish oil.

The relative importance of various subfractions of LDL particles (and the associated composition of the particles), however, is still controversial. While Homma et al. (Ref. 224) suggest that large, light LDL are the fraction associated most closely with atherosclerosis, Austin et al. (Ref. 171) report that the phenotype of small, dense LDL is the fraction most closely related to increased CHD risk. The February 1992 NHLBI consensus development conference (Ref. 255) included among its recommendations for further research the identification of the atherogenic and anti-atherogenic subfractions that may be present in VLDL and HDL; the uncertainty about the relevance of changes in the amounts of subfractions of these two lipoproteins similarly applies to LDL.

In at risk populations, there have been some additional reports of increased LDL cholesterol after fish oil supplementation (Refs. 170, 191, and 251), a concern raised in the proposal. However, most studies have found LDL cholesterol not changed by fish oils (Refs. 174, 189, 205, 209, 219, 258, and 278). Moreover, each of the studies that used a polyunsaturated fat placebo control group found no change in LDL cholesterol (Refs. 203, 209, and 258).

Therefore, FDA concludes that these most recently reviewed studies support the conclusion reached in the proposed rule, that for the general population, there is no significant effect of omega-3 fatty acids on LDL cholesterol. The results of recent studies among at-risk subjects, however, are not in complete agreement with the conclusions in the proposed rule, and suggest that omega-3 fatty acids may not uniformly increase LDL cholesterol. Additional study is needed to determine the conditions under which LDL cholesterol is increased by omega-3 fatty acids.

Among more recent studies in normal healthy subjects found in FDA's updated literature review, about half have found no effect of fish oils or fish on HDL cholesterol (Refs. 202, 206, 217, 219 (after 1.25 and 2.5 g/day EPA plus DHA), 226 (after 1 and 3 g/day EPA plus DHA), 241, and 244), but about half have found increased HDL (Refs. 210, 219 (after 3.75 and 5 g/day EPA plus DHA), 220, 226 (after 6 g/day EPA plus DHA), 235, 253 (compared to baseline, significant compared to olive oil control), 278, 283, and 301), including a metabolic ward study that very carefully controlled for total fat and saturated fat intake (Ref. 253). Weintraub et al. (Ref. 298) found decreased HDL after fish oil compared to saturated fat diet.

FDA attempted to ascertain how those studies that reported an increase in HDL cholesterol after increased intake of omega-3 fatty acids differed from those studies in which no effect was found, however, there was no apparent difference between the studies that reported that omega-3 fatty acids reduced HDL cholesterol and those that reported no change. Most of the studies that found a change used supplements containing substantial amounts (e.g., 30 percent) of saturated fatty acids, raising the possibility that the saturated fatty acids in the supplements were responsible for the increase in HDL (Ref. 17). However, some supplements had low amounts of saturated fatty acids (Ref. 278) or saturated fat in the diet was specifically controlled (Ref. 235), and in one study the control diet was reported to have significantly more saturated fat than the fish diet (Ref. 301), so the saturated fat intake during omega-3 fatty acid supplementation cannot be the factor responsible for increased HDL..

The amounts of omega-3 fatty acids used in those studies that reported increased HDL tended to be high (e.g., more than 5 g EPA plus DHA/day), but some studies that found a change used lower amounts (Ref. 278) and some studies that used high amounts found no change (e.g., Ref. 241 (used 6.7 g EPA plus DHA/day) and 253 (used 8 g/day)). Some studies in which fish was fed, rather than fish oil, found an effect (Refs. 235 and 301), but others did not (Refs. 206 and 244). There was no systematic difference in sample sizes of the studies that found an effect and those that did not; seven of the negative studies reviewed in the proposed rule or in the present document had 30 or more subjects, compared to only one of the positive studies. Small studies ($n = 10$ or fewer) may not have observed a significant difference because of small sample size, but larger studies did not find a significant difference, even though some found a trend toward increased HDL after fish oil supplementation (Ref. 217).

Finally, the enrichment of plasma phospholipids with EPA and DHA tended to be higher for subjects in studies where increased HDL was found than that for subjects in the studies where no change in HDL was found, reflecting the tendency of higher doses to produce increased HDL. In particular, all studies in which the plasma phospholipid EPA value was 3.9 percent or more found increased HDL. However, the studies that fed the highest amounts of EPA but that did not find an effect on HDL did not report data for phospholipid EPA, so it is not clear whether high phospholipid EPA is

uniformly associated with increased HDL. Comparable results were found after inspection of data on phospholipid DHA after supplementation, however, because not all studies reported phospholipid fatty acid values, no conclusion can be drawn about the relationship between phospholipid DHA and iIDL concentration. Notably, recent data suggest a direct correlation between plasma EPA and HDL, but an inverse relationship between plasma DHA and HDL (Refs. 177 and 178), underscoring the importance of reporting these data in future studies.

Among subjects with risk factors for CHD fewer reports found increased HDL (Refs. 191 (for type IV on SuperEPA only), 195, 203, 209 (for type lib), and 219) than found no change (Refs. 170, 174, 189, 191 (for type IIb and type IV on MaxEPA), 209 (type IV), 224, 258, 263, 277, and 299).

Few studies have controlled for effects of PUFA's by giving a PUFA supplement. Two papers found no change in HDL in normal subjects fed fish oil as Promega (Ref. 73) or MaxEPA (Ref. 166) compared to wheat-germ oil or safflower oil (Refs. 73 and 166, respectively). Cobiac et al. (Ref. 20) reported increased HDL for mildly hypertensive subjects fed salmon and sardines in sild oil compared to those given a safflower-olive oil mix, but in comparable subjects, Meland et al. (Ref. 247) found no change in HDL cholesterol after MaxEPA fish oil compared to when the subjects were given a corn-olive oil mix. Very recent results, also for a mildly hypertensive population, found increased HDL after either, ethyl esters of EPA and DHA, or after corn oil (Ref. 177). Thus, for normal and hypertensive subjects, the change in HDL appears to not be a specific effect of omega-3 fatty acids, but may be related nonspecifically to increased PUFA's, either omega-3 fatty acids or omega-6 fatty acids.

In contrast, there are two reports of increased HDL cholesterol in subjects with type lib hyperlipidemia fed fish oil compared to safflower oil (Ref. 166) or a corn-olive oil mix (Ref. 209), and one report of increased HDL in type IIa hyperlipidemics after fish oil or olive oil compared to corn oil (Ref. 286). Others found fish oil did not change HDL in type IV hyperlipidemics (Refs. 166 and 209) or patients with CHD (Ref. 258) compared to PUFA controls.

Therefore, at this time, FDA concludes that there is some evidence that omega-3 fatty acids, in some form and amount and in some selected populations, may increase HDL cholesterol, but that current data are ambiguous because the conditions

under which fish oils reliably increase (total) HDL cholesterol have not been established, either in a specific subpopulation, or in the general population.

When fractions of HDL cholesterol have been reported, an increase has generally been found in the HDL₂ fraction (Refs. 1, 9, 54, 148, 191, 202, 203, 220, 235, 251, and 286), with a comparable decrease in the HDL₃ fraction (Refs. 202, 235, 251, and 286). Interestingly, the two recent reports that failed to find increased HDL₂ both used esterified omega-3 fatty acids rather than the fish oil triglyceride (Refs. 191 and 224), although others using ethyl esters have found increased HDL₂ (Refs. 9 and 286).

These studies suggest that fish oils produce a shift within the HDL fractions toward a lipid-rich, and away from a protein-rich lipoprotein, as well as within the LDL fractions. This shift may occur whether or not there is any change in total HDL cholesterol. FDA noted (56 FR 60663 at 60669) that some studies among normal subjects found increases in the HDL₂ fraction of HDL cholesterol, and that these reports were the most promising changes in blood lipids. New studies published after the period covered in FDA's review of the literature, however, found that both HDL₂, and HDL₃ were correlated with reduced risk of MI (Refs. 185a and 287a), and the NHLBI consensus conference (Ref. 255) concluded that, "The current studies of HDL₂ and HDL₃ levels have not shown consistent associations with CHD." Therefore, data on changes in HDL subfractions after increased consumption of omega-3 fatty acids do not provide a sufficient basis for a health claim, because there is not significant scientific agreement that the endpoints are directly related to risk of CHD. If the risk of CHD becomes linked with particular subfractions of these lipoproteins, these findings in normal subjects may be of great importance.

However, FDA also notes that recently published data from a prospective study demonstrate an effect of aspirin consumption in reducing the incidence of first heart attacks among women (Ref. 243). Another study shows a relationship between spontaneous platelet aggregation *in vitro* and incidence of CHD (Ref. 288). Both studies were conducted in the general population and their results support the hypothesis that platelet aggregation is a useful marker for CHD risk in the general population. Additionally, preliminary data from the Caerphilly Collaborative Heart Disease Study (Ref. 302) supports a relationship between platelet aggregation and the incidence of

ischemic heart disease; final data from this study will be available in the near future. These recently published and forthcoming studies may provide the basis for significant scientific agreement regarding the use of platelet function as a surrogate marker for CHD risk among the general population.

ii. Vessel wall effects.

New human studies on the effects of omega-3 fatty acids on vessel wall effects were discussed in response to comments 35 through 37 of this document. A recent meta-analysis of studies on use of fish oils in the prevention of restenosis concluded that the most plausible interpretation of the results was that there was a small to moderate beneficial effect of fish oils, but that chance could not be ruled out as a cause of the results (Ref. 260). The authors noted a significant heterogeneity in the findings and concluded that data from a large clinical study are necessary to confirm their interpretation. No study of restenosis to date has compared fish oil to an alternate polyunsaturated oil to control for nonspecific effects of PUFA's.

b. Thrombosis and hemostasis

i. Bleeding times

A number of studies have reported data that show no significant effect of fish oils on standardized bleeding time tests (Refs. 179, 218, 253, 268, and 277). However, others have found a significant increase in bleeding time due to fish, oil (Refs. 195, 219, 220, and 278) or salmon (Ref. 297) or have reported increased bleeding as a side effect of treatment (Refs. 189 and 295).

ii. Platelet aggregation.

Consistent with the literature previously reviewed, recent studies show that fish oil tends to decrease platelet aggregations to numerous stimuli including AA (Refs. 179 and 256), adenosine diphosphate (ADP) (Refs. 204, 256, and 297), collagen (Refs. 218, 241, 251, and 297), thrombin (Ref. 241), and PAF (Ref. 251). Only one of these studies controlled for effects due to PUFA's (Ref. 204). The importance of the polyunsaturated fat control is less critical for studies on platelet function than for studies on blood lipids, because nonomega-3 PUFA's (i.e., omega-6 fatty acids derived from plant oils) produce effects in the opposite direction in platelets as omega-3 fatty acids (whereas many of the blood lipid effects of these two classes of fatty acids are in the same direction). Thus, the effects of omega-3 fatty acids on platelet responsiveness are not likely to be produced by PUFA's in general.

The only new study among healthy subjects that reported no difference in responsiveness to ADP used EPA ethyl esters as the source of omega-3 fatty acids (Ref. 179). Furthermore, the data were not shown in this brief report, so it is not clear if there was a trend toward an effect that might not have been statistically significant due to small number of subjects (eight per group). Those studies in healthy subjects reviewed in the proposed rule that did not find statistically significant differences in platelet responsiveness to ADP did have trends in the direction of reduced responsiveness (Refs. 24 and 54).

Other studies found no effect of fish oils on platelet aggregation in response to collagen (Refs. 179, 256 and 277). Each of these studies had a relatively small number of subjects, and there was a trend toward decreased sensitivity toward collagen at a high dose of omega-3 fatty acids in one study (Ref. 277). However, in the recent metabolic ward study (Ref. 256) there was no trend toward decreased sensitivity toward collagen or thrombin. These findings contrast with the results described above (Refs. 218, 241, 251, and 297) and with studies in healthy subjects described in the proposed rule (Refs. 2, 24, 54, 86, 143, and 166).

Studies reporting no effect of fish oils on PAF or AA-induced platelet aggregation (Refs. 179 and 218) may not have had sufficient power to find a statistically significant difference; where the data were reported there was a trend toward decreased sensitivity for both agents (Ref. 218).

iii. Platelet adhesion

A provocative study by Li and Steiner (Ref. 234) showed a 60-percent decrease in the extent to which platelets prepared from subjects fed fish oils adhered to substrates in a laminar flow chamber. The high flow rates used in this experiment showed that the change in adhesiveness of the platelets was due to changes on the platelet surface, and not due to a difference in the amount of material released from platelets that subsequently caused adhesion (i.e., AA). Also, a dose-response relationship was observed, and the time to return to pre-fish oil adhesion values was related to the amount consumed.

However, another study found no effect on fish oils on in vitro platelet adhesion to everted rabbit aorta, although there was a trend toward increased adhesion after 2 and 4 weeks of supplementation (Ref. 264). The reperfusion assay used in this study does not distinguish platelet membrane effects from effects mediated by

substances released from platelets. Neither of these studies used a nonomega-3 PUFA control.

iv. Regulators of bleeding

Two recent studies in normal subjects have reported that omega-3 fatty acids have no effect on the clotting protein fibrinogen (Refs. 183 and 210), although in one of these studies a large supplement of vitamin E was associated with a decrease (Ref. 210). An uncontrolled study in normal subjects found a decrease in fibrinogen after fish oil supplementation (Ref. 278).

