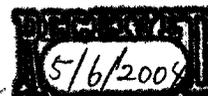


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May 5, 2004

VIA UPS GROUND

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Re: Docket No. 2003Q-0401; Omega-3 Fatty Acids and Coronary Heart Disease Qualified Health Claim

Dear Dr. Shimakawa:

Please find enclosed an original and one copy of the AHRQ reports "Effects of Omega-3 Fatty Acids on Cardiovascular Risk Factors and Intermediate markers of Cardiovascular Disease" and "Effects of Omega-3 Fatty Acids on Cardiovascular Disease." On behalf of the petitioner, please enter both reports in the above docket.

Sincerely,



Andrea G. Ferrenz

Enclosures

2003Q-0401

SUP 3

Effects of Omega-3 Fatty Acids on Cardiovascular Risk Factors and Intermediate Markers of Cardiovascular Disease

Prepared for:

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U.S. Department of Health and Human Services
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Preface

The Agency for Healthcare Research and Quality (AHRQ), through its Evidence-Based Practice Centers (EPCs), sponsors the development of evidence reports and technology assessments to assist public- and private-sector organizations in their efforts to improve the quality of health care in the United States. This report on Effects of Omega-3 Fatty Acids on Cardiovascular Risk Factors and Intermediate Markers of Cardiovascular Disease was requested and funded by the Office of Dietary Supplements, National Institutes of Health. The reports and assessments provide organizations with comprehensive, science-based information on common, costly medical conditions and new health care technologies. The EPCs systematically review the relevant scientific literature on topics assigned to them by AHRQ and conduct additional analyses when appropriate prior to developing their reports and assessments.

To bring the broadest range of experts into the development of evidence reports and health technology assessments, AHRQ encourages the EPCs to form partnerships and enter into collaborations with other medical and research organizations. The EPCs work with these partner organizations to ensure that the evidence reports and technology assessments they produce will become building blocks for health care quality improvement projects throughout the Nation. The reports undergo peer review prior to their release.

AHRQ expects that the EPC evidence reports and technology assessments will inform individual health plans, providers, and purchasers as well as the health care system as a whole by providing important information to help improve health care quality.

We welcome written comments on this evidence report. They may be sent to: Director, Center for Outcomes and Evidence, Agency for Healthcare Research and Quality, 540 Gaither Road, Rockville, MD 20850.

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The authors of this report are responsible for its content. Statements in the report should not be construed as endorsement by the Agency for Healthcare Research and Quality or the U.S. Department of Health and Human Services of a particular drug, device, test, treatment, or other clinical service.

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Structured Abstract

Context. Epidemiologic studies and clinical trials have reported beneficial effects of fish/omega-3 fatty acid consumption on several cardiovascular disease (CVD) outcomes, such as sudden death, cardiac death, and stroke. However, the mechanisms of this benefit are unclear.

Objectives. As the second of a 3-part report on this topic, we performed a systematic review of the literature to assess the effect of consumption of omega-3 fatty acids (eicosapentaenoic acid [EPA; 20:5 n-3], docosahexaenoic acid [DHA; 22:6 n-3], and alpha-linolenic acid [ALA, 18:3 n-3]) on various CVD risk factors and intermediate markers of CVD in healthy people, people with dyslipidemia, diabetes, or known CVD.

Data Sources. We searched Medline, Embase, Cochrane Central Register of Controlled Trials, Biological Abstracts, and Commonwealth Agricultural Bureau databases for potentially relevant studies.

Study Selection. We screened over 7,464 abstracts and retrieved 807 full text articles. We analyzed 123 studies that met inclusion criteria to address the key questions in this report. We included studies in which the amount of fish or omega-3 fatty acid intake was quantified, less than 6 g of omega-3 fatty acid per day was consumed, and of at least 4 weeks' duration.

Data Extraction. From each eligible study, we extracted information about the study design, population demographics, the amount of omega-3 fatty acids (in supplements or diet) or fish consumed, and outcomes. For RCTs, we extracted information about the randomization, allocation, and blinding techniques to assess methodological quality.

Data Synthesis. We examined the effect of omega-3 fatty acids on potential CVD risk factors – including lipoproteins, apolipoproteins, blood pressure, hemoglobin (Hgb) A_{1c}, C-reactive protein (CRP), hemostatic factors, platelet aggregation, and markers of diabetes – and intermediate markers of CVD – including coronary artery restenosis, carotid intima-media thickness (IMT), exercise tolerance testing, and heart rate variability. We also assessed correlations between long-chain omega-3 fatty acids intake and tissue phospholipid levels.

Among the outcomes we analyzed, omega-3 fatty acids demonstrated a consistently large, significant effect on triglycerides. The trials of triglycerides reported a net decrease in triglycerides of about 10% to 33%. The effect was dose dependent, generally consistent in different populations, and was generally larger in studies with higher mean baseline triglyceride levels. In contrast to studies of fish oils, the single study of a plant oil (ALA) found a net increase in triglycerides. The effect of omega-3 fatty acids on other serum lipids was weaker (up to a 6% increase in HDL).

Outcomes for which a small beneficial effect was found with fish oil supplementation include blood pressure (about 2 mm Hg reduction), restenosis rates after coronary angioplasty (14% reduction), exercise tolerance testing, and heart rate variability. For other evaluated outcomes, including measures of glucose tolerance, the effects of omega-3 fatty acids were either small or inconsistent across studies.

Across studies, we found a direct relationships between dose of consumed omega-3 fatty acids and changes in measured levels of EPA+DHA, either as plasma or serum phospholipids, platelet phospholipids, or erythrocyte membrane phospholipids. The correlation between dose and change in level appears to be fairly uniform, where 1 g supplementation of EPA and/or DHA corresponds to approximately a 1% increase in EPA+DHA level.

Conclusions. A large, consistent beneficial effect of omega-3 fatty acids was found only for triglyceride levels. Little or no effect of omega-3 fatty acids was found for a variety of other cardiovascular risk factors and markers of cardiovascular disease. The benefits of omega-3 fatty acids on reducing cardiovascular disease are not well explained by the fatty acids' effects on the cardiovascular risk factors we examined. A strong, linear association was found across studies between omega-3 fatty acid intake and tissue levels.

Heterogeneity of treatment effect was common among studies across the outcomes evaluated. Given the large amount of heterogeneity across studies, many questions remain about the effect of omega-3 fatty acids in improving potential CVD risk factors and intermediate markers of CVD. Few studies addressed questions related to effect modifiers and only limited conclusions could be made regarding these factors. The optimal quantity and type of omega-3 fatty acid, ratio of dietary omega-6 to omega-3, and duration of treatment remain undefined. Future research is needed to address these issues.

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Appendixes and Evidence Tables are provided electronically at <http://www.ahrq.gov/clinic/epcindex.htm>



Evidence Report

Chapter 1. Introduction

This evidence report is 1 of 3 reports prepared by the Tufts-New England Medical Center (Tufts-NEMC) Evidence-based Practice Center (EPC) concerning the health benefits of omega-3 fatty acids on cardiovascular diseases (CVD). These reports are among several that address topics related to omega-3 fatty acids, and that were requested and funded by the Office of Dietary Supplements, National Institutes of Health, through the EPC program at the Agency for Healthcare Research and Quality (AHRQ). Three EPCs - the Tufts-NEMC EPC, the Southern California EPC-RAND, and the University of Ottawa EPC - each produced evidence reports. To ensure consistency of approach, the 3 EPCs collaborated on selected methodological elements, including literature search strategies, rating of evidence, and data table design.

The aim of the reports is to summarize the current evidence on the health effects of omega-3 fatty acids (eicosapentaenoic acid [EPA; chemical abbreviation: 20:5 n-3], docosahexaenoic acid [DHA; 22:6 n-3], alpha-linolenic acid [ALA, 18:3 n-3], and docosapentaenoic acid [DPA, 22:5 n-3]) on the following: CVD, cancer, child and maternal health, eye health, gastrointestinal/renal diseases, asthma, autoimmune diseases, immune-mediated diseases, transplantation, mental health, and neurological diseases and conditions. In addition to informing the research community and the public on the effects of omega-3 fatty acids on various health conditions, it is anticipated that the findings of the reports will also be used to help define the agenda for future research.

The focus of this report is on CVD risk factors and intermediate markers of CVD in humans. The other 2 reports by the Tufts-NEMC EPC focus on CVD outcomes in humans and on arrhythmic electrophysiology in animal and in-vitro studies. In this chapter, the metabolism, physiological functions, and the sources of omega-3 fatty acids are briefly discussed. Subsequent chapters describe the methods used to identify and review studies related to omega-3 fatty acids and CVD - including the analytic framework for this report, findings related to the effects of omega-3 fatty acids on cardiovascular conditions, and recommendations for future research in this area.

Background

Metabolism and Biological Effects of Essential Fatty Acids

Dietary fat is an important source of energy for biological activities in human beings. Dietary fat encompasses saturated fatty acids, which are usually solid at room temperature, and unsaturated fatty acids, which are liquid at room temperature. Unsaturated fatty acids can be further divided into monounsaturated and polyunsaturated fatty acids. Polyunsaturated fatty acids can be classified on the basis of their chemical structure into two groups: omega-3 (n-3) fatty acids and omega-6 (n-6) fatty acids. The *omega-3* or *n-3* notation means that the first double bond from the methyl end of the molecule is in the third. The same principle applies to the *omega-6* or *n-6* notation. Despite their differences in structure, all fats contain the same amount of energy (9 kcal/g or 37 kJ/g).

Of all fats found in food, 2 — ALA and linoleic acid (LA, 18:2 n-6) — cannot be synthesized in the human body, yet are necessary for proper physiological functioning. These 2 fats are

Note: Appendixes and Evidence Tables cited in this report are provided electronically at <http://www.ahrq.gov/clinic/epcindex.htm>.

called essential fatty acids. The essential fatty acids can be converted in the liver to long-chain polyunsaturated fatty acids, which have a higher number of carbon atoms and double bonds. These long-chain polyunsaturated fatty acids retain the omega type (n-3 or n-6) of the parent essential fatty acids.

ALA and LA comprise the bulk of the total polyunsaturated fatty acids consumed in a typical North American diet. Typically, LA comprises 89% of the total polyunsaturated fatty acids consumed, while ALA comprises 9%. Smaller amounts of other polyunsaturated fatty acids make up the remainder¹. Both ALA and LA are present in a variety of foods. For example, LA is present in high concentrations in many commonly used oils, including safflower, sunflower, soy, and corn oil. ALA, which is consumed in smaller quantities, is present in leafy green vegetables and in some commonly used oils, including canola and soybean oil. Some novelty oils, such as flaxseed oil, contain relatively high concentrations of ALA, but these oils are not commonly found in the food supply.

The Institute of Medicine suggests that, for adults 19 and older, an adequate intake (AI) of ALA is 1.1-1.6 g/day, while an adequate daily intake of LA is 11-17 g/day². Recommendations regarding AI differ by age and gender groups, and for special conditions such as pregnancy and lactation.

As shown in Figure 1.1, EPA and DHA can act as competitors for the same metabolic pathways as AA. In human studies, the analyses of fatty-acid compositions in both blood phospholipids and adipose tissue showed similar competitive relationship between omega-3 long-chain polyunsaturated fatty acids and AA. General scientific agreement supports an increased consumption of omega-3 fatty acids and reduced intake of omega-6 fatty acids to promote good health. However, for omega-3 fatty acid intakes, the specific quantitative recommendations vary widely among countries not only in terms of different units — ratio, grams, total energy intake — but also in quantity³. Furthermore, there remain numerous questions relating to the inherent complexities about omega-3 and omega-6 fatty acid metabolism, in particular regarding the inter-relationships between the 2 fatty acids. For example, it remains unclear to what extent ALA is converted to EPA and DHA in humans, and to what extent high intake of omega-6 fatty acids compromises any benefits of omega-3 fatty acid consumption. Without resolution of these 2 foundational questions, it remains difficult to study the importance of omega-6 to omega-3 fatty acid ratio.

Metabolic Pathways of Omega-3 and Omega-6 Fatty Acids

Omega-3 and omega-6 fatty acids share the same pools of enzymes and go through the same oxidation pathways while being metabolized (Figure 1.1). Once ingested, ALA and LA can be elongated and desaturated into long-chain polyunsaturated fatty acids. LA is converted into gamma-linolenic acid (GLA, 18:3 n-6), an omega-6 fatty acid that is a positional isomer of ALA. GLA, in turn, can be converted to the long-chain omega-6 fatty acid, arachidonic acid (AA, 20:4 n-6). ALA can be converted, to a lesser extent, to the long-chain omega-3 fatty acids, EPA and DHA. However, the conversion from parent fatty acids into long-chain polyunsaturated fatty acids occurs slowly in humans, and conversion rates are not well understood. Because of the slow rate of conversion and the importance of long-chain polyunsaturated fatty acids to many physiological processes, humans must augment their level of long-chain polyunsaturated fatty acids by consuming foods that are rich in these important compounds. Meat is the primary food source of AA, while fish is the primary food source of EPA.

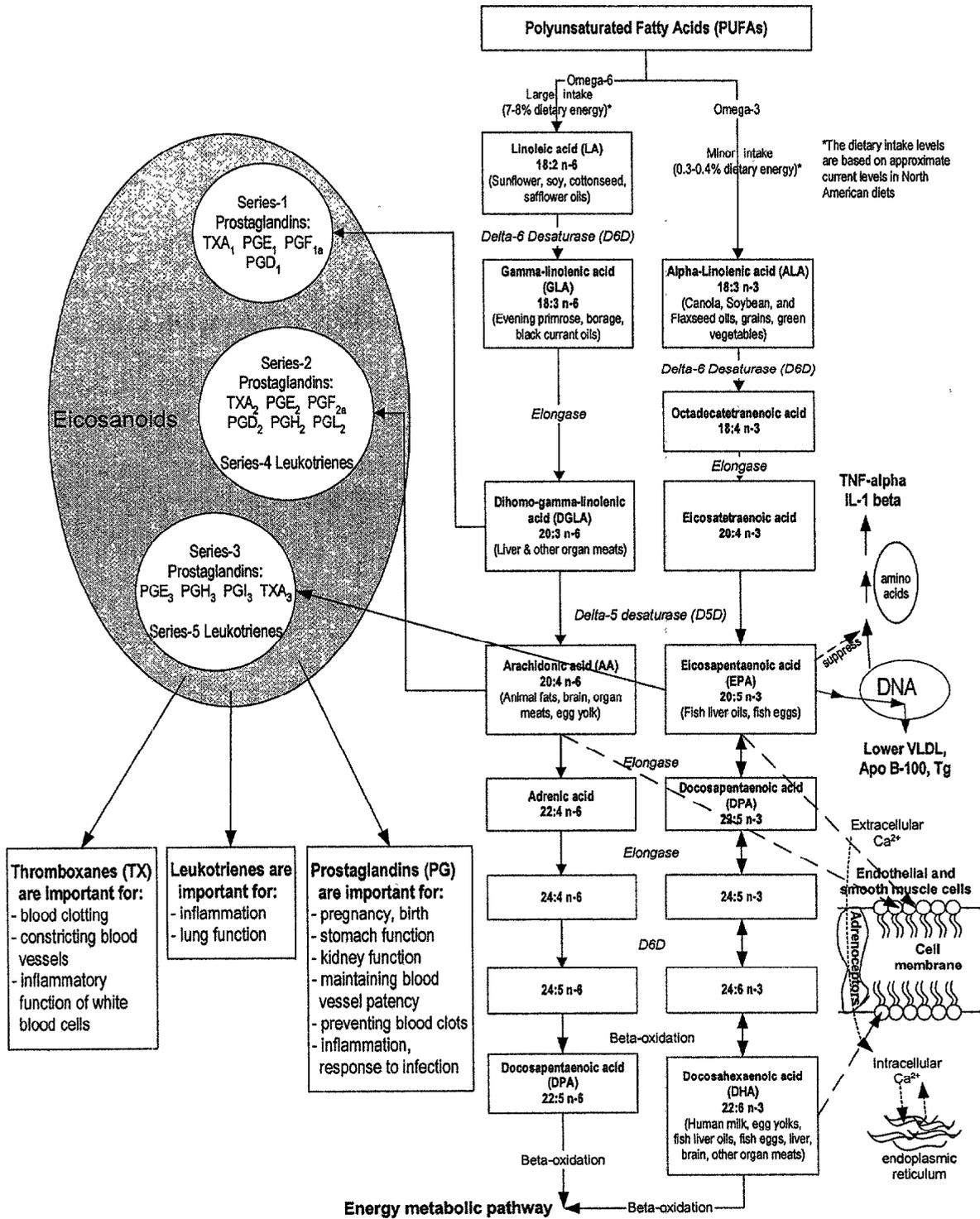
The specific biological functions of fatty acids depend on the number and position of double bonds and the length of the acyl chain. Both EPA and AA are 20-carbon fatty acids and are precursors for the formation of prostaglandins, thromboxane, and leukotrienes — hormone-like agents that are members of a larger family of substances called eicosanoids. Eicosanoids are localized tissue hormones that seem to be one of the fundamental regulatory classes of molecules in most higher forms of life. They do not travel in the blood, but are created in the cells to regulate a large number of processes, including the movement of calcium and other substances into and out of cells, dilation and contraction of muscles, inhibition and promotion of clotting, regulation of secretions including digestive juices and hormones, and control of fertility, cell division, and growth ⁴.

As shown in Figure 1.1, the long-chain omega-6 fatty acid, AA, is the precursor of a group of eicosanoids including series-2 prostaglandins and series-4 leukotrienes. The omega-3 fatty acid, EPA, is the precursor to a group of eicosanoids including series-3 prostaglandins and series-5 leukotrienes. The series-2 prostaglandins and series-4 leukotrienes derived from AA are involved in intense actions (such as accelerating platelet aggregation and enhancing vasoconstriction and the synthesis of inflammatory mediators) in response to physiological stressors. The series-3 prostaglandins and series-5 leukotrienes that are derived from EPA are less physiologically potent than those derived from AA. More specifically, the series-3 prostaglandins are formed at a slower rate and work to attenuate excessive series-2 prostaglandins. Thus, adequate production of the series-3 prostaglandins, which are derived from the omega-3 fatty acid, EPA, may protect against heart attack and stroke as well as certain inflammatory diseases like arthritis, lupus, and asthma ⁴. In addition, animal studies, have demonstrated that omega-3 fatty acids, such as EPA and DHA, engage in multiple cytoprotective activities that may contribute to antiarrhythmic mechanisms ⁵. Arrhythmias are a common cause of “sudden death” in heart disease.

In addition to affecting eicosanoid production as described above, EPA also affects lipoprotein metabolism and decreases the production of other compounds - including cytokines, interleukin 1 β (IL), and tumor necrosis factor α (TNF- α) - that have pro-inflammatory effects. These compounds exert pro-inflammatory cellular actions that include stimulating the production of collagenases and increasing the expression of adhesion molecules necessary for leukocyte extravasation ⁶. The mechanism responsible for the suppression of cytokine production by omega-3 fatty acids remains unknown, although suppression of eicosanoid production by omega-3 fatty acids may be involved. EPA can also be converted into the longer chain omega-3 form of DPA, and then further elongated and oxygenated into DHA. EPA and DHA are frequently referred to as very long chain omega-3 fatty acids. DHA, which is thought to be important for brain development and functioning, is present in significant amounts in a variety of food products, including fish, fish liver oils, fish eggs, and organ meats. Similarly, AA can convert into an omega-6 form of DPA. Studies have reported that omega-3 fatty acids decrease triglycerides (Tg) and very low density lipoprotein (VLDL) in hypertriglyceridemic subjects, with a concomitant increase in high density lipoprotein (HDL). However, they appear to increase or have no effect on low density lipoprotein (LDL). Omega-3 fatty acids apparently lower Tg by inhibiting VLDL and apolipoprotein B-100 synthesis and decreasing post-prandial lipemia ⁷. Omega-3 fatty acids, in conjunction with transcription factors (small proteins that bind to the regulatory domains of genes), target the genes governing cellular Tg production and those activating oxidation of excess fatty acids in the liver. Inhibition of fatty acid synthesis and increased fatty acid catabolism reduce the amount of substrate available for Tg production ⁸.

As noted earlier, omega-6 fatty acids are consumed in larger quantities (>10 times) than omega-3 fatty acids. Maintaining a sufficient intake of omega-3 fatty acids is particularly important since many of the body's physiologic properties depend upon their availability and metabolism.

Figure 1.1. Classical omega-3 and omega-6 fatty acid synthesis pathways and the role of omega-3 fatty acid in regulating health/disease markers.



Population Intake of Omega-3 Fatty Acids in the United States

The major source of omega-3 fatty acids is dietary intake of fish, fish oil, vegetable oils (principally canola and soybean), some nuts including walnuts, and dietary supplements. Two population-based surveys, the third National Health and Nutrition Examination (NHANES III) 1988-94 and the Continuing Food Survey of Intakes by Individuals 1994-98 (CSFII) surveys, are the main source of dietary intake data for the U.S. population. NHANES III collected information on the U.S. population aged ≥ 2 months. Mexican Americans and non-Hispanic African-Americans, children ≤ 5 years old, and adults ≥ 60 years old were over-sampled to produce more precise estimates for these population groups. There were no imputations for missing 24-hour dietary recall data. A total of 29,105 participants had complete and reliable dietary recall. Complete descriptions of the methods used and fuller analyses are available in the report *Effects of Omega-3 Fatty Acids on Cardiovascular Disease*, under "Methods: Method to Assess the Dietary Intake of Omega-3 Fatty Acids in the US population" and "Results: Population Intake of Omega-3 Fatty Acids in the United States". CSFII 1994-96, popularly known as the What We Eat in America survey, addressed the requirements of the National Nutrition Monitoring and Related Research Act of 1990 (Public Law 101-445) for continuous monitoring of the dietary status of the American population. In CSFII 1994-96, an improved data-collection method known as the multiple-pass approach for the 24-hour recall was used. Given the large variation in intake from day-to-day, multiple 24-hours recalls are considered to be the best suited for most nutrition monitoring and will produce stable estimates of mean nutrient intakes from groups of individuals⁹. In 1998, the Supplemental Children's Survey, a survey of food and nutrient intake by children under age of 10, was conducted as the supplement to the CSFII 1994-96. The CSFII 1994-96, 1998 surveyed 20,607 people of all ages with over-sampling of low-income population ($<130\%$ of the poverty threshold). Dietary intake data by individuals of all ages were collected over 2 nonconsecutive days by use of two 1-day dietary recalls.

Table 1.1 reports the NHANES III survey mean intake \pm the standard error of the mean (SEM), as well as, the median and range for each omega-3 fatty acid. Distributions of EPA, DPA, and DHA were very skewed; therefore, the means and standard errors of the means should be used and interpreted with caution. Table 1.2 reports the CSFII survey mean and median intakes for each omega-3 fatty acid, along with SEMs, as reported in Dietary Reference Intakes by the Institute of Medicine².

Table 1.1 Estimates of the mean \pm standard error of the mean (SEM) intake of linoleic acid (LA), alpha-linolenic acid (ALA), eicosapentaenoic acid (EPA), and docosahexaenoic acid (DHA) in the United States population, based on analyses of a single 24-hour dietary recall of NHANES III data

	Grams/day		% Kcal/day	
	Mean \pm SEM	Median (range) ^a	Mean \pm SEM	Median (range) ^a
LA (18:2 n-6)	14.1 \pm 0.2	9.9 (0 - 168)	5.79 \pm 0.05	5.30 (0 - 39.4)
ALA (18:3 n-3)	1.33 \pm 0.02	0.90 (0 - 17)	0.55 \pm 0.004	0.48 (0 - 4.98)
EPA (20:5 n-3)	0.04 \pm 0.003	0.00 (0 - 4.1)	0.02 \pm 0.001	0.00 (0 - 0.61)
DHA (22:6 n-3)	0.07 \pm 0.004	0.00 (0 - 7.8)	0.03 \pm 0.002	0.00 (0 - 2.86)

^a The distributions are not adjusted for the over-sampling of Mexican Americans, non-Hispanic African-Americans, children ≤ 5 years old, and adults ≥ 60 years old in the NHANES III dataset.

Table 1.2 Mean, range, median, and standard error of the mean (SEM) of usual daily intakes of linoleic acid (LA), total omega-3 fatty acids (n-3 FA), alpha-linolenic acid (ALA), eicosapentaenoic acid (EPA), docosapentaenoic acid (DPA) and docosahexaenoic acid (DHA) in the US population, based on CSFII data (1994-1996, 1998)

	Grams/day	
	Mean±SEM	Median±SEM
LA (18:2 n-6)	13.0±0.1	12.0±0.1
Total n-3 FA	1.40±0.01	1.30±0.01
ALA (18:3 n-3)	1.30±0.01	1.21±0.01
EPA (20:5 n-3)	0.028	0.004
DPA (22:5 n-3)	0.013	0.005
DHA (22:6 n-3)	0.057±0.018	0.046±0.013

Dietary Sources of Omega-3 Fatty Acids

Omega-3 fatty acids can be found in many different sources of food, including fish, shellfish, some nuts, and various plant oils. Table 1.3 lists the amount of omega-3 fatty acids in some commonly consumed fish, shellfish, nuts, and edible oils, selected from the USDA website <http://www.nal.usda.gov/fnic/foodcomp> (accessed November 3, 2003; Finfish and Shellfish Products: sr16fg15.pdf; Fats and Oils: sr16fg04.pdf; and Nut and Seed Products: sr16fg12.pdf) ¹⁰

Relationship of Dietary Fat and Cardiovascular Disease

Numerous studies have examined the relationship between dietary fat and CVD. Early epidemiology studies noted very low cardiovascular mortality among the Greenland Inuit as compared to mainland Danes, even though both had very high fat diets ¹¹⁻¹³. Studies in other populations with high fish intake, including South Pacific Islanders, Japanese, and people from the Mediterranean region, also generally found a low prevalence of CVD despite a prevalence of other risk factors, such as hypertension, similar to that found in other populations ¹⁴. However, some epidemiological studies reached the opposite conclusion. The Seven Countries Study, for example, found that coronary heart disease mortality was highest in Eastern Finland, where average fish intake was 60 g per day ¹⁵. This finding may in part be due to a positive association between fish consumption and both cigarette smoking and cholesterol levels in Finland; an association not seen in other countries.

The apparent paradox of low levels of CVD in people with high fat diets was explained by the high consumption of marine sources of very long chain, highly polyunsaturated omega-3 fatty acids ¹⁶. Since these early studies, hundreds of observational and clinical trials have been conducted to analyze the effect of both marine and plant sources of omega-3 fatty acids on CVD and a wide range of CVD risk factors and intermediate markers of CVD, and to define and explain the potential benefits of increased intake of the omega-3 fatty acids.

Omega-3 Fatty Acids and Cardiovascular Disease Risk Factors

A large number of putative risk factors for and intermediate markers of CVD exist, including markers for different aspects of CVD, markers for risk factors of CVD, and markers for other factors related to cardiovascular health. However, the relationship between most of these laboratory measurements and diagnostic tests and aspects of atherosclerosis such as inflammation, are generally unproven. The relationships between these factors and actual clinical

Table 1.3 The omega-3 fatty acid content, in grams per 100 g food serving, of a representative sample of commonly consumed fish, shellfish, and fish oils, and nuts and seeds, and plant oils that contain at least 5 g omega-3 fatty acids per 100 g (from USDA website <http://www.nal.usda.gov/fnic/foodcomp>, 2003).

Food item	EPA	DHA	ALA	Food item	EPA	DHA	ALA
Fish (Raw ^a)				Fish, continued			
Anchovy, European	0.6	0.9	-	Tuna, Fresh, Yellowfin	trace	0.2	trace
Bass, Freshwater, Mixed Sp.	0.2	0.4	0.1	Tuna, Light, Canned in Oil ^e	trace	0.1	trace
Bass, Striped	0.2	0.6	trace	Tuna, Light, Canned in Water ^e	trace	0.2	trace
Bluefish	0.2	0.5	-	Tuna, White, Canned in Oil ^e	trace	0.2	0.2
Carp	0.2	0.1	0.3	Tuna, White, Canned in Water ^e	0.2	0.6	trace
Catfish, Channel	trace	0.2	0.1	Whitefish, Mixed Sp.	0.3	0.9	0.2
Cod, Atlantic	trace	0.1	trace	Whitefish, Mixed Sp., Smoked	trace	0.2	-
Cod, Pacific	trace	0.1	trace	Wolffish, Atlantic	0.4	0.3	trace
Eel, Mixed Sp.	trace	trace	0.4				
Flounder & Sole Sp.	trace	0.1	trace	Shellfish (Raw)			
Grouper, Mixed Sp.	trace	0.2	trace	Abalone, Mixed Sp.	trace	-	-
Haddock	trace	0.1	trace	Clam, Mixed Sp.	trace	trace	trace
Halibut, Atlantic and Pacific	trace	0.3	trace	Crab, Blue	0.2	0.2	-
Halibut, Greenland	0.5	0.4	trace	Crayfish, Mixed Sp., Farmed	trace	0.1	trace
Herring, Atlantic	0.7	0.9	0.1	Lobster, Northern	-	-	-
Herring, Pacific	1.0	0.7	trace	Mussel, Blue	0.2	0.3	trace
Mackerel, Atlantic	0.9	1.4	0.2	Oyster, Eastern, Farmed	0.2	0.2	trace
Mackerel, Pacific and Jack	0.6	0.9	trace	Oyster, Eastern, Wild	0.3	0.3	trace
Mullet, Striped	0.2	0.1	trace	Oyster, Pacific	0.4	0.3	trace
Ocean Perch, Atlantic	trace	0.2	trace	Scallop, Mixed Sp.	trace	0.1	-
Pike, Northern	trace	trace	trace	Shrimp, Mixed Sp.	0.3	0.2	trace
Pike, Walleye	trace	0.2	trace	Squid, Mixed Sp.	0.1	0.3	trace
Pollock, Atlantic	trace	0.4	-				
Pompano, Florida	0.2	0.4	-	Fish Oils			
Roughy, Orange	trace	-	trace	Cod Liver Oil	6.9	11.0	0.9
Salmon, Atlantic, Farmed	0.6	1.3	trace	Herring Oil	6.3	4.2	0.8
Salmon, Atlantic, Wild	0.3	1.1	0.3	Menhaden Oil	13.2	8.6	1.5
Salmon, Chinook	1.0	0.9	trace	Salmon Oil	13.0	18.2	1.1
Salmon, Chinook, Smoked ^b	0.2	0.3	-	Sardine Oil	10.1	10.7	1.3
Salmon, Chum	0.2	0.4	trace				
Salmon, Coho, Farmed	0.4	0.8	trace	Nuts and Seeds			
Salmon, Coho, Wild	0.4	0.7	0.2	Butternuts, Dried	-	-	8.7
Salmon, Pink	0.4	0.6	trace	Flaxseed	-	-	18.1
Salmon, Pink, Canned ^c	0.9	0.8	trace	Walnuts, English	-	-	9.1
Salmon, Sockeye	0.6	0.7	trace				
Sardine, Atlantic, Canned in Oil ^d	0.5	0.5	0.5	Plant Oils			
Seabass, Mixed Sp.	0.2	0.4	-	Canola (Rapeseed)	-	-	9.3
Seatrout, Mixed Sp.	0.2	0.2	trace	Flaxseed Oil	-	-	53.3
Shad, American	1.1	1.3	0.2	Soybean Lecithin Oil	-	-	5.1
Shark, Mixed Sp.	0.3	0.5	trace	Soybean Oil	-	-	6.8
Snapper, Mixed Sp.	trace	0.3	trace	Walnut Oil	-	-	10.4
Swordfish	0.1	0.5	0.2	Wheatgerm Oil	-	-	6.9
Trout, Mixed Sp.	0.2	0.5	0.2				
Trout, Rainbow, Farmed	0.3	0.7	trace				
Trout, Rainbow, Wild	0.2	0.4	0.1				
Tuna, Fresh, Bluefin	0.3	0.9	-				
Tuna, Fresh, Skipjack	trace	0.2	-				

trace = <0.1; - = 0 or no data; Sp. = species.

a Except as indicated.

b Lox.

c Solids with bone and liquid.

d Drained solids with bone.

e Drained solids.

disease and events are generally even more theoretical. Nevertheless, as the science of atherosclerosis advances, our understanding of these relationships is improving.

