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

Summary

Introduction

Numerous studies have examined the relationship between dietary fat and cardiovascular disease (CVD). Most early epidemiology studies noted very low cardiovascular mortality in populations with high fish consumption.[1-4] The apparent benefit of dietary fish is explained by the intake of very long chain, highly polyunsaturated omega-3 fatty acids.[5] 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 risk factors and intermediate markers of CVD, and to define and explain the potential benefits of increased intake of the omega-3 fatty acids. The primary omega-3 fatty acids of interest include eicosapentaenoic acid (EPA, 20:5 n-3) and docosahexaenoic acid (DHA, 22:6 n-3), which are derived primarily from marine sources, and alpha-linolenic acid (ALA, 18:3 n-3), which is derived primarily from plant sources.

This report examines evidence addressing both the association in humans between omega-3 fatty acids and cardiovascular intermediate outcomes and risk factors and the association between omega-3 fatty acids and tissue or plasma levels of omega-3 fatty acids. The three 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. 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 exposure. In addition, because of a lack of clarity regarding the most accurate measure of levels of omega-3 fatty acids in the body, we also address how omega-3 fatty acid intake relates to different measures of tissue and plasma fatty acid levels.

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 or diagnostic tests and aspects of atherosclerosis such as inflammation, are generally unproven. The relationships between these factors and actual clinical disease and events are generally even more theoretical. Based on these limitations and the available data, the effects of omega-3 fatty acid intake on the following risk factors are addressed in this report: total cholesterol; low density lipoprotein cholesterol (LDL); high density lipoprotein cholesterol (HDL); triglycerides (Tg); lipoprotein(a) [Lp(a)]; apolipoprotein (apo) A1; apo B; apo B-100 and LDL apo B; systolic and diastolic blood pressure (BP); hemoglobin (Hgb) A_{1c}; fasting blood sugar (FBS); fasting insulin; C-reactive protein (CRP); fibrinogen; factors VII, VIII, and von Willebrand factor (vWF); and platelet aggregation. In addition, we examine the following intermediate markers of CVD: coronary artery restenosis after

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angioplasty, carotid artery intima-media thickness (IMT), exercise tolerance testing (ETT), and heart rate (HR) variability.

This evidence report is one of three 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. These reports are among several that address topics related to omega-3 fatty acids that were requested and funded by the Office of Dietary Supplements, National Institutes of Health (NIH), through the EPC program at the Agency for Healthcare Research and Quality (AHRQ). Three EPCs—the Tufts-NEMC EPC, the Southern California-RAND EPC, and the University of Ottawa EPC—each produced evidence reports. To ensure consistency of approach, the EPCs collaborated on selected methodological elements, including literature search strategies, rating of evidence, and data table design.

Methods

Key Questions

Four general questions are addressed in this report:

1. What is the effect of omega-3 fatty acids on intermediate markers and risk factors of CVD?
2. What is the effect of different omega-3 fatty acids and different sources of the fatty acids?
3. How does the effect of omega-3 fatty acids differ in different sub-populations and in relation to various confounders?
4. What is the association between intake levels of omega-3 fatty acids and tissue levels?

Literature Search Strategy

We conducted comprehensive literature searches using six databases including MEDLINE®, PreMEDLINE®, EMBASE, Cochrane Central Register of Controlled Trials, Biological Abstracts, and Commonwealth Agricultural Bureau (CAB) Health. Primary searches were performed between December 2002 and February 2003. General updated searches were conducted through April 2003 and highly focused updates were conducted through July 2003. Additional publications were identified from reference lists of review and primary articles, from domain experts, and the other two EPCs.

Selection Criteria and Screening Process

All abstracts identified through the literature search were screened using predetermined eligibility criteria. We identified all English language studies that evaluated any potential source of omega-3 fatty acids in at least five human subjects, regardless of the study outcomes reported in the abstract. We excluded abstracts that included only subjects who had a non-CVD-related condition (e.g., cancer, schizophrenia, or organ transplant), letters, and abstracts.