Studies on subjects at risk for CHD have reported no change (Ref. 203), a decrease (Refs. 276 and 277), and an increase in fibrinogen (Ref. 287). In agreement with its tentative conclusion in the proposed rule, PDA finds that the data on the effects of omega-3 fatty acids on fibrinogen level are ambiguous, because they do not distinguish effects due to PUFA's from effects specific to omega-3 fatty acids.

Plasminogen is an enzyme that dissolves clots. Plasminogen activator is a substance that increases clot dissolving; plasminogen activator is specifically inhibited by another substance, the PAI-1. Thus, a high level of PAI-1 decreases the capability to dissolve clots.

Three recent studies reported increased concentrations of PAI-1 after fish oil supplementation (Refs. 254, 278, and 287), which would appear inconsistent: with a clot-dissolving effect of fish oil. Two of those investigators also found no change in the amount of plasminogen activator (t-PA) after supplemental fish oil (Refs. 254 and 287) including one who used a very specific immunologic assay (Ref. 254), suggesting that fish oils do not increase clot dissolution by increasing the amount of this protein. The third group however, found an increase in the activity of tissue plasminogen activator (Ref. 277), which suggests that fish oils might increase clot dissolution by a different mechanism than affecting the amount of activator. Another group found no effect of cod liver oil on t-PA activity or fibrinolysis measured directly (Ref. 216). These reports are in contradiction to a report of increased fibrinolytic activity after a fish or fish plus fish oil diet (Ref. 183). FDA has not been able to find a reason for this rather marked contradiction. Therefore, in agreement with the conclusion in its proposed rule, FDA finds there is no clear relationship between omega-3 fatty acids and factors involved in dissolving blood clots, or clot dissolution activity.

Numerous investigators (Refs. 174, 191, 210, 220, 235, 241, and 279) have

recently reported that fish oils do not affect the concentration of Lp (a), a lipoprotein correlated with the risk of CHD. One investigator reported that very high levels of fish oils (9 g EPA plus DHA/day) gave a trend toward lower values, but the response may have been due to the PUFA's (Ref. 279). One study reported no effect overall of fish oils on Lp(a) among hypertriglyceridemics, but Lp(a) was reduced in those whose initial values were high (Ref. 174). On the basis of these reports and those reviewed in the proposed rule, FDA concludes that omega-3 fatty acids do not affect the risk of CHD by lowering Lp(a).

v. Blood pressure

Most of the studies not reviewed in the proposed rule that report data on blood pressure after consumption of fish oils have not found a significant change. One study of 50 elderly, healthy subjects reported that fish oils in combination with a salt-restricted diet decreased both systolic and diastolic blood pressure, but that fish oil alone had no effect (Ref. 190). There was a reduction in blood pressure during the run-in period, when the polyunsaturated fat placebo, sunflower oil, was fed.

Most studies on subjects with mild hypertension also have reported no change (Refs. 247, 277, and 289), including one large, randomized, placebo-controlled, multicenter trial of various behavioral changes and dietary supplements (Ref. 289). One study in hypertensives found reduced systolic and diastolic blood pressure comparable to reductions after the hypertension medication propranolol (Ref. 285), and in some cases the combined treatment of fish oil plus propranolol gave a greater decrease than either treatment alone. This study was controlled by olive oil (which is predominantly monounsaturated fatty acids), and therefore does not distinguish effects of omega-3 fatty acids from other PUFA's. Another double-blind randomized, placebo-controlled study in hypertensives whose blood pressures were maintained by medications found comparable blood pressure lowering compared to pretreatment values by fish oil or olive oil placebo (Ref. 299):

One uncontrolled study among hyperlipidemics also found reduced systolic and diastolic blood pressure (Ref. 263), but no effect was found in uncontrolled trials in subjects with end-stage renal disease (Ref. 207) or diabetics (Ref. 215). In a polyunsaturated fat (corn oil) controlled study on subjects with stable claudication (Ref. 203) fish oil and corn

oil both reduced diastolic blood pressure comparably, but systolic blood pressure was only reduced by the corn oil treatment

The results of these studies support the tentative conclusions reached in the proposed rule, that omega-3 fatty acids reduce blood pressure to a small degree in hypertensive people, but that it is not clear if there is any specific effect among normal subjects.

3. Other relevant information

a. Animal studies

Animal studies are especially important for studying effects of long-term consumption of omega-3 fatty acids, where there are few data from human intervention studies. The animal studies cited in the proposed rule related to the ability of omega-3 fatty acids to inhibit the development of atherosclerosis, an area not readily available for study in humans. A more complete discussion of the previously cited studies, with emphasis on those studies in nonhuman primates, is given in response to comment 47 of this document. Other recent animal studies cited in the comments or found during FDA's updated literature search that provide data on the development of atherosclerosis (where atherosclerosis is measured directly) are reviewed here. Also reviewed are studies on effects of omega-3 fatty acids during experimental ischemia, obviously not available for human study.

i. Atherosclerosis

One recent study in rabbits found less atherosclerosis in fish oil-supplemented animals, but there was no control for PUFA's, and the fish oil-treated animals also had reduced serum cholesterol (Ref. 192). Because humans do not have reduced serum cholesterol after fish oil consumption, these results are of questionable relevance to humans. Furthermore, the effect cannot be attributed specifically to omega-3 fatty acids rather than to polyunsaturated fats in general.

Fish oil feeding has also been associated with reduced binding of LDL to the blood vessel endothelium in monkeys (Ref. 193), and purified EPA ethyl ester was reported to reduce susceptibility of LDL to oxidation (Ref. 273), but these studies did not control for PUFA's. The antioxidant levels in the diets with respect to the amount of omega-3 fatty acids may be as important in determining whether or not there is any effect of omega-3 fatty acids on the oxidation of LDL.

Three recent papers describe effects of fish oils fed before surgical grafting of a

vein into an artery, a procedure associated with an accelerated development of atherosclerosis. Two papers (Refs. 275 and 303) each used a polyunsaturated fat control and studied fish oil effects after vein allografts in animals treated with the immunosuppressant cyclosporin. In one study (Ref. 303), six groups of rabbits received one of three amounts of fish oil (giving 29, 87 and 174 mg ERA plus DHA/kg, respectively, similar amounts to those used in most human studies) or comparable amounts of safflower oil. In this study, safflower oil was more effective at reducing cholesterol than fish oil, and there was a trend toward more protection from atherosclerosis in the safflower oil-fed group. In the other study (Ref. 275), rats received, in addition to cyclosporin, either fish oil (containing 210 mg EPA plus DHA/kg), or safflower oil with aspirin, or safflower oil only. The fish oil group had remarkably less atherosclerosis than the other two groups. The contradictory results in these two studies, both of which used the same model of vein allografts with cyclosporin immunosuppression and the same polyunsaturated fat control, may be related to dose and species differences.

A third study of vein allografts in dogs (Ref. 274) found significantly less atherosclerosis in fish oil-fed animals either fed the fish oil alone or in combination with aspirin or a thromboxane synthetase inhibitor. Other animals were treated with aspirin only or a thromboxane synthetase inhibitor only. There was no difference among groups for blood lipids, platelet function or eicosanoid metabolism. This study suggests that mechanisms of atherosclerosis other than those involving blood lipids and platelet function may be affected by omega-3 fatty acids.

These animal models are most relevant to comparable surgical procedures or other invasive procedures (e.g., angioplasty) that would be expected to activate platelets in humans. Use of omega-3 fatty acids in these settings is a drug usage, but provides information on the extent to which omega-3 fatty acids may modify platelet response *in vivo*. The very different results of omega-3 fatty acids in modifying the response to vein allografts in immune-suppressed animals indicates that the actions of omega-3 fatty acids in these settings are not yet well established.

ii. Response to ischemia

One major line of research on omega-3 fatty acids in animals is experimental ischemia (deficiency of blood flow to

the heart). Force et al (Ref. 199) found that rats fed a diet containing a high amount of fish oil (20 percent of the diet) for 6 to 12 weeks had much greater blood flow once the occlusion was removed than rats fed diets enriched in corn oil or lard. There were no differences among diet groups in the amount of tissue damaged by ischemia. Increased blood flow after ischemia has also been reported in a pig model (Ref. 221). This study did not control for polyunsaturated fat and used a lower amount of omega-3 fatty acids, and the differences in blood flow were not as pronounced as in the Force study. Another study found evidence of less tissue damage during reperfusion when rats had been fed a diet with 1.2 percent fish oil compared to other rats fed the same level of corn oil (Ref. 223). Another study in yet a third animal species (Ref. 230) showed that the functional capillary density was preserved during reperfusion in hamsters fed 5 percent fish oil for 4 weeks prior to experimental ischemia.

A reperfusion study in dogs determined the effects of fish oil on the duration of time needed for drug-induced reperfusion following an electrically induced blockage, and on the occurrence of spontaneous reocclusion in the reopened vessel (Ref. 182). High amounts of fish oil (one-third of total calories) for 3 weeks before the surgery resulted in a shorter time needed for the drug-induced reperfusion, but did not affect the time necessary for the electrically mediated occlusion to occur, the occurrence of second occlusion, or the time it took for the second occlusions to appear. This study did not have a polyunsaturated fat control, and even at the high intake only a modest effect of omega-3 fatty acids on platelet function was seen, that being primarily an enhancement of the effects of the fibrinolytic drug.

Another possible consequence of ischemia is arrhythmia, when the heart fails to maintain its normal rhythmic beating. The effects of fish oils on arrhythmia in monkeys are discussed in response to comment 39 of this document. Similarly, data have been reported for experimental ischemia in rats that show that, both fish oil and sunflower oil reduced the occurrence of arrhythmia during occlusion and reperfusion compared to a saturated fat diet (Ref. 246). Another study, done on isolated, cultured rat heart cells (myocytes) showed that EPA, but not AA, prevented a known toxin (ouabain) from disturbing the rhythmic contractions and killing the cells (Ref. 212). The effective amount of EPA was so low that the mode of action was

proposed to be due to production of an active metabolite, rather than due to direct effects of EPA on the cell membranes. This study suggests a specific effect of EPA in stabilizing the heart myocytes during stress. Prevention of arrhythmia by stabilization of these heart cells has been proposed as a mechanism by which omega-3 fatty acids may increase the chances of survival following a heart attack as reported in the Dart study (Ref. 16).

These studies indicate that, in various animal models, dietary fish oils promote greater reestablishment of blood flow in heart tissues following a transient block as occurs in an acute heart attack. Importantly, the results are consistent across many animal species, and in some cases have been shown to be specific for omega-3 fatty acids rather than simply due to any PUFA. Finally, the experimental designs included coronary occlusions in otherwise healthy animals who were not suffering from heart disease, a model relevant for use of omega-3 fatty acids in reducing the risk of CHD rather than in therapy for persons with preexisting heart disease. The studies remain limited in that ischemia was produced by an acute blockage produced by mechanical or electrical means rather than by chronic dietary means, and the response to these different types of block may not be the same.

Other studies have attempted to learn the mechanisms by which the platelet-vessel wall interactions are modified by omega-3 fatty acids. One study (Ref. 240) found that aortas from rats fed fish oil or corn oil did not contract as much in response to agents that cause contraction as aortas from rats fed beef tallow (saturated fat). This was true both before and after oxygen deprivation. The aortas from fish oil-fed rats were more responsive to one of three tested chemical relaxers than aortas from corn oil-fed or beef tallow-fed rats. Another study found, that EPA potentiated the release of an EDRF (Ref. 181), but the effect was thought to be related to the unsaturation of the EPA, because the experiments were carried out in the presence of inhibitors of EPA metabolism.

One research group has recently shown that leukotrienes, chemicals produced from AA, are important in the tissue injury that accompanies reperfusion (Refs. 230 and 232). Since EPA competes with AA for the enzyme that makes leukotrienes from AA, EPA could potentially reduce the amount of leukotriene formed from AA. This same group has shown that leukotrienes promote the adhesion of leukocytes to the vessel wall (Ref. 231), and that

feeding hamsters fish oil at 5 percent of the diet for 4 weeks greatly reduced (over 60 percent) the adhesion of leukocytes to the vessel wall (Ref. 233). The reduced adhesion could be relevant for both the conditions during which atherosclerosis develops (indeed, the stimulus used to elicit leukocyte adhesion was oxidized LDL, a candidate for promoting atherosclerosis in humans), and the acute response to coronary ischemia.