Several measurable factors are generally well accepted to be associated with risk of CVD. These include serum lipoproteins, blood pressure, diabetes mellitus, and related metabolic disorders. Improvement or suppression of these factors has been shown to reduce the risk of CVD. Inflammation is becoming accepted as a cause of atherogenesis, although potential treatments have yet to show reduction of cardiovascular events. Thrombosis and oxidation (free radicals) are also involved in atherogenesis, although their effect on the risk of CVD is less clear (except in people with specific hypercoagulable conditions). Several cardiovascular processes are also risk factors for cardiovascular events. These include atherogenesis, vascular dysfunction, arrhythmias, and cardiac dysfunction among others. These processes generally do not cause symptoms until they are fairly advanced. They may also be reversed, thus potentially reducing cardiovascular morbidity and mortality.

Both in trials and in patient care, surrogate markers for disease or risk of disease are useful measures for tracking people's health. Understanding how omega-3 fatty acids affect these various intermediate markers of CVD can help efforts to explain how omega-3 fatty acids affect clinical CVD. Understanding the relationship between omega-3 fatty acids and intermediate markers would also be helpful in determining who could most benefit (or could be most harmed) from adjusting omega-3 fatty acid intake, and would help efforts to track their effect on cardiovascular risk factors. The following sections briefly summarize the relationship between omega-3 fatty acids and selected risk factors for and intermediate markers of CVD.

Improvement of Lipoproteins

Elevated serum low density lipoprotein (LDL) and depressed high density lipoprotein (HDL), especially when accompanied by elevated triglycerides (Tg), are well-known risk factors for CVD. Studies have reported that omega-3 fatty acids decrease Tg and very low density lipoprotein (VLDL) in hypertriglyceridemic subjects, with a concomitant increase in HDL. However, they appear to increase or have no effect on LDL. Omega-3 fatty acids apparently lower Tg by inhibiting VLDL and apolipoprotein B-100 (apo B-100) synthesis and decreasing post-prandial lipemia⁷. Omega-3 fatty acids, in conjunction with transcription factors (small proteins that bind to the regulatory domains of genes), target the genes governing cellular Tg production and those activating oxidation of excess fatty acids in the liver. Inhibition of fatty acid synthesis and increased fatty acid catabolism reduce the amount of substrate available for Tg production⁸.

Numerous other lipids and associated proteins are involved in lipid metabolism and thus possibly in atherogenesis and CVD; although they are less commonly measured. These include, among others, lipoprotein (a) [Lp(a)]; apolipoproteins (apo) A-I, B-48, B-100, C-III; and free fatty acids.

Reduction of Thrombosis

Blockage of coronary, cerebral and peripheral vessels due to thrombosis is a leading cause of CVD. Omega-3 fatty acids affect the clotting system in a number of ways. EPA competes with AA for the cyclo-oxygenase enzyme, thus reducing thromboxane A₂ (TX), a thrombotic agent. DHA may further inhibit cyclo-oxygenase¹⁷. Omega-3 fatty acids also inhibit TXB₂ production,

platelet aggregation, and platelet adhesion, although much less so than aspirin. Omega-3 fatty acids also lead to endothelial formation of prostaglandin I₃ (PG), PGI₂, and nitrous oxide, all of which reduce vasoconstriction^{17,18}. However, knowledge about the role of omega-3 fatty acids on coagulation factors and fibrinolysis is incomplete.

Many markers of coagulability exist, including the numerous factors involved in the clotting cascade, homocysteine, bleeding time, and platelet aggregation. Except among people with specific hypercoagulable conditions, it is not clear that any of these measures, among others, are predictive of CVD or that modification of their levels modifies risk of CVD.

Reduction of Inflammation, Atherogenesis, and Leukocyte Activity

Awareness of the effect of inflammation on atherogenesis (atheromatous plaque formation) and the risk of cardiovascular events is increasing. Leukocytes (white blood cells) are the blood cells that respond to injury or infection with a protective inflammatory response and an immune response. However, leukocytes are prominent cells in the atheromatous plaque in major blood vessels, which suggests that early plaque formation has an inflammatory component. PGE₂ and leukotriene B₄ (LT) have pro-inflammatory biological actions, and together they can cause vascular leakage and extravasation of fluid. The omega-6 fatty acid, AA, is the progenitor of both PGE₂ and LTB₄ via the cyclo-oxygenase and 5-lipo-oxygenase enzymatic pathways, respectively. EPA is the omega-3 homologue of AA; the 2 fatty acids differ only in that EPA has 1 additional double bond at the third carbon. EPA can thus inhibit AA metabolism competitively via the enzymatic pathways and can suppress production of the omega-6 fatty acid eicosanoid inflammatory mediators. Although EPA promotes the formation of PGE₃ and LTB₅, these eicosanoids are far less active as pro-inflammatory agents than the corresponding derivatives of AA⁸. Furthermore, other pro-inflammatory factors, such as IL-1 β and TNF- α , can be suppressed by the effect of long-chain polyunsaturated fatty acids on lipoprotein metabolism⁶.

C-reactive protein (CRP) is a well-described marker of inflammation and rises in response to injury, infection, and other inflammatory stimuli. In patients with either angina or risk factors for atherosclerosis, increased CRP has been associated with increased relative risk of nonfatal myocardial infarction and overall cardiovascular mortality¹⁹. It is unclear whether reduction in CRP would result in reduced risk of CVD. Trials commonly measure other inflammatory markers including IL-6 and vascular cell adhesion molecule 1 (VCAM-1). Less is known about their association with CVD.

Reduction of Arrhythmia

Cardiac arrhythmias can be fatal, causing sudden death, or can result in stroke, myocardial infarction, congestive heart failure, and peripheral embolisms, among other types of CVD. Animal studies have shown that fatal ventricular fibrillation could be essentially abolished by high-level feeding with omega-3 fatty acids²⁰. Omega-3 fatty acids appear to act in multiple ways to prevent arrhythmias. Various animal and *in vitro* experiments have shown that omega-3 fatty acids directly modulate sodium, potassium, and calcium channels²¹. By incorporating into cell membrane phospholipids, the excitation-contraction coupling that can result in arrhythmia is reduced²². Omega-3 fatty acids also modulate various intracellular enzymes involved in controlling the contraction and relaxation cycles of myocytes²³. EPA and DHA also affect adrenoceptors, membrane proteins whose function in the heart is to transmit the neuroendocrine

message of the catecholamines (adrenaline and its derivatives)²⁴. The activity of DHA is thus similar in principle to that of β -blockers, a group of key cardiovascular drugs used to decrease the cardiac effects of catecholamines. Omega-3 long-chain polyunsaturated fatty acids also appear to act similarly to another group of cardiovascular drugs, calcium channel blockers, by increasing intracellular calcium sequestration and interfering with receptor-operated calcium channels, thus lowering calcium influx²². The effect of omega-3 fatty acids on prostanoids and leukotrienes also theoretically reduces the arrhythmia potential of cardiac myocytes.

The risk of ventricular arrhythmia is most commonly measured by 24 hour ambulatory electrocardiography recordings, in which a continuous electrocardiogram (ECG) is taken for generally 24 hours. Various measures of heart rate variability are calculated, primarily based on the standard deviation (SD) of the duration of time between heart beats. Other common ECG measurements are also followed as indicators of risk of arrhythmia or cardiac ischemia.

Blood Pressure

Hypertension is well recognized as one of the leading causes of CVD. The recent Joint National Committee report (JNC 7) emphasizes the risks of blood pressure that is even slightly elevated above 120/80 mm Hg²⁵. Lifestyle modification, including reduction of sodium and alcohol intake, weight loss, diets high in fruits and vegetables and low-fat dairy products, and exercise has been shown to reduce blood pressure, often as much as medication use. Early investigations into the way in which fatty fish consumption may lower CVD found that omega-3 fatty acids possibly reduce blood pressure²⁶. While the mechanisms for such an effect remain uncertain, the most compelling hypothesis is that by altering the balance between vasoconstrictive TXA₂ and vasodilatory PGI₃, as described in the section on inflammation, overall blood vessel capacitance increases and thus blood pressure falls²⁷. However, the baseline balance of vasoactive and regulatory hormones may be altered in people with frank hypertension or other types of CVD. The question thus arises whether the effect of omega-3 intake on blood pressure is altered in people with hypertension.

Diabetes

Although long-chain omega-3 fatty acids appear to have an overall beneficial effect on CVD, their effect on glucose homeostasis is less clear. Omega-3 fatty acids may, in fact, have a detrimental effect on glucose tolerance²⁸. Theoretical benefits of omega-3 fatty acids to diabetic management include reducing Tg, increasing HDL, increasing glucose-induced insulin secretion, and possibly lowering insulin resistance^{28,29}. However, omega-3 fatty acids may worsen glucose tolerance in patients with clear cut diabetes and may, in fact, worsen insulin resistance²⁸.

Thus, important questions relate to the level of markers of glucose tolerance, such as fasting blood glucose (FBS), glycohemoglobin or hemoglobin A_{1c} (Hgb A_{1c}), and fasting insulin levels, in people with both diabetes and insulin resistance and people without glucose tolerance impairment.

Cardiovascular Diagnostic Tests

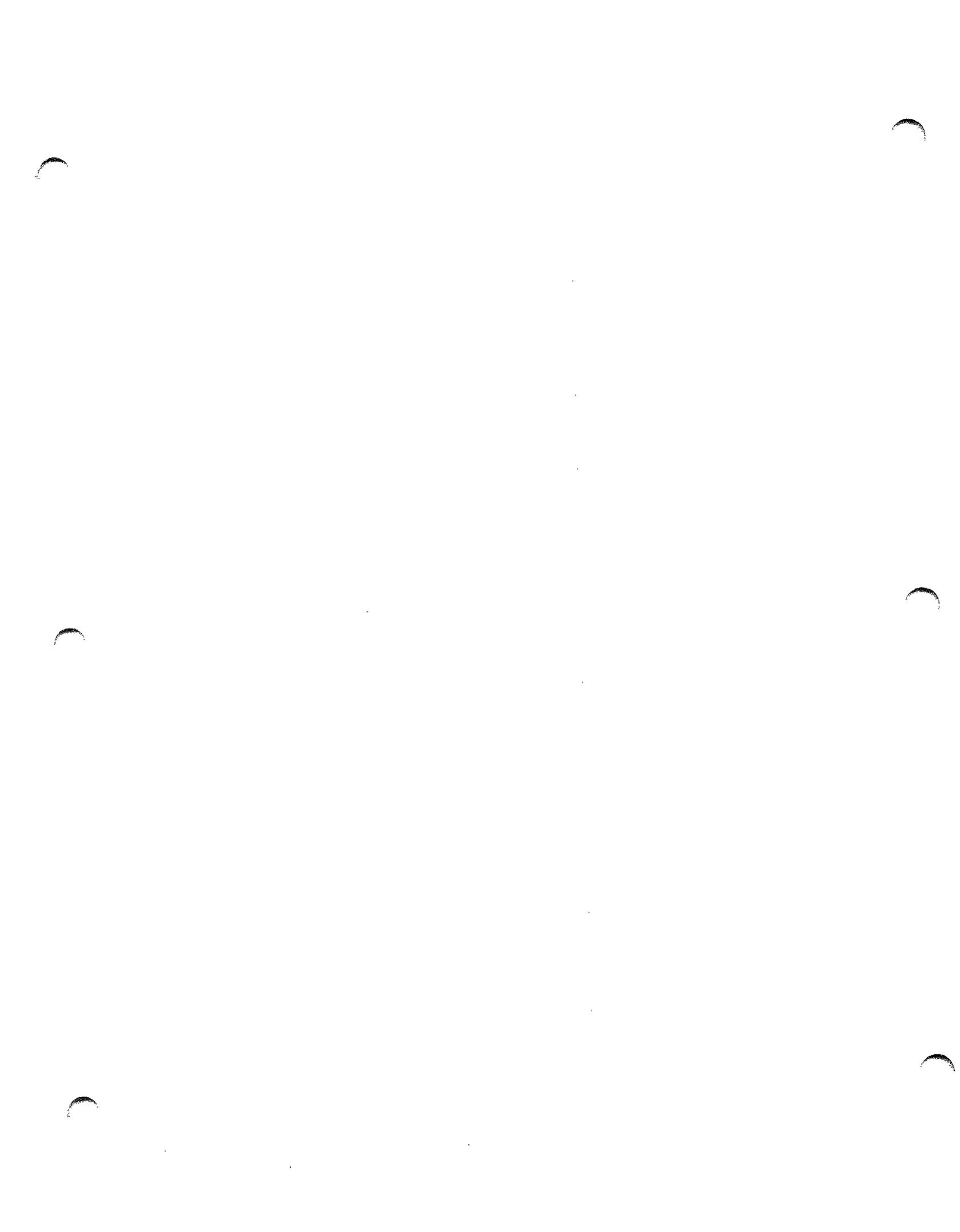
The metabolic effects of omega-3 fatty acids on lipoproteins, thrombosis, inflammation, arrhythmia and blood pressure all have potential effects on blood vessels and the heart, which

eventually can lead to clinical CVD. In addition, there are numerous diagnostic tests of cardiovascular health that are known to be predictive of future cardiovascular events both in people with and without a known history of CVD. Improvements in these diagnostic tests are commonly used as indicators of effective prophylaxis or treatment.

Among the tests of vascular health that have been assessed in omega-3 fatty acid trials are coronary arteriography (to measure coronary vessel stenosis), carotid intima-media thickness (IMT, which measures the thickness of the carotid artery wall, a measure of atherosclerosis), carotid Doppler ultrasonography or magnetic resonance arteriography (to measure carotid and extra-carotid stenosis), ankle brachial index (to measure peripheral blood flow), and endothelium-dependent vasorelaxation (an invasive or minimally invasive test of endothelial function). Other useful diagnostic tests measure heart function, including the exercise tolerance test (treadmill or stress test) and cardiac ultrasonography (which measures heart wall, chamber and valve structure and function).

Association of Omega-3 Fatty Acid Intake and Tissue Levels

The fatty acid composition of the cell membrane is a dynamic system, and the regulatory mechanisms are not fully understood. Since omega-3 fatty acids cannot be synthesized in the human body, the amount of total omega-3 fatty acids stored in adipose tissue is believed to be associated primarily with the amount of long-term omega-3 fatty acid dietary intake³⁰, while the amount incorporated into red blood cell membrane phospholipids is believed to be associated with short-term intake³¹. Studies have consistently shown that populations whose diets are rich in fish (and thus omega-3 fatty acids) have relatively high omega-3 fatty acid content in plasma phospholipids³²⁻³⁵. However, it remains less clear whether there is a reliable dose-response correlation between dietary omega-3 fatty acid intake and fatty acid profiles of plasma phospholipids, LDL fractions of serum phospholipids and cholesteryl esters, and blood cell phospholipids³⁶. Further, the metabolism from ALA - the main source of dietary omega-3 fatty acids - to its longer chain metabolites and then to eicosanoids is not well understood. Thus, the association between fatty acid intake and measurable tissue levels is not straightforward. Further complicating measurement estimates of total body stores of omega-3 fatty acids is that there are numerous measurable levels, including cell membrane phospholipids and triglycerides from the 3 major blood cell lines (erythrocytes, leukocytes and platelets), plasma triglycerides, plasma free fatty acids, and adipose cells. In addition, there is continuous movement of fatty acids between compartments, and each compartment incorporates fatty acids differently. As discussed above, under Metabolic Pathways of Omega-3 and Omega-6 Fatty Acids, omega-3 fatty acid metabolism is in part dependent on omega-6 fatty acid levels, further confounding associations between dietary intake and blood levels.



Chapter 2. Methods

Overview

This evidence report on omega-3 fatty acids and CVD risk factors and intermediate markers of cardiovascular disease (CVD) is based on a systematic review of the literature. To identify the specific issues central to this report, the Tufts-New England Medical Center (Tufts-NEMC) Evidence-based Practice Center (EPC) held meetings and teleconferences with technical experts, including a Technical Expert Panel (TEP) and members of the other EPCs that are reviewing topics related to omega-3 fatty acids. A comprehensive search of the medical literature was conducted to identify studies addressing the key questions. Evidence tables of study characteristics and results were compiled, and the methodological quality and applicability of the studies were appraised. Study results were summarized with qualitative reviews of the evidence, summary tables, and quantitative meta-analyses, as appropriate.

A number of individuals and groups supported the Tufts-NEMC EPC in preparing this report. The TEP served as our science partner. It engaged technical experts, representatives from the Agency for Healthcare Research and Quality (AHRQ), and institutes at the National Institutes of Health (NIH) to work with the EPC staff to refine key questions, identify important issues, and define parameters to the report. Additional domain expertise was obtained through local nutritionists who joined the EPC.

The Tufts-NEMC EPC also worked in conjunction with EPCs at the University of Ottawa and at the Southern California EPC-RAND. Together, the 3 EPCs are mandated to produce evidence reports on 10 topics related to omega-3 fatty acids over a 2-year period. The 3 EPCs coordinated activities with the goal of producing evidence reports of uniform format. Through frequent teleconferences and email contact, approaches toward data presentation, summary and evidence table layout, and study quality and applicability assessment were standardized. In addition, literature searches for all evidence reports were performed by the UO EPC, using identical search terms for studies of omega-3 fatty acids. The 3 EPCs agreed on a common definition of omega-3 fatty acids; however, some variation in definitions and study eligibility criteria were applied that reflected the different topics and key questions addressed. The studies included are described below, under Full Article Inclusion Criteria.

Accompanying reports on omega-3 fatty acids and cardiovascular outcomes, and on the animal and *in vitro* evidence for the effect of omega-3 fatty acids on cardiac electrogenesis, were generated using similar techniques.

Key Questions Addressed in this Report

Four key questions are addressed in this report. Questions 1 and 2 (and their sub-questions) both pertain to the effect of consumption of omega-3 fatty acids (either as treatment or in the diet) and both risk factors and intermediate outcomes. Question 3 pertains primarily to the effect of modifiers on any effects or associations. Question 4 pertains to the association between omega-3 fatty acid intake and tissue and plasma levels. The key questions and their related sub-questions are outlined in detail below.

Note: Appendixes and Evidence Tables cited in this report are provided electronically at <http://www.ahrq.gov/clinic/epcindex.htm>.

Question 1. What is the effect of omega-3 fatty acids (eicosapentaenoic acid [EPA; 20:5 n-3], docosahexaenoic acid [DHA; 22:6 n-3], and alpha-linolenic acid [ALA, 18:3 n-3], supplements, and fish consumption) on cardiovascular risk factors and intermediate markers of cardiovascular disease?

What is their effect on CVD risk factors and intermediate markers of CVD, specifically:

- *Serum lipids (total cholesterol, low density lipoprotein [LDL], high density lipoprotein [HDL], and triglycerides [Tg])*
- *Other CVD risk factors and intermediate markers of CVD*

What is their effect on specific CVD risk factors, specifically:

- *new-onset Type II diabetes mellitus (DM)*
- *new-onset insulin resistance/metabolic syndrome*
- *progression of insulin resistance*
- *new-onset hypertension*
- *blood pressure among hypertensive patients*

What is the relative effect of omega-3 fatty acids on different CVD risk factors and intermediate markers of CVD?

- *Can the intermediate markers and risk factors for CVD be ordered by strength of treatment effect of omega-3 fatty acids?*

Is there a threshold or dose-response relationship between omega-3 fatty acids and intermediate markers and risk factors for CVD?

How does the duration of intervention or exposure affect the treatment effect of omega-3 fatty acids on intermediate markers and risk factors of CVD?

Are treatment effects of omega-3 fatty acids on CVD intermediate markers and risk factors sustained after the intervention or exposure stops?

Question 2. Effect of different omega-3 fatty acids:

What is the effect of different specific omega-3 fatty acids (EPA, DHA, ALA), and different ratios of omega-3 fatty acid components in dietary supplements, on CVD intermediate markers and risk factors?

How does the effect of omega-3 fatty acids on CVD intermediate markers and risk factors differ by source (e.g., dietary fish, dietary oils, dietary plants, fish oil supplement, flax seed supplement)?

Does the ratio of omega-6 fatty acid to omega-3 fatty acid intake affect the effect of omega-3 fatty acid intake on intermediate markers and risk factors of CVD?

Question 3. Sub-population analyses:

How does the effect of omega-3 fatty acids on intermediate markers and risk factors of CVD differ in sub-populations including men, pre-menopausal women, post-menopausal women, and different age groups?

How does baseline dietary intake of omega-3 fatty acids impact the effect of omega-3 fatty acid supplements on intermediate markers and risk factors of CVD?

What are the effects of potential confounders – such as lipid levels, body mass index (BMI), blood pressure, diabetes, aspirin use, hormone replacement therapy, and cardiovascular drugs – on associations?

Does the use of medications for CVD and CVD risk factors (including lipid lowering agents and diabetes medications) impact the effect of omega-3 fatty acids?

Question 4. Omega-3 fatty acid metabolism:

What is the association between intake levels of EPA, DHA, and ALA and blood, tissue, and cell membrane levels?

What is the efficiency of conversion from ALA to EPA/ DHA, EPA/DHA to ALA, DHA to EPA, and EPA to DHA?

Analytic Framework

To guide our assessment of studies that examine the association between omega-3 fatty acids and cardiovascular outcomes, we developed an analytic framework that maps the specific linkages associating the populations of interest, the exposures, modifying factors, and outcomes of interest (Figure 1.2)³⁷. The framework graphically presents the key components of the study questions:

- 1) Who are the participants (i.e., what is the population and setting of interest, including the diseases or conditions of interest)?
- 2) What are the interventions?

- 3) What are the outcomes of interest (intermediate and health outcomes)?
- 4) What study designs are of value?

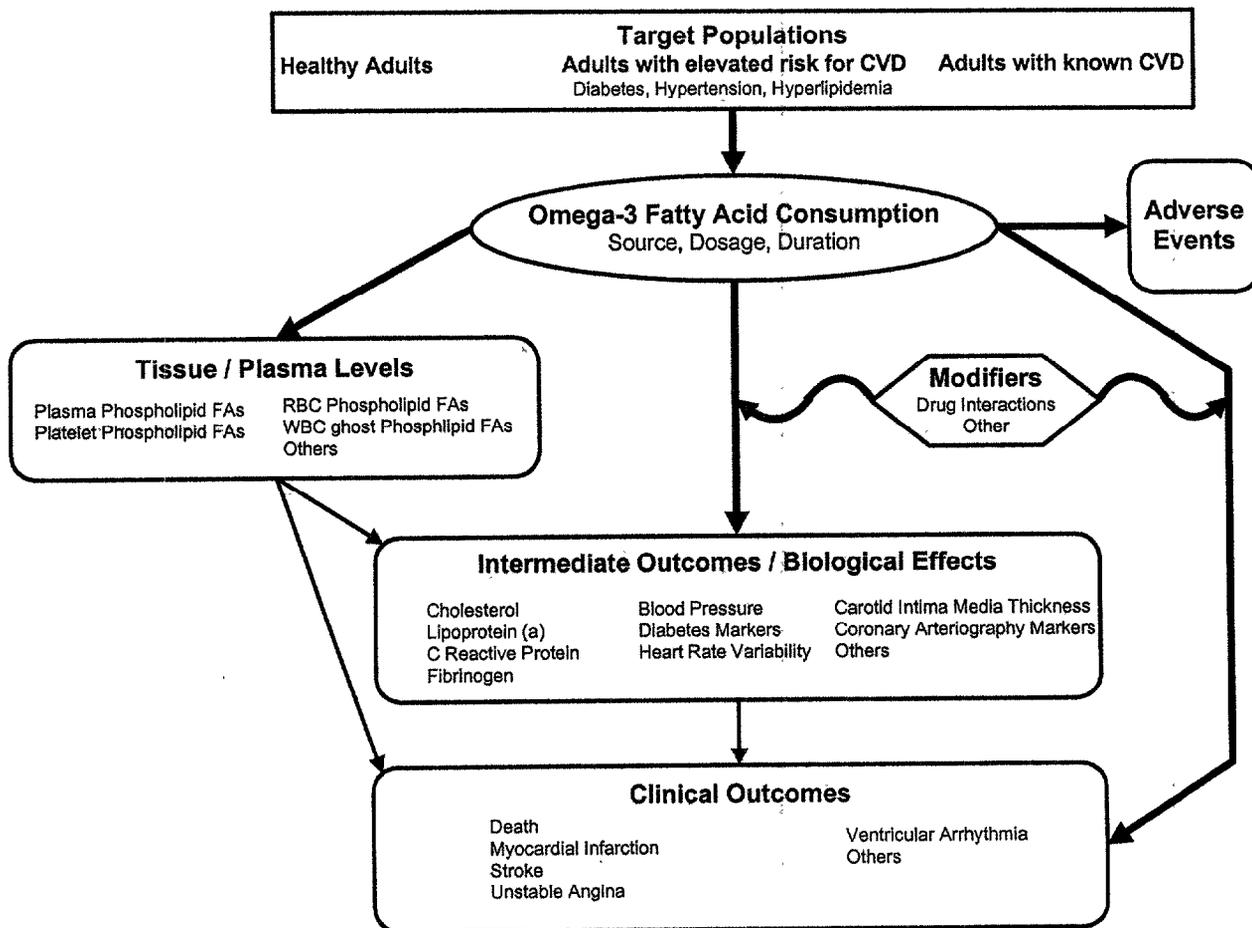
Specifically, this analytic framework depicts the chain of logic that evidence must support to link the intervention (exposure to omega-3 fatty acids) to improved health outcomes.

This report and the accompanying report, *Effects of Omega-3 Fatty Acids on Cardiovascular Disease*, review the evidence addressing the associations or effects in humans. Specifically, this report examines evidence addressing both the association in humans between omega-3 fatty acids and cardiovascular intermediate outcomes or risk factors and the association between omega-3 fatty acids and tissue or plasma levels of omega-3 fatty acids. The accompanying report examines evidence addressing the association between omega-3 fatty acids and clinical cardiovascular outcomes, their efficacy in improving CVD outcomes, and potential adverse effects of omega-3 fatty acid intake in humans.

In both reports, the 3 specific populations of interest are healthy adults with no known CVD or risk factors; adults at increased risk of CVD due specifically to diabetes, hypertension, or hyperlipidemia; and adults with known CVD. The exposure of interest is omega-3 fatty acids. Unlike medications, there are numerous possible sources, types, and possible dosages for omega-3 fatty acids. Thus, questions of interest include how different sources, dosages, and relative proportions of the fatty acids differ in their effects on the outcomes of interest. Included are questions addressing possible differences between the effects of supplements (e.g., fish oil capsules) and dietary sources (e.g., fatty fish), the effect of duration of intervention or exposure, and whether any effect is sustained after stopping treatment.

Theoretically, the most immediate outcome related to omega-3 fatty acid intake is a change in tissue levels of the fatty acids. However, the measurement and interpretation of this effect is complicated by the variety of fatty acids, the different relative intake levels of fatty acids, metabolism of the fatty acids into other fatty acids, the different storage forms, and the wide range of cells into which the fatty acids are incorporated. The question of how omega-3 fatty acid intake relates to different measures of tissue and plasma fatty acid levels is addressed in this

Figure 1.2. Analytic framework for omega-3 fatty acid exposure and cardiovascular disease. This framework concerns the effect of omega-3 fatty acid exposure (as a supplement or from food sources) on cardiovascular disease. Populations of interest are noted in the top rectangle, exposure in the oval, outcomes in the rounded rectangles, and effect modifiers in the hexagon. Thick connecting lines indicate associations and effects reviewed in this and the accompanying report. Lists noted in a smaller font indicate the specific factors reviewed. CVD indicates cardiovascular disease; FA, fatty acid; RBC, red blood cell (erythrocyte); WBC, white blood cell (leukocyte).



report. Once it is understood how to best estimate body stores of omega-3 fatty acids, it will then be of interest in future reviews to understand how levels of body stores affect cardiovascular outcomes.

Although the most important questions relating to omega-3 fatty acids pertain to their effects on clinical outcomes (and potential adverse events), collecting data on long-term cardiovascular effects is relatively difficult. As a result, the bulk of the available evidence generally pertains to the efficacy in trials of interventions on intermediate outcomes and biological effects. This evidence is summarized in this report.

The effects of omega-3 fatty acids on CVD risk factors, intermediate markers of CVD and clinical outcomes can be related to one another in two ways. First, by reducing risk factors for CVD, such as blood pressure, or putative markers of the risk factors, such as C-reactive protein, omega-3 fatty acids can directly reduce the overall risk of cardiovascular events. Second, omega-3 fatty acids can have a direct or indirect beneficial effect on specific intermediate markers of

CVD, such as coronary stenosis, which would result in a lowered risk of cardiovascular events. In this report, we investigate how the effects of omega-3 fatty acids on risk factors and intermediate markers can be modified by various factors, including concomitant drugs, demographic features (e.g., sex, age), baseline diet, and subject characteristics (e.g., lipid levels, weight, blood pressure).

The analytic framework does not directly address the level of evidence that is necessary to evaluate each of the effects. Large randomized controlled trials that are adequately blinded and otherwise free of substantial bias provide the best evidence to prove causation between intervention and outcome. However, this study design is not always available (or possible). Crossover trials have the advantage of controlling fully for biases due to differences between study arms but may introduce bias due to incomplete washout of first treatment effect. In addition, they are generally small and have a narrow range of subjects. Uncontrolled trials and observational studies provide lesser degrees of evidence that are usually hypothesis-generating regarding causation. The current analysis relies as much as possible on high quality, randomized controlled trials, using evidence from other studies when data are relatively sparse.