Upon review of full articles we excluded studies of children (under age 19 years), studies of daily omega-3 fatty acid doses of more than 6 g per day, studies of less than 4 weeks duration, crossover studies with less than 4 weeks washout between treatments, and studies that did not report complete data on outcomes of interest. We also excluded studies that did not report either the specific dose of omega-3 fatty acids or the amount of fish consumed and studies that reported only associations between omega-3 fatty acid tissue levels and risk factors. Specific sources of omega-3 fatty acid 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 the control.

Because of the large number of studies available for analysis, for most outcomes of interest we confined 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 five subjects). We limited our review of studies examining 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.

Data Extraction

Each eligible study was fully extracted by a single reviewer. 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, number enrolled and analyzed, reasons for withdrawals, description of omega-3 fatty acid and control interventions or diets, intermediate and clinical outcomes, adverse events, results, and whether each study addressed each of the key questions. In addition, each study was categorized based on applicability and study quality.

Grading Study Quality

In order to improve consistency among omega-3 fatty acid reports by the three EPCs, we used three measures of study quality to evaluate the evidence:

- The Jadad Score, which captures items related to adequacy of randomization, double blinding, and dropouts on a scale of 0 to 5.[6]

- Adequacy of allocation concealment as either adequate, inadequate, or unclear using the definitions described by Schulz et al.[7]
- Generic quality grade of either A, B, or C.[8]

  A–Least bias; results are valid. A study that mostly adheres to the commonly held concepts of high quality; no reporting errors; 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, above.

  C–Significant bias that may invalidate the results. A study with serious errors in design, analysis, or reporting.

Applicability

In this report, the focus is on the U.S. population. We categorized studies based on the study eligibility criteria into four populations: generally healthy people, people with CVD, people with diabetes, and people with dyslipidemia. A study could be categorized into multiple populations, as appropriate.

We further categorized studies within a target population into one of three levels of applicability.[8]

- 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 U.S. population.
- II–Sample is representative of a relevant sub-group of the target population, but not the entire population.
- III–Sample is representative of a narrow subgroup of subjects only, and is not applicable to other subgroups.

Qualitative and Statistical Analyses

Most outcomes evaluated were continuous variables. For these outcomes, summary tables report three sets of data pertaining to results: 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. Coronary artery restenosis studies provided rate data on a dichotomous variable (restenosis or no restenosis). For these studies, we report three equivalent sets of data: the control rate, the relative risk of restenosis, and the 95 percent confidence interval of the relative risk. In addition, we performed a random effects model meta-analysis of the relative risk.[9]

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 compositions were calculated for each treatment arm. Data were extracted for fatty acid composition of plasma or serum phospholipids, platelet membrane phospholipids, and erythrocyte membrane phospholipids (and, from one study each, granulocyte 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.[10] Changes in non-omega-3-fatty-acid arms or control groups were not included in meta-regression analyses.

Results

We screened over 7,464 abstracts. Based on this screen, we retrieved 807 full articles, 344 of which reported on CVD risk factors and intermediate markers of potential interest and met initial eligibility criteria. Within the 344 articles, there were 197 randomized trials that analyzed outcomes of interest in this report. We evaluated 123 articles that met final eligibility criteria regarding 23 potential risk factors and intermediate markers of CVD and tissue levels of omega-3 fatty acids. The majority of analyzed studies evaluated fish or other marine oils (EPA+DHA); few evaluated plant oils (EPA+DHA or ALA). 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.

Lipids

Abnormal levels of serum lipids, primarily LDL, HDL, and Tg have long been recognized as independent risk factors for CVD. We analyzed the effect of omega-3 fatty acids on these and other serum lipids that have been associated with risk of CVD, including: Lp(a) which consists of an LDL core covalently bound to a plasminogen-like glycoprotein, apolipoprotein(a); apo AI, the major apolipoprotein of HDL; apo B, a ligand for the receptor that clears the lower density lipoprotein particles from the bloodstream; and two forms of one of its subtypes: total apo B-100, which is associated with lipoprotein particles of hepatic origin; and LDL apo B, which represents the portion of total blood apo B-100 that is associated with the LDL subfraction.