These animal data suggest mechanisms by which omega-3 fatty acids could affect the development of atherosclerosis or the response of heart tissue after a transient occlusion of its blood flow. Both modes of action could make important contributions to the risk of CHD and, therefore, merit additional study. The reperfusion studies and the myocyte toxicity study have demonstrated specificity of the effect as to omega-3 fatty acids. However, the increase in reperfusion volume is not sufficient to ensure a reduced risk of CHD death. Omega-3 fatty acids may not affect the extent of tissue damaged during an occlusion, or the tendency for a second, spontaneous occlusion. Additionally, omega-3 fatty acids may not affect tissue vulnerability during reperfusion. Those studies where CHD deaths or second occlusions have been recorded used large amounts of fish oils, and do not indicate whether amounts of omega-3 fatty acids found in the diet would have the same effects. Thus, there are many possible avenues suggested by these animal studies for beneficial effects of omega-3 fatty acids on the development of CHD, but there are also important limitations in the study designs and models used that prevent drawing conclusions from these data about the importance of omega-3 fatty acids in reducing the risk of human CHD.

b. Safety concerns

i. Diabetes

Three additional papers (Refs. 170, 222, and 304) and one major review (Ref. 238) on the effects of fish oils in diabetics were published after the time period reviewed in the proposal. All three new studies found increased LDL cholesterol after fish oil consumption in type II diabetics. However, effects oil fasting glucose varied, with no change (Ref. 170), a transient increase (Ref. 222) or an increase (Ref. 304) reported. Although fasting insulin concentration was unchanged after fish oil (Refs. 170 and 304), postprandial, insulin response usually, but not always (Ref. 170), has been reported as reduced (Refs. 238 and 304).

These effects of fish oils on blood glucose appear to depend on the amount of fish oils fed. One study found no change in fasting blood glucose levels among type n diabetics treated with 3.0 g/day EPA plus DHA for 2 weeks (Ref. 170). Two other studies that used 3 or 2.7 g/day EPA plus DHA for 6 and 8 weeks (Refs. 222 and 79) only found transient increases in blood glucose halfway through their respective supplementation periods. A fifth study (Ref. 12) that used 3.0 g/day EPA plus DHA for 3 weeks found comparable increases in fasting blood glucose when either fish oil or safflower oil was fed, so the increase cannot be attributed specifically to the omega-3 fatty acids. Similarly, Vessby and Boberg (Ref. 157) fed 3 g/day EPA plus DHA and did not find a difference in fasting glucose or glycosylated hemoglobin after fish oil supplementation compared to baseline; they did find a significant difference compared to the olive oil treatment that produced changes in the opposite direction from fish oil. Studies on type II diabetics that reported increased glucose used higher amounts (4.5 to 8 g/day) of omega-3 fatty acids (Refs. 52, 55, 128, and 304). Thus, FDA concludes that glycemic control among diabetics remains a valid safety concern, but notes that restriction of the amount of supplemental omega-3 fatty acids may suitably address this concern.

ii. Increase in LDL cholesterol

Many studies published after 1987 with data for LDL or apoB report increased LDL cholesterol or apoB after fish oils, in hypercholesterolemic or hypertriglyceridemic subjects (Refs. 1, 26, 32, 55, 60, 61, 63, 73, 75, 94, 114, 119, 120, 129, 146, and 166). Virtually all the studies with 10 or more subjects supplemented with 5 g/day EPA plus DHA or more found increased LDL. Some studies on normal subjects (all of which were reviewed in the proposed rule) also report increased LDL or apoB after fish oil consumption (Refs. 54, 127, and 156). Many studies that found no effect may not have had sufficient sample size to detect a difference due to treatment. FDA concludes that increased LDL cholesterol among some populations already at increased risk of CHD remains a valid safety concern, but because most reports of increased LDL are in studies where large amounts of fish oils are given, it is possible that restriction of the amount of supplemental omega-3 fatty acids and/or changes in the fatty acid composition of omega-3 fatty acid supplements may suitably address this concern.

III. Overall Summary and Conclusions

FDA concludes that there is some evidence that supports a relationship between omega-3 fatty acids and CHD, but that the totality of scientific evidence available at this time does not provide an adequate basis for a health claim. In some cases, there is not significant scientific agreement that the changes that are specific to omega-3 fatty acids will reduce the risk of CHD. In other cases, the data do not demonstrate that the effect is specifically due to the omega-3 fatty acids and not due to other dietary variables. For yet other cases, the data are ambiguous because effects of omega-3 fatty acids are not consistently observed, which suggests that other variables are important in determining whether or not an effect is seen. Therefore, the agency does not consider the evidence sufficiently strong to draw a firm conclusion about the relationship between omega-3 fatty acids and risk of CHD, and therefore is not authorizing the claim at this time.

In the course of developing this regulation, FDA has identified some areas where greater agreement is needed that the effects produced by omega-3 fatty acids are directly related to the risk of CHD. Many surrogate markers have been hypothesized, on the basis of limited evidence, to be related to specific diseases, including CHD, but few have withstood the continued scrutiny of scientific investigation. Also, some markers may have scientific validity, but may not be applicable for use in the general population, because of technical limitations. Thus, FDA asserts that only when a surrogate marker for a disease has been accepted as a risk factor for the general population, as indicated by a statement by an unbiased, nationally representative authoritative scientific or medical body, can the agency authorize a health claim based on the relationship of a nutrient to the surrogate marker of the disease. Examples of potential surrogate measures for which validation is needed are in vitro platelet aggregation, in vitro platelet adhesion, elevated fasting triglycerides postprandial triglycerides (recently considered at the NHLBI consensus development conference, Ref. 255), and subtractions of LDL and/or HDL.

In some cases additional research is needed to determine whether hypothesized subpopulations, e.g., those with high LDL:HDL ratio and high triglycerides, are at increased risk of disease. The pronounced triglyceride lowering effects of omega-3 fatty acids

might well have a protective effect against CHD in such a subpopulation.

There are other areas where additional research is needed to show, for agreed endpoints, that the effects are consistently produced, or are specifically due to omega-3 fatty acids. These areas require additional data to establish that the effect of omega-3 fatty acids is specific, or to further define the conditions under which omega-3 fatty acids have their effects. For example, data are needed to show a reduction in MI or CHD mortality among individuals fed supplemental omega-3 fatty acids (specifically) compared to a group fed omega-6 PUFA'S. The critical failing of some recent studies associating omega-3 fatty acids and CHD is that specificity was not obtained. Future studies should carefully control for known confounders, particularly dietary variables.

Finally, the available data suggest that some set of conditions or population may exist for which omega-3 fatty acids will increase HDL. Additional research should be able to define the conditions under which omega-3 fatty acids have this effect.

Interested parties may choose to petition the agency for approval of other health claims about omega-3 fatty acids. For example, additional data may be developed to support an omega-3 fatty acids/hypertension health claim petition. Because the blood pressure-lowering effect of omega-3 fatty acids appears most marked against a background of very low dietary intakes of omega-3 fatty acids, the role of omega-3 fatty acids in the total diet would need clarification before such a petition could be adequately supported. Similarly, limitations of the effects of omega-3 fatty acids on the magnitude and duration of change in blood pressure, the quantitative amounts of omega-3 fatty acids required for the effects, and characterization of the sensitive subpopulation would require discussion in a petition. A petition should also address apparent conflicting pieces of information, e.g., high blood pressure among populations that have high intakes of omega-3 fatty acids. Safety concerns raised in this final rule will, of course, require resolution prior to the authorization of any petitioned claim.

IV. Decision Not to Authorize a Health Claim Relating Ingestion of Omega-3 Fatty Acids to Reduced Risk of CHD

In evaluating the scientific evidence, FDA considered: (1) The strength of the association of omega-3 fatty acids with CHD or surrogate markers for CHD, (2) the consistency of findings among the

many studies, (3) the specificity of the outcome to omega-3 fatty acids, (4) the presence or absence of a dose-response relationship, and (5) the biologic plausibility of an association. FDA has determined that there is inadequate evidence to show that increased consumption of omega-3 fatty acids will reduce the risk of CHD.

FDA sought to determine whether there was significant scientific agreement among qualified experts that the totality of publicly available scientific evidence supported the claim that omega-3 fatty acids reduce the risk of heart disease. FDA reviewed the position taken in numerous Federal Government reports and other authoritative scientific reports and evaluated the publicly available scientific evidence that has become available since those reports were written. The decision to deny a health claim is based on the conclusions reached following review of the following sources of information: (1) "The Surgeon General's Report on Nutrition and Health;" (2) the National Research Council's "Diet and Health: Implications for Reducing Chronic Disease Risk," and (3) the National Cholesterol Education Program's Report of the Expert Panel on Detection, Evaluation and Treatment of High Blood Cholesterol in Adults. Each of these reports concluded that there was inadequate evidence of a relationship between consumption of omega-3 fatty acids and CHD. FDA has reviewed again all of the relevant cross-sectional data from which the hypothesized relationship between omega-3 fatty acids and CHD originated, and all clinical intervention data published since these Federal Government and other authoritative reports were issued to determine whether the additional evidence is adequate to support a health claim for omega-3 fatty acids.

The LSRO report reached a different conclusion from the other authoritative reports by finding a relationship between omega-3 fatty acids and CHD. The report used only selected evidence, and often did not distinguish effects specifically due to omega-3 fatty acids from effects due to PUFA's in general. The description of international epidemiologic findings of a relationship between fish consumption and CHD, similarly, was not shown to be specific to omega-3 fatty acids. In some instances, FDA disagreed with the interpretation of the studies reviewed by LSRO, or with LSRO's conclusions. Finally, the LSRO report also based its conclusions about the usefulness of omega-3 fatty acids, in part, on changes in blood lipid parameters that are not

generally agreed to be risk factors for CHD. Therefore, FDA finds numerous reasons for not accepting all of the findings of the LSRO report. FDA's conclusions regarding the relationship between omega-3 fatty acids and CHD rely instead on FDA's independent review of the publicly available scientific information, and are consistent with the Federal Government and other comprehensive and authoritative reports except for the LSRO report.

The surveys, cross-sectional studies, and nonintervention prospective studies do not provide adequate support for a relationship between consumption of omega-3 fatty acids and CHD. Only a few studies found an association between fish intake and CHD, while others have found no association; thus, there was no consistency of findings. None of the studies that reported a relationship distinguished fish consumption from other factors associated with fish consumption, and therefore none demonstrates specificity. Even in those studies that reported a relationship between fish consumption and CHD, it is not clear that the observed effects were due to the omega-3 fatty acids in fish. Also, the omega-3 fatty acid content of the fish diet associated with reduced CHD in these studies was so low that the importance of omega-3 fatty acids is questionable, thus weakening the biologic plausibility of the relationship.

The data from intervention studies also do not establish a relationship between omega-3 fatty acids and risk of CHD. The most compelling type of evidence to support a diet-disease relationship is a prospective, double-blind, placebo-controlled intervention study, with CHD morbidity and mortality as endpoints. To date, there is only one such trial (Ref. 16). The results of that study showed that increased consumption of fish does not reduce the risk of a second heart attack but may reduce the risk that the attack will be fatal. This study provides evidence for a protective effect of fish consumption against second heart attacks. However, as with the nonintervention study data, this study did not provide evidence to attribute the benefit to omega-3 fatty acid intake rather than some other factor associated with fish consumption (specificity).

Less persuasive than prospective studies in which CHD per se is measured, but still very useful, are prospective clinical trials in which surrogate markers for CHD are measured. Recent studies have not found beneficial effects from omega-3 fatty acids on total cholesterol or LDL

cholesterol in normal, healthy persons, or among persons at risk for CHD. Numerous studies, including some large or multicenter studies, have reported these results, demonstrating consistency in the findings and providing the agency confidence that they were not spurious. The data on HDL cholesterol are ambiguous. There appear to be other factors in the dietary interventions besides the omega-3 fatty acids that determine whether or not supplementation with fish or fish oils raises HDL.

An increase in bleeding times and a decrease in platelet aggregation have been observed frequently, but not always, after supplemental omega-3 fatty acids in normal healthy individuals as well as in diseased persons. Additionally, there is evidence that platelet adhesion is reduced by omega-3 fatty acids. The effects of decreased platelet aggregation and platelet adhesion appear to be related to the intake of omega-3 fatty acids in a dose-response relationship. What has not been established, however, is that in vitro platelet aggregation or platelet adhesion are bona fide surrogate risk factors for CHD in the general population.

Omega-3 fatty acids have been shown to reduce blood pressure in hypertensive people to a small degree, which may bear on a relationship between omega-3 fatty acids and CHD. This effect was not of large magnitude, but it is specific to omega-3 fatty acids, it has been reported by a number of investigators, a dose response was found, and the effect is biologically plausible through at least two mechanisms. However, it has not been established that omega-3 fatty acids reduce blood pressure in normal subjects (lack of consistency, weak effect, absence of dose-response relationship). Additionally, it has not been demonstrated that the magnitude and duration of changes in blood pressure observed in short-term studies will persist during long-term consumption of omega-3 fatty acids, or that these changes result in a reduced risk of CHD.

Finally, the potential that omega-3 fatty acids may increase LDL cholesterol and/or apoB among diabetics and hyperlipidemics, and the potential that omega-3 fatty acids may worsen control of blood glucose in diabetics are significant safety concerns that must be addressed before a claim may be made that consumption of omega-3 fatty acids by the general population will reduce the risk of CHD.