Literature Search Strategy

We conducted a comprehensive literature search to address the key questions related to CVD and to the metabolism of omega-3 fatty acids (Appendix A.1, available electronically at <http://www.ahrq.gov/clinic/epcindex.htm>). Relevant studies were identified primarily through search strategies conducted in collaboration with the UO EPC. The Tufts-NEMC EPC used the Ovid search engine to conduct preliminary searches on the Medline database. The final searches used 6 databases including Medline from 1966 to week 2 of February 2003, PreMedline February 7, 2003, Embase from 1980 to week 6 of 2003, Cochrane Central Register of Controlled Trials 4th quarter of 2002, Biological Abstracts 1990 - December 2002, and Commonwealth Agricultural Bureau (CAB) Health from 1973 to December 2002. Subject headings and text words were selected so that the same set could be applied to each of the different databases with their varying attributes. Supplemental search strategies were conducted as needed. Additional publications were referred to us by the TEP and the other 2 EPCs. Details about selected terms used in the search strategy are discussed below.

Omega-3 Fatty Acids Search Strategy

A wide variety of search terms were used to capture the many potential sources of omega-3 fatty acids. Search terms used include the specific fatty acids, fish and other marine oils, and specific plant oils (flaxseed, linseed, rapeseed, canola, soy, walnut, mustard seed, butternut, and pumpkin seed). These terms were used in all search strategies.

Cardiovascular Search Strategy

The primary search strategy was designed to address both the clinical and intermediate outcomes of CVD in humans (Appendix A.1). In order to identify CVD outcomes in human studies, the search was divided into 3 categories consisting of controlled trials, other studies, and reviews. These 3 categories were further divided into English and non-English subsets.

Diabetes

Because specific terms referring to diabetes had been omitted from the primary search strategy, a supplemental search strategy was conducted on March 29, 2003. The diabetes supplemental search strategy included relevant search terms for diabetes. This search strategy resulted in an additional 410 citations for screening (Appendix A.2).

Supplemental Searches

Because some studies evaluated the effect of nuts on CVD outcomes without specifying in the abstract the type of nuts used in the study, we performed a supplemental Medline search on July 30, 2003 using the term “nut” as a text word for studies of CVD (Appendix A.3). Furthermore, upon noting that a number of relevant articles were missing from our search strategy, we performed a supplemental search on July 1, 2003. This search included terms specific to the CVD risk factor and intermediate markers outcomes of interest (Appendix A.4).

Overall

The number of citations for the final results of the database searches is approximate. Because the 5 main databases used in the search employ different citation formats, duplicate publications were encountered. The UO EPC eliminated most of the duplicate publications, however, because of many different permutations it was impossible to identify all of them. We eliminated duplicate publications as we encountered them.

Ongoing automatic updates of Medline searches were conducted using the CVD search strategy. The last automatic update was on April 19, 2003. The UO EPC conducted a final update search of the other databases on April 10, 2003.

Study Selection

Abstract Screening

All abstracts identified through the literature search were screened manually. At this stage, eligibility criteria were loosely defined to include all English language primary experimental or observational studies that evaluated any potential source of omega-3 fatty acids in at least 5 human subjects, irrespective of the study outcomes reported in the abstract. We excluded abstracts that clearly included only subjects who had a non-CVD-related condition (such as cancer, schizophrenia, or organ transplant), letters and abstracts.

Full Article Inclusion Criteria

Articles that passed the abstract screening process were retrieved and the full articles were screened for eligibility. Articles were rejected during this round based on the following criteria: review articles, inappropriate human population, pediatric studies and those conducted on

subjects less than 19 years old, no mention of omega-3 fatty acid dietary supplements or fish consumption, daily dose of omega-3 fatty acid greater than 6 g, fewer than 5 subjects in omega-3 fatty acid arm(s), prospective interventional studies of less than 4 weeks duration, crossover studies with less than 4 week washout between treatments, and no appropriate outcome of interest reported. Studies that reported only the tissue level of omega-3 fatty acid without explicitly reporting the amount of omega-3 fatty acid consumed were also excluded. Studies that reported only lipid data among the outcomes of potential interest with fewer than 20 subjects were excluded during screening because of the large number of such studies and limited resources. In addition, with the exception of studies of Mediterranean diets and studies that reported fish servings, studies were excluded if no specific data were reported about omega-3 fatty acid consumption. Specific sources of omega-3 fatty acids considered acceptable included fish oils, dietary fish, canola (rapeseed) oil, soybean oil, flaxseed or linseed oil, walnuts or walnut oil, and mustard seed oil. Other sources were eligible if omega-3 fatty acid levels were reported to be greater than control. For each study that was rejected, the reason(s) for rejection was noted.

The exclusion criterion of more than 6 g per day for non-adverse event clinical outcomes was based on discussions with the TEP, in which it was agreed that omega-3 fatty acid intake above this amount is impractical and has little relevance on health care recommendations. Therefore, the inclusion criterion for the maximum daily intake was set at 6 g per day. The definition of dose of omega-3 fatty acids varied greatly across studies. Thus, the maximal allowable dose may have applied to total daily omega-3 fatty acid, total EPA plus DHA, or a total of other combinations of omega-3 fatty acids. The total did not refer to total fish oil. Short duration studies (less than 4 weeks) and crossover studies with washout periods less than 4 weeks were excluded since, it was agreed, a metabolic steady-state of omega-3 fatty acids is likely not achieved for about 4 weeks.

Sometimes there were multiple publications of the same study reporting interim results or different outcomes. We identified and grouped articles belonging to the same overall study and used data from the latest publication, supplemented by data from earlier publications, as appropriate.

In addition, a list of approximately 100 potential markers of CVD (e.g., coronary intima media thickness) and risk factors (e.g., hypertension, C-reactive protein) was reviewed in detail. Because of limited time and resources, 22 factors were chosen from this list for definite inclusion. A second list of factors was evaluated for possible inclusion if time and resources allowed (see Table 3.1 in Results section). Studies that reported on none of these factors were rejected.

Because of the large number of studies available for analysis, for most outcomes of interest we decided to confine analysis to the largest randomized trials for each outcome evaluated. For outcomes with few studies, all studies were included regardless of study design or sample size (minimum of 5 subjects). We used a lower sample size threshold for crossover studies because these studies are more strongly powered for a given number of subjects than parallel studies. We generally aimed for approximately 20 to 25 studies for analysis. For studies of platelet aggregation, we used the additional inclusion criterion that platelet aggregation data must be presented in a numerical format; articles that reported platelet aggregation results only graphically were not analyzed. This additional criterion was used because of the particular difficulty in estimating data from graphs for this outcome and because of the large number of specific outcomes reported in each study. Specific criteria used are listed in Table 3.1 and

described in each outcome section in Chapter 3.

Incorporation of omega-3 fatty acids into phospholipids is very commonly reported by studies, often as proof of treatment compliance. Again because of limited time and resources, we limited our review of studies examining omega-3 fatty acid incorporation (or the association between dietary omega-3 fatty acid intake and tissue levels of omega-3 fatty acids) to the larger randomized trials that met eligibility criteria for either intermediate or clinical outcomes. We based this decision on the assumption that this sample of studies should not be biased. In addition, because the primary research question concerns correlation between dietary intake and blood levels of omega-3 fatty acids, for these analyses we have included only prospective, intervention trials to avoid biases and inaccuracies inherent to retrospective or survey-based studies. We have limited measurable levels to those most commonly reported and most practically measured, including erythrocyte, platelet cell membrane, and plasma phospholipids.

Data Extraction Process

An electronic data extraction form and database were created specifically for the evaluation of studies of omega-3 fatty acids and intermediate and clinical outcomes (Appendix B, available electronically at <http://www.ahrq.gov/clinic/epcindex.htm>). Data were entered into the form by selecting single or multiple choice buttons or as free text, as appropriate. The form allowed direct input of data into a Microsoft Access database and further manipulation of extracted data in both Microsoft Excel and Word.

As the data extraction form was being developed, all members of the EPC were trained to use the electronic form and software. In an iterative process, in which groups of studies were extracted by all trainees, the data entry form was improved, consensus was reached on definitions, and issues specific to omega-3 fatty acid studies were addressed. After this process, each study was screened for eligibility criteria and for outcomes using the electronic form. Each eligible study was then fully extracted by a single researcher. During weekly meetings, data extraction problems were addressed. Occasional sections were re-extracted to ensure that uniform definitions were applied across extracted studies. Problems and corrections were noted through spot checks of extracted data and during the creation of summary and evidence tables. A second reviewer independently verified the data in the summary tables using the original article.

Items extracted included: study design, blinding, randomization method, allocation concealment method, country, funding source, study duration, eligibility criteria, sample characteristics (including comorbid conditions, concomitant medications, baseline diet, and demographics), number enrolled and analyzed, reasons for withdrawals, description of omega-3 fatty acid and control interventions or diets (including amount of specific fatty acids), risk factor, intermediate markers, and clinical outcomes, adverse events (which are discussed in the report, *Effects of Omega-3 Fatty Acids on Cardiovascular Disease*), results (including baseline value, final value, within-treatment change, or between-treatment difference, and variance, as reported), and whether each study addressed each of the key questions. In addition, each study was categorized based on applicability and study quality as described below.

Meta-Regression

To examine the association between the level of intake of omega-3 fatty acids and tissue levels, the change in omega-3 fatty acid and arachidonic acid (AA 20:4 n-6) compositions were calculated for each study arm. Data were extracted for fatty acid composition of plasma or serum phospholipids, platelet membrane phospholipids, and erythrocyte membrane phospholipids, granulocyte membrane phospholipids, and monocyte membrane phospholipids. For each tissue type, data from each treatment arm were combined in a meta-regression on the change of EPA+DHA composition compared to mean dose of EPA+DHA received in each treatment arm.³⁸ Changes in non-omega-3-fatty-acid arms or control groups were not included in meta-regression analyses.

We performed simple linear regressions with the weighted least squares method, weighting each study arm by the square root of its sample size³⁹. The equation of the meta-regression line is reported for each blood marker. R^2 , or the goodness of fit, for the regression line is also reported. Data are presented both in summary tables and graphically in scatter plots in which the sources of the omega-3 fatty acid treatments are distinguished by different symbols.

Grading Evidence

Studies accepted in evidence reports have been designed, conducted, analyzed, and reported with various degrees of methodological rigor and completeness. Deficiencies in any of these processes may lead to biased reporting or interpretation of the results. While it is desirable to grade individual studies to inform the reader of these reports about the degree of potential bias, the grading of the quality of evidence is not straightforward. Despite many attempts, even for a single type of study design, most factors commonly used in quality assessment of randomized controlled trials have not been found to be consistently related to the direction or magnitude of the reported effect size⁴⁰. There is still no uniform approach to reliably grade published studies based on the information reported in the literature. Different EPCs have used a variety of approaches to grade study quality in past evidence reports.

Common Elements for Grading the Methodological Quality of Randomized Controlled Trials in Evidence Reports

As part of the overall omega-3 fatty acid project, the 3 collaborating EPCs agreed to use the Jadad Score and adequacy of random allocation concealment as elements to grade individual randomized controlled trials^{41,42}. We also agreed that individual EPCs might add other elements to this core set, as we deemed appropriate. All EPCs agreed that studies should not be graded using a single numerical quality score, as this has been found to be unreliable and arbitrary⁴³.

The Jadad Score assesses the quality of randomized controlled trials using 3 criteria: adequacy of randomization, double blinding, and drop outs⁴¹. A study that fully meets all 3 criteria gets a maximum score of 5 points. Adequacy of allocation concealment was assessed using the criteria described by Schulz et al., as adequate, inadequate, or unclear⁴².

Generic Summary Quality Grade for Studies

The Jadad and Schulz scores address only some aspects of the methodological quality of randomized controlled trials. Potential biases due to reporting and analytic problems in the study are ignored. In this evidence report, we applied a 3-category grading system (A, B, C) to each randomized trial. We have used this grading system in most of our previous EPC evidence reports, as well as in several evidence based clinical practice guidelines⁴⁴. This scheme defines a generic grading system for study quality that is applicable to each type of study design (i.e., randomized controlled trial, cohort study, case-control study):

- A Least bias; results are valid. A study that mostly adheres to the commonly held concepts of high quality, including the following: a formal randomized study; clear description of the population, setting, interventions and comparison groups; appropriate measurement of outcomes; appropriate statistical and analytic methods and reporting; no reporting errors; less than 20% dropout; clear reporting of dropouts; and no obvious bias.
- B Susceptible to some bias, but not sufficient to invalidate the results. A study that does not meet all the criteria in category A. It has some deficiencies but none likely to cause major bias. Study may be missing information making assessment of the limitations and potential problems difficult.
- C Significant bias that may invalidate the results. A study with serious errors in design, analysis, or reporting. These studies may have large amounts of missing information or discrepancies in reporting.

Studies that reported multiple results of interest to this report could receive different quality grades for different outcomes if there were reporting or methodological issues with specific outcomes but not others. We did not grade the few non-randomized studies that were analyzed.

Applicability

Applicability addresses the relevance of a given study to a population of interest. Every study applies certain eligibility criteria when selecting study subjects. Most of these criteria are explicitly stated (i.e., disease status, age, sex). Some may be implicit or due to unintentional biases, such as those related to study country, location (e.g., community vs. specialty clinic), or factors resulting in study withdrawals. The question of whether a study is applicable to a population of interest (such as Americans) is distinct from the question of the study's methodological quality. For example, due to differences in the background diets an excellent study of Japanese men may be very applicable to people in Japan, but less applicable to Japanese-American men, and even less applicable to African-American men. The applicability of a study is thus dictated by the questions and populations that are of interest to those analyzing the studies.

In this report, the focus is on the US population, as specified in the Scope of Work for this series of evidence reports. We also address specific subgroups within that population (i.e., healthy Americans, Americans with CVD, and Americans with diabetes or dyslipidemia), as specified. To capture the potential applicability of studies to the different populations of interest as defined in the scope of work we define the following target population categories:

- GEN General population. Typical healthy people similar to Americans without known CVD, diabetes or dyslipidemia.

- CVD Cardiovascular disease population. Subjects with a history of or currently with cardiac, peripheral vascular, or cerebrovascular disease, as defined by the author. In addition studies of hypertensive patients were included.

- DM Diabetic population. Subjects with any type of diabetes, including type I (DM I), type II (DM II), insulin dependent (IDDM) and non-insulin dependent (NIDDM), as defined by the authors.

- DysLip Population with dyslipidemia, either elevated total cholesterol, LDL, or Tg, or low levels of HDL, as defined by the authors.

One study was classified as CVD Risk because it included a combination of subjects with known CVD, diabetes, dyslipidemia and other potential CVD risk factors. In addition, some studies received multiple classifications (CVD/DM or DM/DysLip), when inclusion criteria included multiple conditions.

Even though a study may focus on a specific target population, limited study size, eligibility criteria and the patient recruitment process may result in a narrow population sample that is of limited applicability, even to the target population. To capture this parameter, we categorize studies within a target population into 1 of 3 levels of applicability⁴⁴:

- I Sample is representative of the target population. It should be sufficiently large to cover both sexes, a wide age range, and other important features of the target population including baseline dietary intake broadly similar to that of the US population.

- II Sample is representative of a relevant sub-group of the target population, but not the entire population. For example, while the Nurses Health Study is the largest such study and the results are highly applicable to women, it is nonetheless representative only of women. A fish oil study in Japan, where the background diet is very different from that of the US, would also fall into this category.

- III Sample is representative of a narrow subgroup of subjects only, and not well applicable to other subgroups. For example, a study of male college students or a study of a population on a highly controlled diet.

In the summary tables, each study receives a combined applicability grade comprised of the target population (GEN, CVD, DM, and DysLip) and the 3-level grade (I, II, III).

Sample Size

The study sample size provides a quantitative measure of the weight of the evidence. In general, large studies provide more precise estimates of effect and associations. In addition, large studies are more likely to be generalizable; however, large size alone does not guarantee broad applicability.

Reporting Results

Most outcomes evaluated were continuous variables, such as lipid level or intima-media thickness. For these outcomes, summary tables report 3 sets of data: the mean (or median) baseline level in the omega-3 fatty acid arm; the net change of the outcome, and the reported *P* value of the difference between the omega-3 fatty acid arm and control. The net change of the outcome is the difference between the change in the omega-3 fatty acid arm and the change in the control arm, or:

$$\text{Net change} = (\text{Omega } 3_{\text{Final}} - \text{Omega } 3_{\text{Initial}}) - (\text{Control}_{\text{Final}} - \text{Control}_{\text{Initial}}).$$

The great majority of articles reported these 4 values and *P* values. While some studies reported adjusted and unadjusted within-arm and between-arm (net) differences, to maintain consistency across studies we calculated the unadjusted net change using the above formula for all studies when the data were available. To provide a rough estimate of the effect of omega-3 fatty acids when median values were reported (as for lipoprotein (a)), we used the above formula with the median values, recognizing that the resultant net change is not mathematically valid. When data were available at multiple time points, we extracted data on only the time point at the end of omega-3 fatty acid intervention. Data from other time points are discussed in the text.

We included only the reported *P* values for the net differences. We did not calculate any *P* values, but, when necessary, used provided information on the 95% confidence interval or standard error of the net difference to determine whether the *P* value was less than .05. We included any reported *P* value less than .10. Reported *P* values above .10 and values reported as “non-significant” were included as NS, non-significant.

Coronary artery restenosis studies provided rate data on a dichotomous variable (restenosis or no restenosis). For these studies, we report 3 equivalent sets of data: the control rate (the rate of restenosis in the control group, a standard measure of the underlying severity of illness in the study population), the relative risk of restenosis, and the 95% confidence interval. In addition we performed a random effects model meta-analysis⁴⁵.

All exceptions and caveats are described in footnotes.

Evidence and Summary Tables

We report the evidence in 2 complementary forms:

Evidence tables offer a detailed description of studies we analyzed that address each of the key questions. These tables provide detailed information about the study design, patient characteristics, inclusion and exclusion criteria, interventions and comparison groups evaluated, and outcomes. Baseline and follow-up data for each analyzed outcome are reported in the Results column. A study, regardless of how many interventions or outcomes were reported, appears once in the evidence tables. The studies are ordered alphabetically by the first author's last name and study year.

Summary tables succinctly report on each study using summary measures of the main outcomes. These tables were developed by condensing information from the evidence tables and are designed to facilitate comparisons and synthesis across studies. Summary tables include important concise information regarding study size, intervention and control, study population (e.g., general population or CVD), outcome measures, methodological quality and applicability. Studies are grouped by omega-3 fatty acid source (EPA/DHA oils, plant oils, fish and Mediterranean diets, and combinations – comparisons – of different sources). Then studies are ordered first by omega-3 fatty acid dose and second by omega-3 fatty acid study arm size (both largest to smallest). A study with outcomes may appear multiple times in different summary tables.

Methodological Limitations

Due to practical limitations of time and resources, many constraints were applied to the available data, as described above. In consultation with the TEP and NIH representatives, we prioritized the original list of questions to focus on those of greatest interest to the scientific and medical communities and for which data were likely to be available. Likewise, the list of specific CVD risk factors that we examined was reduced to those that members of the TEP agreed have the greatest clinical relevance and are most clearly related to CVD. Therefore, a large number of commonly evaluated markers were not included. For example, tissue plasminogen activator (TPA), plasminogen activator inhibitor (PAI), and LDL oxidation were not included because their levels are not clearly associated with clinical CVD outcomes, or the meaning of a change in their levels is not well understood, or there is much variability in how the factor is measured and interpreted, among other reasons. In addition, the TEP attempted to focus on those factors which are most relevant to clinical practice.

The decision about which specific outcomes to evaluate from the list of potential outcomes was based on an evaluation of the available evidence. CVD risk factors and intermediate markers with more limited evidence, possibly due to publication bias, or that were primarily evaluated in small or non-randomized or uncontrolled trials were generally omitted; although data on particular outcomes of interest, such as C-reactive protein and exercise tolerance testing, were included despite limited data.

Finally, because of the large number of studies, only the highest quality, larger studies were analyzed. While we attempted to find data to answer all the key questions, only those studies included in the main analyses were evaluated in thorough detail. This has implications for questions regarding populations, covariates, comparison of omega-3 fatty acid sources, and other sub-questions. However, it is unlikely that any of the missed studies were critical to our understanding of the key questions, since only the smaller, lower quality studies would have been missed.

It is also important to note that for almost all analyzed outcomes, the available data are biased toward positive results. Many articles reported that omega-3 fatty acid treatment did not affect levels of various outcomes, but did not report supporting data. These studies were not evaluated for the reported outcomes.



Chapter 3. Results

In this chapter, we review the results of our literature search and summarize findings from studies that passed our screening and selection process. Studies examining the relationship between omega-3 fatty acids - eicosapentaenoic acid (EPA, 20:5 n-3), docosahexaenoic acid (DHA, 22:6 n-3), and alpha linolenic acid (ALA, 18:3 n-3) - and selected risk factors of cardiovascular disease (CVD) are summarized first, followed by studies that examine the correlation between omega-3 fatty acid intake and tissue levels of fatty acids.

Summary of Studies Found

Through the literature search we identified and screened over 7,464 abstracts indexed as English language articles concerning humans. We retrieved and screened 807 full text articles for potentially relevant human data. Of these, we rejected 463 articles for the reasons listed in the section "Listing of Excluded Studies" under "Rejected Studies". Of the remaining 344 articles, we analyzed risk factor and other outcome data from 123 (Table 3.1, "References and Included Studies" under "Included Studies"). The 221 non-rejected studies that were not analyzed are listed in the section "Listing of Excluded Studies" under "Studies Not Analyzed Because of Non-Randomized Design or Small Size". For most outcomes, we analyzed only the approximately 20 to 30 largest randomized trials. These trials were selected based on criteria described both in Table 3.1 and in the sections describing each risk factor included in this chapter.

We compiled an Evidence Table that provides detailed information about each study we analyzed (Appendix C, available electronically at <http://www.ahrq.gov/clinic/epcindex.htm>). The summary tables present specific information about each of the studies that we analyzed for a given risk factor or outcome. Information presented in the summary tables include: study design and size, amount of omega-3 fatty acid consumption, baseline level of the relevant risk factor, net change of risk factor level (change in omega-3 fatty acid arm less change in control arm), reported statistical significance of the net change, study quality, study population, and applicability for each study.

Most studies that we analyzed evaluated fish or other marine oils (as supplements, dietary fish, or oil spreads); few evaluated plant oils (as supplements, dietary oils, or oil spreads). Furthermore, few studies compared doses of similar omega-3 fatty acids, compared different omega-3 fatty acids, reported on potential covariates such as age and sex, analyzed effects based on duration of intake, or repeated measurements after subjects had stopped omega-3 fatty acid supplementation. Only 13 articles (reporting on 12 trials) reported any data related to either baseline dietary or experimental dietary intake of both omega-3 fatty acid and omega-6 fatty acid intake to allow an estimate of mean daily omega-6 to omega-3 fatty acid ratio⁴⁶⁻⁵⁸. However, no study analyzed the relationship between evaluated outcomes and either omega-6 to omega-3 fatty acid consumption ratio or combined omega-6 and omega-3 fatty acid consumption amounts. Any available data relating to relative amounts of omega-6 fatty acid consumption could not be evaluated separately from different doses or types of omega-3 fatty acids.

Each risk factor is discussed separately in the following, largely arbitrary, order:

- Lipids (total cholesterol, low density lipoprotein [LDL], high density lipoprotein [HDL], triglycerides, lipoprotein (a) [Lp(a)], apolipoproteins [apo] AI, B, B-100, and LDL apo B)
- Blood pressure
- Measures of glucose metabolism (hemoglobin A_{1c} [Hgb A_{1c}], fasting blood sugar [FBS], and fasting insulin)
- C-reactive protein (CRP)
- Measures of hemostasis (fibrinogen, factors VII and VIII, von Willebrand factor [vWF], and platelet aggregation)
- Non-serum diagnostic tests (coronary artery restenosis [following angioplasty], carotid intima-media thickness [IMT], exercise tolerance testing [ETT], and heart rate variability).

The final section of this chapter summarizes studies that examine the correlation between omega-3 fatty acid intake and tissue levels, including plasma or serum phospholipid levels, platelet phospholipids, erythrocyte membrane phospholipids, granulocyte membrane phospholipids, and monocyte membrane phospholipids.

Table 3.1 Numbers of studies of omega-3 fatty acids and cardiovascular risk factors

CVD Risk Factor	Total Studies Meeting Minimum Eligibility Criteria	Total Randomized Studies	Eligibility Criteria for Analysis ^a		Analyzed Studies
Lipids	182 ^b	108	RCT ≥ 60	Xover ≥ 40	25
Total Cholesterol	169	98	RCT ≥ 60	Xover ≥ 40	23
Low Density Lipoprotein	119	70	RCT ≥ 60	Xover ≥ 40	15
High Density Lipoprotein	141	81	RCT ≥ 60	Xover ≥ 40	19
Triglycerides	164	100	RCT ≥ 60	Xover ≥ 40	19
Lipoprotein (a)	23	14	RCT ≥ 5	Xover ≥ 5	14
Apolipoprotein A-1	61	37	RCT ≥ 20	Xover ≥ 15	27
Apolipoprotein B	52	29	RCT ≥ 20	Xover ≥ 10	25
Apolipoprotein B-100	11	10	RCT ≥ 5	Xover ≥ 5	10
Blood pressure	103	71	RCT ≥ 15 DM	Xover ≥ 10 DM	6 ^c
Hemoglobin A_{1c}	32	22	RCT ≥ 10	Xover ≥ 10	18
Blood sugar, fasting	57	34	RCT ≥ 25	Xover ≥ 15	17
Fasting insulin	21	15	RCT ≥ 5	Xover ≥ 5	15
C-reactive protein	5	4		All	5
Fibrinogen	59	34	RCT ≥ 15	Xover ≥ 10	24
Factor VII	40	25	RCT ≥ 15	Xover ≥ 10	19
Factor VIII	13	5	RCT ≥ 5	Xover ≥ 5	5
von Willebrand factor	20	9	RCT ≥ 5	Xover ≥ 5	9
Platelet aggregation	84	39	RCT ≥ 15	Xover ≥ 10	11 ^d
Coronary arteriography	17	14	RCT ≥ 5	Xover ≥ 5	12 ^e
Carotid intima-media thickness	4	1		All	4
Exercise tolerance test	6	3		All	6
Heart rate variability	3	2		All	3
Sub-Total^f	327	197			123
Risk Factors Not Analyzed					
Apolipoprotein C-III	3	1			
Remnant-like particles	2	0			
Free fatty acids or Non-esterified fatty acids	7	5			
Diabetes incidence	1	0			
Microalbuminuria	4	3			
Homocysteine	4	2			
Factor XII	4	1			
Bleeding time	48	21			
Interleukin 6	2	1			
VCAM-1^g	2	1			
Creatine kinase	5	4			
Echocardiography	1	1			
Endothelial function	11	8			
ECG parameters	4	3			
Heart rate, resting	23	16			
Ankle brachial index	1	1			
Total (Analyzed and not analyzed)	346				

- a RCT ≥, minimum number of subjects in a parallel randomized controlled trial; Xover ≥, minimum number of subjects in a cross-over study; DM = diabetes mellitus.
- b Minimum of 20 subjects consuming omega-3 fatty acids.
- c We analyzed only studies of diabetic patients.
- d We analyzed only studies with platelet aggregation data reported in text or table. We did not analyze studies that reported outcomes only in figures.
- e We analyzed only studies that reported the number (or percent) of patients who had restenosis.
- f Individual study numbers do not add up to totals because many articles reported more than 1 outcome.
- g Vascular cell adhesion molecule 1

Lipids: Total Cholesterol

(Table 3.2)

Abnormal levels of serum lipids, primarily low density lipoprotein (LDL), high density lipoprotein (HDL), and triglycerides (Tg) have long been recognized as risk factors for CVD. Of interest is whether consuming omega-3 fatty acids as part of a therapeutic lifestyle change would improve lipid levels, or at least would not be detrimental. Recent National Cholesterol Education Program (NCEP) guidelines recommend a goal for fasting total cholesterol of less than 200 mg/dL in all adults, with lower levels recommended for people at elevated risk for CVD, including diabetics, smokers, people with hypertension or a family history of premature CVD, or who are beyond middle age⁵⁹.

Lipid levels are the most commonly measured CVD risk factor in trials of omega-3 fatty acid consumption. We found 182 studies that met eligibility criteria and reported data on the effect of omega-3 fatty acids on lipid levels in at least 20 subjects (See Table 3.1). Of these, we analyzed the 25 randomized trials with lipid data for at least 60 subjects in parallel trials and 40 subjects in crossover trials who consumed omega-3 fatty acids. It is important to note that because we analyzed only the largest randomized trials, we did not capture many smaller studies of diabetic patients.

Among these studies, 169 reported data on total cholesterol levels. We analyzed the 23 largest randomized trials.