We found 182 studies that met eligibility criteria and reported data on the effect of omega-3 fatty acids on cholesterol or Tg levels in at least 20 subjects. 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. The strongest, most consistent effect of omega-3 fatty acids was found among the 19 studies of Tg. Most studies reported a net decrease in Tg of about 10 percent to 33 percent. The effect was dose-dependent and generally consistent among healthy subjects and patients with CVD, at elevated risk of CVD, or dyslipidemia. Across studies, the effect of omega-3 fatty acids on triglyceride levels was generally

greater in those studies with higher baseline mean triglyceride levels. However, the single study of a plant (rapeseed and linseed) oil found a non-significant but large net increase in Tg. Limited data suggest that the effect is not related to sex, age, baseline Tg level, weight, background diet, or lipid treatment. The effect of duration of intervention or exposure is unclear and there were no data regarding sustainment of effect. The effect of omega-3 fatty acids on other serum lipids was weaker. The 23 analyzed studies of total cholesterol and the 19 studies of HDL found heterogeneous results, but mostly found small, non-significant net increases in levels of both lipids. The 15 analyzed trials of LDL fairly uniformly found small net increases in LDL level. The effect of plant oils on these lipoproteins was possibly weaker, but was similar to the effect of marine oils. No differences in effect were seen by population across studies and in one study that performed a sub-analysis of diabetic subjects. One study found a larger net increase in total cholesterol among subjects on a higher fat diet compared to those on a lower fat diet, but this effect was not seen for other lipids. A single study reported a steady increase in HDL levels over time (from 6 weeks to 12 months) with fish oil. No other studies found an effect of time on lipids. No other covariates were reported to interact with fish oil effects on lipids.

No consistent effect was found across the 14 randomized studies of Lp(a) (among a total of 23 studies examined), although one study reported a small but significant effect in subjects with elevated baseline Lp(a) levels compared to those with lower levels. Among 61 studies of apo AI, we analyzed the 27 randomized studies of apo AI with data on at least 20 subjects in parallel trials and 15 subjects in crossover trials who consumed omega-3 fatty acids. The studies generally found no effect or a net decrease in level with omega-3 fatty acid consumption. Among 52 studies of total apo B there was little consistency of effect in the 25 randomized studies with data on at least 20 subjects in parallel trials and 10 subjects in crossover trials who consumed omega-3 fatty acids. The four available studies of apo B-100 and the six of LDL apo B came to opposite conclusions in that the former all found small net changes in apo B-100—mostly net decreases—but most of the latter found large, significant net increases in LDL apo B with omega-3 fatty acid consumption.

Blood Pressure

We reviewed a recent publication that performed a meta-regression of the effect of fish oils on blood pressure.[11] This study found a small but significant reduction in both systolic and diastolic blood pressure of about 2 mm Hg with fish oil consumption. The effect was stronger in older and hypertensive populations. Because the meta-regression excluded diabetic populations, we evaluated the six randomized studies of diabetics and found similar results. One study reported that neither sex nor Hgb A_{1c} levels were related to the fish oil effect on blood pressure. No study analyzed plant oils.