In conclusion, there are numerous effects of omega-3 fatty acids that may

be related to the risk of CHD, e.g., reduction in fasting and postprandial triglycerides, reductions in platelet aggregation and adhesion, changes in the composition of lipoproteins.

However, at this time these endpoints are not generally agreed to be closely related to the risk of CHD. In other areas, additional data are needed to show that effects related to fish consumption are specifically due to the omega-3 fatty acids in the fish, and to define the conditions under which omega-3 fatty acids consistently increase HDL. These avenues may provide a reasonable basis for a future petition for a health claim relating omega-3 fatty acids to the risk of CHD.

V. Environmental Impact

The agency has determined under 21 CFR 25.24(a)(11) that this action is of a type that does not individually or cumulatively have a significant effect on the human environment. Therefore, neither an environmental assessment nor an environmental impact statement is required.

VI. Economic Impact

In its food labeling proposals of November 27, 1991 (56 FR 60366 et seq.), FDA stated that the food labeling reform initiative, taken as a whole, would have associated costs in excess of the \$100 million threshold that defines a major rule. Thus, in accordance with Executive Order 12291 and the Regulatory Flexibility Act (Pub. L. 96-354), FDA developed one comprehensive regulatory impact analysis (RIA) that presented the costs and benefits of all of the food labeling provisions taken together. That RIA was published in the *Federal Register* of November 27, 1991 (56 FR 60856), and along with the food labeling proposals, the agency requested comments on the RIA.

FDA has evaluated more than 300 comments that it received in response to the November 1991 RIA. FDA's discussion of these comments is contained in the agency's final RIA published elsewhere in this issue of the *Federal Register*. In addition, PDA will prepare a final regulatory flexibility analysis (RFA) subsequent to the publication of the food labeling final rules. The final RFA will be placed on file with the Dockets Management Branch (HFA-305), Food and Drug Administration, rm. 1-23, 12420 Parklawn Dr., Rockville, MD 20857, and a notice will be published in the *Federal Register* announcing its availability.

In the final RIA, FDA has concluded, based on its review of available data and

comments, that the overall food labeling reform initiative constitutes a major rule as defined by Executive Order 12291. Further, the agency has concluded that although the costs of complying with the new food labeling requirements are substantial, such costs are outweighed by the public health benefits that will be realized through the use of improved ~~nutrititional~~^{nutritional} information provided by food labeling.

VII. References

The following references have been placed on display in the Dockets Management Branch (address above) and may be seen by interested persons between 9 a.m. and 4 p.m., Monday through Friday.

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List of Subjects in 21 CFR Part 101

Food labeling. Reporting and recordkeeping requirements.

Therefore, under the Federal Food, Drug, and Cosmetic Act and under authority delegated to the Commissioner of Food and Drugs, 21 CFR part 101 is amended as follows:

PART 101—FOOD LABELING

1. The authority citation for 21 CFR part 101 continues to read as follows:

Authority: Secs. 4, 5, 6 of the Fair Packaging and Labeling Act (15 U.S.C. 1453, 1454, 1455); secs. 201, 301, 402, 403, 409, 701 of the Federal Food, Drug, and Cosmetic Act (21 U.S.C. 321, 331, 342, 343, 348, 371).

2. Section 101.71 is amended by adding new paragraph (f) to read as follows:

§ 101.71 Health claims: claims not authorized.

* * * * *

(f) Omega-3 fatty acids and coronary heart disease.

Dated: October 30, 1992.

David A. Kessler,
Commissioner of Food and Drugs.

Louis W. Sullivan,
Secretary of Health and Human Services.

Note: The following tables will not appear in the annual Code of Federal Regulations.

BILLING CODE 4160-01-F

TABLE 1
Epidemiological Studies: Omega 3 Fatty Acids and CHD

Study	Study Design	Subjects	Methods	Results	Comments
Beswick et al. (Ref. 167)	Dietary Counseling compared with survey data and platelet activity 6 months later. Men from Cardiff, Wales with history of myocardial infarction.	56 men, ages 36 to 71 (mean=58). 13 excluded for medication, 1 for inadequate blood sample. 18 smokers, 34 patients on cardiovascular medication, 19 on B blockers, 8 on antihypertensive drugs, and 24 on angina medication.	Questionnaire to estimate polyunsaturated to saturated fat ratio, percent calories from fat, and EPA intake. Platelet activity based on ADP added for aggregation of platelets in blood and plasma.	Significant inverse correlation between fat ratio and platelet activity. Nonsignificant trend for decreased platelet aggregation with increased EPA intake.	Study inconclusive on role of EPA in platelet activity. Small sample. In vitro assay not repeated for accuracy. Nutrient intake estimated. Health behaviors, medications, and other factors which may have influenced results not presented. Study population ill.
Bulliyaa et al. (Ref. 185)	Dietary and serologic survey in Nellore and Chittor districts of India.	100 individuals in fish consuming populations and 109 individuals in nonfish consuming populations.	Dietary survey. Total serum and HDL—cholesterol, TG, and phospholipid measured. Clotting and bleeding times observed. Age, weight, pulse rate, blood pressure tabulated.	Total serum cholesterol was lower in the coastal village (152.7 mg/dL) than in the nonfish consuming village (214.9 mg/dL). HDL—cholesterol was higher in the coastal village.	Dietary survey methods not presented in paper. Dietary intake was presented as correlational data. The potential confounding effect of other components of diet on cholesterol level warrants explanation. Lower total cholesterol level in the fish eating population was observed.
Bjerregaard and Dyerberg, (Ref. 176).	Analysis of CHD mortality rates, Greenland.	All deaths due to CHD in Greenland from 1968 to 1983.	A register of deaths by cause was developed using information from death certificates, parish registers, and civil registration records. The register was computerized and brought under the management of the Danish Board of Health from 1975 to 1983.	Age standardized mortality rates per 10,000 person-years for CHD in men in Greenland settlements (5.3) were half those of men in Denmark (10.0). There was an increasing rate of CHD from settlements to towns in Greenland. The difference in rates of CHD in women were less apparent, with lower rates in Denmark than in towns in Greenland.	Genetic protection, exercise and confounding factors such as tobacco use cannot be eliminated as factors in this observation. These data support earlier observations of lower CHD rates in male Greenlanders versus Danes, but not for females. Note, the 50 percent difference is approximately the same as reported by Kromhout for comparing men eating no fish and those eating approximately 30 g/day.

TABLE 1--CONTINUED

Study	Study Design	Subjects	Methods	Results	Comments
Bonaa et al. (Ref. 178)	Dietary and serologic survey of residents of Tromso, Norway	156 subjects selected subjects from a survey population of 21,826 for an intervention trial.	Food consumption questionnaire, with separate questions for fatty and lean fish, also alcohol. Also, 2 unannounced 24-hour diet recalls. Analysis of covariance and multiple linear regression.	Fish consumption was inversely correlated with TG's, but no significant correlations between fish and cholesterol or apoprotein measures. EPA correlated with HDL until TG's were controlled. EPA correlated with TG even after HDL was controlled. DHA inversely correlated with HDL and apoA1	Divergent relationships between EPA and HDL, and DHA and HDL, may explain discrepancies in the literature regarding the effect of various supplements on HDL
Gerasimova et al. (Ref. 205)	Dietary and serologic survey of residents of Moscow and the Chukot peninsula.	Randomly selected men; Moscow n= 650 Chukot n= 261.	HDL by ultracentrifugation. A subsample was used for HDL phospholipids and apoproteins. 24-hour recall for dietary data.	Chukot residents had ↓ cholesterol, TG, LDL; ↑ HDL, ↑ consumption of omega-3 fatty acids, plasma lipid EPA.	Correlation between increased consumption of omega-3 fatty acids and serologic measures is consistent with the hypothesis of a relationship between omega-3 fatty acids and CHD, but many other dietary and behavioral factors could also be correlated and were not examined in this survey.
Innis et al. (Ref. 225)	Dietary and serologic survey.	Sample was selected as part of an unrelated dietary survey. 185 Canadian Inuit and 24 Vancouver residents.	Phospholipid fatty acid analysis.	Mean chain length and unsaturation index of the lipids in the two populations was very similar. The Inuit had greater EPA and lower AA than the Vancouver population. NS cholesterol	Observational data. Supports a dietary origin of phospholipids. Not directly relevant to CHD.

TABLE 1—CONTINUED

Study	Study Design	Subjects	Methods	Results	Comments
Popeski et al. (Ref. 266)	Retrospective survey of pregnancy induced hypertension in Inuit women and diet survey of women in these communities	Hypertension study: 300 medical records from Inuit women in 7 communities in the Northwest Territories. Diet survey 27 Inuit women in the 7 communities above.	Retrospective study: Blood pressure measurements 6 hours before delivery, incidence of pregnancy induced hypertension. Diet survey: Reported diet, hunter interviews, cord serum phospholipid from 16 infants born in 6 months.	Communities with higher marine oil consumption have significantly lower diastolic pressure in their women in the last 6 hours of pregnancy. Pregnancy associated hypertension was 2.6 times more common in communities with low fish consumption.	Ecologic data. Generates hypothesis for a relationship between the consumption of fish and blood pressure during pregnancy. Prospective study or clinical trial of diet and pregnancy needed.
Seidelin et al. (Ref. 281)	Correlation study of adipose tissue fatty acids and extent of coronary artery stenosis.	40 consecutive autopsies from subjects age 52 to 90 years.	Coronary artery disease was quantified by semi automatic image analysis. Degree of stenosis was used to divide subjects into three groups for correlations. Umbilical adipose lipids were extracted with methanol-chloroform, trans-methylated esters by gas chromatography.	No correlation between extent of stenosis and 16:10, 18:0, 18:2n-6, 16:1n-9 or 18:1n-9, but a significant inverse correlation with 22:6n-3. Stenosis correlated with extent of body weight.	Limited data are presented for a limited number of subjects, i.e., no data for other fatty acids of interest are presented, e.g., EPA, AA.
Van Houwelingen et al. (Ref. 294)	Sample of clinical parameters from 40 healthy men selected from cohort in longitudinal study, Zutphen, Holland.	Men in low (n=15) and high (n=25) fish consumption groups. Low consumption group ate an average of 2 g fish/day while high consumption group ate an average of 33 g fish/day.	Individuals in this study where interviewed 4 times in 25 years using cross-check dietary methods for habitual fish consumption. Of 79 men selected for the study, 40 completed it. Blood collected for fatty acid composition, PAI activity, collagen-induced platelet aggregation, ATP release, and TXB ₂ .	There was significantly higher serum phospholipid concentration of omega-3 fatty acids EPA and DHA acid and no significant difference in collagen-induced platelet aggregation, cutaneous bleeding time, ATP-release in whole blood or platelet number between the two groups.	Small sample reflects inconsistency of fish consumption over time. Dose of omega-3 in high fish consumption group lower than in most clinical trials.

TABLE 1--CONTINUED

Study	Study Design	Subjects	Methods	Results	Comments
Van Houwelingen et al. (Ref. 293).	Sample of clinical parameters from 61 elderly male volunteers from longitudinal cohort. Zutphen, Holland.	61 healthy elderly male volunteers ages 67 to 82. Lowest quartile of fish consumption ate 0 g/day. Highest quartile ate 27 g/day.	Cross-check dietary history was taken to assess the habitual intake of polyunsaturated fatty acids. Blood was collected under controlled conditions for measurement of phospholipids, TG's, and cholesterol esters.	Dietary history seemed to correlate with serum linoleic acid. The correlation between dietary history using the cross check method for fish consumption and serum level of fish-related fatty acids, however, seems less reliable.	The discrepancy between dietary history and serum level of fish-related fatty acid may be the result of large variation in the level of these fatty acids in the same foods.