Table 3.2 Effects of omega-3 fatty acids on total cholesterol (mg/dL) in randomized trials (6 weeks to 2 years)

Author, Year	Omega-3 Fatty Acid Arm ^a			Control		Results ^b			Quality ^c			Applicability ^d
	N	Source	g/d	N	Source	Base	Net Δ	P	Summary	Jadad	Allocation Conceal	
DHA/EPA Oils												
Cairns, 1996	325	Fish oil	ED 5.4	328	Corn oil	227	-3	NS	B	3	Un	CVD II
Bonaa, 1992	71	Fish oil	ED 5.1	74	Corn oil	251	+2	NS	B	4	Un	DysLip I
Lungershausen, 1994	42 ^e	Fish oil	ED 4.9	42 ^e	Corn oil	221	+2	NS	B	3	Un	CVD II
Bairati, 1992b	66	Fish oil	ED 4.5	59	Olive oil	240	-1	NS	B	5	Un	CVD II
Grimsgaard, 1997	75	Purified EPA	E 3.8	77	Corn oil	231	-10	.004 ^f	A	5	Un	GEN I
	72	Purified DHA	D 3.7			232	-3	NS ^f				
Nilsen, 2001 ^g	75	Fish oil	ED 3.4	75	Corn oil	214	+9	NS	B	3	Un	CVD II
Eritsland, 1995b	260	Fish oil	ED 3.3	251	No oil	252	-2	NS	B	2	Ad	CVD II
Brox, 2001 ^h	38	Cod liver oil	ED 3.3	37	No oil	319	-19	NS	C	1	Un	DysLip I
	37	Seal oil	ED 2.6			308	0	NS				
Franzen, 1993	92 ⁱ	Fish oil	ED 3.1	83 ⁱ	Olive	219	+2	nd	C	5	Ad	CVD II
Osterud, 1995	26	Cod liver oil	ED 3.1	28	No oil	203	+1	NS	B	2	Un	GEN I
	27	Seal/Cod oil	ED 2.8			204	+9	NS				
	27	Seal oil	ED 2.4			199	+2	NS				
	26	Whale oil	ED 1.7			197	+10	NS				
Leigh-Firbank, 2002	55 ^e	Fish oil	ED 3.0	55 ^e	Olive oil	255	-2	NS	B	3	Un	DysLip I
Sacks, 1994	60 ^j	Fish oil	ED 2.4	66 ^j	Olive oil	190	+4	NS	C	3	Un	GEN I

Continued

Table 3.2 Effects of omega-3 fatty acids on total cholesterol (mg/dL) in randomized trials (continued)

Author, Year	Omega-3 Fatty Acid Arm ^a			Control		Results ^b			Quality ^c			Applicability ^d	
	N	Source	g/d	N	Source	Base	Net Δ	P	Summary	Jadad	Allocation Concealment		
DHA/EPA Oils (continued)													
Sirtori, 1998	459	Fish oil	ED 1.7 ^k	450	Olive oil	234 ^L	-1 ^L	NS	B	4	Ad	CVD risk ^m I	
von Schacky, 1999	89 ⁿ	Fish oil	ED 1.7 ^o	86 ^p	Plant oil	237	+6	NS	C	5	Ad	CVD II	
GISSI, 1999	2836	Fish oil	ED 0.9	2828	No oil	210	+2	NS	B	3	Un	CVD II	
	2830	Fish oil ^q		2830	Vitamin E	211	+4						
Leng, 1998	37 ^r	Fish oil	ED 0.045 ^s	36 ^t	Sunflower oil	233	+2	NS	C	4	Ad	CVD II	
Plant Oils													
Natvig, 1968	289 ^u	Linseed oil	A ~5	316 ^u	Sunflower oil	246	+1	NS	C	2	Un	GEN III DM ^w III	
	47 ^v			51 ^v		250	+5	NS					
Borchgrevink, 1966	100	Linseed oil	A ~5	100	Corn oil	289	+13	nd	C	2	Un	CVD III	
Fish and Mediterranean Diets													
Singh, 2002	499	Indo-Mediterranean	T 1.8	501	NCEP I ^x	221	-20	<.0001	C	2	Un	CVD risk ^y III	
Hanninen, 1989	19	Fish (3.8/week)	ED 0.9	18	0.4 Fish/week	176	-8	nd	B	2	Un	GEN III	
	22	Fish (2.3/week)	ED 0.5			157	+2	nd					
	21	Fish (1.5/week)	ED 0.4			158	-7	nd					
	20	Fish (0.9/week)	ED 0.2			170	+3	nd					
de Lorgeril, 1994	171 ^z	Mediterranean/Canola margarine	A 0.8% Kcal	168 ^{aa}	Regular	240	-1	NS	C	2	Un	CVD II	
Combinations													
Mori, 1994	17	Fish oil & Fish diet ^{bb}	ED 5.2	18	Olive/Palm/Safflower 40% fat diet	235 ^{cc}	+7 ^{dd}	NS	B	2	Un	CVD II	
	16	Fish oil	ED 4.2				+19 ^{dd}	NS					
	17	Fish diet ^{bb} & Placebo oil	ED 3.0				+13 ^{dd}	NS					
	17	Fish oil	ED 2.1				+21 ^{dd}	<.05					
	18	Fish diet ^{bb} & Placebo oil	ED 3.0	17	Oil 30% fat	+1 ^{dd}	NS						
Finnegan, 2003	31	Fish oil margarine/ Fish oil	ED 1.7	30	Sunflower margarine	212	+14	NS	A	4	Un	DysLip I	
	30	Fish oil margarine	ED 0.8				211	+4					NS
	30	Rapeseed/Linseed margarine	A 4.5				217	+2					NS

nd = no data

a A = ALA; D = DHA; E = EPA; ED = EPA+DHA; T = Total omega-3 fatty acids.

b Base = baseline level in treatment arm; Net Δ = net difference in change in omega-3 fatty acids arm compared with the change in control arm, see Methods; P = P value of difference between treatment and control arms; NS = not statistically significant.

c A = good quality; B = fair quality; C = poor quality; Jadad = Jadad Score (0-5, based on randomization, double blinding, and dropouts); Ad = adequate allocation concealment; In = inadequate allocation concealment; Un = allocation concealment unclear. See Methods.

d CVD = history of cardiovascular disease; DM I/II = diabetes mellitus type 1 or 2; DysLip = dyslipidemia; GEN = general, healthy population; N/IDDM = (non-) insulin dependent diabetes mellitus. I = broadly applicable; II = applicable to sub-group; III = narrow applicability. See Methods.

e Cross-over study.

f P=.04 for difference in effect of EPA and DHA.

- g Only subjects who did not change statin treatment are included here.
- h Data missing from article provided by study author.
- i Maximum. Total analyzed was 172, not 175 (92+83).
- j 84 at baseline.
- k 2.6 g/day for first 2 months, then 1.7 g/day for 4 months.
- L Estimate from graph.
- m Dyslipidemia and one or more of: hypertension, diabetes, or glucose intolerance.
- n 111 at baseline.
- o 3.4 g/day for first 3 months, then 1.7 g/day for 21 months.
- p 112 at baseline.
- q Plus vitamin E 300 mg.
- r Baseline data based on N=52.
- s Plus 280 mg gamma linolenic acid (omega-6 fatty acid).
- t Baseline data based on N=50.
- u 33 missing data for one or both tests.
- v 7 missing data for one or both tests.
- w Sub-analysis.
- x National Cholesterol Education Program step I prudent diet.
- y One or more of: hypercholesterolemia, hypertension, diabetes, angina pectoris or myocardial infarction.
- z Baseline data based on 289 subjects.
- aa Baseline data based on 295 subjects.
- bb Omega-3 fatty acid from fish diet is approximate, assuming that each of 4 different fish was consumed equally.
- cc Mean baseline value for all subjects combined.
- dd Estimated from graph.

Overall Effect ^{48,49,52,53,60-78}

Across the 23 studies there was a wide range of effects of omega-3 fatty acids on total cholesterol, although in most studies the net effect was small and generally of an increase in total cholesterol. Most studies found net increases of between 0% and 6% (approximately 0 to 14 mg/dL). Only 3 studies found that the changes in total cholesterol in subjects on omega-3 fatty acids were significantly different than control. Notably, the directions of the treatment effects were not consistent across these studies.

Sub-populations

Only 5 of the studies included generally healthy subjects, 3 of which were all male^{66,67,72}. Net effects were generally small but inconsistent in direction. Most of the studies included subjects with a variety of types of CVD. There was no clear consistent effect among the 12 studies. Two studies evaluated subjects at increased risk of CVD with different sets of treatments and came to different conclusions. Sirtori et al. found no effect with fish oil in approximately 900 individuals with dyslipidemia and either hypertension, diabetes or glucose intolerance⁷⁷. Singh et al. reported a large, highly significant reduction in total cholesterol with an Indo-Mediterranean diet in approximately 1,000 people with either hypercholesterolemia, hypertension, diabetes, angina or myocardial infarction⁷⁶. However, this study found that subjects on the Indo-Mediterranean diet lost significantly more weight (3 kg) than those on the control diet. In addition, they reported uniform highly significant effects on all serum markers despite widely ranging effects. A number of statistical calculation errors were also found.

While no study evaluated a population of all diabetic subjects, Natvig et al., in an early Norwegian trial of linseed oil supplements, reported a sub-analysis of the 98 diabetic subjects and found that the effect of linseed oil was similar in both all subjects and specifically in diabetic

subjects, but that total cholesterol decreased by a small amount more in the diabetic subjects⁷². The difference was not significant.

Covariates

No subgroup analyses based on covariates were reported. Two studies performed regressions. Bairati et al. reported no change in total cholesterol effect after adjusting for age, sex, baseline lipid level, lipid treatment, body mass index and alcohol use⁶⁰. Mori et al. performed a regression adjusting for change in weight and found a highly significant “group effect” increase in total cholesterol with omega-3 fatty acids ($P < .001$)⁷¹. This study also found larger relative net increases in total cholesterol among subjects on a 40% fat diet, but no net effect (and a decrease in absolute change) in subjects on a 30% fat diet. No clear difference was seen between the 5 studies that included only men and the remaining studies^{61,66,67,71,72}.

Dose and Source Effect

Three studies compared different sources – and doses – of marine oil supplements^{62,66,74}. Grimsgaard et al. found a significantly greater decrease in total cholesterol with purified EPA than DHA in healthy, middle-aged men⁶⁶. Brox et al. found a substantially greater decrease in total cholesterol with higher omega-3 fatty acid dose cod liver oil supplement than seal oil supplement in healthy subjects with elevated total cholesterol; although they imply that the difference was not statistically significant⁶². Osterud et al. found varying degrees of net increases of total cholesterol with different marine oil supplements in healthy subjects⁷⁴. No clear pattern was evident among different doses of omega-3 fatty acids and dose effect of marine oil supplements was evident across the studies.

Hanninen et al. compared 5 fish diets⁶⁷. No significant effect on total cholesterol was seen with any diet and there was no dose effect based on frequency of fish consumption.

Among subjects on a higher fat diet, there was no clear difference in effect based on source of EPA+DHA among men studied by Mori et al.⁷¹. Despite an apparent larger net increase in total cholesterol among subjects consuming both fish oil margarine and fish oil supplements compared to those consuming only fish oil margarine or rapeseed and linseed margarine, Finnegan et al. found no differences in effect among the treatments⁵³.

The 4 studies of ALA all reported net increases in total cholesterol, but there was no apparent difference compared to fish and fish oil studies.

Exposure Duration

In 7 studies, total cholesterol levels varied by similar amounts in treatment and control arms at multiple time points^{49,53,67,69,73,75,77}. No differences in effect were seen at times ranging from 5 weeks to 2 years. No effect across studies is evident based on duration of intervention or exposure.

Sustainment of Effect

No study reported data on an effect after ceasing omega-3 fatty acid treatment.

Lipids: Low Density Lipoprotein

(Table 3.3)

Among the lipids commonly measured, the level of low density lipoprotein (LDL) is generally of most concern when determining CVD risk and whether to initiate therapy. The NCEP guidelines note that the relationship between LDL levels and CVD risk is continuous over a broad range of LDL levels from low to high⁵⁹. Recommended goals for LDL level depend on an individual's CVD risk factors. Risk factors include diabetes, smoking, hypertension, family history of premature CVD, and being beyond middle age. With no or one risk factor, LDL goal is less than 160 mg/dL; with 2 or more risk factors, LDL goal is less than 130 mg/dL. People who already have CVD or who have diabetes are recommended to achieve an LDL of less than 100 mg/dL. As with total cholesterol, of interest is whether consuming omega-3 fatty acids as part of a therapeutic lifestyle change would improve LDL levels, or at least would not be detrimental.

Of the 25 randomized trials with lipid data for at least 60 subjects in parallel trials and 40 subjects in crossover trials who consumed omega-3 fatty acids 15 reported data on LDL (See Table 3.1).

Overall Effect^{48,49,52,53,60,63-66,68-71,76,79}

The effect of omega-3 fatty acid consumption was fairly uniform across studies. Most found a net increase in LDL with treatment, although the range of effects varied substantially. Most studies found net increases of LDL of 10 mg/dL or less, although the complete range of mean net effects was a decrease of 19 mg/dL to an increase of 21 mg/dL. As with a number of other outcomes, Singh et al. found a discordant result⁷⁶. In this case, they reported a large, highly significant reduction in LDL with an Indo-Mediterranean diet in subjects at risk for CVD. However, as previously noted, this study found a difference in weight loss between the 2 interventions and reported uniform highly significant effects on all serum markers despite widely ranging effects; also, a number of statistical calculation errors were found.

Sub-populations

Only a single study included generally healthy subjects and no study included exclusively diabetics. Most of the studies included subjects with CVD. There was no clear difference among the 10 studies of CVD populations compared to the 3 dyslipidemia studies or single study of healthy subjects.

Covariates

No subgroup analyses based on covariates were reported. Two studies performed regressions. Bairati et al. reported that the effect of fish oil supplements on LDL (a net increase) was reduced and became borderline non-significant ($P = .06$) after adjusting for age, sex, baseline lipid level, lipid treatment, body mass index and alcohol use⁶⁰. Mori et al. performed a regression adjusting for change in weight and found a highly significant "group effect" increase in LDL with omega-3 fatty acids ($P < .001$)⁷¹. In contrast to their findings for total cholesterol, they reported similar effects on LDL among subjects on a 40% fat diet and on a 30% fat diet.

Table 3.3 Effects of omega-3 fatty acids on low density lipoprotein (mg/dL) in randomized trials (6 weeks to 2 years)

Author, Year	Omega-3 Fatty Acid Arm ^a			Control		Results ^b			Quality ^c			Applicability ^d
	N	Source	g/d	N	Source	Base	Net Δ	P	Summary	Jadad	Allocation Concealment	
DHA/EPA Oils												
Cairns, 1996	325	Fish oil	ED 5.4	328	Corn oil	148	+3	NS	B	3	Un	CVD II
Bonaa, 1992	70	Fish oil	ED 5.1	68	Corn oil	177	+7	NS	B	4	Un	DysLip I
Lungers-hausen, 1994	42 ^e	Fish oil	ED 4.9	42 ^e	Corn oil	156	+7	NS	B	3	Un	CVD II
Bairati, 1992b	66	Fish oil	ED 4.5	59	Olive oil	158	+12	<.05	B	5	Un	CVD II
Grimsgaard, 1997	75	Purified EPA	E 3.8	77	Corn oil	157	-5	NS	A	5	Un	GEN I
	72	Purified DHA	D 3.7			157	0	NS				
Eritsland, 1995b	260	Fish oil	ED 3.3	251	No oil	177	+4	NS	B	2	Ad	CVD II
Franzen, 1993	92 ^f	Fish oil	ED 3.1	83 ^f	Olive	151	+9	nd	C	5	Ad	CVD II
Leigh-Firbank, 2002	55 ^e	Fish oil	ED 3.0	55 ^e	Olive oil	175	+13	.03	B	3	Un	DysLip I
Angerer, 2002	87	Fish oil	ED 1.7	84	Fatty acid	157	+6	NS	B	4	Ad	CVD II
GISSI, 1999	2836	Fish oil	ED 0.9	2828	No oil	137	+3	NS	B	3	Un	CVD II
	2830	Fish oil ^g	ED 0.9	2830	Vitamin E	138	+5					
Leng, 1998	37 ^h	Fish oil	ED 0.045 ⁱ	36 ^j	Sunflower oil	107	+6	NS	C	4	Ad	CVD II
Fish and Mediterranean Diets												
Singh, 2002	499	Indo-Mediterranean T	1.8	501	NCEP I ^k	141	-19	<.0001	C	2	Un	CVD risk ^L III
de Lorgeril, 1994	171 ^m	Mediterranean/Canola margarine	A 0.8% Kcal	168 ⁿ	Regular	175	+3	NS	C	2	Un	CVD II
Combinations												
Mori, 1994	17	Fish oil & Fish diet ^o	ED 5.2	18	Olive/Palm/Safflower 40% fat diet	157 ^p	+11 ^q	NS	B	2	Un	CVD II
	16	Fish oil	ED 4.2				+21 ^q	<.01				
	17	Fish diet ^o & Placebo oil	ED 3.0				+10 ^q	NS				
	17	Fish oil	ED 2.1				+16 ^q	<.05				
	18	Fish diet ^o & Placebo oil	ED 3.0	17	Oil 30% fat		+12 ^q	<.05				
Finnegan, 2003	31	Fish oil margarine/ Fish oil	ED 1.7	30	Sunflower margarine	132	+13	NS	A	4	Un	DysLip I
	30	Fish oil margarine	ED 0.8			132	0	NS				
	30	Rapeseed/Linseed margarine	A 4.5			137	-2	NS				

nd = no data

^a A = ALA; D = DHA; E = EPA; ED = EPA+DHA; T = Total omega-3 fatty acids.

^b Base = baseline level in treatment arm; Net Δ = net difference in change in omega-3 fatty acids arm compared with the change in control arm, see Methods; P = P value of difference between treatment and control arms; NS = not statistically significant.

^c A = good quality; B = fair quality; C = poor quality; Jadad = Jadad Score (0-5, based on randomization, double blinding, and dropouts); Ad = adequate allocation concealment; In = inadequate allocation concealment; Un = allocation concealment unclear. See Methods.

^d CVD = history of cardiovascular disease; DM I/II = diabetes mellitus type 1 or 2; DysLip = dyslipidemia; GEN = general, healthy population; N/IDDM = (non-) insulin dependent diabetes mellitus. I = broadly applicable; II = applicable to subgroup; III = narrow applicability. See Methods.

^e Cross-over study.

^f Maximum. Total analyzed was 172, not 175 (92+83).

- g Plus vitamin E 300 mg.
- h Baseline data based on N=52.
- i Plus 280 mg gamma linolenic acid (omega-6 fatty acid).
- j Baseline data based on N=50.
- k National Cholesterol Education Program step I prudent diet.
- L One or more of: hypercholesterolemia, hypertension, diabetes, angina pectoris or myocardial infarction.
- m Baseline data based on 289 subjects.
- n Baseline data based on 295 subjects.
- o Omega-3 fatty acid from fish diet is approximate, assuming that each of 4 different fish was consumed equally.
- p Mean baseline value for all subjects combined.
- q Estimated from graph.

Dose and Source Effect

Mori et al. found no difference in effect among men consuming various doses of EPA+DHA either as supplements or as dietary fish⁷¹. Finnegan et al. noted a particularly large increase in LDL in the fish oil margarine/fish oil supplement arm compared to other arms, but the differences were not statistically significant⁵³. Grimsgaard found no difference in effect on LDL level between purified EPA and purified DHA⁶⁶.

The 2 studies of ALA reported smaller net changes in LDL, but it is not clear that this represents a real difference in effect.

Exposure Duration

In 3 studies, LDL levels varied by similar amounts in treatment and control arms at multiple time points^{49,53,69}. No differences in effect were seen at times ranging from 8 weeks to 2 years. No effect across studies is evident based on duration of intervention or exposure.

Sustainment of Effect

No study reported data on an effect after ceasing omega-3 fatty acid treatment.

Lipids: High Density Lipoprotein

(Table 3.4)

High density lipoprotein (HDL) plays a primary function in removing lipids from the bloodstream to be processed in the liver. Therefore, people with reduced levels of HDL are at increased risk of CVD independent of LDL or Tg levels. The new NCEP guidelines categorize an HDL level of less than 40 mg/dL as low, implying an increased risk of CVD⁵⁹. Commonly used and well-tolerated drugs for dyslipidemia generally have at most a modest effect on HDL levels. Lifestyle changes, including physical exercise and low saturated fat diets are generally recommended to help increase HDL. Of interest is whether consuming omega-3 fatty acids as part of a therapeutic lifestyle change would help improve HDL levels, or at least that it would not be detrimental.

Of the 25 randomized trials with lipid data for at least 60 subjects in parallel trials and 40 subjects in crossover trials who consumed omega-3 fatty acids 19 reported data on HDL (See Table 3.1).

Table 3.4 Effects of omega-3 fatty acids on high density lipoprotein (mg/dL) in randomized trials (6 weeks to 2 years)

Author, Year	Omega-3 Fatty Acid Arm ^a				Control		Results ^b			Quality ^c			Applicability ^d
	N	Source	g/d		N	Source	Base	Net Δ	P	Summary	Jadad	Allocation Conceal	
DHA/EPA Oils													
Cairns, 1996	325	Fish oil	ED	5.4	328	Corn oil	40	0	NS	B	3	Un	CVD II
Bonaa, 1992	70	Fish oil	ED	5.1	69	Corn oil	51	-1	NS	B	4	Un	DysLip I
Lungershausen, 1994	42 ^e	Fish oil	ED	4.9	42 ^e	Corn oil	40	+1	NS	B	3	Un	CVD II
Grimsgaard, 1997	75	Purified EPA	E	3.8	77	Corn oil	51	+1	NS ^f	A	5	Un	GEN I
	72	Purified DHA	D	3.7			53	+3	.0005 ^f				
Bairati, 1992b	66	Fish oil	ED	4.5	59	Olive oil	40	+4	<.05	B	5	Un	CVD II
Nilsen, 2001 ^g	119	Fish oil	ED	3.4	120	Corn oil	42	+5	<.05	B	3	Un	CVD II
Eritsland, 1995b	260	Fish oil	ED	3.3	251	No oil	41	+1	NS	B	2	Ad	CVD II
Brox, 2001 ^h	38	Cod liver oil	ED	3.3	37	No oil	50	0	NS	C	1	Un	DysLip I
	37	Seal oil	ED	2.6			50	+4	NS				
Franzen, 1993	92 ⁱ	Fish oil	ED	3.1	83 ⁱ	Olive	43	+2	nd	C	5	Ad	CVD II
	26	Cod liver oil	ED	3.1			48	+3	NS				
Osterud, 1995	27	Seal/Cod oil	ED	2.8	28	No oil	53	+4	<.05	B	2	Un	GEN I
	27	Seal oil	ED	2.4			51	+2	NS				
	26	Whale oil	ED	1.7			49	+5	<.005				
Leigh-Firbank, 2002	55 ^e	Fish oil	ED	3.0	55 ^e	Olive oil	37	0	NS	B	3	Un	DysLip I
Sacks, 1994	60 ^j	Fish oil	ED	2.4	66 ^j	Olive oil	46	+2	NS	C	3	Un	GEN I
Angerer, 2002	87	Fish oil	ED	1.7	84	Fatty acid	51	-3	NS	B	4	Ad	CVD II
GISSI, 1999	2836	Fish oil	ED	0.9	2828	No oil	42	0	NS	B	3	Un	CVD II
	2830	Fish oil ^k			2830	Vitamin E	42	0					
Leng, 1998	37 ^l	Fish oil	ED	0.045 ^m	36 ⁿ	Sunflower oil	45	+1	NS	C	4	Ad	CVD II
Fish and Mediterranean Diets													
Singh, 2002	499	Indo-Mediterranean T		1.8	501	NCEP I ^o	45	+2	<.0001	C	2	Un	CVD risk ^p III
de Lorgeril, 1994	171 ^q	Mediterranean/Canola margarine	A	0.8% Kcal	168 ^r	Regular	45	-1	NS	C	2	Un	CVD II
Combinations													
Mori, 1994	17	Fish oil & Fish diet ^s	ED	5.2	18	Olive/Palm/Safflower 40% fat diet	48 ^t	+3 ^u	<.01	B	2	Un	CVD II
	16	Fish oil	ED	4.2				+2 ^u	<.05				
	17	Fish diet ^s & Placebo oil	ED	3.0				+3 ^u	<.001				
	17	Fish oil	ED	2.1				+4 ^u	<.01				
	18	Fish diet ^s & Placebo oil	ED	3.0	17	Oil 30% fat	+3 ^u	<.05					
Finnegan, 2003	31	Fish oil margarine/ Fish oil	ED	1.7	30	Sunflower margarine	52	+2	NS	A	4	Un	DysLip I
	30	Fish oil margarine	ED	0.8			53	+3	NS				
	30	Rapeseed/Linseed margarine	A	4.5			50	+1	NS				

nd = no data

^a A = ALA; D = DHA; E = EPA; ED = EPA+DHA; T = Total omega-3 fatty acids.

- b Base = baseline level in treatment arm; Net Δ = net difference in change in omega-3 fatty acids arm compared with the change in control arm, see Methods; P = P value of difference between treatment and control arms; NS = not statistically significant.
- c A = good quality; B = fair quality; C = poor quality; Jadad = Jadad Score (0-5, based on randomization, double blinding, and dropouts); Ad = adequate allocation concealment; In = inadequate allocation concealment; Un = allocation concealment unclear. See Methods.
- d CVD = history of cardiovascular disease; DM I/II = diabetes mellitus type 1 or 2; DysLip = dyslipidemia; GEN = general, healthy population; N/IDDM = (non-) insulin dependent diabetes mellitus. I = broadly applicable; II = applicable to subgroup; III = narrow applicability. See Methods.
- e Cross-over study.
- f P = .009 for difference in effect of EPA and DHA.
- g All subjects regardless of whether statin treatment changed during study.
- h Data missing from article provided by study author.
- i Maximum. Total analyzed was 172, not 175 (92+83).
- j 84 at baseline.
- k Plus vitamin E 300 mg.
- l Baseline data based on N=52.
- m Plus 280 mg gamma linolenic acid (omega-6 fatty acid).
- n Baseline data based on N=50.
- o National Cholesterol Education Program step I prudent diet.
- p One or more of: hypercholesterolemia, hypertension, diabetes, angina pectoris or myocardial infarction.
- q Baseline data based on 289 subjects.
- r Baseline data based on 295 subjects.
- s Omega-3 fatty acid from fish diet is approximate, assuming that each of 4 different fish was consumed equally.
- t Mean baseline value for all subjects combined.
- u Estimated from graph.

Overall Effect ^{48,49,52,53,60,62-66,68-71,73-76,79}

The effect of omega-3 fatty acid consumption was generally consistent across the 19 studies. Most found a small net increase in HDL with treatment of up to 3 to 5 mg/dL, although 7 found a small net decrease or no effect in at least one tested study arm. Six of the studies reported that the net increase in HDL was statistically significant.

Sub-populations

Across studies, there is no clear difference in effect among the 11 studies of CVD populations, the 4 studies of dyslipidemic patients, the 3 studies of healthy subjects, or the study of Indians at increased risk of CVD. No study included only diabetic patients.

Covariates

No subgroup analyses based on covariates were reported. Two studies performed regressions. Bairati et al. reported that the effect of fish oil supplements on HDL (a net increase) was reduced and became borderline non-significant ($P = .06$) after adjusting for age, sex, baseline lipid level, lipid treatment, body mass index and alcohol use⁶⁰. Mori et al. performed a regression adjusting for change in weight and found a highly significant "group effect" increase in HDL with omega-3 fatty acids ($P < .001$)⁷¹. In contrast with their findings for total cholesterol, they reported similar effects on HDL among subjects on a 40% fat diet and those on a 30% fat diet.

Dose and Source Effect

Three studies compared different sources – and doses – of marine oil supplements^{62,66,74}. Grimsgaard et al. found a small difference in effect between purified EPA and DHA, but the net increase in HDL was significantly larger in men consuming DHA than those consuming EPA⁶⁶. In studies by Brox et al. and Osterud et al., somewhat different net effects were seen with the different types of oils; however, neither study reported on whether the oils differed from each other on their effect on HDL^{62,74}. No dose effect of marine oil supplements was evident across the studies.

Mori et al. found no difference in effect among men consuming various doses of EPA+DHA either as supplements or as dietary fish⁷¹. All doses and sources of omega-3 fatty acids resulted in significant increases in HDL. Finnegan et al. reported no difference in effect with different omega-3 fatty acid treatments⁵³.

Only 2 studies tested ALA supplementation, with minimal effect.

Exposure Duration

Five studies reported data on time trends of HDL levels. Leng et al., de Lorgeril et al. and Finnegan et al. reported no difference in HDL levels at multiple time periods between 8 weeks and 2 years.^{49,53,69} In contrast, Nilsen et al. reported a steady increase in HDL in patients with recent myocardial infarctions who started fish oil supplements at 6 weeks (+8%), 6 months (+14%), and 12 months (+19%); patients on corn oil had variable HDL levels (-0.3%, +4%, and +7%, respectively). Sacks et al. reported that HDL levels were unchanged at 3 months in healthy subjects taking fish oil supplements compared to control – decreasing by about 1.5 mg/dL in both – but that HDL returned to baseline at 6 months, resulting in a small net difference compared to control. No clear effect across studies is evident based on duration of intervention or exposure.

Sustainment of Effect

No study reported data on an effect after ceasing omega-3 fatty acid treatment.

Lipids: Triglycerides

(Table 3.5, Figures 3.1 and 3.2)

Elevated levels of triglycerides (Tg) are increasingly being recognized as a risk factor for CVD, independent of other serum lipids. Elevated Tg are most frequently seen in patients with the metabolic syndrome, although various secondary and genetic factors can raise Tg. The recent NCEP guidelines recommend a goal for fasting Tg of less than 150 mg/dL⁵⁹. Fish oil's ability to lower Tg is considered one of the leading mechanisms by which omega-3 fatty acid consumption lowers CVD risk⁸⁰.

Of the 25 randomized trials with lipid data for at least 60 subjects in parallel trials and 40 subjects in crossover trials who consumed omega-3 fatty acids 19 reported data on Tg (See Table 3.1).

Overall Effect ^{48,49,52,53,60,63-68,70,71,73,74,76,77,79,81}

With few exceptions, Tg levels in the 19 studies decreased by substantial amounts in subjects taking omega-3 fatty acids, both in absolute amount and compared to control groups. The changes in Tg were generally highly significant.