Glucose Tolerance

To evaluate the effect of omega-3 fatty acids on glucose tolerance, an important risk factor for CVD among people with diabetes or insulin resistance, we evaluated Hgb A_{1c}, an indicator of long-term serum glucose levels. We also evaluated fasting blood sugar (FBS) and fasting insulin levels, which are suggestive of insulin resistance in people with normal glucose levels. Overall, there was no consistent effect of omega-3 fatty acids on glucose tolerance. Among 32 studies of Hgb A1c there was no substantial significant effect of omega-3 fatty acid consumption, regardless of study population in the 18 randomized trials with data on at least 10 subjects who consumed omega-3 fatty acids in either parallel trials or crossover trials. Among the 57 studies of FBS, we found a wide range of net effects of omega-3 fatty acids on fasting blood sugars across the 17 randomized studies with data on at least 25 subjects in parallel trials and 15 subjects in crossover trials who consumed omega-3 fatty acids among the 57 studies with data on FBS. The heterogeneity was present regardless of the makeup of the study population, although the range of effect was widest among diabetic patients. The 15 randomized trials of fasting insulin levels were very heterogeneous. The heterogeneity found in the nine studies of generally euglycemic populations was similar to that found in the studies of diabetics and obese subjects.

Inflammation and Thrombosis

CRP is an acute phase reactant that is thought to represent an integrated assessment of the overall state of activation of the inflammatory system. A growing body of studies suggests that elevations in CRP levels detected by the high sensitivity assay predict a poor cardiovascular prognosis. The five available studies of CRP found no effect with fish oil supplementation or dietary fish.

Thrombosis plays an important role in atherosclerosis and CVD. There are numerous measurable factors to assess clotting potential. Of these, we analyzed fibrinogen, a liver protein necessary for clotting that has been found to be both increased in patients with ischemic heart disease and a predictor of cardiovascular events; factors VII, VIII, and vWF, important factors in the extrinsic coagulation system; and *in vitro* platelet aggregation. No consistent effect was found among the 24 randomized trials (among 59 available studies) of fibrinogen with data on at least 15 subjects in parallel trials and 10 subjects in crossover trials who consumed omega-3 fatty acids. Nor was a consistent effect found among the 19 randomized trials of factor VII with at least 15 subjects in parallel trials and 10 subjects in crossover trials who consumed omega-3 fatty acids, or the five available randomized trials of factor VIII. The nine available randomized trials of vWF mostly found a small, non-significant decrease in level with omega-3 fatty acid consumption. The results among the 11 analyzed studies of

platelet aggregation were heterogeneous depending on aggregating agent, dose of agent, and measurement metric used, however, in most studies no effect was found with omega-3 fatty acid intake. We found 84 studies that met eligibility criteria and reported data on the effect of omega-3 fatty acids on platelet aggregation. Of these, we analyzed the randomized trials with data on at least 15 subjects in parallel trials and 10 subjects in crossover trials who consumed omega-3 fatty acids and that also reported platelet aggregation in tabular or text format. Studies that presented platelet aggregation data in graphical format only were not analyzed.

Coronary Artery Restenosis

We performed a meta-analysis of the 12 randomized trials that reported restenosis rates after coronary angioplasty. All 12 trials evaluated fish oils. We found heterogeneity of results across studies but an overall trend toward a net reduction of relative risk of 14 percent with fish oil intake. Two studies reported no significant difference in effect in men and women. Five additional non-randomized studies were not analyzed.

Carotid Artery Intima-Media Thickness

The four available studies of carotid IMT were heterogeneous. The randomized trial found no effect of fish oil, but two cross-sectional studies found that dietary omega-3 fatty acid was correlated with thinner IMT. The cohort study of plant oil margarine was inconclusive.

Exercise Tolerance Testing

The six available studies of exercise tolerance testing suggest that fish oil consumption may benefit exercise capacity among patients with coronary artery disease, although the effect may be small.

Heart Rate Variability

Three analyses of two study populations of heart rate variability concluded that fish oil supplementation among patients with recent myocardial infarction and dietary fish consumption in healthy people improves heart rate variability, which may reduce the incidence of ventricular arrhythmias. However, fish oil supplementation did not improve heart rate variability in the same healthy population.