TABLE 2
Intervention Trials of Omega-3 Fatty Acids and CHD

Study	Study Design	Subjects	Methods	Results	Comments
Agren et al. (Ref. 168)	Randomized parallel trial of fish, fish plus reduced fat (especially saturated fat) or usual diet (one fish meal per 2 weeks) for 15 weeks.	62 Normal, healthy female students.	Plasma lipids and lipoproteins at baseline, 7 and 15 weeks.	Controls: NS TG, cholesterol, apoA _i , apoB. Fish eaters: NS versus baseline TG, cholesterol, apoB; ↓ apoA _i ; ↓ TG versus controls. Fish plus low fat: ↓ TG, cholesterol, apoA _i , apoB.	This study shows the importance of the balance of the diet, particularly regarding saturated fat, in determining the blood lipid response to omega-3 fatty acids. Changes were slight unless a low fat low saturated fat diet was used. Study would have been stronger with a control group eating a low fat low saturated fat intake to the nonfish control.
Annuzzi et al. (Ref. 170).	Randomized double-blind crossover trial of 10 g fish oil/ day (3.0 g EPA plus DHA, MaxEPA) versus olive, no wash out, 2 weeks each treatment.	Eight female NIDDM, without liver, kidney or any other disease known to influence lipid and/or carbohydrate metabolism.	One of the patients was treated by diet only; other on Glibenclamide (4) and metformin (3). The patients were under dietary control and they were in the metabolic ward.	↓ TG, VLDL; ↑ LDL; NS cholesterol, HDL FFA; NS LDL-TG, HDL-TG; NS fasting glucose, average glucose insulin response, sensitivity.	Duration period was very short and no fatty acid analysis on neither fish oil nor olive oil diet. There was no washout period between cross-over of the study.
Bairati et al. (Ref. 172)	Randomized, double-blind placebo-controlled trial of 15 g fish oil/day (MaxEPA, 4.5 g EPA plus DHA) versus olive oil from 3 weeks pre angioplasty to 6 months; concurrent aspirin.	119 subjects undergoing first, successful, computer quantified percutaneous transluminal coronary angioplasty, evaluated also at 6 months.	Angiographic assessment, quantified by computer. diet by food frequency. Restenosis defined in four was for analysis.	By three of four definitions of restenosis, and multivariate analysis to control for exclusions, fish oil reduced restenosis. NS ECG evidence of myocardial ischemia, but trend toward ↓ fish oil, also on exercise testing. Significant difference also according to dietary omega-3 fatty acids (highest, middle versus lowest tertile) although highest tertile intake was only 0.15 g/day, and dietary odds ratio comparable to fish oil odds ratio.	Comparable compliance. Olive oil control doesn't control for polyunsaturated fatty acids. The comparable magnitude of the effects of dietary omega-3 fatty acids and fish oil supplementation suggest long-term, low dose effects are as strong as short-term, larger amounts. No associations of restenosis with other dietary variables, total fat, classes of fat, cholesterol, or dietary seafood. Differences between groups use of blood pressure medications (see Bairati et al. Ref. 173) was not discussed.

TABLE 2--CONTINUED

Study	Study Design	Subjects	Methods	Results	Comments
Bairati et al. (Ref. 173).	Randomized double-blind, placebo-controlled trial with 15 g/day MaxEPA (4.5 g EPA plus DHA) versus olive oil.	125 patients undergoing first percutaneous transluminal coronary angioplasty, evaluated also at 6 months.	Recumbent blood pressure, blood lipids by commercial kits.	Blood pressure increased in the olive oil control group, and was unchanged in the fish oil group, possibly because greater number of patients in the olive oil group discontinued concurrent blood pressure medications. Fish oil ↓ TG's, NS in cholesterol, LDL or HDL versus control, but the change from baseline was different between groups with ↑ LDL and HDL in fish oil.	Multiple linear regression analysis used to control use of blood pressure medications reduced differences in LDL and HDL to borderline significance ($p=0.06$), and inclusion of TG resulted in NS change in HDL.
Beil et al. (Ref. 174)	Randomized double-blind trial with 10.5 g/day fish oil (3.15 g EPA plus DHA, MaxEPA) versus 5.25 g/day fish oil plus 5.25 g oleic acid (low fish oil) versus 10.5 g oleic acid (placebo) 6 weeks.	30 Patients with primary hyper-triglyceridemia. Patients off lipid lowering drugs for 6 weeks, no beta blockers, diuretics or hormones. Fat restricted diet (30 percent, polyunsaturated fat: saturated fat ratio 1.5:1 cholesterol <250 mg/day).	Lipids by standard methods, Lp(a) by commercial kit.	↓ TG in high fish oil group versus placebo; NS cholesterol, LDL, HDL apoB; NS Lp(a), post-hoc analysis shows ↓ Lp(a) in those initially greater than 10 mg/dL.	Although randomized, there were large differences in initial Lp(a) levels, with only 1 of 10 in the placebo group over 6 mg/dL versus 6 and 8 of 10 in the low and high fish oil groups, respectively. Therefore, on the basis of Lp(a) the randomization was inadequate. Oleic acid does not control for polyunsaturated fatty acids.
Bhathena et al. (Ref. 175)	Nonblinded, longitudinal design, 10 week run-in on placebo (15 g mixed fat); 10 weeks with 15 g fish oil (anchovy oil, 6.5 g EPA plus DHA); 8 weeks of 15 fish oil plus 200 mg vitamin E	40 Healthy females, no history of metabolic disease, no medication, no smoking.	Diet for the placebo and test group was controlled, fish was eliminated from the menu. Forty percent of energy comes from dietary fat which was more than the level of current dietary guideline recommended.	Fish oil ↑ fasting glucose; ↓ TG, insulin, glucagon, GH, somatomedin-C; NS cholesterol, cortisol, dihydroepiandrosterone sulphate. Fish oil plus vitmain E gave no further change in glucose, TG, glucagon cortisol or cholesterol, but ↓ GH, insulin, increased somatomedin-C to placebo levels, and produced a ↓ in DHEA-S.	Unusual source and high level of omega-3 fatty acids. Study design doesn't allow conclusions about omega-3 fatty acid-specific effects.

TABLE 2—CONTINUED

Study	Study Design	Subjects	Methods	Results	Comments
Bonaa et al. (Ref. 178).	Randomized, double-blind placebo-controlled trial of 6 g/day Norek Hydro (4.5 g ethyl esters of EPA plus DHA) versus corn oil, 6 month observational run in, 10-week intervention.	146 healthy subjects.	Fasting blood samples at beginning and end of intervention, standard, commercial assays for lipids and apoproteins. Multiple linear regression.	Fish oil ↓ TG; In both groups NS cholesterol, LDL, apoB, ↑ HDL; ↑ apoA _i in corn oil group. In fish oil group ↑ phospholipid EPA correlated with HDL, but not in corn oil group, whereas in corn oil group DHA was inversely correlated to HDL.	No change in dietary fat, alcohol or protein. Both groups had a small, significant weight gain. The divergent results underscore the need for studies on individual omega-3 fatty acids, that may help explain inconsistencies in results of fish oil effects on HDL.
Bordet et al. (Ref. 179).	Randomized, dose-response study to 300, 900, 2700 mg EPA (ethyl ester, MND-21, Mochida, Japan) 4 weeks plus 4-week washout.	32 healthy females.	Bleeding times by Simplate II.	NS platelet aggregation sensitivity to ADP, collagen, PAF; ↑ sensitivity to a-tc NS bleeding time.	Differences from other studies may be due to absence of DHA, and need for longer studies to allow DHA incorporation.
Brown and Roberts (Ref. 183).	Randomized 3 X 3 crossover of fish (0.6 g EPA plus DHA/day) versus fish plus fish oil (2.0 g EPA plus DHA/day) versus control (fish free) 6 weeks each with 6-week washout.	12 healthy females.	Clotting times on 2 samples taken 4 days apart at the end of each diet treatment. Fibrinolytic activity in pooled samples from each individual assayed by euglobulin activity versus fibrin.	↓ leukocytes on fish, fish plus fish oil diets. Platelet count ↓ on fish oil; NS fibrinogen, ↑ fibrinolytic activity on both fish and fish plus fish oil.	The authors review other reports on fibrinolytic activity and note the inconsistency in findings.
Brown and Wahle, (Ref. 184).	Crossover trial of 15 g fish oil (MaxEPA, 4.5 g/day EPA plus DHA) with or without 400 IU vitamin E for 4 weeks each with 2-week washout between.	11 healthy females.	Thiobarbituric acid reactive substances, total tocopherol by fluorometric assay, whole blood aggregation by electrial impedance after collagen stimulus.	NS conjugated dienes, creatine kinase, or TBARS in the platelet poor plasma; ↑ total plasma TBARS on both, but less with added vitamin E; ↑ glucose on fish oil without vitamin E.	Small number of subjects; considerable variance in many measures with the exception of plasma TBARS. The interaction between glucose suggests a mechanism to address potential adverse effect in diabetics.

TABLE 2--CONTINUED

Study	Study Design	Subjects	Methods	Results	Comments
Clarke et al. (Ref. 189).	Noncontrolled supplementation, 1 g fish oil/day (0.3 g EPA plus DHA, MaxEPA) increasing in 1 g increments monthly to 5 g/day for months 5 and 6.	7 male and 4 female adolescents with FHL type II (5 type Iia, 6 type Iib).	Usual low cholesterol, low saturated fat diets, 2 received colstipol.	NS TG's cholesterol, LDL, HDL. Increased nose bleeds during fish oil treatment versus before and after.	Numbers of observation at each time per subjects not given for blood lipids measurements, so fish oil amounts for "after" treatment not clear. Platelet count and other biochemestries described as normal, but no data of effects if fish oil are described.
Cobiac et al. (Ref. 190).	Randomized double-blind placebo-controlled, 2 week run-in on restricted Na intake plus 8 to 1 g sunflower oil capsules and 8 to 600 mg slow release NaCl. Then 4 weeks on fish oil (8 g HIMEGA, 4.2 g EPA plus DHA) on either lo or normal Na, and crossover to alternate Na for 4 weeks.	50 Elderly, healthy subjects.	Blood pressure in sitting position.	↓ sys, dias blood pressure on Na restricted diet plus fish oil; NS on fish oil only; NS on sunflower oil (the run-in treatment).	Note that sodium restriction alone had no effect. 55 elderly subjects started the experiment but only 50 completed the study. No explanation was given why some subjects were dropped out. Dietary intake and compliance were not controlled. No fatty acid analysis of the diet and/or the control and test oils. No washout period in between cross-over.

TABLE 2--CONTINUED

Study	Study Design	Subjects	Methods	Results	Comments
Davidson et al. (Ref. 191).	Three experiments: 1. Dose response study with fish oil, (7.2, 5.4, 3.6 g EPA plus DHA/day, SuperEPA) for 6 weeks sequentially, 6-week washout between doses; highest dose crossover to olive oil 2. Crossover of MaxEPA at 4.8 g EPA plus DHA/day each, 6 weeks, 6-week washout 3. Uncontrolled supplementation of cases from a 4 year period, 148 subjects treated for 6 week periods with various fish oil.	1. 16 Type II-B hyperlipidemic patients. 2. 12 Hyper triglyceridemic type IV. 3. 148 Patients of different hyperlipidemias.	1. Stable AHA phase I diet for 3 months prior to and during study. 3. Stable on AHA phase I or Phase II diets.	1. ↓ TG, cholesterol on 7.2, 5.6 g EPA plus DHA/day, NS cholesterol on 3.6 g EPA plus DHA/day; NS HDL; cholesterol ↑ on olive oil. 2. SuperEPA ↓ cholesterol more than MaxEPA; MaxEPA ↑ LDL, HDL, HDL, versus SuperEPA; NS HDL. 3. Each MaxEPA, SuperEPA, Promega ↓ TG's, cholesterol; ↑ HDL in Type IIb on SuperEPA only; ↓ LDL in familial hypercholesterolemia after SuperEPA, NS Lp(a).	Olive oil control increased TG cholesterol versus run-in diet. Design doesn't control for polyunsaturated fatty acids. Larger decrease in cholesterol by SuperEPA may be due to its reduced content of saturated fatty acids. Capsule counts were used to assess compliance.
Eritsland et al. (Ref. 195).	Randomized to aspirin (300 mg/g) for 1 week, followed by 4 week on fish oil (Norak, 85 percent ethyl esters of EPA plus DHA, 3.4 g/day) or 4 week on fish oil followed by 1 week on aspirin.	22 female patients with stable CHD.	4 week run-in. Beta blockers used by 9 patients, nitrates allowed. Usual diet.	↓ TG's by fish oil; ↓ cholesterol by fish oil plus aspirin; ↑ HDL in fish oil only.	2 minor bleeding episodes on aspirin, none on fish oil. No was out between treatments, small number of subjects makes differences in response to fish oil only and fish oil plus aspirin questionable.

TABLE 2--CONTINUED

Study	Study Design	Subjects	Methods	Results	Comments
Fahrer et al. (Ref. 196).	Self selected to treatment of fish (1.5 to 2.0 g EPA plus DHA/day), fish oil (Sanomega s18, 3.1 g EPA plus DHA/day), normal diet for 2 months.	21 female, 21 male healthy volunteers.	No run-in, fish was pink salmon, tuna, herring, mackerel, pilchard. Fish consumption recorded in daily diary.	↓ TG's in both fish and fish oil groups; NS cholesterol, HDL; decrease in TG correlated with increase in EPA.	Baseline blood lipid values were comparable in the self selected groups.
Ferretti et al. (Ref. 197).	Nonblinded, longitudinal design, 10 week run-in on placebo (15 g mixed fat); 10 weeks with 15 g fish oil (anchovy oil, 6.5 g EPA plus DHA); 8 weeks of 15 fish oil plus 200 mg vitamin E.	40 nonsmoking females.	PGE-M by a stable isotope dilution method developed in the authors' laboratories.	Fish oil alone and fish oil supplemented with vitamin E produced comparable results on average, with the mean values µg PGE-M excreted per 24 hours in control, fish oil and fish oil plus vitamin E of 15.41 ± 2.12 , 12.51 ± 1.78 and 12.77 ± 1.85 , respectively. Paired t-test showed a significant ($p < 0.002$) reduction between baseline and fish oil treatment.	PGE-M is the sum of PGE ₁ plus PGE ₂ , derived from AA. The EPA-derived PGE ₂ was not measured. Dietary intake was well controlled. Prolonged use of fish oil supplementation was not recommended. The range of baseline values was from 3.8 to 60.9 µg/24 hours. There was no apparent relationship between initial values and magnitude of the change, and there were many individuals who had substantial differences for the two fish oil treatments. Thus, the significance of an average change of about 20 percent is not clear.
Force et al (Ref. 200).	12 g fish oil (n=8) (6 g EPA plus DHA, Promegre) or 16 g fish oil (n=6) (8 g EPA plus DHA), 6 weeks. After 6 weeks on fish oil only, concurrent increasing daily dosages of ASA (50, 100, 225, 1,300 mg) 2 weeks each.	14 females, 2 males, clinically stable but advanced CHD.	Gas chromatography-mass spectroscopy	Fish oil ↓ TXA, 38 percent, with ASA ↓ 97 percent at each dose; fish oil and ASA each ↓ PGI ₂ (ASA more than fish oil); fish oil ↑ PGI ₂ , but ASA did not increase PGI ₂ .	This is a study on the mechanism of action of fish oil. No concurrent placebo control. Dietary intake was not controlled.