Table 3.5 Effects of omega-3 fatty acids on triglycerides (mg/dL) in randomized trials (6 weeks to 2 years)

Author, Year	Omega-3 Fatty Acid Arm ^a			Control		Results ^b		Quality ^c			Applicability ^d	
	N	Source	g/d	N	Source	Base	Net Δ	P	Summary	Jadad		Allocation Conceal
DHA/EPA Oils												
Cairns, 1996	325	Fish oil	ED 5.4	328	Corn oil	235	-64	<.05	B	3	Un	CVD II
Bonaa, 1992	72	Fish oil	ED 5.1	72	Corn oil	124	-23	<.01	B	4	Un	DysLip I
Lungershausen, 1994	42 ^e	Fish oil	ED 4.9	42 ^e	Corn oil	150	-19	<.01	B	3	Un	CVD II
Bairati, 1992b	66	Fish oil	ED 4.5	59	Olive oil	204	-80	<.0001	B	5	Un	CVD II
Grimsgaard, 1997	75	Purified EPA	E 3.8	77	Corn oil	109	-23	.0001 ^f	A	5	Un	GEN I
	72	Purified DHA	D 3.7			110	-29	.0001 ^f				
Nilsen, 2001 ^g	61	Fish oil (men)	ED 3.4	61	Corn oil	140	-50	.001	B	3	Un	CVD II
	12	Fish oil (women)	ED 3.4	13		123	-71	.07				
Eritsland, 1995b	260	Fish oil	ED 3.3	251	No oil	172	-32	<.0001	B	2	Ad	CVD II
Franzen, 1993	92 ^h	Fish oil	ED 3.1	83 ^h	Olive	158	-34	nd	C	5	Ad	CVD II
	26	Cod liver oil	ED 3.1			113	-28	<.05				
	27	Seal/Cod oil	ED 2.8			114	-21	NS				
	27	Seal oil	ED 2.4			106	-14	NS				
Osterud, 1995	26	Whale oil	ED 1.7	28	No oil	97	-9	NS	B	2	Un	GEN I
Leigh-Firbank, 2002	55 ^e	Fish oil	ED 3.0	55 ^e	Olive oil	221	-74	<.001	B	3	Un	DysLip I
Maresta, 2002	125	Fish oil	ED 2.6 ⁱ	132	Olive oil	160	+5	NS	B	3	Un	CVD II
Sirtori, 1998	459	Fish oil	ED 1.7 ^j	450	Olive oil	294 ^k	-63	<.0001	B	4	Ad	CVD risk ^L I
Angerer, 2002	87	Fish oil	ED 1.7	84	Fatty acid	194	-22	NS	B	4	Ad	CVD II
GISSI, 1999	2836	Fish oil	ED 0.9	2828	No oil	163	-8	<.05	B	3	Un	CVD II
	2830	Fish oil ^m	ED 0.9	2830	Vitamin E	160	-6					
Fish and Mediterranean Diets												
Singh, 2002	499	Indo-Mediterranean	T 1.8	501	NCEP I ⁿ	163	-22	<.0001	C	2	Un	CVD risk ^o III
Hanninen, 1989	19	Fish (3.8/week)	ED 0.9	18	0.4 Fish/week	81	-14	nd ^p	B	2	Un	GEN III
	22	Fish (2.3/week)	ED 0.5			81	-12	nd ^q				
	21	Fish (1.5/week)	ED 0.4			60	-8	nd ^q				
	20	Fish (0.9/week)	ED 0.2			69	+4	NS				
de Lorgeril, 1994	171 ^r	Mediterranean/Canola margarine	A 0.8% Kcal	168 ^s	Regular	190	-19	NS	C	2	Un	CVD II
Combinations												
Mori, 1994	17	Fish oil & Fish diet ^t	ED 5.2	18	Olive/Palm/Safflower	154 ^u	-65 ^k	<.001	B	2	Un	CVD II

Author, Year	Omega-3 Fatty Acid Arm ^a			Control		Results ^b			Quality ^c			Applicability ^d
	N	Source	g/d	N	Source	Base	Net Δ	P	Summary	Jadad	Allocation Concealment	
	16	Fish oil	ED 4.2		40% fat diet	-56 ^k	<.01					
	17	Fish diet ^t & Placebo oil	ED 3.0			-32 ^k	<.001					
	17	Fish oil	ED 2.1			-21 ^k	<.05					
	18	Fish diet ^t & Placebo oil	ED 3.0	17	Oil 30% fat	-37 ^k	<.01					
Finnegan, 2003	31	Fish oil margarine / Fish oil	ED 1.7	30	Sunflower margarine	142	-10	NS	A	4	Un	DysLip I
	30	Fish oil margarine	ED 0.8			146	+6	NS				
	30	Rapeseed/Linseed margarine	A 4.5			147	+23	NS				

nd = no data

a A = ALA; D = DHA; E = EPA; ED = EPA+DHA; T = Total omega-3 fatty acids.

b Base = baseline level in treatment arm; Net Δ = net difference in change in omega-3 fatty acids arm compared with the change in control arm, see Methods; P = P value of difference between treatment and control arms; NS = not statistically significant.

c A = good quality; B = fair quality; C = poor quality; Jadad = Jadad Score (0-5, based on randomization, double blinding, and dropouts); Ad = adequate allocation concealment; In = inadequate allocation concealment; Un = allocation concealment unclear. See Methods.

d CVD = history of cardiovascular disease; DM I/II = diabetes mellitus type 1 or 2; DysLip = dyslipidemia; GEN = general, healthy population; N/IDDM = (non-) insulin dependent diabetes mellitus. I = broadly applicable; II = applicable to subgroup; III = narrow applicability. See Methods.

e Cross-over study.

f Non-significant difference in effect between EPA and DHA.

g Only subjects who did not change statin treatment are included here.

h Maximum. Total analyzed was 172, not 175 (92+83).

i 5.1 g for 1 month before and 1 month after PTCA, then reduced to 2.6 g for an additional 5 months.

j 2.6 g/day for first 2 months, then 1.7 g/day for 4 months.

k Estimate from graph.

L Dyslipidemia and one or more of: hypertension, diabetes, or glucose intolerance.

m Plus vitamin E 300 mg.

n National Cholesterol Education Program step I prudent diet.

o One or more of: hypercholesterolemia, hypertension, diabetes, angina pectoris or myocardial infarction.

p P<.02 change from baseline.

q P<.10 change from baseline.

r Baseline data based on 289 subjects.

s Baseline data based on 295 subjects.

t Omega-3 fatty acid from fish diet is approximate, assuming that each of 4 different fish was consumed equally.

u Mean baseline value for all subjects combined.

Sub-populations

The 3 studies of healthy subjects, whose mean Tg levels were normal, generally found net decreases in Tg levels of about 10% to 25%. Eleven studies included subjects with a variety of types of CVD, all with mean Tg levels above 150 mg/dL. With the exception of Maresta et al., the 11 studies reported net decreases in Tg of between about 10% to 30%, most of which were statistically significant⁸¹. There was no obvious difference between the study by Maresta et al. of patients undergoing PTCA and other studies to explain the discordant finding.

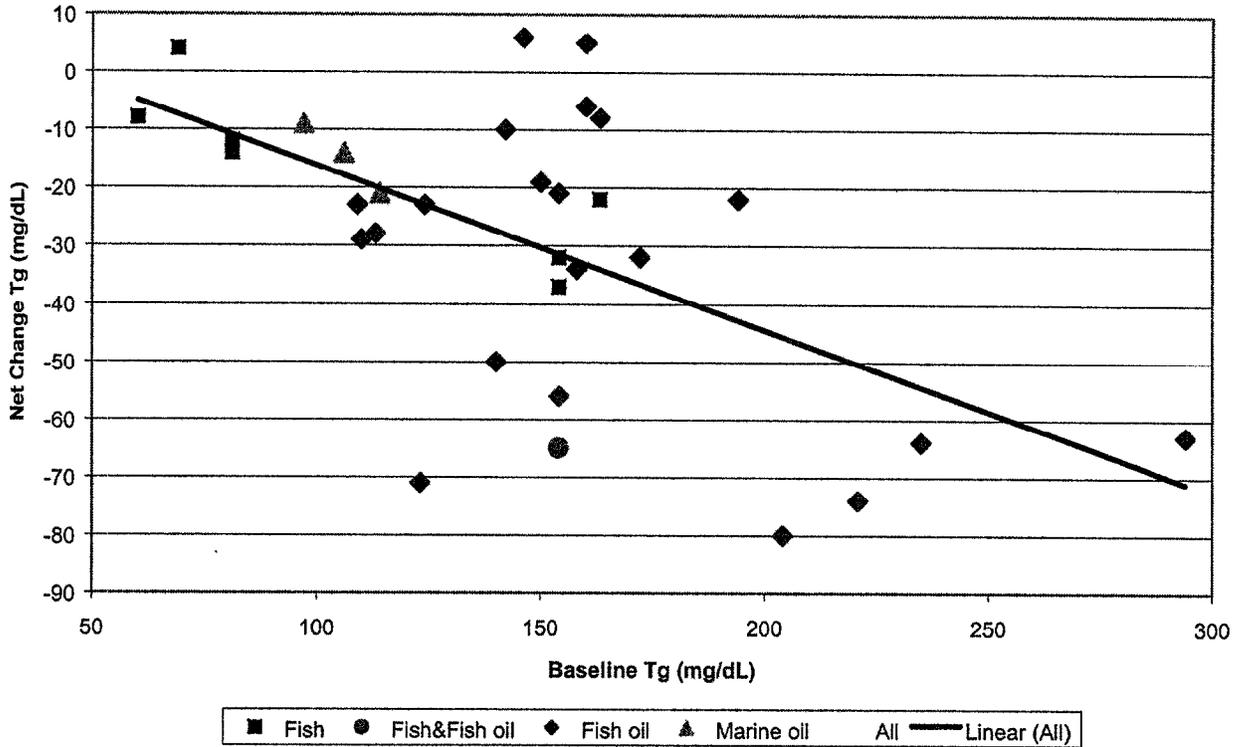
Two studies evaluated subjects at increased risk of CVD with different sets of treatments. Both of these studies found large, significant reductions in Tg. Two of 3 studies of dyslipidemic

patients reported large net decreases in Tg of 20% or 33%. Finnegan et al., in a study of moderately hyperlipidemic patients, found different effects of omega-3 fatty acid consumption on Tg depending on dose and source⁵³. No study evaluated a population of only diabetic subjects.

Covariates

Nilsen et al. found similar decreases in Tg among men and women, where the difference in significance level can be ascribed mostly to sample size⁷³. Two studies that performed regressions both found no substantial change in the significant Tg reduction after adjusting for age, sex, baseline lipid level, lipid treatment, body mass index and alcohol use⁶⁰ or change in weight⁷¹. Grimsgaard et al. reported the effect of purified EPA and DHA on Tg in quartiles of baseline Tg⁶⁶. While the authors did not discuss whether the effect of omega-3 fatty acids was associated with baseline Tg level, there does appear to be a trend toward greater reduction of Tg in subjects with higher baseline Tg. Those in the lowest quartile had a net reduction of approximately 7 mg/dL (10 – 14%); those in the middle two quartiles had net reductions of between 15 mg/dL and 27 mg/dL (14 – 30%); and those in the highest quartile (128 mg/dL – 319 mg/dL) had net decreases in Tg of about 50 mg/dL (about 28%). Across studies, the average net decrease in Tg level was larger in studies with higher mean baseline levels, as indicated by Figure 3.1, in which the meta-regression is not adjusted for dose of omega-3 fatty acid or study size. After adjusting for dose and the study variance, the association across studies remains statistically significant. In a separate analysis comparing different percentages of fat in the diet, Mori et al. also found nearly identical effects in subjects on 30% or 40% fat diets who were consuming similar amounts of omega-3 fatty acids⁷¹.

Figure 3.1 Meta-regression of baseline triglyceride (Tg) level versus net change in Tg. Each point represents an individual study or study arm. Marine oils include non-fish animal sources including Minke whale and seal. Regression not adjusted for dose of omega-3 fatty acid or study size.

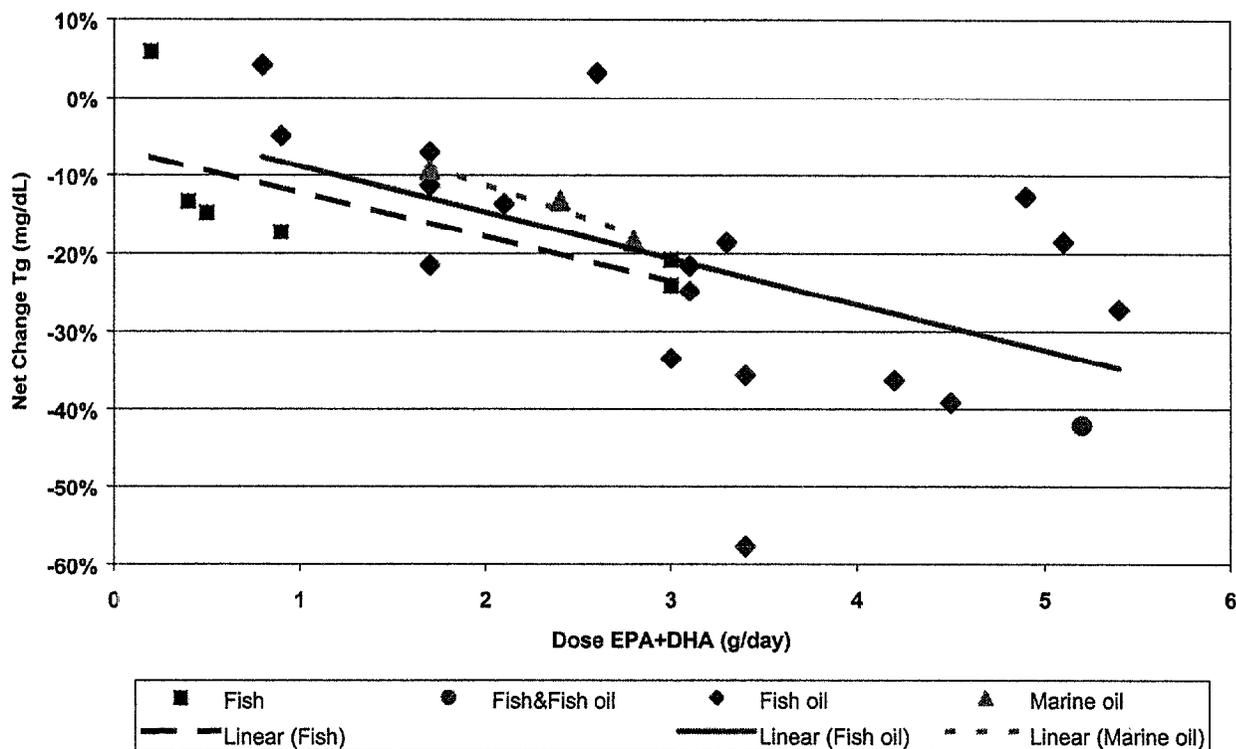


Dose and Source Effect

The 4 studies that compared different doses of marine oil supplements found that the greatest net decrease in Tg level occurred in study arms receiving the highest dose of EPA+DHA, although none of the articles reported whether there was a significant trend within the study. Across studies there was a clear trend toward greater percent decrease in Tg with higher doses, regardless of source (Figure 3.2). At least a 10% reduction in Tg was found in most studies with doses of at least 1.7 g per day of marine oil supplementation. Most study arms with doses of at least 3 g per day of marine oil supplements resulted in at least a 20% reduction in Tg. Among the studies of dietary fish, only the 2 arms with high omega-3 fatty acid fish diets in Mori, et al. achieved at least a 20% reduction of Tg⁷¹.

Grimsgaard et al., overall, found no difference in effect between purified EPA and purified DHA, although the net decreases in Tg were consistently greater in the DHA group than in the EPA group across quartiles of baseline Tg⁶⁶. Across studies, and within the Mori et al. study⁷¹, the source of the EPA+DHA, whether as a supplement or from dietary fish, does not appear to make a difference. In contrast, the effect of ALA is uncertain. The single study that evaluated pure ALA supplementation, Finnegan et al., found increases in Tg levels in subjects on both 4.5 g and 9.5 g per day of ALA margarine (the latter dose is not included in the summary table)⁵³. Both Singh et al. and de Lorgeril et al. provided ALA in the context of a Mediterranean diet, which also included higher dietary fish intake^{49,76}.

Figure 3.2 Meta-regression of dose of EPA+DHA intake versus net change in triglycerides (Tg). Each point represents an individual study or study arm. Separate simple regressions were performed for each oil source type (except for the individual study arm of combined fish and fish oil). Marine oils include non-fish animal sources including Minke whale and seal. Regression not adjusted for baseline Tg or study size.



Exposure Duration

The effect of duration of intervention or exposure was somewhat inconsistent among the 4 studies that reported data on Tg levels at different time points in studies of omega-3 fatty acids. Hanninen et al. found progressive decreases of Tg at 5 and 12 weeks in group of subjects consuming higher amounts of fish⁶⁷. Similarly, Nilsen et al found progressive decreases in men, but not in a small group of women, at 6 weeks, 6 months and 12 months⁷³. Sirtori et al. found that the effect of lower dose fish oil supplementation to reduce Tg occurred by 2 months and remained stable at 4 and 6 months⁷⁷. In contrast, Finnegan et al. reported a significant decrease (15%) in mean Tg levels after 2 months which was not sustained at 6 months in the EPA+DHA arms⁵³. Across studies, there is no apparent correlation between study duration and fish oil supplement effect, even after grouping studies by fish oil dosage.

Sustainment of Effect

No study reported data on an effect after ceasing omega-3 fatty acid treatment.

Lipoprotein(a)

(Table 3.6)

Lipoprotein(a) [Lp(a)] consists of an LDL core covalently bound to a plasminogen-like glycoprotein, apolipoprotein(a)⁸². Elevated levels of Lp(a) are an independent risk factor for atherosclerotic disease, possibly by promoting thrombosis. Lp(a) levels are largely determined by genetic polymorphism, specifically the number of K-IV repeats. Steroid hormones, and thus menopause, affect levels. There is a very large range of Lp(a) levels, from less than 0.1 mg/dL to more than 300 mg/dL and the distribution can be highly skewed. Treatments available to lower Lp(a) levels include niacin and hormone replacement therapy (in post-menopausal women).

We found 23 studies that met eligibility criteria and reported data on the effect of omega-3 fatty acids on Lp(a) levels (See Table 3.1). Of these, we analyzed the 14 randomized trials. All but 2 were parallel trials. The source of fatty acids was marine oil supplements in 12 studies, dietary fish in 1 study and Mediterranean diet in 1 study.

Overall Effect ^{49,55,58,62,83-92}

Across the 14 studies there is no consistent effect on Lp(a) levels of omega-3 fatty acid consumption compared to control. In approximately one-third of the studies the omega-3 fatty acid study arms had a net increase in Lp(a) level compared to control; in the remaining studies the net decrease in Lp(a) level was generally small and non-significant. Only 2 studies reported a statistically significant difference between the effect of omega-3 fatty acid and control, both of which found a net decrease in Lp(a). However, the variability of Lp(a) levels among subjects within all the studies resulted in wide confidence intervals which limited the likelihood of statistically significant findings.

Sub-populations

The 5 studies that evaluated generally healthy subjects found no consistent effect of omega-3 fatty acids on Lp(a). Marckmann et al. found a large net increase of Lp(a) with fish oil supplement use and Deslypere et al. found a large net increase of Lp(a) in 1 of 3 treatment arms^{85,89}. The remaining studies (and study arms) reported generally small effects, which were not uniform in direction. Five studies evaluated subjects with known CVD, one of which included only patients with hypertriglyceridemia on simvastatin. The apparent large decrease in Lp(a) in the latter study, Durrington et al., occurred because the median Lp(a) level rose by less in the fish oil supplement group than the corn oil group⁸⁶. Again no consistent effect was seen. In the only study of diabetic subjects, Luo et al. found a statistically significant net reduction of Lp(a) of about 20% with fish oil supplementation⁸⁸. The 4 studies of subjects with dyslipidemia (including the one with subjects with CVD on simvastatin) all found that subjects on marine oil supplements had a net decrease in Lp(a) compared to control; however, none of the changes was significant.

Table 3.6 Effects of omega-3 fatty acids on lipoprotein (a) (mg/dL) in randomized trials (4 weeks to 14 months)

Author, Year	Omega-3 Fatty Acid Arm ^a			Control		Results ^b		Quality ^c			Applicability ^d
	N	Source	g/d	N	Source	Base	Net Δ P	Summary	Jadad	Allocation Conceal	
DHA/EPA Oils											
Destypere, 1992	14	Fish oil	T 3.4	14	Olive oil	22.5	+10.3 NS	B	2	Un	GEN I
	15	Fish oil	T 2.2			27.2	+2.3 NS				
	15	Fish oil	T 1.1			22.1	+4.9 NS				
Alaswad, 1999	11	Fish oil	ED 3.4	12	Calcium gluconate	7.8	-1.1 NS	B	2	Un	DysLip II
Prisco, 1994	10	Fish oil	ED 3.4	10	Olive oil	10.2	-0.9 NS	B	3	Un	GEN II
Eritsland, 1995a	214 ^e	Fish oil	ED 3.3	219 ^e	No oil	[5.5]	[0] NS	B	2	Ad	CVD II
	66 ^f			50 ^f		[29.7]	[-1.5] .02				
Brox, 2001 ^g	38	Cod liver oil	ED 3.3	37	No oil	18.5	-1.7 NS	C	1	Un	DysLip I
	37	Seal oil	ED 2.6			16.3	-1.9 NS				
Durrington, 2001	30	Fish oil	ED 3.2	29	Corn oil	[10.5]	[-6.8] NS	A	4	Un	CVD DysLip II
Conquer, 1999	9	Seal oil	ED 3.0	10	Evening primrose	1.6	+0.1 NS	A	4	Un	GEN II
Swahn, 1998	26	Fish oil	ED 2.9	27	Corn oil	30.8	-0.7 NS	B	5	Un	CVD II
Hamazaki, 1996	13	DHA-rich Fish oil	ED 1.7-2.0 ^h	11	Corn oil	120	0 NS	B	4	Un	GEN II
Luo, 1998	10 ⁱ	Fish oil	ED 1.8	10 ⁱ	Sunflower	17	-3 <.02	B	3	Un	DM II II
Marckmann, 1997	22	Fish oil margarine	T 0.9	24	Sunflower margarine	[3.6]	[+3.0] NS	B	3	Un	GEN II
Nenseter, 2000	34	Fish powder	ED 0.2	36	Cellulose	[13.5]	[-0.8] NS	B	3	Un	DysLip I
Fish and Mediterranean Diets											
de Lorgeril, 1994	171 ^j	Mediterranean/ Canola margarine	A 0.8% Kcal	168 ^k	Regular	28	+6 NS	C	2	Un	CVD II
Schaefer, 1996	11	High fish	ED 0.7% Kcal	11	Low fish	38	-3 NS	C	1	Un	GEN I

a A = ALA; D = DHA; E = EPA; ED = EPA+DHA; T = Total omega-3 fatty acids.

b Base = baseline level in treatment arm. Numbers in brackets are median values.; Net Δ = net difference in change in omega-3 fatty acids arm compared with the change in control arm; for studies with numbers in brackets, the net difference was estimated by subtracting the final median value from the baseline median value; see Methods; P = P value of difference between treatment and control arms; NS = not statistically significant.

c A = good quality; B = fair quality; C = poor quality; Jadad = Jadad Score (0-5, based on randomization, double blinding, and dropouts); Ad = adequate allocation concealment; In = inadequate allocation concealment; Un = allocation concealment unclear. See Methods.

d CVD = history of cardiovascular disease; DM I/II = diabetes mellitus type 1 or 2; DysLip = dyslipidemia; GEN = general, healthy population; N/IDDM = (non-) insulin dependent diabetes mellitus. I = broadly applicable; II = applicable to subgroup; III = narrow applicability. See Methods.

e Baseline Lp(a) < 20 mg/dL.

f Baseline Lp(a) ≥ 20 mg/dL.

g Data missing from article provided by study author.

h Depending on body weight.

i Cross-over study.

j Baseline data based on 289 subjects.

k Baseline data based on 295 subjects.

Eritsland et al. found that the effect on Lp(a) was not related to age or sex⁸⁷. The 2 studies that excluded pre-menopausal women both found small, non-significant, net reductions in mean Lp(a) with fish oil supplements or fish diet^{58,83}. The 4 studies of men generally found small, non-significant, net increases in Lp(a)^{84,85,89,91}. No study included only women.

Covariates

As shown in the summary table, Eritsland et al. found a differential effect of omega-3 fatty acids based on baseline Lp(a) level in patients referred for coronary artery bypass graft surgery⁸⁷. Those with Lp(a) in the upper quintile (≥ 20 mg/dL) had a small but significant absolute and net reduction in Lp(a), while the remaining subjects did not. A similar comparison between subjects with elevated baseline Tg (≥ 245 mg/dL) and those with lower Tg found no difference in effect.

Dose and Source Effect

Only 2 studies directly compared different doses of fish oil supplements or different oils. Deslypere et al. reported no effect on Lp(a) at any of 3 doses of fish oil supplements, although the mean Lp(a) level rose by almost 50% after 1 year in subjects on the highest dose⁸⁵. Brox et al. found no difference between similar doses of cod liver oil and seal oil supplements⁶². Across studies no differences could be discerned based on marine oil dose or omega-3 fatty acid-rich diet.

Exposure Duration

Two studies reported Lp(a) data at different time periods. de Lorgeril et al. found no difference in effect on Lp(a) at 8, 52, and 104 weeks in a study of Mediterranean diet⁴⁹. Prisco et al. also found no difference in effect at 2 and 4 months in a study of fish oil supplements⁹¹. Across studies there is no apparent relationship between effect and duration of intervention or exposure.

Sustainment of Effect

Both Prisco et al. and Deslypere et al. reported no difference between Lp(a) levels while subjects were on fish oil supplements and at multiple time points up to 6 months after stopping supplementation^{85,91}.

Apolipoprotein A-I

(Table 3.7)

Apolipoprotein A-I (apo A-I) is the major apolipoprotein of HDL. It serves as a cofactor for enzymes that metabolize HDL in plasma. Apo A-I levels are strongly correlated with HDL cholesterol levels, but ratios of HDL to apo A-I do vary. While the effect of omega-3 fatty acids on lipoprotein-associated cholesterol and apolipoprotein assays are of interest, unlike cholesterol

levels, apolipoprotein assays, which are antibody specific and are not standardized, are not as amenable to cross-study comparisons. Furthermore, there are no data to suggest that apolipoprotein levels are more predictive of CVD risk than lipoprotein cholesterol levels.

We found 61 studies that met eligibility criteria and reported data on the effect of omega-3 fatty acids on apo A-I levels (See Table 3.1). Of these, we analyzed the 27 randomized trials with data on at least 20 subjects in parallel trials and 15 subjects in crossover trials who consumed omega-3 fatty acids.

Table 3.7 Effects of omega-3 fatty Acids on apolipoprotein A-I (mg/dL) in randomized trials (4 weeks to 2 years)

Author, Year	Omega-3 Fatty Acid Arm ^a			Control		Results ^b			Quality ^c			Applicability ^d
	N	Source	g/d	N	Source	Base	Net Δ	P	Summary	Jadad	Allocation Conceal	
DHA/EPA Oils												
Wilt, 1989	38 ^e	Fish oil	ED 6.0	38 ^e	Safflower	151	-4	NS	B	4	Un	DysLip III
Green, 1990	27 ^e	Fish oil	ED 5.2	27 ^e	Corn/Olive	113	-10	NS	B	4	Un	DysLip II
Bonaa, 1992	71	Fish oil	ED 5.1	74	Corn oil	155	-7	<.05	B	4	Un	DysLip I
Balestrieri, 1996	14 ^e	Fish oil	ED 5.1	38 ^e	Olive oil	116	-2	NS	B	3	Un	DysLip III
Jensen, 1989	18 ^e	Cod liver oil	ED 4.6	18 ^e	Olive oil	134	-6	NS	B	4	Un	IDDM II
Sirtori, 1992	12 ^e	Fish oil	ED 4.5	12 ^e	No oil	132	-4	nd	C	2	Un	DysLip II
Schechtman, 1989 ^f	18 ^e	Fish oil	ED 4.0	18 ^e	Safflower	117	-5	NS	B	2	Un	DysLip II
Schechtman, 1988 ^f	13 ^e	Fish oil	ED 4.0	13 ^e	Safflower	114	-10	NS	B	2	Un	NIDDM II
Grimsgaard, 1997	75	Purified EPA	E 3.8	77	Corn oil	138	-4	.02 ^g	A	5	Un	GEN I
	72	Purified DHA	D 3.7			138	+2	NS ^g				
Harris, 1997	22	Fish oil	ED 3.4	20	Corn oil	132	+1	NS	B	3	Un	DysLip II
Nordoy, 1998	21	Fish oil	ED 3.4	20	Corn oil	142	+1	NS	B	4	Un	DysLip I
Deslypere, 1992	14	Fish oil	T 3.4	14	Olive oil	137	-17	NS	B	2	Un	GEN III
	15	Fish oil	T 2.2			139	-7	NS				
	15	Fish oil	T 1.1			137	-9	NS				
Chan, 2002	12	Fish oil	ED 3.4	12	Corn oil	118	+5	NS ^h	B	3	Un	DysLip II
	11	Fish oil & Atorvastatin		12	Corn oil & Atorvastatin	128	+3					
Eritsland, 1995b	178	Fish oil	ED 3.3	174	No oil	124	+2	NS	B	2	Ad	CVD II
Brox, 2001 ⁱ	38	Cod liver oil	ED 3.3	37	No oil	160	0	NS	C	1	Un	DysLip I
	37	Seal oil	ED 2.6			160	+10 ^j	NS				
Durrington, 2001	30	Fish oil	ED 3.2	29	Corn oil	90	-6	NS	A	4	Un	CVD DysLip II
McGrath, 1996	23 ^e	Fish oil	ED 3.0	23 ^e	Olive oil	119	+2	NS	A	4	Un	DM II II
Nikkila, 1991	32 ^e	Fish oil	ED 2.4	32 ^e	Corn oil	109	-2	NS	B	3	Un	CVD DysLip II
Luo, 1998	10 ^e	Fish oil	ED 1.8	10 ^e	Sunflower	148	+1	NS	B	3	Un	DM II II
Marckmann, 1997	23	Fish oil margarine	T 0.9	24	Sunflower margarine	149	-2	NS	B	3	Un	GEN II

Continued

Table 3.7 Effects of omega-3 fatty Acids on apolipoprotein A-I (mg/dL) in randomized trials (continued)

Author, Year	Omega-3 Fatty Acid Arm ^a			Control		Results ^b			Quality ^c			Applicability ^d
	N	Source	g/d	N	Source	Base	Net Δ	P	Summary	Jadad	Allocation Concealment	
Fish and Mediterranean Diets												
Hanninen, 1989	19	Fish (3.8/week)	ED 0.9	18	0.4 Fish/week	113	0	NS	B	2	Un	GEN III
	22	Fish (2.3/week)	ED 0.5			117	+2	NS				
	21	Fish (1.5/week)	ED 0.4			121	-9	NS				
	20	Fish (0.9/week)	ED 0.2			118	0	NS				
Agren, 1988	14	Fish (3.7/week)	ED 0.8	12	0.25 Fish/week	126	-14	<.01	B	3	Un	GEN III
	15	Fish & low SFA				123	-3	NS				
Agren, 1991	20	Fish (5/week)	ED 0.75	23	Regular ^k	149	+8	NS	B	2	Un	GEN III
	20	Fish (5/week) ^k				159	0	NS				
de Lorgeril, 1994	171 ^L	Mediterranean/Canola margarine	A 0.8% Kcal	168 ^m	Regular	124	-12	NS	C	2	Un	CVD II
Combinations												
Cobiac, 1991	13	Fish oil	ED 4.6	6	Olive, Palm, Safflower oil	117 ⁿ	+1	NS	B	2	Un	GEN II
	12	Fatty Fish diet	ED 4.5			120 ⁿ	0	NS				
Silva, 1996	20	Fish oil	ED 3.6	--	--	159	-28 ^o	nd ^p	B	3	Un	DysLip II
	15	Soya oil	A 0.8 ^q			184	-33 ^o	nd ^p				
Agren, 1996	14	Fish oil	ED 2.3	14	No oil	125	-8	NS	B	3	Un	GEN III
	14	Algae DHA oil	D 1.7			128	+1	NS				
	13	Fatty Fish diet	ED 1.1			120	+1	NS				

nd = no data

a A = ALA; D = DHA; E = EPA; ED = EPA+DHA; T = Total omega-3 fatty acids.

b Base = baseline level in treatment arm; Net Δ = net difference in change in omega-3 fatty acids arm compared with the change in control arm, see Methods; P = P value of difference between treatment and control arms; NS = not statistically significant.

c A = good quality; B = fair quality; C = poor quality; Jadad = Jadad Score (0-5, based on randomization, double blinding, and dropouts); Ad = adequate allocation concealment; In = inadequate allocation concealment; Un = allocation concealment unclear. See Methods.

d CVD = history of cardiovascular disease; DM I/II = diabetes mellitus type 1 or 2; DysLip = dyslipidemia; GEN = general, healthy population; N/IDDM = (non-) insulin dependent diabetes mellitus. I = broadly applicable; II = applicable to subgroup; III = narrow applicability. See Methods.

e Cross-over study.

f Unclear if 2 studies by Schectman et al.^{93,94} are independent of each other. Possible overlap of up to 6 subjects with NIDDM and hypertriglyceridemia.

g P=.0008 for difference in effect of EPA and DHA.

h Main effect.

i Data missing from article provided by study author.

j Only 2 significant digits reported. 10 mg/dL is smallest unit of change possible.

k Recommended aerobic exercise for 30 minutes 3 times a week.