Correlation of Intake of Omega-3 Fatty Acids With Tissue Levels

Meta-regression revealed direct relationships between dose of consumed omega-3 fatty acids and changes in levels of EPA and DHA, either as plasma or serum phospholipids, platelet phospholipids, or erythrocyte membranes. Among the 60 studies analyzed for other outcomes that reported data on percent phospholipid levels, we analyzed the 30 randomized trials with data on at least 25 subjects in parallel trials and 20 subjects in crossover trials who consumed omega-3 fatty acids. 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 one percent increase in EPA+DHA level. Granulocyte and monocyte membrane phospholipid levels also increased after omega-3 fatty acid supplementation in individual studies.

Discussion

Overall, there is strong evidence that fish oils have a strong beneficial effect on Tg that is dose-dependent and similar in various populations. There is also evidence of a very small beneficial effect of fish oils on blood pressure and possible beneficial effects on coronary artery restenosis after angioplasty, exercise capacity in patients with coronary atherosclerosis, and possibly heart rate variability, particularly in patients with recent myocardial infarctions. No consistent beneficial effect is apparent for other analyzed CVD risk factors or intermediate markers. However, there is also no consistent evidence of a detrimental effect of omega-3 fatty acids on glucose tolerance. The correlation between intake of omega-3 fatty acids and tissue levels is fairly uniform in different measured tissues.

There are little available data, however, on how the effect of omega-3 fatty acids on CVD risk factors and intermediate markers may differ depending on people's underlying conditions and risk of CVD, amount of omega-3 fatty acid consumed, duration of consumption, or source or type of omega-3 fatty acids. In particular, few studies analyzed data based on CVD risk or compared doses or types of omega-3 fatty acids. Thus, conclusions regarding these areas are all weak and based on limited data. With the exceptions of studies confined to men or to specific populations of interest (e.g., diabetics), studies generally did not base eligibility criteria on factors of particular interest here. Most conclusions that we were able to draw were based on across-study comparisons (particularly for different populations), which cannot account for confounders. Furthermore, the potential effect of ALA is unknown.

Our analyses were further limited by factors inherent to evaluation of CVD risk factors and intermediate markers. While some of these markers have indeed been demonstrated to be important markers or risk factors for CVD, it is unclear whether all of the factors are. The measurement techniques for a number of the outcomes evaluated also have not been standardized, which complicates interpretation of individual study findings and limits the ability to compare studies. Thus, the meaning in terms of CVD risk of omega-3 fatty acids on various putative risk factors and intermediate outcomes is uncertain.

Given the limitations of the current evidence, we have several recommendations for future research. Future studies on CVD risk factors and intermediate outcomes should address the questions of possible different effects of omega-3 fatty acids

in different sub-populations and different effects related to different covariates, including dose and duration of intake. More multi-center trials are needed to assess the effect of ALA, independent of EPA+DHA, on CVD risk factors and intermediate outcomes. Additional research is needed to clarify the effect of omega-3 fatty acids on markers of glucose tolerance. The omega-6/omega-3 ratio of subjects' total diet (including supplements) should be estimated, reported, and analyzed for its effect on outcomes. Attempts should be made to determine the effect of higher fish intake on the consumption of other foods in the diet, specifically meat and cheese (sources of saturated fat). Future prospective cohort studies and diet trials on fish consumption should pay special attention to collecting data with regard to fish consumed, including the type of fish and method of preparation.

Availability of the Full Report

The full evidence report from which this summary was taken was prepared for the Agency for Healthcare Research and Quality (AHRQ) by the Tufts-New England Medical Center Evidence-based Practice Center, Boston, MA, under Contract No. 290-02-0022. It is expected to be available in March 2004. At that time, printed copies may be obtained free of charge from the AHRQ Publications Clearinghouse by calling 800-358-9295. Requesters should ask for Evidence Report/Technology Assessment No. 93, *Effects of Omega-3 Fatty Acids on Cardiovascular Risk Factors and Intermediate Markers of Cardiovascular Disease*. In addition, Internet users will be able to access the report and this summary online through AHRQ's Web site at www.ahrq.gov.

Suggested Citation

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