TABLE 2--CONTINUED

Study	Study Design	Subjects	Methods	Results	Comments
Franceschini et al. (Ref. 202).	Uncontrolled supplementation study of 6 g/day fish oil (Norsk hydro, 2.8 g EPA plus 1.7 g DHA), 6 weeks.	5 healthy subjects.	HDL subfractions assayed by nondenaturing polyacrylamide gel electrophoresis.	NS cholesterol, HDL, ↑ HDL ₂ , ↓ HDL ₃ , ↑ HDL ₂ /HDL ₃ mass ratio.	Dietary intake was not controlled. Compliance was checked by plasma PL fatty acid composition. Small study.
Gans et al. (Ref. 203).	Randomized double-blind study (fish oil, source not specified, 1.8 g EPA plus 1.2 g DHA/day) versus corn oil, 4 months.	Stable claudication patients; 37 enrolled, 16 per group completed.	Supine blood pressure at rest and 1, 6, 10 min post exercise. Fibrinogen by commercial kit.	↓ Diastolic by both groups, ↓ systolic only in CO group; ↑ RBC deformability; NS cholesterol, LDL; fibrinogen; ↑ HDL, HDL ₂ ; ↓ TG's; NS pain, walking distance.	Wide variations of age (18 to 80 years). Dietary intake was not controlled. Compliance was checked by plasma PL fatty acid composition. Blood pressure was lowered in both groups.
Gazso et al. (Ref. 104).	Randomized double-blind placebo-controlled Efamol-marine (1.2 g EPA) versus Efamol (v olive oil) 12 capsules/day 6 weeks.	17 normal healthy 6 males, 11 females 6-Efamol marine, 5-Efamol, 6-olive oil.	ic conversion, MDA, platelet aggregation to ADP.	↓ Platelet aggregation by Efamol-marine; ↓ MDA in all groups.	Groups differ in the endpoints at beginning of the experiment, so it is difficult to interpret changes. The MDA ↓ may be due to the vitamin E added.
Gerhard et al. (Ref. 206).	3-period crossover of three fish diets; Dover sole (2 g EPA plus DHA), Salmon (4 g EPA plus DHA), or sablefish (3.4 g EPA plus DHA), 18 d each with 3-week washout between.	21 normo-triglyceridemic females.	ApoB standardized to Centers for Disease Control standards. VLDL and LDL precipitated with magnesium phosphotungstate, HDL enzymatically in the supernatant.	Salmon, sablefish diets ↑ cholesterol, apoB, LDL. Sole ↓ HDL, ↑ LDL; Sablefish ↓ HDL.	Diets were comparable for total fat, saturated fat. Study design doesn't allow conclusions about omega-3 fatty acid-specific effects.
Goren et al. (Ref. 207).	Uncontrolled supplementation study, 150 to 200 mg/kg (EPAGIS) (0.86 to 2.3 g EPA plus DHA/day) 8 weeks.	16 Patients with end stage renal disease, 6 type Iib, 1 type Iia, 8 type IV.	Blood lipids before and after supplementation. cholesterol by microenzymatic method. Apoproteins by turbidity assays.	↓ TG; NS cholesterol, ↓ cholesterol/HDL in a subset of excessively hyperlipidemic subjects; NS blood pressure, platelet counts, apoA:apoB.	Fish oil dosage was adjusted to the body weight of chronic renal failure young patients (7 to 18 years).

TABLE 2--CONTINUED

Study	Study Design	Subjects	Methods	Results	Comments
Green et al. (Ref. 209).	Randomized double-blind placebo-controlled crossover 15 g fish oil (EPAGIS, 4.3 g EPA plus DHA) versus corn:olive oil mix, 8 weeks each treatment with 4 week wash out.	27 Hyperlipidemic subjects, 15 type Iib, 12 type IV.	Cholesterol, HDL, TG by commercial kits. Apoprotein by immunoturbidity assay.	Overall NS cholesterol, LDL. Type IIb: ↓ TG, cholesterol, LDL, HDL. Type IV: ↓ TG; NS cholesterol, LDL, HDL. Both: NS platelet count, platelet aggregation, RBC deformability, apoB, apoA _i ; ↓ blood viscosity.	Substantial amount of other omega-3 fatty acids in this supplement (0.50 g 18:3, 0.45 g 18:4 and 0.42 g 22:5) per day. Patients were on weight-maintenance diet but no cal percent or wt percent of each component was given. Compliance was shown on blood lipid analysis.
Haglund et al. (Ref. 210).	Double-blind crossover study 30 mL/day of low vitamin E fish oil (9.6 g EPA plus DHA/day, Eskimo-3 or Inuit-3) or same oil supplemented with 1.5 IU vitamin E, 3 week.	12 Normal healthy subjects.	Lipids, glucose, Lp(a), fibrinogen.	Both fish oil ↓ TG's; ↑ HDL, glucose; NS cholesterol, Lp(a), apoB, insulin. Low vitamin E fish oil ↑ MDA, fructosamine; ↓ vitamin E. High vitamin E fish oil ↓ TG's, fibrinogen.	High vitamin E produced some effects independently of the fish oil, underscoring the need the control for polyunsaturated fatty acids, and have adequate vitamin E in the test substances.
Hamazaki et al. (Ref. 215).	Uncontrolled supplementation study, 1.8 g EPA ethyl ester/day, (laboratory purified), 6 months.	16 Diabetics, 5 IDDM, 11 NIDDM.	Albumin by radioimmunoassay with a commercial kit. HbA ₁₀ by high performance liquid chromatography.	NS TG, cholesterol, glucose, HbA ₁₀ , systolic diabolistic blood pressure, blood viscosity; ↓ albumin excretion, hematocrit.	NS body weight. Compliance was checked by blood EPA level. Dietary intake was not controlled. In IDDM fasting glucose was reduced, and barely missed statistical significance. Study design doesn't allow conclusions about omega-3 fatty acid-specific effects.
Hansen et al. (Ref. 216).	Crossover trial of 25 mL cod liver oil/day (6.25 g EPA plus DHA), 8 weeks versus no supplement.	20 healthy female and 20 healthy male subjects.	Whole blood clot (produced by added thrombin) lysis time to complete lysis. t-PA by commercial ELISA kit. Fibrinogen by spectrophotometric assay.	NS fibrinogen, fibrinolytic activity, t-PA; ↓ TXB ₂ .	Study design doesn't allow conclusions about omega-3 fatty acid-specific effects.

TABLE 2-CONTINUED

Study	Study Design	Subjects	Methods	Results	Comments
Harats et al. (Ref. 217).	Parallel untreated controls versus 10 g MasEPA/day (3 f EPA plus DHA), 4 weeks.	a. Smokers 6 MaxEPA, 5 controls. b. Smokers 3 control, 4 fish oil only, 4 fish oil plus 400 mg smokers. c. Nonsmokers.	40 hour smoking abstinence and overnight fast prior to blood draw; 90 minutes 4 to 6 cigarettes smoked, for second blood draw.	↓ TG; NS cholesterol, HDL; fish oil ↑ TBARS in plasma presmoking ↑ TBARS after smoking.	TBARS in plasma, and LDL, more responsive to cigarettes than fish oils. Most, but not all of the increase due to fish oil alone could be blocked by the added vitamin E.
Harris et al. (Ref. 218).	Uncontrolled supplementation trial of fish oils plus aspirin; 325, 80, 80 mg aspirin for 3 days; 4 day wash out; 2 weeks on 4.5 g EPA plus DHA (SuperEPA); 325, 80 80 mg aspirin plus SuperEPA for 3 days.	8 healthy males.	Bleeding times by Simplate II. Platelet aggregation to AA, collagen, PAF and AA in combination with the other agonists.	Bleeding NS on fish oil; fish oil plus aspirin same as aspirin only; fish oil and fish oil plus aspirin NS on platelet sensitivity to AA, collagen, PAF, but fish oil ↓ extent of aggregation to collagen.	Medications were controlled but diet was not controlled. Short-term study with a small number of subjects may explain inconsistencies with other comparable studies. The study may not have adequate statistical power to determine whether bleeding time increases of aspirin and fish oil are additive or greater than additive.
Harris and Windsor (Ref. 220).	Uncontrolled supplementation study on postprandial lipemia with of fish oil (2.2 g EPA plus DHA, Dale Alexander Omega-3), random assignment or emulsion for 4 weeks.	12 male and 4 female healthy normolipidemic subjects.	Bleeding times by Simplate. Background diets had 32 to 36 percent fat with 12 to 14 percent as saturated, 12 to 13 percent as monounsaturated, and 6 percent as polyunsaturated. Test meal provided 1 g fat/kg (61 percent of total calories: 32 percent of calories from saturated fatty acids; 13 percent from monounsaturated fatty acids, 7 percent from omega-3 fatty acids. Two hour blood samples through 10 hours post meal.	↓ TG's VLDL; NS cholesterol, LDL, apoB, apoA1, HDL ₃ , vitamin E, Lp(a); ↑ HDL, HDL ₂ . ↑ Bleeding time; NS RBC deformability. Postprandial lipemia reduced about 40 percent.	No medications. No difference between capsules and emulsion in test meal, possibly because most fat was from other diet components.

TABLE 2--CONTINUED

Study	Study Design	Subjects	Methods	Results	Comments
Harris et al. (Ref. 219).	Randomized dose-response, 1.25 to 5 g EPA plus DHA/day (Promega) 6 months.	28 Hyperlipidemic patients.	Blood lipids, Simplate II for bleeding times.	↓ TG in dose-related manner 1 month and 6 months, except lowest dose NS at 6 months, ↓ VLDL on all but lowest dose; NS cholesterol, LDL, HDL, HDL ₂ , except 2.5 g/day at 6 months ↑ LDL, HDL). ↑ Bleeding times on 2.5, 5 g/g RBC deformability largely unaffected.	Discrepancies among studies, methodologies were discussed. Irregularities may be due in part to small sample size. 4-week washout returned most values to pretreatment levels.
Hendra et al. (Ref. 222).	Randomized, double-blind, placebo-controlled trial of 10 g/day MaxEPA (3 g EPA plus DHA) versus olive oil, 6 weeks.	80 Noninsulin-dependent diabetic subjects.	Fibrinogen by Clauss, HDL by precipitation, LDL by calculation.	Transient ↑ glucose; NS HbA ₁ . ↓ TG; NS cholesterol, HDL; ↑ LDL (versus baseline). ↓ Spontaneous platelet aggregation, but NS response to induced aggregation. ↓ blood pressure in both treatments.	Large, carefully controlled study in an at-risk population. Olive oil control doesn't allow conclusions about omega-3 fatty acid-specific effects.
Homma et al. (Ref. 224).	Uncontrolled supplementation test of 2.7 g/day purified EPA ethyl ester (source not specified), 12 weeks.	15 outpatients.	Ad libitum diets. Blood samples after 12 hour fast. Plasma lipids by ultracentrifugation every 4 weeks.	↓ cholesterol, TG, apoB, small dense LDL; ↑ large light LDL, lipid transfer protein activity; NS HDL, HDL, HDL ₃ , apoA ₁ , apoC, apoE.	Authors state the relative atherogenicity of large light LDL and small dense LDL is controversial.
Jensen et al. (Ref. 226).	Sequential dose-response with 1, 3, 6 g EPA plus DHA (Shaklee EPA), 4 weeks each with 3-week washout between.	14 healthy males and 4 healthy females.	1 month run-in on fish free diet, otherwise diet not controlled. Bleeding times by Simplate II. Lipids by auto laboratory method.	↓ TG; VLDL on 6 g dose; ↑ HDL and HDL/LDL ratio on 6 g dose, but baseline HDL changed; NS cholesterol, LDL.	Changes in baseline HDL not shown.
Kremer et al. (Ref. 228).	Randomized double-blind placebo (olive oil) controlled trial of 3.25 or 6.5 g EPA plus DHA/day (ethyl esters, Pharmacaps), 24 weeks.	49 with rheumatoid arthritis completed this study.	IL-1 by bioassay.	↓ IL-1 38 percent 41 percent and 55 percent in olive oil, low and high fish oil groups, respectively; NS IL-2 in both fish oil groups.	Actual doses were adjusted per kg body weight.