L Baseline data based on 289 subjects.

m Baseline data based on 295 subjects.

n Units were not reported

o Pre-post difference (not compared to control).

p NS between treatments.

q No data on ALA amount. We assumed 7 g ALA per 100 g oil. 12 g oil.

Overall Effect 48,49,52,62,66,67,85,86,88,89,93-109

Across the 27 studies, effects of omega-3 fatty acids on apo A-I levels were generally heterogeneous but small. Most studies found a small net change in apo A-I with omega-3 fatty acid consumption. Three-quarters of studies found net changes between -5% and +5% (-7 to

+10 mg/dL). No study found a large net increase in apo A-I level. A small number of studies found larger net decreases of up to 18% reductions (-33 mg/dL).

Sub-populations

Eight studies evaluated healthy people, all single-sex groups (7 male^{66,85,89,95,97,100,110}, 1 female⁹⁶), mostly of university students. Four studies evaluated diabetic patients. Thirteen studies evaluated patients with dyslipidemia, 2 of which were also of patients with CVD. There was one additional study of patients with CVD. There were no clear patterns of treatment effect or differences in effect among the sub-populations.

Covariates

Silva et al. reported that sex, body mass index, hypertension, and non-insulin dependent diabetes did not affect the fish oil or soya oil supplement effect on lipid parameters including apo A-I in hyperlipidemic subjects¹⁰⁷. No other study evaluated correlations or sub-analyses based on apo A-I. Agren et al. (1988) compared the effect of daily fish with daily fish with a low saturated fat diet in male university students⁹⁵. Among subjects on a fish and low saturated fat diet, apo A-I levels remained essentially unchanged compared to those on a regular diet. In contrast, subjects on a fish diet who were not told to lower their saturated fat intake had a significant net decrease in apo A-I that was among the largest net decreases across studies. However, no comparison was made between the 2 treatment groups, nor were any explanations for the difference examined or discussed. Three studies compared fish oil to placebo oil supplements in dyslipidemic patients who were all taking either atorvastatin or simvastatin^{98,99,106}. The effects of fish oil supplementation on apo A-I were small in all 3 studies. The effects were not uniform in direction.

Dose and Source Effect

Neither Deslypere et al. nor Hanninen et al. reported a dose dependent effect on apo A-I of either fish oil supplements or different frequencies of fish meals^{67,85}. No dose effect was seen across studies of EPA+DHA either.

Five studies compared different sources of omega-3 fatty acids. Grimsgaard et al. found a small but significant net decrease in apo A-I with purified EPA compared to a smaller, non-significant, net increase with purified DHA; the difference between the 2 omega-3 fatty acids was statistically significant ($P = .008$)⁶⁶. Brox et al. compared 2 sources of marine oil supplements: cod liver and seal oil⁶². No effect was found with either treatment. Cobiac et al. found no treatment effect with either fish oil supplementation or with a fatty fish diet¹⁰⁰. Silva et al. found similarly large, significant reductions in apo A-I level in subjects taking either fish oil or soya oil supplements; however, no non-omega-3 fatty acid was used as a control¹⁰⁷. Agren et al. (1996) compared fish oil supplementation, algae DHA oil supplementation, and fatty fish diet and also found no difference in effect on apo A-I among the groups⁹⁷.

Exposure Duration

Two studies reported apo A-I levels at multiple time points. Neither Hanninen et al. nor de Lorgeril et al. found any time-related effects of omega-3 fatty acids on apo A-I, at 5 and 12 weeks, and 8, 52, and 104 weeks, respectively^{49,67}.

Sustainment of Effect

Three studies followed subjects after stopping the intervention. Jensen et al. and Deslypere et al. found no change in apo A-I levels 8 weeks and 6 months, respectively, after stopping fish oil supplements^{85,103}. In contrast, Agren et al. (1988) reported that 5 months after a 15 week trial of dietary fish apo A-I levels remained at lowered levels in the fish diet group who had no limitation of saturated fat; however, they do not indicate what these students' diets were at subsequent follow-up⁹⁵.

Apolipoprotein B, Apolipoprotein B-100, and LDL Apolipoprotein B

(Tables 3.8 and 3.9)

Apolipoprotein (apo) B has 2 major subtypes, B-100 and B-48. Apo B-100 is associated with lipoprotein particles of hepatic origin, specifically very low, intermediate, and low density lipoproteins (VLDL, IDL, LDL). Its major function is to serve as a ligand for the receptor that clears these particles from the bloodstream. During the conversion of VLDL to LDL in the circulation, only apo B-100 remains on LDL. Measures of LDL apo B represent the portion of total blood apoB-100 that is associated with the LDL subfraction. There is 1 apo B-100 molecule per LDL particle. A discordance in LDL apoB-100 and LDL cholesterol levels implies a change in the composition of the LDL particle. Total apo B is thus indicative of VLDL, IDL and LDL levels, while apo B-100 and LDL apo B are indicative specifically of LDL levels. While the effect of omega-3 fatty acids on lipoprotein-associated cholesterol and apolipoprotein assays are of interest, unlike cholesterol levels, apolipoprotein assays, which are antibody specific and are not standardized, are not as amenable to cross-study comparisons. Furthermore, there are no data to suggest that apolipoprotein levels are more predictive of CVD risk than lipoprotein cholesterol levels.

We found 52 studies that met eligibility criteria and reported data on the effect of omega-3 fatty acids on total apo B levels, and 11 studies that reported data on either apo B-100 or LDL apo B (See Table 3.1). Of these, we analyzed the 25 randomized trials of apo B that had data on at least 20 subjects in parallel trials and 10 subjects in crossover trials who consumed omega-3 fatty acids. We also analyzed the 10 studies of apo B-100 or LDL apo B, all of which were randomized.

Overall Effect

Total apo B (Table 3.8)^{48,49,53,66,67,71,85,86,88-90,93,95-101,103-106,108,109}. Across the 25 studies, we found little consistency in the effect of omega-3 fatty acids on apo B levels. About half the

studies found a small net increase and half a small net decrease in apo B levels. Only 2 studies found significant changes in individual study arms, but Deslypere et al. found a significant decrease and Mori et al. found a significant increase ^{71,85}.

Table 3.8 Effects of omega-3 fatty acids on apolipoprotein B (mg/dL) in randomized trials (4 weeks to 2 years)

Author, Year	Omega-3 Fatty Acid Arm ^a			Control		Results ^b			Quality ^c			Applicability ^d
	N	Source	g/d	N	Source	Base	Net Δ	P	Summary	Jadad	Allocation Conceal	
DHA/EPA Oils												
Wilt, 1989	38 ^e	Fish oil	ED 6.0	38 ^e	Safflower	112	+5	NS	B	4	Un	DysLip III
Green, 1990	27 ^e	Fish oil	ED 5.2	27 ^e	Corn/Olive	122	-5	NS	B	4	Un	DysLip II
Bonaa, 1992	71	Fish oil	ED 5.1	74	Corn oil	153	-1	NS	B	4	Un	DysLip I
Balestrieri, 1996	14 ^e	Fish oil	ED 5.1	14 ^e	Olive oil	205	+1	NS	B	3	Un	DysLip III
Jensen, 1989	18 ^e	Cod liver oil	ED 4.6	18 ^e	Olive oil	109	+6	NS	B	4	Un	IDDM II
Sirtori, 1992	12 ^e	Fish oil	ED 4.5	12 ^e	No oil	167	0	NS	C	2	Un	DysLip II
Schechtman, 1988	13 ^e	Fish oil	ED 4.0	13 ^e	Safflower	99	+7	NS	B	2	Un	NIDDM II
Grimsgaard, 1997	75	Purified EPA	E 3.8	77	Corn oil	101	-5	NS	A	5	Un	GEN I
	72	Purified DHA	D 3.7			100	-3	NS				
Nordoy, 1998	21	Fish oil	ED 3.4	20	Corn oil	108	+1	NS	B	4	Un	DysLip I
Deslypere, 1992	14	Fish oil	T 3.4	14	Olive oil	89	-8	<.05	B	2	Un	GEN III
	15	Fish oil	T 2.2			85	-1	NS				
	15	Fish oil	T 1.1			91	-2	NS				
Chan, 2002	12	Fish oil		12	Corn oil	128	-4	NS ^f	B	3	Un	DysLip II
	11	Fish oil & Atorvastatin	ED 3.4	12	Corn oil & Atorvastatin	134	-8					
Durrington, 2001	30	Fish oil	ED 3.2	29	Corn oil	96	+5	NS	A	4	Un	CVD DysLip II
McGrath, 1996	23 ^e	Fish oil	ED 3.0	23 ^e	Olive oil	95	+1	NS	A	4	Un	DM II II
Nikkila, 1991	32 ^e	Fish oil	ED 2.4	32 ^e	Corn oil	122	+3	NS	B	3	Un	CVD DysLip II
Luo, 1998	10 ^e	Fish oil	ED 1.8	10 ^e	Sunflower	138	+10	NS	B	3	Un	DM II II
Marckmann, 1997	23	Fish oil margarine	T 0.9	24	Sunflower margarine	113	+1	NS	B	3	Un	GEN II
Nenseter, 2000	34	Fish powder	ED 0.2	36	Cellulose	133	+2	NS	B	3	Un	GEN II
Fish and Mediterranean Diets												
Hanninen, 1989	19	Fish (3.8/week)	ED 0.9	18	0.4 Fish/week	93	-9	nd	B	2	Un	GEN III
	22	Fish (2.3/week)	ED 0.5			78	-2	nd				
	21	Fish (1.5/week)	ED 0.4			80	-5	nd				
	20	Fish (0.9/week)	ED 0.2			78	0	nd				
Agren, 1988	14	Fish (3.7/week)	ED 0.8	12	0.25 Fish/week	70	-2	NS	B	3	Un	GEN III
	15	Fish & low SFA				63	-3	NS				
Agren, 1991	20	Fish (5/week)	ED 0.75	15	Regular	64	+2	NS	B	2	Un	GEN III
	20	Fish (5/week) ^g		23	Regular ^g	67	+5	NS				
de Lorgeril, 1994	171 ^h	Mediterranean/Canola margarine	A 0.8% Kcal	168 ⁱ	Regular	152	-1	NS	C	2	Un	CVD II

Table 3.8 Effects of omega-3 fatty acids on apolipoprotein B (mg/dL) in randomized trials (continued)

Author, Year	Omega-3 Fatty Acid Arm ^a			Control		Results ^b			Quality ^c			Applicability ^d	
	N	Source	g/d	N	Source	Base	Net Δ	P	Summary	Jadad	Allocation Concealment		
	Combinations												
Mori, 1994	17	Fish oil & Fish diet ^j	ED 5.2	18	Olive/Palm/Safflower 40% fat diet	143 ^k	+5 ^L	NS	B	2	Un	GEN II	
	16	Fish oil	ED 4.2				+9 ^L	<.05					
	17	Fish oil	ED 2.1				+12 ^L	<.05					
	17	Fish diet ^j & Placebo oil	ED 3.0				+6 ^L	NS					
	18	Fish diet ^j & Placebo oil	ED 3.0	17	Oil 30% fat diet	+1 ^L	NS						
Cobiac, 1991	13	Fish oil	ED 4.6	6	Olive, Palm, Safflower oil	99 ^m	+6	NS	B	2	Un	GEN II	
	12	Fatty Fish diet	ED 4.5				100 ^m	-1					NS
Agren, 1996	14	Fish oil	ED 2.3	14	No oil	72	-3	NS	B	3	Un	GEN III	
	14	Algae DHA oil	D 1.7				71	-3					NS
	13	Fatty Fish diet	ED 1.1				75	0					NS
Finnegan, 2003	31	Fish oil margarine/ Fish oil	ED 1.7	30	Sunflower margarine	176 ⁿ	+1	NS	A	4	Un	DysLip I	
	30	Fish oil margarine	ED 0.8				174 ^o	+3					NS
	30	Rapeseed/Linseed margarine	A 4.5				178 ^p	+1					NS

nd = no data

a A = ALA; D = DHA; E = EPA; ED = EPA+DHA; T = Total omega-3 fatty acids.

b Base = baseline level in treatment arm; Net Δ = net difference in change in omega-3 fatty acids arm compared with the change in control arm, see Methods; P = P value of difference between treatment and control arms; NS = not statistically significant.

c A = good quality; B = fair quality; C = poor quality; Jadad = Jadad Score (0-5, based on randomization, double blinding, and dropouts); Ad = adequate allocation concealment; In = inadequate allocation concealment; Un = allocation concealment unclear. See Methods.

d CVD = history of cardiovascular disease; DM I/II = diabetes mellitus type 1 or 2; DysLip = dyslipidemia; GEN = general, healthy population; N/IDDM = (non-) insulin dependent diabetes mellitus. I = broadly applicable; II = applicable to subgroup; III = narrow applicability. See Methods.

e Cross-over study.

f Main effect.

g Recommended aerobic exercise for 30 minutes 3 times a week.

h Baseline data based on 289 subjects.

i Baseline data based on 295 subjects.

j Omega-3 fatty acid from fish diet is approximate, assuming that each of 4 different fish was consumed equally.

k Mean baseline value for all subjects combined.

L Estimated from graph.

m Units were not reported.

n Reported as 1.76 mmol/L.

o Reported as 1.74 mmol/L.

p Reported as 1.78 mmol/L.

Apo B-100 (Table 3.9, top)^{50,52,62,107} and LDL apo B (Table 3.9, bottom)^{93,94,108,111-113}

The 4 studies of apo B-100 found a range of effects with omega-3 fatty acid consumption. Two found a decreases in level of less than 5%; the other 2 studies found net increases of 2% and 15%. In contrast, large, significant net increases in LDL apo B were found in 4 of 6 studies (20 to 45 mg/dL).

Table 3.9 Effects of omega-3 fatty acids on apolipoprotein B-100 and LDL apolipoprotein B (mg/dL) in randomized trials (1 month to 14 months)

Author, Year	Omega-3 Fatty Acid Arm ^a			Control		Results ^b			Quality ^c			Applicability ^d
	N	Source	g/d	N	Source	Base	Net Δ	P	Summary	Jadad	Allocation Concealment	
Apo B-100												
DHA/EPA Oils												
Eritsland, 1995b	178	Fish oil	ED 3.3	174	No oil	182	+3	NS	B	2	Ad	CVD II
Brox, 2001 ^e	38	Cod liver oil	ED 3.3	37	No oil	200	-10 ^f	NS	C	1	Un	DysLip I
	37	Seal oil	ED 2.6			200	-10 ^f	NS				
DeLany, 1990 ^g	5	Fish oil	ED 2	5	No oil	62	+9	NS	C	1	Un	GEN III
Combinations												
Silva, 1996	20	Fish oil	ED 3.6	--	--	188	-3 ^h	nd ⁱ	B	3	Un	DysLip II
	15	Soya oil	A 0.8 ^j			222	-5 ^h	nd ⁱ				
LDL Apo B												
DHA/EPA Oils												
Deck, 1989	8 ^k	Fish oil	ED 4.6	8 ^k	Corn oil	96	+25	<.05	B	5	Un	DysLip II
Sirtori, 1992	12 ^k	Fish oil	ED 4.5	12 ^k	No oil	157	+2	NS	C	2	Un	DysLip II
Schectman, 1989 ^L	15 ^k	Fish oil	ED 4.0	15 ^k	Safflower	92	+20	nd ^m	C	2	Un	DysLip II
Schectman, 1988 ^L	13 ^k	Fish oil	ED 4.0	13 ^k	Safflower	82	+9	<.05	B	2	Un	NIDDM II
Radack, 1990	10	Fish oil	ED 2.2	8	Olive oil	100	+45	<.05	B	5	Un	DysLip II
	7	Fish oil	ED 1.1			95	+29	<.05				
Radack, 1991	33 ^k	Fish oil	ED 2.0	33 ^k	Safflower oil	249	-6	NS	B	5	Ad	CVD II

nd = no data

a A = ALA; D = DHA; E = EPA; ED = EPA+DHA; T = Total omega-3 fatty acids.

b Base = baseline level in treatment arm; Net Δ = net difference in change in omega-3 fatty acids arm compared with the change in control arm, see Methods; P = P value of difference between treatment and control arms; NS = not statistically significant.

c A = good quality; B = fair quality; C = poor quality; Jadad = Jadad Score (0-5, based on randomization, double blinding, and dropouts); Ad = adequate allocation concealment; In = inadequate allocation concealment; Un = allocation concealment unclear. See Methods.

d CVD = history of cardiovascular disease; DM I/II = diabetes mellitus type 1 or 2; DysLip = dyslipidemia; GEN = general, healthy population; N/IDDM = (non-) insulin dependent diabetes mellitus. I = broadly applicable; II = applicable to sub-group; III = narrow applicability. See Methods.

e Data missing from article provided by study author.

f Only 2 significant digits reported. 10 mg/dL is smallest unit of change possible.

g Possibly not randomized ("[S]ubjects were divided into... treatment groups so that initial mean cholesterol concentration of each group was similar.").

h Pre-post difference (not compared to control).

i NS between treatments.

j No data on ALA amount. Assume 7 g ALA per 100 g oil. 12 g oil.

k Cross-over study.

L Unclear if 2 studies by Schectman et al.^{93,94} are independent of each other. Possible overlap of up to 6 subjects with NIDDM and hypertriglyceridemia.

m Increase in LDL apo B within fish oil arm was significant compared to baseline (P<.05).

Sub-populations

Total apo B. The heterogeneity of effects seen across all studies is apparent among the 10 studies of healthy populations (8 of which were in men^{66,67,71,85,89,95,97,100} and one of which was in women⁹⁶), the 10 studies of dyslipidemic populations (subjects in 2 of which also had CVD), and

the 3 studies of CVD populations (including those studies with subjects with dyslipidemia). The 4 studies of diabetics, one of which included insulin-dependent diabetics, all found small, non-significant, net increases in total apo B.

Apo B-100 and LDL apo B. The 2 apo B-100 studies of dyslipidemic patients reported small net decreases in apo B-100, while the study of patients undergoing coronary bypass surgery showed a small net increase and the study of healthy, male college students found a larger net increase in apo B-100. The 5 LDL apo B studies of dyslipidemic or diabetic subjects found generally large increases in LDL apo B, while the single study of hypertensive subjects showed a small net decrease.

Covariates

Total apo B. Nenseter et al. performed a subanalysis based on age of the effect of a low-omega-3 fatty acid fish powder⁹⁰. Subjects between ages 30 and 52 years had a significantly greater rise in apo B level compared to subjects 53 to 70 years old; furthermore age negatively correlated with the rise in apo B ($r = -0.40$, $P < .04$). The authors also imply that the effect was not correlated with sex. Mori et al. performed a regression adjusting for change in weight and found a highly significant "group effect" increase in apo B with omega-3 fatty acids ($P < .01$)⁷¹. Agren et al. (1988), in a study of male university students, found no difference in effect between 2 fish diets that differed in the amount of low saturated fats⁹⁵. Three studies compared fish oil to placebo oil supplements in dyslipidemic patients who were all taking either atorvastatin or simvastatin^{98,99,106}. The effects of fish oil supplements on apo B were small in all. They were not uniform in direction.

Apo B-100 and LDL apo B. Silva et al. reported that any effect of fish oil and soya oil supplements on apo B was not correlated with sex, BMI, hypertension, or diabetes in hyperlipidemic patients¹⁰⁷. Schectman et al. found that changes in LDL apo B did not correlate with baseline differences in diet or with individual changes in diet or body weight⁹³. Other studies did not correlate findings with possible covariates. The small number of studies limits hypothesis generating of possible effect mediators across studies.

Dose and Source Effect

Total apo B. Among studies of fish oil supplements, Deslypere et al. found a significant net decrease in apo B in subjects on the highest dose of omega-3 fatty acids but smaller non-significant net decreases with smaller doses⁸⁵. Among the individual study arms, apo B levels fell in the arm with a higher dose of fish oil but rose in the lower dose arms (and the olive oil arm). No dose effect was seen across fish oil supplement studies. Among studies of dietary fish, Hanninen et al. reported a trend in effect related to different frequencies of fish meals⁶⁷. Subjects most frequently consuming fish had the largest, significant reduction in apo B (compared to baseline). Subjects with intermediate frequencies of fish consumptions (average of 1.5 and 2.3 meals per week) had smaller reductions in apo B with P values (compared to baseline) of less than .10. Subjects on only about 1 fish meal per week had a non-significant increase in apo B.

Five studies compared different sources of omega-3 fatty acids. Grimsgaard et al. found no difference in effect between purified EPA and purified DHA⁶⁶. Mori et al. compared a variety of doses of fish oil supplements and combinations of dietary fish and supplemental fish oil, along with higher and lower percentage fat diets⁷¹. Overall, significant net increases in apo B were

seen in the subjects who consumed fish oil supplements and were on non-fish diets, but smaller, non-significant increases were seen in the subjects who were on fish diets, regardless of fish oil supplementation or percent fat in the diet. Cobiac et al. similarly found that subjects on fish oil supplement had a net increase in apo B while those on dietary fish had almost no change¹⁰⁰. While neither change was statistically significant, there was a trend toward a difference between the 2 treatments ($P = .10$). In contrast, Agren et al. (1996) reported small non-significant net reductions in apo B with fish oil and algae DHA oil supplementation and no effect with fatty fish diet; although they do not comment on potential differences between groups⁹⁷. Finally, Finnegan et al. reported no effects on apo B and no differences among people consuming different omega-3 fatty acids from margarine and/or supplements⁵³.

Apo B-100 and LDL apo B. Neither Brox et al. nor Silva et al. found a difference in effect of different omega-3 fatty acids on apo B-100 levels^{62,107}. Radack et al. (1990) found a similar large increase in LDL apo B in 2 groups of hypertriglyceridemic patients consuming different doses of fish oil supplements¹¹³. While the increase was greater in the group consuming a higher dose of fish oil, no analysis was done to compare the effect in the 2 arms.

Exposure Duration

Total apo B. While the authors do not describe an effect of duration of fish consumption, the data at 5 and 12 weeks in Hanninen et al. may suggest that any effects of dietary fish on apo B do not occur until after 5 weeks⁶⁷. At 5 weeks there were essentially no changes in apo B in any of the study arms, compared to significant and near significant reductions in arms with more frequent fish consumption. In de Lorgeril et al. a Mediterranean and ALA margarine diet had no effect on apo B at 8 weeks, 1 year, and 2 years.

Apo B-100 and LDL apo B. In their study of apo B-100, DeLany et al. found that while there was no difference in effect between 5 g fish oil supplementation and no oil at 5 weeks, there was a significant increase over time at 0, 2, and 5 weeks in subjects on fish oil supplements⁵⁰. However, this analysis included 5 subjects who took 20 g fish oil supplements. There was also a small increase in apo B-100 levels in subjects not consuming oil supplements. Radack et al. (1990) reported no change in LDL apo B level between measurements at 8, 12, and 20 weeks¹¹³.

Sustainment of Effect

Total apo B. Three studies followed subjects after stopping the intervention. Both Jensen et al. and Agren et al. (1988) found no change in apo B levels 8 weeks and 5 months, respectively, after stopping fish oil supplements^{95,103}. Deslypere et al. found that 6 months after stopping supplements apo B levels rose to similar levels in all groups except those who had been on the lowest dose fish oil, although no analysis was performed on follow-up data⁸⁵.

Apo B-100 and LDL apo B. Although Radack et al. (1990) measured LDL apo B levels 4 weeks after stopping treatment¹¹³, no study reported whether changes in apo B-100 or LDL apo B levels were sustained.

Blood Pressure

(Tables 3.10 and 3.11)

Hypertension is a well-known risk factor for atherosclerosis and cardiovascular disease. Recently the Seventh Report of the Joint National Committee on Prevention, Detection, Evaluation, and Treatment of High Blood Pressure (JNC 7) noted that the relationship between blood pressure and risk of cardiovascular events is continuous, consistent and independent of other factors.²⁵ The benefits to lowering blood pressure are evident even in people with “pre-hypertension” (blood pressure of 120-139/80-89).

We found 103 studies that met eligibility criteria and reported data on the effect of omega-3 fatty acids on blood pressure (See Table 3.1). In addition, we found a recent systematic review with a meta-regression of the blood pressure response to fish oil supplementation¹¹⁴. This thorough review touched on most of the major questions addressed by the current report, therefore this section relies primarily on the findings of Geleijnse et al. However, they explicitly excluded studies of diabetic patients. Therefore, we analyzed the 6 randomized trials with data on blood pressure in diabetic patients that had a minimum of 15 patients in parallel trials and 10 patients in crossover trials who consumed omega-3 fatty acids.

Meta-Regression¹¹⁴

Geleijnse et al. collected trials of fish oil supplementation and blood pressure through March 2001. Eligibility criteria were: (1) randomized design, (2) adult study population, and (3) publication after 1966. Trials were excluded if they included sick or hospitalized patients, including kidney disease and diabetic patients, or if the intervention was shorter than 2 weeks duration. A total of 36 trials with 50 omega-3 fatty acid study arms were analyzed. Of note, 6 of these studies did not meet our eligibility due to high omega-3 fatty acid dose (3), short washout period in crossover trial (2), or short study duration (1).

The range of trial duration was 3 to 52 weeks and doses of omega-3 fatty acids were less than 1.0 g/day in 1 trial, 1.0 to 1.9 g/day in 5 trials, 2.0 to 2.9 g/day in 4 trials, and 3.0 to 15.0 g/day in 26 trials.

The mean net reduction (controlling for placebo arms) in systolic and diastolic blood pressure, weighted for study size, was -2.1 mm Hg (95% confidence interval $-3.2, -1.0$) and -1.6 mm Hg ($-2.2, -1.0$), respectively. The mean reductions in systolic and diastolic blood pressures were somewhat smaller in the 22 double blinded studies. Data on univariate and multivariate weighted meta-regression analyses performed on study subgroups based on mean age, sex, mean baseline blood pressure, and mean body mass index are reported. Briefly, systolic and diastolic blood pressure reductions were significantly larger in older (mean age ≥ 45 years) than younger populations, and in hypertensive (blood pressure $\geq 140/90$ mm Hg) compared to normotensive populations. A lack of studies in women precluded adequate analysis based on sex. Body mass index was not associated with blood pressure response to fish oil supplementation. In addition, trial duration and fish oil dose were not associated with effect.

Overall Effect in Diabetes Studies ¹¹⁵⁻¹²⁰

Across the 6 studies of diabetic patients, there were generally small, non-significant effects of fish oil supplements on systolic (Table 3.10) and diastolic (Table 3.11) blood pressure. Overall, these study results were similar to the findings of the meta-regression among non-diabetic populations in their small, but generally inconsistent net effects. One study reported a small significant reduction in mean diastolic pressure (-2 mm Hg) and 2 reported significant reductions in mean systolic pressure (-3 and -6 mm Hg).

Covariates

Haines et al., who found non-significant small net increases in blood pressure, reported that neither sex nor Hgb A_{1c} levels were related to the effect of fish oil supplements on blood pressure ¹¹⁵. No study analyzed data based on age. Across studies there was no clear difference

Table 3.10 Effects of omega-3 fatty acids on systolic blood pressure (mm Hg) in randomized trials of diabetic subjects (6 weeks to 1 year)

Author, Year	Omega-3 Fatty Acid Arm ^a			Control		Results ^b			Quality ^c			Applicability ^d
	N	Source	g/d	N	Source	Base	Net Δ	P	Summary	Jadad	Allocation Conceal	
	DHA/EPA Oils											
Haines, 1986	19	Fish oil	ED 4.6	22	Olive oil	135	+1	NS	B	2	Ad	IDDM II
Rossing, 1996 ^e	14	Cod liver oil	ED 4.6	15	Olive oil	141	-3	NS	A	3	Un	IDDM II
Woodman, 2002 ^e	17	Purified EPA	E 3.8	16	Olive oil	137	0	NS	B	3	Un	DM II II
	17	Purified DHA	D 3.7			139	+7	NS				
Lungershausen, 1997	16	Fish oil	ED 3.4	16	Corn oil	139	-6	.04	B	4	Un	DM I&II II
Hendra, 1990	37	Fish oil	ED 3.0	37	Olive oil	145	+1	NS	B	4	Un	DM II I
Jain, 2002	25	Fish oil	ED 0.6	15	"Placebo"	127	-3	.0003	C	2	Un	DM II II

a A = ALA; D = DHA; E = EPA; ED = EPA+DHA; T = Total omega-3 fatty acids.

b Base = baseline level in treatment arm; Net Δ = net difference in change in omega-3 fatty acids arm compared with the change in control arm, see Methods; P = P value of difference between treatment and control arms; NS = not statistically significant.

c A = good quality; B = fair quality; C = poor quality; Jadad = Jadad Score (0-5, based on randomization, double blinding, and dropouts); Ad = adequate allocation concealment; In = inadequate allocation concealment; Un = allocation concealment unclear. See Methods.

d CVD = history of cardiovascular disease; DM I/II = diabetes mellitus type 1 or 2; DysLip = dyslipidemia; GEN = general, healthy population; N/IDDM = (non-) insulin dependent diabetes mellitus. I = broadly applicable; II = applicable to sub-group; III = narrow applicability. See Methods.

e Mean 24 hour ambulatory blood pressure.

Table 3.11 Effects of omega-3 fatty acids on diastolic blood pressure (mm Hg) in randomized trials of diabetic subjects (6 weeks to 1 year)

Author, Year	Omega-3 Fatty Acid Arm ^a			Control		Results ^b			Quality ^c			Applicability ^d
	N	Source	g/d	N	Source	Base	Net Δ	P	Summary	Jadad	Allocation Conceal	
	DHA/EPA Oils											
Haines, 1986	19	Fish oil	ED 4.6	22	Olive oil	81	+2	NS	B	2	Ad	IDDM II

Rossing, 1996 ^e	14 Cod liver oil	ED 4.6	15 Olive oil	82	-1	NS	A	3	Un	IDDM II
Woodman, 2002 ^e	17 Purified EPA	E 3.8	16 Olive oil	76	0	NS	B	3	Un	DM II II
	17 Purified DHA	D 3.7		72	+1	NS				
Lungershausen, 1997	16 Fish oil	ED 3.4	16 Corn oil	81	+1	NS	B	4	Un	DM I&II II
Hendra, 1990	37 Fish oil	ED 3.0	37 Olive oil	85	-3	NS	B	4	Un	DM II I
Jain, 2002	25 Fish oil	ED 0.6	15 "Placebo"	82	-2	.0003	C	2	Un	DM II II

a-e See Table 3.10

among populations with type I or type II diabetes, and there were insufficient data to comment on age, sex, menopausal status, race, weight or other variables.

Dose and Source Effect

No study compared different doses of omega-3 fatty acids. Woodman et al. compared purified EPA and purified DHA and found a net fall in mean 24 hour ambulatory systolic blood pressure in subjects on EPA and a net increase in diastolic pressure; however, there was no statistical difference between the 2 treatments¹²⁰. Across studies, there is no apparent difference in effect on systolic blood pressure based on fish oil supplement dose. However, the largest, and significant, reductions in diastolic pressure were found in the 2 studies with the smallest fish oil supplementation doses.

Exposure Duration

In 3 studies no differences in effect are noted based on duration of intervention or exposure at 3 and 6 weeks¹¹⁵, 6 and 12 weeks¹¹⁸, or 6 and 12 months¹¹⁹.

Sustainment of Effect

No study reported blood pressures after subjects stopped treatment.

Hemoglobin A_{1c}

(Table 3.12)

Chronically elevated serum glucose levels, which occur in diabetes, result in elevated levels of glucose binding to hemoglobin. This bound product, hemoglobin A_{1c} (Hgb A_{1c}), or glycohemoglobin, is used to measure long-term control of diabetes.

We found 32 studies that met eligibility criteria and reported data on the effect of omega-3 fatty acids on Hgb A_{1c} levels (See Table 3.1). Of these, we analyzed the 18 randomized trials with data on at least 10 subjects in either parallel trials or crossover trials who consumed omega-3 fatty acids.

Overall Effect^{77,85,88,93,102,103,106,115,117-126}

Across the 18 studies, omega-3 fatty acids had a very small, if any, effect on Hgb A_{1c} levels compared to control. The range of net effects across the studies was -0.4% to +1.0%. Only 1

study reported a statistically significant reduction in Hgb A_{1c}; however, this study by Jain et al. found one of the smaller net changes of all studies ¹¹⁷.

Sub-populations

As expected, the large majority of studies evaluating Hgb A_{1c} included diabetic patients. Fourteen studies analyzed diabetic populations, 3 of which were also dyslipidemic. An additional 2 studies analyzed dyslipidemic patients; 1 included patients with untreated hypertension; and 1 evaluated healthy monks.

While none of the 4 studies of dyslipidemic patients had net reductions in Hgb A_{1c} levels, given the small differences in almost all studies, there are no clear difference in effect in the different populations, including diabetic patients.

Covariates

Schectman et al. found that the effect of fish oil supplements on Hgb A_{1c} did not correlate with baseline differences in diet or with individual changes in diet or body weight ⁹³. Toft et al. and Westerveld et al. reported no change in effect of fish oil supplements on Hgb A_{1c} after adjustment for body weight ^{125,126}. Likewise, Haines et al reported no relationship between effect on Hgb A_{1c} and sex ¹¹⁵. Three studies were notable for including only men ^{85,88}, or because all subjects were taking simvastatin ¹⁰⁶. The effect found in these studies was not clearly different than that found in studies.

Table 3.12 Effects of omega-3 fatty acids on hemoglobin A_{1c} (%) in randomized trials (4 weeks to 1 year)

Author, Year	Omega-3 Fatty Acid Arm ^a			Control		Results ^b			Quality ^c			Applicability ^d
	N	Source	g/d	N	Source	Base	Net Δ	P	Summary	Jadad	Allocation Conceal	
DHA/EPA Oils												
Haines, 1986	19	Fish oil	ED 4.6	22	Olive oil	11.1	+0.2	NS	B	2	Ad	IDDM II
Jensen, 1989	18 ^e	Cod liver oil	ED 4.6	18 ^e	Olive oil	9.5	+0.1	NS	B	4	Un	IDDM II
Rossing, 1996	14	Cod liver oil	ED 4.6	15	Olive oil	8.8	-0.3	NS	A	3	Un	IDDM II
Schectman, 1988	11 ^e	Fish oil	ED 4.0	11 ^e	Safflower	7.9	+0.1	NS	B	2	Un	NIDDM II
Woodman, 2002	17	Purified EPA	E 3.8	16	Olive oil	7.1	+0.2	NS	B	3	Un	DM II II
	18	Purified DHA	D 3.7			7.5	0	NS				
Toft, 1995	38	Fish oil	ED 3.4	40	Corn oil	5.7	+0.1	NS	A	5	Ad	CVD II
Harris, 1997	22	Fish oil	ED 3.4	18	Corn oil	5.3	0	NS	B	3	Un	DysLip II
Nordoy, 1998	21	Fish oil	ED 3.4	20	Corn oil	5.8	+0.2	NS	B	4	Un	DysLip I
Lungershausen, 1997	16	Fish oil	ED 3.4	16	Corn oil	8.5	+0.2	NS	B	4	Un	DM II
Deslypere, 1992	14	Fish oil	T 3.4	14	Olive oil	4.8	+0.2	NS	B	2	Un	GEN III
	15	Fish oil	T 2.2			4.9	-0.1	NS				
	15	Fish oil	T 1.1			5.0	-0.1	NS				
Bonnema, 1995	14	Fish oil	ED 3.3	14	Olive oil	8.0	+1.0	NS	A	3	Ad	IDDM II
McVeigh, 1993	23 ^e	Fish oil	ED 3.0	23 ^e	Olive oil	9.6	+0.2	NS	A	4	Un	DM II II
Pedersen, 2003	23	Fish oil	ED 2.6	21	Corn oil	8.2	0.0	NS	A	3	Un	DysLip DM II II
Luo, 1998	10 ^e	Fish oil	ED 1.8	10 ^e	Sunflower	8.8	-0.4	NS	B	3	Un	DM II II
Westerveld, 1993	8	Purified EPA	E 1.8	8	Olive oil	8.2 ^f	-0.4 ^g	NS	C	3	Un	NIDDM II

	8	Purified EPA	E	0.9		7.6	+0.4	NS					
Sirtori, 1998	203	Fish oil	ED	1.7 ^h	211	Olive oil	7.3	+0.6	NS	B	4	Ad	DysLip NIDDM I
Jain, 2002	25	Fish oil	ED	0.6	15	"Placebo"	8.0	-0.1	.009	C	2	Un	DM II II
Fish and Mediterranean Diets													
Dunstan, 1997	26	Fatty Fish	T	3.6	23	No fish	8.2	+0.3	.06	B	2	Un	DysLip NIDDM I

- a A = ALA; D = DHA; E = EPA; ED = EPA+DHA; T = Total omega-3 fatty acids.
- b Base = baseline level in treatment arm; Net Δ = net difference in change in omega-3 fatty acids arm compared with the change in control arm, see Methods; P = P value of difference between treatment and control arms; NS = not statistically significant.
- c A = good quality; B = fair quality; C = poor quality; Jadad = Jadad Score (0-5, based on randomization, double blinding, and dropouts); Ad = adequate allocation concealment; In = inadequate allocation concealment; Un = allocation concealment unclear. See Methods.
- d CVD = history of cardiovascular disease; DM I/II = diabetes mellitus type 1 or 2; DysLip = dyslipidemia; GEN = general, healthy population; NIDDM = (non-) insulin dependent diabetes mellitus. I = broadly applicable; II = applicable to subgroup; III = narrow applicability. See Methods.
- e Cross-over study.
- f According to text. In table, baseline Hgb A_{1c} = 8.6%
- g Per data in text. Per data in table, net change = -0.8%
- h 2.6 g/day for first 2 months, then 1.7 g/day for 4 months.

Dose and Source Effect

Two studies compared different doses of fish oil supplements. Deslypere et al., in a 1 year study of healthy Belgian monks, reported no difference in the effect of 3 doses of fish oil or olive oil⁸⁵. Westerveld et al. also reported no difference in the effect of 2 different doses of fish oil, purified EPA, or olive oil in non-insulin dependent diabetics¹²⁶. Across studies, there was no apparent dose effect of fish oil supplements. The only study of dietary fish found a lack of effect similar to the fish oil supplement studies. Woodman et al. compared purified EPA to DHA in type II diabetics¹²⁰. No difference was noted between the 2 treatments.

Exposure Duration

Two studies reported treatment effect at multiple time points. In Haines et al. there was a transient drop in Hgb A_{1c} by 0.6% (0.5% net) at 3 weeks which reverted to baseline at 6 weeks¹¹⁵. The change was not statistically significant. Rossing et al. found no difference in effect between 6 and 12 months¹¹⁹. Across studies there was no apparent effect of treatment duration.

Sustainment of Effect

Jensen et al., in a crossover study, found that Hgb A_{1c} remained unchanged 8 weeks after stopping oil supplementation.

Fasting Blood Sugar (Table 3.13)

Elevated fasting blood sugar (FBS) is a risk factor or indicator of diabetes. People with diabetes or with altered glucose tolerance have a highly elevated risk of CVD. As discussed in the introduction, the effect of omega-3 fatty acids on diabetic control is unclear.

We found 57 studies that met eligibility criteria and reported data on the effect of omega-3 fatty acids on FBS levels (See Table 3.1). Of these, we analyzed the 17 randomized trials with data on at least 25 subjects in parallel trials and 15 subjects in crossover trials who consumed omega-3 fatty acids.

Overall Effect ^{52,53,68,76,77,103,116,117,120,123,125,127-132}

The effect of omega-3 fatty acids on FBS was inconsistent across the 17 studies. Four studies found large and/or near-significant net increases in FBS compared to control; 3 found large and/or significant net decreases in FBS and the rest found small non-significant changes. Across the studies, the net effect ranged between a decrease of 29 mg/dL over 8 weeks and an increase of 25 mg/dL over 6 weeks. Interpretation of the overall data is further complicated by inconsistent patterns of effect within individual study arms. In omega-3 fatty acid arms and in control arms, FBS increased from baseline in half the arms and either decreased or remained unchanged in the other half.

Sub-populations

Seven studies evaluated diabetic populations, 2 of which also had dyslipidemia; an additional 5 studies evaluated patients with dyslipidemia. Three studies included subjects who had CVD or were at increased risk for CVD (due to either diabetes or dyslipidemia). Two studies were of healthy populations.

The findings within the diabetic populations were inconsistent. The largest net decrease in FBS was found by Jensen et al. in the only study of insulin-dependent diabetics ¹⁰³, while the largest net increase in FBS with omega-3 fatty acids was seen in Woodman et al. in one of the studies of type II diabetics ¹²⁰. Furthermore in each of the 3 groups of subjects on fish oil supplements in these 2 studies, FBS rose by approximately 10 or 20 mg/dL; the large difference

Table 3.13 Effects of omega-3 fatty acids on fasting blood sugar (mg/dL) in randomized trials (4 weeks to 2 years)

Author, Year	Omega-3 Fatty Acid Arm ^a			Control		Results ^b			Quality ^c			Applicability ^d
	N	Source	g/d	N	Source	Base	Net Δ	P	Summary	Jadad	Allocation Conceal	
DHA/EPA Oils												
Jensen, 1989	18 ^e	Cod liver oil	ED 4.6	18 ^e	Olive oil	178	-29	NS	B	4	Un	IDDM II
Mori, 2000	19	Purified EPA	E 3.8	20	Olive oil	91	+2	.06	B	4	Un	DysLip II
	17	Purified DHA	D 3.7			92	-3	NS				
Woodman, 2002	17	Purified EPA	E 3.8	16	Olive oil	134	+25	.002	B	3	Un	DM II II
	18	Purified DHA	D 3.7			149	+18	.002				
Mackness, 1994	41	Fish oil	ED 3.4	38	Corn oil	91	+2	NS	A	3	Un	DysLip I
Toft, 1995	38	Fish oil	ED 3.4	40	Corn oil	99	+2	.06	A	5	Ad	CVD II
Grundt, 1995	28	Fish oil	ED 3.4	28	Corn oil	85	0	NS	B	2	Un	DysLip II
Eritsland, 1995b	255	Fish oil	ED 3.3	245	No oil	86	+1	NS	B	2	Ad	CVD II

Leigh-Firbank, 2002	55 ^e Fish oil	ED 3.0	55 ^e Olive oil	99	+3	NS	B	3	Un	DysLip I
Hendra, 1990	37 Fish oil	ED 3.0	37 Olive oil	202	+14	NS	B	4	Un	DM II I
McVeigh, 1993	23 ^e Fish oil	ED 3.0	23 ^e Olive oil	184	+7	NS	A	4	Un	DM II II
Sirtori, 1998	203 Fish oil	ED 1.7 ^f	211 Olive oil	149	+2	NS	B	4	Ad	DysLip NIDDM I
Jain, 2002	25 Fish oil	ED 0.6	15 "Placebo"	139	-10	.004	C	2	Un	DM II II
Fish and Mediterranean Diets										
Mori, 1999	17 Fatty Fish ^g	T 3.7	16 No fish ^g	95	+4 ^h	NS	B	2	Un	CVD II
	14 Fatty Fish ⁱ		16 No fish ⁱ	94	-1 ^h	NS				
Dunstan, 1998	14 Fatty Fish ^j	T 3.6	11 No fish ^j	180	-4	NS	B	2	Un	DysLip NIDDM I
	12 Fatty Fish ^k		12 No fish ^k	160	+5	NS				
Singh, 2002	499 Indo-Mediterranean	T 1.8	501 NCEP I ^L	108	-5	<.0001	C	2	Un	CVD risk ^m III
Combinations										
Freese, 1997a	16 Fish oil	ED 5.2	--	85	+5 ⁿ	nd ^o	C	3	Un	GEN II
	22 Linseed oil	A 5.9	--	86	-1 ^p	nd ^o				
Finnegan, 2003	31 Fish oil margarine/ Fish oil	ED 1.7	30 Sunflower margarine	97	-3	NS	A	4	Un	DysLip I
	30 Fish oil margarine	ED 0.8		97	-3	NS				
	30 Rapeseed/Linseed margarine	A 4.5		99	-5	NS				

nd = no data

a A = ALA; D = DHA; E = EPA; ED = EPA+DHA; T = Total omega-3 fatty acids.

b Base = baseline level in treatment arm; Net Δ = net difference in change in omega-3 fatty acids arm compared with the change in control arm, see Methods; $P = P$ value of difference between treatment and control arms; NS = not statistically significant.

c A = good quality; B = fair quality; C = poor quality; Jadad = Jadad Score (0-5, based on randomization, double blinding, and dropouts); Ad = adequate allocation concealment; In = inadequate allocation concealment; Un = allocation concealment unclear. See Methods.

d CVD = history of cardiovascular disease; DM I/II = diabetes mellitus type 1 or 2; DysLip = dyslipidemia; GEN = general, healthy population; N/IDDM = (non-) insulin dependent diabetes mellitus. I = broadly applicable; II = applicable to subgroup; III = narrow applicability. See Methods.

e Cross-over study.

f 2.6 g/day for first 2 months, then 1.7 g/day for 4 months.

g Weight-maintaining diet.

h Estimate from graph.

i Weight-loss diet.

j Moderate exercise.

k Light exercise.

L National Cholesterol Education Program step I prudent diet.

m One or more of: hypercholesterolemia, hypertension, diabetes, angina pectoris or myocardial infarction.

n Pre-post difference (not compared to control); $P < .05$ compared to baseline.

o NS between treatments.

p Pre-post difference (not compared to control); not significantly different than baseline.

in net effect is due to the difference in effect of the olive oil control (+49 mg/dL and -7 mg/dL, respectively). In the remaining studies of diabetics, the change in FBS was in the same direction in omega-3 fatty acid arms and control arms; in 6 omega-3 study arms FBS rose from 10 mg/dL to 23 mg/dL; in 4 arms FBS fell from -2 mg/dL to -16 mg/dL. In studies of diabetics, factors other than omega-3 fatty acid consumption – such as those related to population characteristics, other treatments, or study design – appear to have had a greater effect on change in FBS than the omega-3 fatty acid treatment itself.

Among the 7 studies of dyslipidemic populations, 2 of which were also diabetic, all found a small non-significant net effect of omega-3 fatty acids on FBS that ranged from -4 to +5 mg/dL. Only Dunstan et al. found large changes in individual omega-3 fatty acid arms, which were related primarily to exercise level and were similar to the changes in the no fish control arms ¹²⁷.

The 4 studies of CVD patients or people with an elevated risk of CVD all found small absolute and net changes in FBS with omega-3 fatty acid consumption. Only Singh et al. found a significant net change and had a relatively large absolute change (-8 mg/dL) in FBS, although notably about 20% of the subjects were diabetic, two-thirds were vegetarian, and those subjects on the Indo-Mediterranean diet on average lost 3 kg more weight than controls ⁷⁶. In addition, this study reported uniform, highly significant effects on all serum markers despite widely ranging effects. A number of statistical calculation errors were also found.

The single study of a healthy population, by Freese et al., found small differences in FBS with 2 different omega-3 fatty acid treatments (in opposite directions) ¹²⁸.

Covariates

Schectman et al. found that changes in FBS did not correlate with baseline differences in diet or with individual changes in diet or body weight ⁹³. Two studies of diabetics reported data on associations between effect and other variables. Hendra et al. reported that the change in FBS was unrelated to change in weight ¹¹⁶. Woodman et al. reported that the significant effect compared to olive oil was unchanged after adjusting for age, sex, and BMI ¹²⁰. In Mori, et al. (1999), a study of obese hypertensive subjects, the direction of the absolute and net changes in FBS appear related to whether subjects were on a weight-reduction diet or not (those on a weight maintaining diet had increases in FBS, while those on a weight-reduction diet had reductions in FBS); however, they reported no interaction between fish diet and weight loss on FBS ¹³¹. No patterns across studies are evident based on reported data on covariates.

Dose and Source Effect

No study directly compared doses of the same source of omega-3 fatty acids. In comparisons of EPA and DHA, Woodman et al. reported no difference in effect on FBS ¹²⁰; however, Mori et al. (2000) reported a trend toward increasing FBS with EPA, but no change with DHA ¹³². Freese et al. reported a significant increase from baseline in FBS with fish oil supplementation compared to no change with linseed oil; however the difference between the 2 treatments was reported to be non-significant ¹²⁸. In a comparison of multiple sources of omega-3 fatty acids, Finnegan et al. found no significant differences in effect between various doses of either fish oils or plant oils ⁵³. Across studies, there was no discernable difference in effect based on either fish oil dose or omega-3 fatty acid source among diabetic or dyslipidemic populations.

Exposure Duration

Two studies measured FBS levels at multiple time points. Hendra et al. found that FBS rose with fish oil supplements at both 3 and 6 weeks, although the net difference with control was significant only at 3 weeks ¹¹⁶. In a longer study that measured FBS at 2, 4, and 6 months, Finnegan et al. found no treatment effect at any time period ⁵³. The heterogeneity does not appear to be related to study duration.

Sustainment of Effect

Jensen et al., in a crossover study which found that FBS rose by large amounts in both the high-dose cod liver oil and olive oil supplement arms, found that FBS fell back near baseline levels 8 weeks after stopping oil supplementation, although none of the levels were significantly different from each other¹⁰³. Freese et al., who compared fish oil to linseed oil supplements, reported that FBS, which had risen in the fish oil arm, returned to baseline during a 12 week follow-up period¹²⁸.

Fasting Insulin

(Table 3.14)

In people with normal glucose levels (euglycemia), elevated fasting insulin levels are suggestive of insulin resistance, a precursor to type II diabetes and an independent risk factor for CVD. The value of insulin levels in those with insulin resistance, including insulin resistance related to obesity, and diabetes ("hyperglycemia"), is questionable. The effect of omega-3 fatty acids on insulin resistance and fasting insulin levels is also unclear.

We found 21 studies that met eligibility criteria and reported data on the effect of omega-3 fatty acids on fasting insulin levels (See Table 3.1). Of these, we analyzed the 15 randomized trials. All but 3 of the trials were also analyzed for data on FBS or Hgb A_{1c}.

Overall Effect^{52,53,68,77,88,89,106,120,122,125,129,131-134}

Baseline levels of fasting insulin varied broadly across studies. In general, studies of non-insulin-dependent diabetics and obese subjects (under "Studies of "Hyperglycemic" Subjects") had higher mean insulin levels than dyslipidemic, hypertensive, or healthy patients (under Studies of "Euglycemic" Subjects). However, within each population grouping the range of insulin levels remained broad. Mean insulin levels varied within studies also. In 6 studies, baseline insulin levels differed between omega-3 fatty acid arms and control arms by 20% to 60%. Among these, Toft et al. reported a significant difference at baseline and Chan et al. reported no significant difference; the remaining studies did not comment^{125,133}. In an attempt to standardize across studies, given the large variation in insulin levels, we calculated net differences in terms of percent change from baseline instead of absolute changes.

Table 3.14 Effects of omega-3 fatty acids on fasting insulin (pmol/L) in randomized trials (4 weeks to 9 months)

Author, Year	Omega-3 Fatty Acid Arm ^a			Control		Results ^b			Quality ^c			Applicability ^d
	N	Source	g/d	N	Source	Base ^e	Net % Δ	P	Summary	Jadad	Allocation Concealment	
Studies of "Euglycemic" Subjects												
DHA/EPA Oils												
Mori, 2000	19	Purified EPA	E 3.8	20	Olive oil	9	+28%	.04	B	4	Un	DysLip II
	17	Purified DHA	D 3.7			10	+29%	.001				
Toft, 1995	38	Fish oil	ED 3.4	40	Corn oil	52 ^f	-1%	NS	A	5	Ad	CVD II
Grundt, 1995	28	Fish oil	ED 3.4	28	Corn oil	66	-15%	NS	B	2	Un	DysLip II
Nordoy, 1998	21	Fish oil	ED 3.4	20	Corn oil	12 ^g	-28%	NS	B	4	Un	DysLip I
Eritsland, 1995b	255	Fish oil	ED 3.3	245	No oil	125	-1%	NS	B	2	Ad	CVD II
Leigh-Firbank, 2002	55 ^h	Fish oil	ED 3.0	55 ^h	Olive oil	72	-1%	NS	B	3	Un	DysLip I
Marckmann, 1997	23	Fish oil margarine	T 0.9	24	Sunflower margarine	64 ⁱ	-8%	NS	B	3	Un	GEN II
Fish and Mediterranean Diets												
Mori, 1999	17	Fatty fish ^j	T 3.7	16	No fish ^j	12	+14%	NS	B	2	Un	CVD ^k II
	14	Fatty fish ^l		16	No fish ^l	13	-18%	NS				
Combinations												
Finnegan, 2003	31	Fish oil margarine/ Fish oil	ED 1.7	30	Sunflower margarine	42	0%	NS	A	4	Un	DysLip I
	30	Fish oil margarine	ED 0.8			57 ^m	-16%	NS				
	30	Rapeseed/Linseed margarine	A 4.5			49 ^m	-19%	NS				
Studies of "Hyperglycemic" Subjects												
DHA/EPA Oils												
Woodman, 2002	17	Purified EPA	E 3.8	16	Olive oil	98	+4%	NS	B	3	Un	DM II II
	18	Purified DHA	D 3.7			115	+3%	NS				
Chan, 2003	12	Fish oil	ED 3.1	12	Corn oil	285 ⁿ	+12%	NS	A	4	Un	GEN ^o III
Rivellese, 1996	8	Fish oil	ED 2.6 ^p	8	Olive oil	75 ^q	+29%	NS	A	3	Un	DysLip NIDDM II
Luo, 1998	10 ^h	Fish oil	ED 1.8	10 ^h	Sunflower	84	+15%	NS	B	3	Un	DM II II
Sirtori, 1998	203	Fish oil	ED 1.7 ^r	211	Olive oil	116	-11%	NS	B	4	Ad	DysLip NIDDM I
Fish and Mediterranean Diets												
Dunstan, 1997	14	Fatty fish ^s	T 3.6	11	No fish ^s	78	-25% ^t	.08	B	2	Un	DysLip NIDDM I
	12	Fatty fish ^u		12	No fish ^u	78	-28% ^t	.05				

a A = ALA; D = DHA; E = EPA; ED = EPA+DHA; T = Total omega-3 fatty acids.

b Base = baseline level in treatment arm; Net % Δ = net percent difference in change in omega-3 fatty acids arm compared with the change in control arm, see Methods; P = P value of difference between treatment and control arms; NS = not statistically significant.

c A = good quality; B = fair quality; C = poor quality; Jadad = Jadad Score (0-5, based on randomization, double blinding, and dropouts); Ad = adequate allocation concealment; In = inadequate allocation concealment; Un = allocation concealment unclear. See Methods.

d CVD = history of cardiovascular disease; DM I/II = diabetes mellitus type 1 or 2; DysLip = dyslipidemia; GEN = general, healthy population; N/IDDM = (non-) insulin dependent diabetes mellitus. I = broadly applicable; II = applicable to subgroup; III = narrow applicability. See Methods.

- e Studies with a greater than 20% difference between treatment and control are noted.
- f Mean insulin in control arm = 64 pmol/L. Reported to be significantly different than treatment arm.
- g Mean insulin in control arm = 9 pmol/L. No data on whether significantly different than treatment arm.
- h Cross-over study.
- i Mean insulin in control arm = 53 pmol/L. No data on whether significantly different than treatment arm.
- j Weight-maintaining diet.
- k Overweight.
- L Weight-loss diet.
- m Mean insulin in control arm = 37 pmol/L. No data on whether significantly different than treatment arm.
- n Mean insulin in control arm = 215 pmol/L. Reported as not significantly different from treatment arm.
- o Obese men.
- p 2.6 g/day for first 2 months, then 1.7 g/day for 1 month.
- q Mean insulin in control arm = 121 pmol/L. No data on whether significantly different than treatment arm.
- r 2.6 g/day for first 2 months, then 1.7 g/day for 4 months.
- s Moderate exercise.
- t Percent decrease based on baseline level in fish diet arm, derived from regression analysis.
- u Light exercise.

Across the 15 studies there were a wide range of apparent treatment effects ranging from net changes of -28% to $+29\%$ (or -22 pmol/L in Dunstan et al.¹²² to $+34$ pmol/L in Chan et al.¹³³). Approximately one-third of the omega-3 fatty acid study arms had net percent changes of either greater than $+10\%$, between -10% and $+10\%$, or less than -10% .

Sub-populations

Nine of the studies reported data on essentially euglycemic populations. The remaining 6 studies evaluated diabetic or obese populations in which the fasting insulin level may be of less value. While the studies with hyperglycemic subjects all had elevated mean fasting insulin levels, there was a wide range of mean insulin levels in the studies of euglycemic subjects.

Among the studies of euglycemic subjects, the heterogeneity of effect was similar to the heterogeneity seen across all studies. The heterogeneity was particularly apparent among the studies of dyslipidemic patients.

Covariates

Among the studies of euglycemic subjects, Mori et al. (1999) reported no interaction between dietary fish intake and weight loss on insulin levels¹³¹. However, a weight loss diet resulted in a reduction of insulin levels, regardless of fish consumption. In addition, there was a net decrease in insulin levels in subjects who were on a weight loss diet with fish compared to a net increase in insulin in subjects who were on a weight-maintaining diet. Otherwise, studies did not attempt to correlate the effect on insulin of covariates. The 3 studies that either included only euglycemic men^{89,132} or excluded pre-menopausal women¹³¹ had a wide range of effects on insulin levels. Thus, no potential sex effect could be seen.

No study of hyperglycemic subjects reported a correlation between insulin and covariates. As in studies of euglycemic subjects the effects on insulin found among the 2 studies of hyperglycemic men^{88,133} and the study that excluded pre-menopausal women¹²⁰ were heterogeneous.

Dose and Source Effect

Finnegan et al. compared plant oil margarine to 2 doses of fish oil (as margarine and as both margarine and supplement) and to omega-6 fatty acid margarine⁵³. None of the differences in insulin levels was statistically significant and the article does not comment on the relative effects of different treatments. However, dyslipidemic subjects on ALA margarine had an absolute and net decrease in fasting insulin, while subjects on low dose fish oil had a small absolute increase in insulin that was less than the increase in the control group, and subjects on high dose fish oil had an increase in insulin similar to controls. Across the studies, the effect on insulin does not appear to be associated with fish oil dose.

Both Mori et al. (2000) and Woodman et al. compared purified EPA to DHA, although in different populations^{120,132}. No difference was noted between the 2 treatments in both studies.

Exposure Duration and Sustainment of Effect

Only Finnegan et al. measured insulin levels at multiple time points⁵³. They reported no treatment-time interaction with insulin levels at 2, 4, and 6 months. No study measured insulin levels after ceasing omega-3 fatty acid consumption.

C-Reactive Protein

(Table 3.15)

C-reactive protein (CRP) is an acute phase reactant produced in the liver. It is thought to represent an integrated assessment of the overall state of activation of the inflammatory system. Recently, a high sensitivity assay for measuring CRP has been developed that can detect levels of CRP below what was previously considered the 'normal' range. A growing body of studies suggest that elevations in CRP levels detected by the high sensitivity assay predict a poor cardiovascular prognosis¹³⁵.

All eligible studies that reported on the effect of omega-3 fatty acids on CRP levels were included; 5 studies qualified. Four were randomized trials of oil supplements or diet; 1 was a retrospective cross-sectional analysis of usual diet.

Overall Effect^{56,99,136-138}

No study found a significant effect of omega-3 fatty acid consumption on CRP level. However, CRP levels increased relative to subjects who were on control oils in most study arms among the 4 randomized trials. In contrast, the cross-sectional study did find that CRP levels were lower among subjects who ate fish regularly (fish score >4) but the difference was not statistically significant.

Sub-populations and Covariates

No study directly compared the effect of omega-3 fatty acids with placebo in different populations. There was no clear difference in effect across studies based on population. Baseline CRP levels varied across studies; although the reason for the different CRP levels is not apparent. Madsen et al. reported that when the 11 subjects with baseline CRP greater than 2 mg/L were analyzed separately, no difference in effect was seen with fish oil supplementation (as in all subjects) ¹³⁷. Likewise, the effect of omega-3 fatty acids does not appear to differ across studies based on average baseline CRP.

The trial by Chan et al. was a factorial study with fish oil supplements and atorvastatin (40 mg/day) in obese men who had a substantially higher baseline CRP than a separate group of 10 lean men (0.49 mg/L) ¹³⁹. While atorvastatin did significantly reduce CRP levels (by 0.73 mg/L) there was no interaction with fish oil.