TABLE 2--CONTINUED

Study	Study Design	Subjects	Methods	Results	Comments
Li and Steiner (Ref. 234).	Dose-response, parallel design: 4.8, 9.6, or 14.4 g EPA plus DHA/day, (source not specified), 3 weeks.	5 Normal healthy subjects each dose.	Platelet adhesion measured ex vivo in laminar flow chamber, using purified substrates.	↓ Platelet adhesion to collagen I and fibrinogen, near maximal response at 3 g EPA/day; speed of return to baseline values in the washout was directly related to dose.	This procedure reduces formation of thrombi, dilutes platelet derived factors. Measures direct interaction of platelets with surface matrix. Study design doesn't allow conclusions about omega-3 fatty acid-specific effects.
Lindgren et al. (Ref. 235).	Metabolic ward crossover of salmon versus prudent diet (30 percent fat). 20 day run-in, 40 days each diet. Salmon diet provided 2.1 percent of calories as omega-3 fatty acids, (approximately 5 g/day EPA plus DHA).	9 normolipidemic females.	Plasma proteins measured by competitive ELISA except apoA, by radioimmunoassay.	NS cholesterol, LDL, HDL _{2a} , HDL _{3a} , apoB, apoE, Lp(a); ↓ TG, HDL _{3a} , HDL _{3b} , apoA ₁ , apoA ₂ ; ↑ HDL, HDL _{2b} .	Carefully designed metabolic ward study, using practical level of omega-3 fatty acids, and Fat (saturated fatty acids and omega-6 polyunsaturated fatty acids) carefully controlled. Details on effects of lipoprotein subfractions. Two assay methods for Lp(a) gave same result.
Mallo et al. (Ref. 241).	Uncontrolled supplementation with fish oil, (6.7 g EPA plus DHA/day, EPAX-5000), 6 weeks and 4-week washout.	Normolipemic subjects with very high (?) and very low i.e., undetectable Lp(a) levels (7).	4 week run-in. normal diets. Platelet aggregation to collagen, thrombin.	↓ TG, platelet aggregation; NS cholesterol, LDL, HDL, Lp(a).	Comparable lipid and platelet responses for the low Lp(a) and high Lp(a) groups.
Marckmann et al. (Ref. 244).	observational, sequential diets of fish (3.4 g/day EPA plus DHA, 10 d) uncontrolled (18 d), and meat (10 d).	12 healthy females.	Clotting fibrinogen assay, t-PA and PAI-1 antigens by ELISA.	NS cholesterol, HDL, TG, fibrinogen; TG ↓ on both diets; ↓ PAI-1 and t-PA antigen, PAI activity and ↑ t-PA activity on meat, but NS on fish.	Since both diets produced changes with respect to the initial diets (that were uncontrolled) it is difficult to ascribe any change to the omega-3 fatty acids. However, the changes on the meat diet are more in line with reduced CHD risk than the lack of change on the fish diet.

TABLE 2--CONTINUED

Study	Study Design	Subjects	Methods	Results	Comments
Meland et al. (Ref. 247).	Double-blind, randomized multi-center placebo (corn oil- olive oil mix) controlled trial, 20 mL MaxEPA/day (6.8 g EPA plus DHA), 6 weeks.	40 females mild hypertension.	Calibrated instruments at 8 centers. Time of day for measurements was controlled.	NS blood pressure, cholesterol; ↓ TG's on fish oil; ↓ cholesterol/HDL ratio in both groups.	Power to detect a 5 mm blood pressure difference was 96 percent; a 10 percent cholesterol/HDL ratio decrease in placebo was nearly more than that after fish oil ($p<0.07$). 11 of 14 subjects on fish oil guessed their assignment correctly.
Meydani et al (Ref. 248).	Uncontrolled supplementation study 2.4 g/day EPA plus DHA (Promega), 3 months.	25 males.	Blood at 1, 2, 3 months.	↓ TG's; ↑ lipid peroxides.	6 IU vitamin E may not be adequate.
Molvig et al. (Ref. 250).	Randomized, double-blind Placebo- controlled trial of 1.6, 3.2 g EPA plus DHA (Pikasol) versus fatty acid blend, 7 weeks.	25 Healthy subjects and 8 IDDM subjects.	Isolated monocyte cell cultures. TNF and IL-1 by commercial ELISA kits.	↓ IL-1B immunoreactivity on high dose only; NS after low dose. NS TNF- α ; ↓ proliferative response.	Placebo had 20 percent polyunsaturated, 38 percent monounsaturated fatty acids. Spontaneous and LPS- stimulated leucotriene B, and PGE, secretion did not differ among groups at baseline or after 7 weeks of treatment. IL-1 returned to baseline with 3- week washout.
Mori et al. (Ref. 251).	Matched (age, weight) and randomized to 15 g MaxEPA/day (4.5 g EPA plus DHA) or olive oil, 4 weeks.	32 females with peripheral vascular disease.	No aspirin for 14 days prestudy platelet aggregation to PAF, collagen.	↑ cholesterol, LDL, HDL ₂ ; ↓ TG by fish oil, (but olive oil ↓ cholesterol, LDL); ↓ platelet aggregation by fish oil, but olive oil ↑ aggregation.	Compliance by capsule count. Changes in control make interpretation difficult. Olive oil does not control for polyunsaturated fatty acids.
Mori et al. (Ref. 252).	Matched groups randomly assigned to 15 g/day MaxEPA (4.5 g EPA plus DHA), olive oil, or olive oil plus cholesterol.	27 normolipidemic insulin-dependent female diabetics.	HDL by heparin, manganese chloride precipitation, followed by separate precipitation of subfractions. LDL by calculation.	NS cholesterol, LDL, HDL; ↑ HDL ₂ , ↓ HDL ₃ , TG.	Study design doesn't allow conclusions about omega-3 fatty acid-specific effects.

TABLE 2--CONTINUED

Study	Study Design	Subjects	Methods	Results	Comments
Mueller et al. (Ref. 253).	Randomized double-blind crossover trial of 8 g EPA plus DHA (Promega) versus olive oil, 21 d.	12 Healthy adults.	Bleeding times by Simplate II before and after administration of 325 mg aspirin. Excludes subjects with platelet or coagulation disorders, thrombocytopenia, ethanol.	Fish oil-NS bleeding time versus baseline but ↑ versus olive oil both before ($p < 0.02$) and after (NS) aspirin. ↓ TG on fish oil, platelet count, WBC count; NS cholesterol, LDL, HDL.	Trend toward ↑ HDL versus baseline, but olive oil in same direction, some order effects confound results.
Muller et al. (Ref. 253a).	Multicenter observational trial of 135 g canned mackerel paste (4.7 g/day EPA plus DHA) or meat paste.	84 healthy females.	Published methods for factor X, antiplasmin, plasminogen. Fibrinogen by Clause.	NS fibrinogen, other blood coagulation measures (only ↑ factor X), or fibrinolysis measures; meat ↓ plasminogen.	Compliance by lithium excretion. Study design doesn't allow conclusions about omega-3 fatty acid-specific effects.
Mullertz et al. (Ref. 254).	Uncontrolled supplementation, 0.55 g EPA plus DHA/day (Pikasol), 21 days.	7 Healthy adults.	Normal diets, PAI-1 and u-PA by ELISA kits.	↓ α-Tocopherol; NS cholesterol, TG's; ↑ PAI-1; NS t-PA, u-PA.	Suggests that differences reported for PAI-1 are due to the assay used, with the double antibody assay used in this study, and the monoclonal antibody used by Emeis et al. providing specificity. Concludes that fish oil decreases fibrinolytic activity.
Nelson et al. (Ref. 256).	Metabolic ward crossover of salmon, prudent diet (30 percent fat). 20 day run-in, 40 days each diet. Salmon diet gave 2.1 percent of calories as omega-3 fatty acids, (approximately 5 g/day EPA plus DHA).	9 normolipidemic females.	Platelet aggregation to ADF, AA, collagen, thrombin: threshold and maximum response. Bleeding time by Simplate II.	NS bleeding time; salmon diet ↓ platelet counts NS platelet response to collagen, thrombin but ↓ sensitivity to ADP.	Carefully designed metabolic ward study, using practical level of omega-3 fatty acids, and Fat (saturated fatty acids and omega-6 polyunsaturated fatty acids) carefully controlled.

TABLE 2—CONTINUED

Study	Study Design	Subjects	Methods	Results	Comments
Nikkilila (Ref. 258).	Randomized, double-blind, placebo (corn oil) controlled, crossover, 2.4 g EPA plus DHA/day as ethyl ester (EPA X 6000EE, Almarin), two 4-week periods with a 4-week washout between, followed by open study of 3.6 g EPA plus DHA/day for 4 weeks.	32 females with CHD, increased TG and decreased HDL, 62 percent were overweight.	Lipid lowering diet for 4 weeks prior to study.	NS cholesterol, LDL, HDL, HDL/cholesterol ratio, apoA ₁ , apoB; ↓ TG's; During open phase those with severe hypertriglyceridemia had ↑ HDL/cholesterol.	HDL inversely related to TG in study group pretreatment.
Nye et al. (Ref. 259).	Randomized, fish oil and its placebo were double-blind 1. Aspirin 300 mg plus dipyridamole 75 mg. 2. 3.6 g EPA plus DHA (MaxEPA). 3. Olive oil, up to 1 year.	79 females, 29 males post PTCA referred for angina, none had grafts.	Angiography (blind) at one year or before in those with anginal symptoms; restenosis defined as a loss of 50 percent or more of the gain produced by PTCA.	NS angina (trend toward less in A/D and fish oil groups D restenosis by EPA (11 percent versus 30 percent for olive oil) MaxEPA not different in any regard versus A/D NS in any blood lipids in a subset (n= 42).	No deaths in any group through 1 year, 93 percent follow-up rate. Results suggest that MaxEPA is as useful or moreso than aspirin/dipyridamole.
Oh et al. (Ref. 261).	Randomized crossover of 4 normal eggs versus 4 omega-3 fatty acid-enriched eggs/day (4.5 g EPA plus DHA/day), 2 week run-in, 4 weeks each treatment.	9 female and 3 male healthy volunteers.	Recumber blood pressure; VLDL by ultracentrifugation, HDL by manganese-heparin precipitation.	Omega-3 fatty acid-enriched eggs did not ↑ cholesterol, but regular eggs did. Omega-3 fatty acid-enriched eggs ↓ TG in one group.	One of the groups used butter to prepare eggs, changing the P:S ratio. Pooled data were not given despite absence of order effects for most variables. ↓ LDL in one group; NS HDL in either treatment. Systolic blood pressure ↓ in both groups, diastolic only in one.
Olivieri et al. (Ref. 263).	Uncontrolled supplementation trial of 20 mL fish oil/day (3.0 g EPA plus DHA, source not specified), 8 weeks.	20 hyperlipidemic 16 female, 4 male.	No hypolipidemic drugs for 15 days pre trial. blood pressure by blinded nurse.	↓ Systolic diastolic blood pressure, TG; NS cholesterol, HDL, vitamin E; ↑ glutathione peroxidase activity in RBCs and platelets, ↓ MDA.	Design doesn't allow conclusions about omega-3 fatty acid-specific effects.

TABLE 2—CONTINUED

Study	Study Design	Subjects	Methods	Results	Comments
Owens and Cave, (Ref. 264).	Observational study, 15 g/day MaxEPA (4.5 g EPA plus DHA) 4 weeks.	6 normal females.	Simplate II for bleeding time. Platelet adhesion in Baumgartner chamber using everted rabbit aorta. Prothrombin time, WBC and platelet count by automated methods.	NS WBC, prothrombin time, platelet adhesion, bleeding time.	Trend toward increased adhesion with duration of feeding. Assay method measures platelet changes, but does not assay vessel wall changes. Study design doesn't allow conclusions about omega-3 fatty acid-specific effects.
Rapp et al. (Ref. 268).	Uncontrolled supplementation study of MaxEPA at 6 percent of calories (16 to 21.3 g EPA plus DHA/day), 6 to 120 days.	11 patients, 9 female, 2 male with obstructive atherosclerosis scheduled for peripheral vascular surgery.	Excludes subjects with habitual fish intake. 15 endarterectomy specimens. Control specimens from 18 nonfish consuming subjects undergoing vascular reconstruction.	Fish oil increased content of omega-3 fatty acids in atherosclerotic lesion linearly with duration of feeding, although plasma enrichment of omega-3 fatty acids plateaued by 2 to 3 weeks; ↓ cholesterol; NS TG's, platelet counts, bleeding times.	Shows incorporation of omega-3 fatty acids into plaque, especially DHA. Relevance to CHD not known. Not a specific effect of omega-3 fatty acids, but would be expected to polyunsaturated fatty acids. High amount of omega-3 fatty acids.
Saynor and Gillot (Ref. 276).	Uncontrolled long-term supplementation with 20 mL/day MaxEPA during year 1, 10 mL/day thereafter.	365 During 1 month to 40 at 84 months. 47 percent had survived a heart attack, 49 percent had angina.	Total cholesterol by enzymatic assay. HDL after precipitation.	↓ TG; ↓ cholesterol only for initial high cholesterol; ↑ HDL for total group; NS LDL; ↓ fibrinogen.	Large attrition makes it difficult to ascribe changes to fish oil (responders to treatment are more likely to stay in the study). Lack of blinding also may have contributed to bias. Some data were presented for all subjects only, other data only for subsets. Estimates of deviation from mean values not shown. Lack of control prevents conclusions regarding effects to omega-3 fatty acids.
Schmidt et al. (Ref. 277).	Dose-response study 1.3, 4, 9 g EPA plus DHA/day (Pikasol), 3 periods of 6 weeks/amount. Randomized to increasing or decreasing dose. 6-week washout.	10 healthy females.	Simplate II for bleeding times; t-PA, PAI by commercial kits; fibrinogen by thrombin clotting time.	NS cholesterol, LDL, platelet aggregation; ↑ HDL, bleeding time on 4 and 9 g/day, PAI and t-PA antigen after 9 g/day; ↓ TG, fibrinogen.	Design doesn't allow conclusions about omega-3 fatty acid-specific effects.