Table 3.15 Effects of omega-3 fatty acids on C-reactive protein (mg/L) in studies (4 wk to 3 mo or cross-sectional)

Author, Year	Omega-3 Fatty Acid Arm ^a			Control		Results ^b			Quality ^c			Applicability ^d
	N	Source	g/d	N	Source	Base	Net Δ	P	Summary	Jadad	Allocation Concealment	
RCTs												
DHA/EPA Oils												
Madsen, 2003	20	Fish oil	ED 5.9	20	Olive oil	[1.07]	[-0.15]	NS	B	3	Un	GEN I
	20	Fish oil	ED 1.7			[0.69]	[+0.02]	NS				
Chan, 2002	12	Fish oil	ED 3.4	12	Corn oil	2.11	+0.05	NS ^e	B	3	Un	DysLip II
Plant oils												
Junker, 2001	18	Rapeseed oil diet	T 2.5% ^f	40	Olive or Sunflower	0.5 ^g	+0.11 ^h	NS	C	1	Un	GEN I
Fish and Mediterranean Diets												
Mezzano, 2001	21	Mediterranean	T 1.6	21	Red meat	4.9	+1.7	NS	C	1	In	GEN III
Cross-Sectional												
Diets												
Madsen, 2001	43	Fish Score 5-6		24	Fish Score 2-4	2.3	-0.1 ⁱ	NS	--	--	--	CVD II
	83	Fish Score 7-8				1.9	-0.5 ⁱ					
	102	Fish Score 9-10				2.1	-0.3 ⁱ					
	127	Fish Score 11-12				2.2	-0.1 ⁱ					

a A = ALA; D = DHA; E = EPA; ED = EPA+DHA; T = Total omega-3 fatty acids.

b Base = baseline level in treatment arm (numbers in square brackets are median values or net differences of median values); Net Δ = net difference in change in omega-3 fatty acids arm compared with the change in control arm, see Methods; Cohort Δ = difference in CRP between cohort and reference cohort (cross-sectional); P = P value of difference between treatment and control arms; NS = not statistically significant.

c A = good quality; B = fair quality; C = poor quality; Jadad = Jadad Score (0-5, based on randomization, double blinding, and dropouts); Ad = adequate allocation concealment; In = inadequate allocation concealment; Un = allocation concealment unclear. See Methods.

d CVD = history of cardiovascular disease; DM I/II = diabetes mellitus type 1 or 2; DysLip = dyslipidemia; GEN = general, healthy population; N/IDDM = (non-) insulin dependent diabetes mellitus. I = broadly applicable; II = applicable to subgroup; III = narrow applicability. See Methods.

e Statistical significance based on 23 subjects on Omacor and 25 on placebo, half of whom were also on atorvastatin.

- f Kcal.
- g Median.
- h Net difference of median values of rapeseed compared to average change in 2 control groups.
- i Difference between cohort and low-fish cohort (fish score 2-4). Back-calculated from reported ln(CRP).

Dose and Source Effect

No study compared different sources of omega-3 fatty acids. Any differences in effect due to differing sources across studies could not be appreciated among the few studies. The cross-sectional study did not find an association between fish score (amount of fish in diet) and CRP level.

Exposure Duration

Junker et al. evaluated CRP levels at both 2 and 4 weeks. No differences were noted between baseline and either 2 or 4 weeks⁵⁶. Mezzano et al. evaluated CRP levels at 30 days and 90 days (and also at 60 days after 30 days of added red wine). CRP was unchanged at all observation points.

Sustainment of Effect

No study re-examined CRP after subjects stopped taking omega-3 fatty acids.

Fibrinogen (Table 3.16)

Fibrinogen, a liver protein necessary for clotting, has been found to be both increased in patients with ischemic heart disease and a predictor of cardiovascular events. It is unknown whether reducing fibrinogen levels would alter cardiovascular risk. In addition, there is currently no standardized measurement technique.

We found 59 studies that met eligibility criteria and reported data on the effect of omega-3 fatty acids on fibrinogen levels (See Table 3.1). Of these, we analyzed the 24 randomized trials with data on at least 15 subjects in parallel trials and 10 subjects in crossover trials who consumed omega-3 fatty acids.

Overall Effect ^{46,56,69,74,85,89,90,100,115,116,138,140-152}

Across the 24 studies there was no consistent effect on fibrinogen levels of omega-3 fatty acid consumption compared to control. Approximately half the omega-3 fatty acid study arms resulted in a net increase in fibrinogen level compared to control; in the other half there was either a net decrease or no effect on fibrinogen level. Only 4 studies reported a statistically significant difference between the effect of omega-3 fatty acid and control. In 3 of these, the net decrease of fibrinogen ranged from approximately 5% to 20%. One study reported a significant net increase of fibrinogen of 11%.

Sub-populations

Thirteen of the studies evaluated generally healthy subjects. No consistent effect was found specifically in this population. Four studies evaluated subjects with known CVD: 2 studies of patients with stable claudication (Gans et al. and Leng et al.)^{69,144}, one of patients who were undergoing coronary bypass (Eritsland et al.)¹⁴², and one of subjects with hypertension (Toft et al.)¹⁵². All 4 studies found no effect of omega-3 fatty acids on fibrinogen levels. Seven studies included subjects with diabetes and/or dyslipidemia. Again, there was no consistent effect. However, the largest (significant) net decrease in fibrinogen was found by Radack et al. in a group of 10 subjects with hyperlipoproteinemia types IIb or IV on a moderate dose of fish oil supplement¹⁵¹. A significant net increase in fibrinogen was seen by Haines et al. among 19 subjects with insulin-dependent diabetes on a high dose of fish oil supplement, although the effect was not related to Hgb A1c level.¹¹⁵

In the study of patients undergoing coronary bypass, Eritsland et al. found that the (lack of) effect of omega-3 fatty acids on fibrinogen was unchanged after adjusting for multiple factors including age and sex¹⁴². Seven studies included only men^{46,85,100,138,140,147,149}. The distribution of effects was similar in this subset of studies as in the whole set. Three of these studies of men and an one additional study included only younger adults (generally less than 30 or 40 years old)^{46,138,140,146}. These studies had results similar to studies of broader age ranges or of older subjects. Overall, the studies provided insufficient data on race or ethnicity to allow analysis of these subpopulations. Almost half the studies were performed in Scandinavia and Finland; most of the remaining are from northern Europe and Australia. Notably the study by Radack et al., which

Table 3.16 Effects of omega-3 fatty acids on fibrinogen (g/L) in randomized trials (4 weeks to 2 years)

Author, Year	Omega-3 Fatty Acid Arm ^a			Control		Results ^b			Quality ^c			Applicability ^d
	N	Source	g/d	N	Source	Base	Net Δ	P	Summary	Jadad	Allocation Conceal	
	DHA/EPA Oils											
Hansen, 1989	40 ^e	Cod liver oil	ED 5.8	40 ^e	No oil	2.4	-0.1	NS	C	1	Un	GEN I
Haines, 1986	19	Fish oil	ED 4.6	22	Olive oil	2.7	+0.3	<.05	B	2	Ad	IDDM II
Misso, 1995	12 ^e	Fish oil	ED 3.6	12 ^e	Olive oil	3.0	+0.2	NS	C	2	Un	GEN II
Hansen, 1993a	11	Fish oil Tg ^f	ED 3.6	10	Corn oil	2.4	+0.3 ^g	nd	B	4	Un	GEN II
	10	Fish oil EE ^h	ED 3.4			2.4	-0.1 ^g	nd				
Toft, 1997	38	Fish oil	ED 3.4	38	Corn oil	2.2	+0.2	NS	A	5	Ad	CVD II
Grundt, 1999	28	Ethyl ester ⁱ	ED 3.4	28	Corn oil	2.9	-0.1	NS	B	2	Un	DysLip II
Nordoy, 2000	21	Fish oil	ED 3.4	20	Corn oil	3.0	+0.1	NS	B	4	Un	DysLip I
Deslypere, 1992	14	Fish oil	T 3.4	14	Olive oil	2.3	-0.1	NS	B	2	Un	GEN III
	15	Fish oil	T 2.2			2.3	-0.3	NS				
	15	Fish oil	T 1.1			2.0	+0.1	NS				
Eritsland, 1995c	254	Fish oil	ED 3.3	249	No oil	2.6	-0.1	NS	B	2	Ad	CVD II
Osterud, 1995	26	Cod liver oil	ED 3.1	28	No oil	2.6	0.0	NS	B	2	Un	GEN I
	27	Seal/Cod oil	ED 2.8			2.5	+0.1	NS				
	27	Seal oil	ED 2.4			2.6	0.0	NS				
	26	Whale oil	ED 1.7			2.6	-0.1	NS				
Hendra, 1990	37	Fish oil	ED 3.0	37	Olive oil	3.2	+0.2	NS	B	4	Un	DM II I
Gans, 1990	16	Fish oil	ED 3.0	16	Corn oil	3.3	+0.1	NS	A	3	Ad	CVD II

Radack, 1989	10	Fish oil	ED	2.2	8	Olive oil	3.2	-0.6 ^j	<.05	B	3	Un	DysLip	II
	7	Fish oil	ED	1.1			2.9	0.0	NS					
Marckmann, 1997	23	Fish oil margarine	T	0.9	24	Sunflower margarine	2.4	-0.05	NS	B	3	Un	GEN	II
Nenseter, 2000	34	Fish powder	ED	0.2	36	Cellulose	3.0	-0.2	NS	B	3	Un	DysLip	I
Leng, 1998	37 ^k	Fish oil	ED	0.045 ^L	36 ^m	Sunflower oil	3.4	+0.04	NS	C	4	Ad	CVD	II
Plant Oils														
Allman-Farinelli, 1999	15	Flaxseed oil diet	A	10% ⁿ	14	Safflower oil	2.1	+0.1	NS	B	2	Un	GEN	II
Junker, 2001	18	Rapeseed oil	T	2.5% ^o	40	Olive or Sunflower oil	2.3	+0.1 ^p	NS	C	1	Un	GEN	I
Fish and Mediterranean Diets														
Muller, 1989	40	Mackerel paste	ED	4.7	42	Meat paste	2.7	-0.02	NS	B	1	Un	GEN	II
Dunstan, 1999	14	Fatty fish ^q	T	3.6	23	No fish	2.9	+0.2 ^r	NS	B	2	Un	DysLip NIDDM	I
	12	Fatty fish ^s					3.3	+0.1 ^r	NS					
Mezzano, 2001	21	Mediterranean	T	1.6	21	Red meat	2.3	-0.3	.03	C	1	In	GEN	III
Combinations														
Freese, 1997b	24	Fish oil	ED	5.2	--	--	3.1	-0.06 ^t	nd ^u	C	3	Un	GEN	II
	22	Linseed oil	A	5.9			3.1	+0.05 ^t	nd ^u					
Cobiac, 1991	13	Fish oil	ED	4.6	6	Olive, Palm, Safflower oil	2.35	+0.4	NS	B	2	Un	GEN	II
	12	Fatty fish diet	ED	4.5			2.65	-0.15	<.05					
Agren, 1997	14	Fish oil	ED	2.3	14	No oil	3.6	+0.3	NS	B	3	Un	GEN	III
	14	Algae DHA oil	D	1.7			3.4	+0.1	NS					
	13	Fatty fish diet	ED	1.1			3.4	+0.3	NS					

nd = no data

a A = ALA; D = DHA; E = EPA; ED = EPA+DHA; T = Total omega-3 fatty acids.

b Base = baseline level in treatment arm; Net Δ = net difference in change in omega-3 fatty acids arm compared with the change in control arm, see Methods; P = P value of difference between treatment and control arms; NS = not statistically significant.

c A = good quality; B = fair quality; C = poor quality; Jadad = Jadad Score (0-5, based on randomization, double blinding, and dropouts); Ad = adequate allocation concealment; In = inadequate allocation concealment; Un = allocation concealment unclear. See Methods.

d CVD = history of cardiovascular disease; DM I/II = diabetes mellitus type 1 or 2; DysLip = dyslipidemia; GEN = general, healthy population; NIDDM = (non-) insulin dependent diabetes mellitus. I = broadly applicable; II = applicable to subgroup; III = narrow applicability. See Methods.

e Cross-over study.

f Triglycerides.

g P=.09 between treatments.

h Ethyl esters.

i No data on source.

j P<.05 compared to 1.1 g/day.

k Baseline data based on N=52.

L Plus 280 mg gamma linolenic acid (omega-6 fatty acid).

m Baseline data based on N=50.

n ALA = 10% of daily fatty acid intake.

o Kcal.

p Difference compared to average change in 2 control groups.

q Moderate exercise.

r Estimate from graph. Not clear which control group compared to (or combined). Possibly adjusted for age and sex.

s Light exercise.

t Pre-post difference (not compared to control).

u NS between treatments.

showed the largest benefit from omega-3 fatty acids and was the only study to show a dose effect (see below), was the only study performed in the United States¹⁵¹.

Covariates

Eritsland et al., Haines et al. and Toft et al. found no association of effect of omega-3 fatty acids on fibrinogen with various factors including sex, baseline and change in weight, baseline blood pressure, change in lipids or insulin, or cardiovascular, lipid or antithrombotic drug use among patients with cardiovascular disease^{115,142,152}. Mezzano et al. found no interaction of wine consumption with a Mediterranean diet in a multiphase trial¹³⁸. No differences were found among studies with run-in phases of either high- or low-fat diets. No study quantified baseline fish consumption. Radack et al. reported that the relative effect of higher dose fish oil supplements was greater with higher baseline fibrinogen values ($r = -0.59, P < .01$)¹⁵¹.

Dose and Source Effect

Two studies compared different doses of the same omega-3 fatty acid supplements. Radack et al. found that subjects with dyslipidemia who took 6 g of fish oil supplements (2.2 g EPA+DHA) for 20 weeks had a relatively large, statistically significant net reduction in fibrinogen¹⁵¹. This effect was significantly greater than in the subjects who took 3 g of fish oil (1.1 g EPA+DHA), who had no effect. Deslypere et al., however, found no difference in effect across 3 doses of fish oil supplements (3.4 g, 2.2 g, and 1.1 g EPA+DHA) in monks who took fish oils for 1 year. Across all studies the effect is not related to omega-3 fatty acid dosage.

Hansen et al. (1993a) reported a possible trend toward greater effect of fish oil ethyl esters than fish oil triglycerides¹⁴⁷. Osterud et al. found no difference among different marine oils⁷⁴. Two studies evaluated ALA oils. Both found no effect with dietary flaxseed oil or rapeseed oil supplements^{46,56}.

Three studies compared fish oil supplements with other sources of omega-3 fatty acids^{100,140,143}. Cobiac et al. found a small significant reduction in fibrinogen only among the subjects consuming dietary fish; however the significance of the difference between the 2 treatments was not reported¹⁰⁰. Overall, there were no clear differences in effect of different sources of omega-3 fatty acids.

Exposure Duration

Across studies, there was no apparent effect on fibrinogen of duration of consumption of omega-3 fatty acids in studies that reported data from 2 weeks to 2 years. Seven studies reported fibrinogen levels at various time points^{56,69,85,115,138,149,151}. Although mean fibrinogen levels varied with time in most studies, no study found a difference in effect related to time.

Sustainment of Effect

Two studies, which both found no effect of omega-3 fatty acids on fibrinogen levels, reported no further change after stopping treatment. Deslypere et al. reported no difference in fibrinogen levels up to 6 months after 1 year of treatment⁸⁵. Freese et al. likewise found no difference 4 weeks after finishing 4 weeks of treatment¹⁴³.

Factor VII, Factor VIII, and von Willebrand Factor (Tables 3.17, 3.18, and 3.19)

Omega-3 fatty acids affect the clotting system in a number of ways in animal and *in vitro* models. Factors VII and VIII and von Willebrand factor (vWF) are factors in the extrinsic coagulation system that have been suggested to play a crucial role in the initiation of blood coagulation in atherosclerotic disease, particularly in diabetes¹⁵³. Although the mechanism is not well-established, high vWF levels help to predict cardiovascular events, although the vWF level is not powerfully predictive in the individual at risk¹⁵⁴. However, different laboratories use different methods to measure coagulation factors including antigen or activity level, percent compared to a standard or concentration, and other variations. This makes comparisons across studies difficult.

We found 44 studies that met eligibility criteria and reported data on the effect of omega-3 fatty acids on factor VII, factor VIII, and/or vWF (40, 13, and 20 studies, respectively; See Table 3.1). Of these, we analyzed the 23 randomized trials that met additional criteria. For factor VII, we analyzed studies that had data on at least 15 subjects in parallel trials or 10 subjects in crossover trials who consumed omega-3 fatty acids (19 studies). For factor VIII and vWF, we analyzed all randomized trials (5 and 9 studies, respectively).

Overall Effect

Factor VII (Table 3.17)^{46,56,74,89,90,115,116,138,140-143,145-147,149,150,152,155}. There is little consistency in effect across the 19 studies of factor VII activity. In general, the net change in factor VII in subjects consuming omega-3 fatty acids is small (7% change from baseline or less), although a nearly equal number of studies found net increases as found net decreases in levels.

Table 3.17 Effects of omega-3 fatty acids on factor VII activity (%) in randomized trials (4 weeks to 9 months)

Author, Year	Omega-3 Fatty Acid Arm ^a			Control		Results ^b			Quality ^c			Applicability ^d
	N	Source	g/d	N	Source	Base	Net Δ	P	Summary	Jadad	Allocation Conceal	
	DHA/EPA Oils											
Hansen, 1989	40 ^e	Cod liver oil	ED 5.8	40 ^e	No oil	90	+2	NS	C	1	Un	GEN I
Haines, 1986	19	Fish oil	ED 4.6	22	Olive oil	79	+5	NS	B	2	Ad	IDDM II
Hansen, 1993a	11	Fish oil Tg ^f	ED 3.6	10	Corn oil	87	-1	NS	B	4	Un	GEN II
	10	Fish oil EE ^g	ED 3.4			83	-2	NS				
Toft, 1997	38	Fish oil	ED 3.4	38	Corn oil	105	+1	NS	A	5	Ad	CVD II
Grundt, 1999	28	Ethyl ester ^h	ED 3.4	28	Corn oil	119	-5	NS	B	2	Un	DysLip II
Nordoy, 2000	21	Fish oil	ED 3.4	20	Corn oil	132	-2	NS	B	4	Un	DysLip I
Eritsland, 1995c	90	Fish oil	ED 3.3	107	No oil	109	-6	NS	B	2	Ad	CVD II
Osterud, 1995	26	Cod liver oil	ED 3.1	28	No oil	1.16 ⁱ	0	NS	B	2	Un	GEN I
	27	Seal/Cod oil	ED 2.8			1.21 ⁱ	+0.03	NS				
	27	Seal oil	ED 2.4			1.23 ⁱ	-0.08	NS				
	26	Whale oil	ED 1.7			1.20 ⁱ	-0.01	NS				
Hendra, 1990	37	Fish oil	ED 3.0	37	Olive oil	94	+22	.02	B	4	Un	DM II I
Berrettini, 1996	20	Fish oil	ED 2.6	19	Corn oil	116	0 ^j	NS	B	3	Un	CVD II

Marckmann, 1997	23	Fish oil margarine	T	0.9	24	Sunflower margarine	104	0	NS	B	3	Un	GEN II
Nenseter, 2000	34	Fish powder	ED	0.2	36	Cellulose	121	+1	NS	B	3	Un	DysLip I
Plant Oils													
Allman-Farinelli, 1999	15	Flaxseed oil diet	A	10% ^k	14	Safflower oil	83	+3 ^j	NS	B	2	Un	GEN II
Junker, 2001	18	Rapeseed oil	T	2.5% ^L	40	Olive or Sunflower oil	101	+4 ^m	NS	C	1	Un	GEN I
Fish and Mediterranean Diets													
Muller, 1989	40	Mackerel paste	ED	4.7	42	Meat paste	99	-0.5	NS	B	1	Un	GEN II
Dunstan, 1999	14	Fatty fish ⁿ	T	3.6	23	No fish	112	+1 ^o	NS	B	2	Un	DysLip NIDDM I
	12	Fatty fish ^p					113	+5 ^o	<.05				
Mezzano, 2001	21	Mediterranean	T	1.6	21	Red meat	78	-4	.03	C	1	In	GEN III
Combinations													
Freese, 1997b	24	Fish oil	ED	5.2	--	--	89	+6 ^q	nd ^r	C	3	Un	GEN II
	22	Linseed oil	A	5.9			90	+5 ^q	nd ^r				
Agren, 1997	14	Fish oil	ED	2.3	14	No oil	93	0	NS	B	3	Un	GEN III
	14	Algae DHA oil	D	1.7			98	-6	NS				
	13	Fatty Fish diet	ED	1.1			94	-2	NS				

nd = no data

a A = ALA; D = DHA; E = EPA; ED = EPA+DHA; T = Total omega-3 fatty acids.

b Base = baseline level in treatment arm; Net Δ = net difference in change in omega-3 fatty acids arm compared with the change in control arm, see Methods; *P* = *P* value of difference between treatment and control arms; NS = not statistically significant.

c A = good quality; B = fair quality; C = poor quality; Jadad = Jadad Score (0-5, based on randomization, double blinding, and dropouts); Ad = adequate allocation concealment; In = inadequate allocation concealment; Un = allocation concealment unclear. See Methods.

d CVD = history of cardiovascular disease; DM I/II = diabetes mellitus type 1 or 2; DysLip = dyslipidemia; GEN = general, healthy population; N/IDDM = (non-) insulin dependent diabetes mellitus. I = broadly applicable; II = applicable to subgroup; III = narrow applicability. See Methods.

e Cross-over study.

f Triglycerides.

g Ethyl esters.

h No data on source.

i Factor VIIc activity in U/mL.

j Estimated from graph.

k ALA = 10% of daily fatty acid intake.

L Kcal.

m Difference compared to average change in 2 control groups.

n Moderate exercise.

o Estimate from graph. Not clear which control group compared to (or combined). Possibly adjusted for age and sex.

p Light exercise.

q Pre-post difference (not compared to control).

r NS between treatments.

Factor VIII (Table 3.18)^{46,84,85,115,138}. Five studies reported data on factor VIII activity. (It is unclear whether Conquer et al. measured factor VIII activity or antigen⁸⁴.) There is no consistent effect across studies, with some finding a net increase and some a net decrease in factor VIII level.

Table 3.18 Effects of omega-3 fatty acids on factor VIII activity (%) in randomized trials (6 weeks to 1 year)

Author, Year	Omega-3 Fatty Acid Arm ^a			Control		Results ^b			Quality ^c			Applicability ^d
	N	Source	g/d	N	Source	Base	Net Δ	P	Summary	Jadad	Allocation Concealment	
DHA/EPA Oils												
Haines, 1986	19	Fish oil	ED 4.6	22	Olive oil	123	+8	NS	B	2	Ad	IDDM II
Deslypere, 1992	14	Fish oil	T 3.4	14	Olive oil	77	-1	NS	B	2	Un	GEN III
	15	Fish oil	T 2.2			73	-2	NS				
	15	Fish oil	T 1.1			81	+4	NS				
Conquer, 1999	9	Seal oil	ED 3.0	10	Evening primrose	0.85 ^e	+0.12	NS	A	4	Un	GEN II
Plant Oils												
Allman-Farinelli, 1999	15	Flaxseed oil diet	A 10% ^f	14	Safflower oil	82	-5 ^g	NS	B	2	Un	GEN II
Fish and Mediterranean Diets												
Mezzano, 2001	21	Mediterranean	T 1.6	21	Red meat	68	-5	.006	C	1	In	GEN III

a A = ALA; D = DHA; E = EPA; ED = EPA+DHA; T = Total omega-3 fatty acids.

b Base = baseline level in treatment arm; Net Δ = net difference in change in omega-3 fatty acids arm compared with the change in control arm, see Methods; P = P value of difference between treatment and control arms; NS = not statistically significant.

c A = good quality; B = fair quality; C = poor quality; Jadad = Jadad Score (0-5, based on randomization, double blinding, and dropouts); Ad = adequate allocation concealment; In = inadequate allocation concealment; Un = allocation concealment unclear. See Methods.

d CVD = history of cardiovascular disease; DM I/II = diabetes mellitus type 1 or 2; DysLip = dyslipidemia; GEN = general, healthy population; N/IDDM = (non-) insulin dependent diabetes mellitus. I = broadly applicable; II = applicable to subgroup; III = narrow applicability. See Methods.

e Factor VIII in U/mL (unclear whether activity or antigen).

f ALA = 10% of daily fatty acid intake.

g Estimated from graph.

von Willebrand Factor (Table 3.19)^{46,69,84,85,89,147,149,150,156}. Nine studies reported data on various measurements of vWF using different measurement methods. Some studies were not explicit about the specific measurement used. Most studies found a net decrease in vWF level (of up to a 13% reduction from baseline), although in only 1 study was the difference with placebo reported to be statistically significant.

Table 3.19 Effects of omega-3 fatty acids on von Willebrand factor in randomized trials (4 weeks to 2 years)

Author, Year	Omega-3 Fatty Acid Arm ^a			Control		Results ^b			Quality ^c			Applicability ^d	
	N	Source	g/d	N	Source	Base	Net Δ	Unit	P	Summary	Jadad		Allocation Concealment
DHA/EPA Oils													
Seljefflot, 1998	22	Fish oil	ED 4.8	19	Fatty acids	127	-17	% ^e	.03	B	4	Un	DysLip II
Hansen, 1993a	11	Fish oil Tg ^f	ED 3.6	10	Corn oil	100	-13	% ^g	nd	B	4	Un	GEN II
	10	Fish oil EE ^h	ED 3.4			121	-16						
Nordoy, 2000	21	Fish oil	ED 3.4	20	Corn oil	101	-5	% ⁱ	NS	B	4	Un	DysLip I
Deslypere,	14	Fish oil	T 3.4	14	Olive oil	133	-1		NS	B	2	Un	GEN III

1992	15	Fish oil	T	2.2		141	-2	% ^j	NS					
	15	Fish oil	T	1.1		137	+7		NS					
Conquer, 1999	9	Seal oil	ED	3.0	10	Evening primrose	6.9	-0.5	µg/mL ^k	NS	A	4	Un	GEN II
Marckmann, 1997	22	Fish oil margarine	T	0.9	24	Sunflower margarine	86	-6	% ^e	NS	B	3	Un	GEN II
Leng, 1998	37 ^L	Fish oil	ED	0.045 ^m	36 ⁿ	Sunflower oil	118	+7	IU/dL ^o	NS	C	4	Ad	CVD II
Plant Oils														
Allman-Farinelli, 1999	15	Flaxseed oil diet	A	10% ^p	14	Safflower oil	96	-6 ^q	% ^r	NS	B	2	Un	GEN II
Fish and Mediterranean Diets														
Muller, 1989	40	Mackerel paste	ED	4.7	42	Meat paste	1.02	0	IU ^e	NS	B	1	Un	GEN II

nd = no data

- a A = ALA; D = DHA; E = EPA; ED = EPA+DHA; T = Total omega-3 fatty acids.
- b Base = baseline level in treatment arm; Net Δ = net difference in change in omega-3 fatty acids arm compared with the change in control arm, see Methods; P = P value of difference between treatment and control arms; NS = not statistically significant.
- c A = good quality; B = fair quality; C = poor quality; Jadad = Jadad Score (0-5, based on randomization, double blinding, and dropouts); Ad = adequate allocation concealment; In = inadequate allocation concealment; Un = allocation concealment unclear. See Methods.
- d CVD = history of cardiovascular disease; DM I/II = diabetes mellitus type 1 or 2; DysLip = dyslipidemia; GEN = general, healthy population; N/IDDM = (non-) insulin dependent diabetes mellitus. I = broadly applicable; II = applicable to subgroup; III = narrow applicability. See Methods.
- e By enzyme-linked immunosorbent assay (ELISA).
- f Triglycerides.
- g Factor VIII-related antigen, by ELISA.
- h Ethyl esters.
- i Activity.
- j Plasma content, by ELISA.
- k By rocket immunoelectrophoretic procedure.
- L Baseline data based on N=52.
- m Plus 280 mg gamma linolenic acid (omega-6 fatty acid).
- n Baseline data based on N=50.
- o Concentration, by ELISA.
- p ALA = 10% of daily fatty acid intake.
- q Estimated from graph.
- r Antigen, by ELISA.

Sub-populations

Factor VII. A small, inconsistent effect across studies was found among the 10 studies of a general population, the 3 studies of populations with CVD, and the 4 studies of people with dyslipidemia. The only statistically significant effects – both net increases in factor VII – were seen in 2 of the 3 studies of diabetic patients (one of which included only diabetics with dyslipidemia). The large increase in factor VII found by Hendra et al. in a 6 week study of fish oil versus olive oil supplements in non-insulin dependent diabetics was noted to be unexpected in light of a large decrease in Tg level¹¹⁶.

Factor VIII. The single study of insulin dependent diabetics found a larger net increase of factor VIII than the studies of general populations, although the difference in this study was not significant. No study measured factor VIII in CVD or dyslipidemic populations.

von Willebrand Factor. With the exception of a low-dose arm in 1 study, the 6 studies of general populations found either net decreases or no effect in vWF, although none was statistically significant. The single study of a CVD population was the only study to find an

overall net increase in vWF level, although Leng et al. was also an anomaly in that the oil analyzed was primarily gamma-linolenic acid (GLA, 18:3 n-6), an omega-6 fatty acid, with a small amount of EPA⁶⁹. The only study to find a large, statistically significant decrease in vWF was 1 of the 2 studies of dyslipidemic patients. No study evaluated diabetic patients.

Covariates

Factor VII. Haines et al. found no association between change in factor VII with fish oil supplementation and either sex or Hgb A_{1c} in insulin dependent diabetics¹¹⁵. In contrast, in a study of non-insulin dependent diabetics, Dunstan et al. reported a significant positive association between the changes in factor VII and fasting blood sugar with a fatty fish diet; however, dietary fish significantly affected factor VII levels only in subjects who were not in a moderate exercise program¹⁴¹. Eritsland et al. reported no change in (lack of) effect of fish oil supplements in patients undergoing coronary bypass surgery after controlling for multiple factors including age, sex, weight, blood pressure, diabetes and CVD medications¹⁴².

In possible contrast to the rest of the studies, only 1 of the 6 studies of male subjects, 3 of which were of younger men, found a net increase in factor VII; however all effects were small^{46,89,138,140,147,149}. One study in which all subjects were on simvastatin¹⁵⁰ found a non-significant effect of fish oil supplements similar to other studies.

Factor VIII. Haines et al. found no relationship between effect of fish oil supplementation in insulin dependent diabetics who were taking aspirin on factor VIII and either sex or Hgb A_{1c}¹¹⁵. All other studies were in men, most of whom were under age 40 years. There were no other data relating to other covariates.

von Willebrand Factor. No study reported on correlations between effect on vWF and covariates. Notably, though, only 2 of the studies included women^{69,150}. The effect of fish oil supplements in patients on simvastatin was similar to the effect of fish oil alone in other studies¹⁵⁰.

Dose and Source Effect

Factor VII. No study compared different doses of the same omega-3 fatty acid source. Across studies there does not appear to be a dose effect. Four studies compared different sources of omega-3 fatty acids. Hansen et al. (1993a) found no difference between fish oil triglycerides and fish oil ethyl esters¹⁴⁷. Osterud et al. reported no difference in effect of different marine oils⁷⁴. Freese et al. compared similar doses of fish oil and linseed oil supplements and found no difference between the 2 oils¹⁴³. Agren et al. also did not report a difference in effect among fish oil supplementation, algae DHA oil supplementation, and fatty fish diet¹⁴⁰.

Factor VIII. Only Deslypere et al. compared different doses of fish oil supplements⁸⁵. They reported no difference in effect of fish oil on factor VIII related to dose. None of the studies of fish oil supplements showed more than a marginal decrease in factor VIII level. In contrast, the single study of a flaxseed oil diet found a non-significant, approximately 6% net decrease in factor VIII activity and the single study of Mediterranean diet found a highly significant, approximately 7% net reduction in factor VIII activity. In the latter study, Mezzano et al. also found significant reductions in factor VII activity and fibrinogen levels, in contrast to most other studies¹³⁸. They found no association between the effect on factor VIII and either ABO blood type (which is related to factor VIII level) or CRP, as a marker of inflammation.