TABLE 2--CONTINUED

Study	Study Design	Subjects	Methods	Results	Comments
Schmidt et al. (Ref. 270).	Uncontrolled supplementation with 4 g EPA plus DHA/day (Pikasol), 6 weeks.	10 Untreated hypertensives.	Supine blood pressure.	NS cholesterol, LDL, HDL, TG, platelet aggregation to collagen, ADP, systolic, diastolic blood pressure, bleeding time; ↓ cholesterol/HDL ratio, fibrinogen, monocyte chemotaxis.	Design doesn't allow conclusions about omega-3 fatty acid-specific effects. Absence of significant change in plasma TG despite 25 percent decrease suggests inadequate sample size. Before and after compared by Pratt's test.
Schmidt et al. (Ref. 279).	Uncontrolled supplementation with 1.3 to 9.0 g EPA plus DHA/day (Pikasol, MaxEPA or cod liver oil), most for 6 weeks, angina subjects for 12 weeks.	Various at-risk subjects with angina (14), IDDM (10), hyperlipidemia (17), hypertension (10), and healthy subjects (46).	Normal diets. Lp(a) by two-site immunoradiometric test kit.	NS Lp(a) in any group.	Reports Lp(a) data for subjects from 5 previous Schmidt reports (Refs. 133 through 135), and the current refs above. Design doesn't allow conclusions about omega-3 fatty acid-specific effects.
Shapiro et al. (Ref. 203).	Uncontrolled supplementation with 10 g MaxEPA/day (5.4 g EPA plus DHA/day), 6 weeks, 10-week washout.	10 normolipidemic healthy females.	3 Samples per time point, 2 to 3 days apart.	↑ cholesterol, LDL, HDL, vitamin E, retinol versus presupplementation and washout; ↓ TG versus washout.	Multiple samples per treatment reduces day-to-day fluctuations, magnitude of changes: Cholesterol 6 percent; LDL 9 percent; HDL 11 percent versus average of pretreatment and washout values.
Singer et al. (Ref. 205).	Randomized to propranolol (P), or fish oil (2.9 g EPA plus DHA/day, source not specified), for 36 weeks, or (P) only (12 weeks) then P plus fish oil (12 weeks) then P plus olive oil placebo (12 weeks). Each followed by 4 week washout.	47 female patients with mild essential hypertension.	Two baseline blood pressure measure 4 weeks apart, blood pressure measured in triplicate at fixed time and post 2 hours of rest each 12 weeks.	P ↓ systolic, diastolic blood pressure, recumbent and upright; fish oil ↓ systolic, diastolic blood pressure in recumbent and upright; Some additive effects of P plus fish oil.	Olive oil control doesn't control for polyunsaturated fatty acids. Study duration and multiple measure (each 12 weeks) shows blood pressure lowering effect is persistent.

TABLE 2--CONTINUED

Study	Study Design	Subjects	Methods	Results	Comments
Sirtori et al. (Ref. 286).	Randomized, three-arm crossover of 6 g fish oil (Norsk Hydro, 4.5 g EPA plus DHA ethyl esters) versus olive oil (middle arm for each sequence) versus corn oil for 6 weeks each, 1 month run-in and 4 week wash-out between each arm with low saturated fat diet.	12 Type Iia hyperlipidemics.	Lipids by enzymatic assays, apoproteins by immunoturbidity assay. Platelet aggregation versus AA, TXB ₂ by radioimmunoassay. Superoxide by spectrophotometry.	Fish oil ↓ cholesterol, LDL, ↑ HDL; Olive oil ↓ LDL, ↑ HDL; Corn oil ↓ HDL, apoB. Platelet aggregation ↓ by all three oils. Fish oil ↓ superoxide in monocytes.	Excellent design. Divergent results from another recent study with comparable design (Bonnaa et al. (Ref. 178) that used the same amount and form of fish oil supplement and same control (corn oil), suggesting that responses to supplemental oils may be different for different sub-populations. Absence of change in platelet aggregation may also be a population specific finding.
Spannagl et al. (Ref. 287).	Uncontrolled supplementation with 8.1 g EPA plus DHA/day (PGE-technology), 4 weeks.	13 (3 male 10 female) near normoglycemic type I diabetics.	Fibrinogen by turbidity assay. t-PA, PAI by test kits.	NS clotting tests, t-PA; ↑ PAI, fibrinogen; ↓ TG.	Design doesn't allow conclusions about omega-3 fatty acid-specific effects. Fairly high amount of omega-3 fatty acids in this nonnormal population.
Trial of Hypertension Prevention Collaborative Research Group (Ref. 289).	Randomized life style interventions and double-blind, placebo-controlled nutritional supplement interventions including 3.0 g omega-3 fatty acids/day (source not specified), 6 months.	2182 female and male with diastolic blood pressure 80 to 89 mm Hg.	Sitting blood pressure after 5 minute rest. Measurements for baseline, 3 and 6 months were made in triplicate on 3 different days. On the fish oil arm there were 161 active and 157 control subjects.	NS systolic, diastolic blood pressure.	Large, multicenter design with many internal comparisons.
Vandongen et al. (Ref. 291).	2 week run-in, observational trial of 15 g/day MaxEPA (4.5 g EPA plus DHA) versus no supplement.	22 female insulin-dependent diabetics.	Double precipitations for HDL subfractions.	↑ cholesterol, LDL, HDL, HDL ₂ ; ↓ TG, HDL ₃ .	Study design doesn't allow conclusions about omega-3 fatty acid-specific effects.

TABLE 2--CONTINUED

Study	Study Design	Subjects	Methods	Results	Comments
Virella et al. (Ref. 295).	Double-blind placebo (olive oil) controlled study of 2.4 g EPA (DHA not given) 6 weeks.	6 normal subjects.	Blood samples at baseline, 3, 6, 14, 20, 28. Humoral immune response.	↓ TG in 3/4 on fish oil; variable in vivo response to tetanus toxoid booster; ↓ in vitro response to tetanus toxoid, ↓ IL-2 release.	Small number of subjects limits conclusions. Increase in bleeding reported in 2/4.
Vohwinkel et al. (Ref. 296).	Randomized, double-blind crossover of 6 g EPA plus DHA/day (source not specified) versus olive oil, 3 weeks.	48 Healthy subjects.	Glucose tolerance tests to 100 g oligosaccharides.	↑ Fasting glucose, insulin at 4 hours post load. Response of glucose to load affected differently by fish oil, depending on initial insulin response; among low responders fish oil increased insulin response and decreased glucose; among high insulin responders, fish oil reduced insulin response and lowered glucose response.	Complex results according to insulin responsiveness. Olive oil doesn't control for effects of polyunsaturated fatty acids.
Wander and Patton, (Ref. 297).	3-period crossover of three fish diets: Dover sole (2 g EPA plus DHA), Salmon (4 g EPA plus DHA), or sablefish (3.4 g EPA plus DHA), 18 day each with 3-week washout between.	23 normo-triglyceridemic females.	Bleeding time by Simplate. Platelet aggregation versus collagen, ADP. TXB ₂ by radioimmunoassay.	Salmon ↑ bleeding time. Sablefish ↓ platelet aggregation to collagen; Both sablefish and salmon ↓ aggregation to ADP, ↓ TXB ₂ .	Diets were comparable for total fat, saturated fat. Study design doesn't allow conclusions about omega-3 fatty acid-specific effects.
Weintraub et al. (Ref. 298).	Metabolic ward study. Crossover to 3 isocaloric diets:saturated fat; omega-6 fatty acids and omega-3 fatty acids (3.4 g/day EPA plus DHA), 25 days each with 5 to 7 day wash-out.	8 normolipidemic females.	Vitamin A fat load test in fasted subjects; HDL by precipitation, LDL by calculation. Lipolysis assay using human milk lipoprotein lipase.	Omega-3 diet ↓ cholesterol, TG, LDL, HDL, platelet count versus saturated fat. Omega-6 diet ↓ cholesterol, TG, LDL NS fasting glucose, postprandial insulin. Both omega-3 and omega-6 reduced postprandial lipemia versus saturated fat. NS in lipemia between omega-6 and omega-3.	Excellent design studies both chronic and acute fat effects. Many postprandial fat effects were larger on the omega-3 than the omega-6 diets, but not statistically significant.

TABLE 2—CONTINUED

Study	Study Design	Subjects	Methods	Results	Comments
Wing et al. (Ref. 299).	Double-blind, placebo (olive oil) controlled crossover trial of 15 g fish oil (Lipitac), 4.5 g EPA plus DHA, 8 weeks each.	20 Treated hypertensives maintained on blood pressure medications.	Supine and standing blood pressure. HDL after manganese chloride/heparin precipitation.	Blood pressure lower comparable on olive oil and fish oil. ↓ TG in fish oil; NS HDL on either treatment.	Study design doesn't allow conclusions about omega-3 fatty acid-specific effects.
Wojenski et al. (Ref. 300).	Sequential treatments with ethyl oleate (placebo), 6 g Res-Q1000 (3.6 g EPA plus DHA/day), or 4.0 g ethyl EPA. Washouts between phases of 5 weeks, 4 months, respectively, and 8 weeks posttreatment.	9 healthy female volunteers.	Bleeding time by Simplatate II; HDL by hospital automated method, platelet aggregation to ADP, collagen. TXB ₂ by radioimmunoassay. Fibrinogen binding by (125) ^I -Fibrinogen versus saline.	Bleeding time ↑ on ethyl EPA; Platelet count ↓ on Res-Q1000 and ethyl EPA; Ethyl EPA ↓ cholesterol, TG, platelet aggregation; NS fibrinogen binding.	No aspirin or ibuprofen. Evidence for a greater effect by the ethyl ester than for a comparable amount of omega-3 fatty acids in a mixed TG supplement.
Wolmarans et al. (Ref. 301).	Crossover comparison of red meat to fish, (6.1 g EPA plus DHA/day) 3 week baseline, 6 week treatment, 6 week posttreatment and 3-month washout.	Healthy subjects, 12 females, 16 males.	Habitual diet.	Fish diet ↓ cholesterol, LDL, VLDL; ↑ HDL.	NS total fat but ↓ saturated fat on fish diet. EPA was 1.91 g/day versus 0.06 g/day in baseline and 0.01 g/day on meat; total omega-3 fatty acids were 6.1 g/day on fish, and 0.9 g/day otherwise.

TABLE 2--CONTINUED

Study	Study Design	Subjects	Methods	Results	Comments
Zambon et al. (Ref. 304).	Randomized crossover trial of fish oil 15 g/day SuperEPA (8 g EPA plus DHA ethyl esters), with and without glyburide 8 weeks on fish oil, 4 weeks on and 4 weeks off glyburide. Baseline treatment was glyburide alone, 4 weeks.	10 females with NIDDM.	Regular diets. Insulin by radioimmunoassay. Automated glucose analysis. Cholesterol by enzymatic methods.	Fish oil ↑ fasting glucose, NS fasting insulin, ↓ postprandial insulin. Fish oil ↑ LDL, NS cholesterol, HDL	High amount of omega-3 fatty acids may produce effects on glucose metabolism not seen with lower amounts. Effects are consistent with other reports, but absence of polyunsaturated fat control limits inferences about specificity of the effects.

Abbreviations used: apoA, apoprotein A (a protein in high-density lipoprotein) apoE, apoprotein E (a protein in many lipoproteins, most notable VLDL and HDL); ASA, acetylsalicylic acid; ATP, adenosine triphosphate; CDC, Centers for Disease Control; CHD, coronary heart disease; ELISA, enzyme-linked immunosorbant assay; MDA, malondialdehyde; NIDDM, noninsulin dependent diabetes mellitus; NS, not statistically significantly different; PGE-M, prostaglandin-M; TEARS, thiobarbituric acid reactive substances; TG's, triglycerides; TXB, thromboxane.