Chapter 4. Discussion

In this chapter, we summarize findings from our review of studies examining the effect of omega-3 fatty acids on cardiovascular disease (CVD) risk factors and intermediate markers of CVD, discuss limitations of our review, and offer recommendations for future research.

Overview

Through a structured literature review process, we screened over 7,464 abstracts and retrieved and screened 807 full text articles that addressed omega-3 fatty acids and CVD risk factors and intermediate markers of CVD. After narrowing the list of outcomes of interest and applying specific eligibility criteria, we analyzed 123 articles that examined the effects of eicosapentaenoic acid (EPA, 20:5 n-3), docosahexaenoic acid (DHA, 22:6 n-3), and alpha linolenic acid (ALA, 18:3 n-3) on one of the following risk factors or intermediate markers:

- Lipids (total cholesterol, low density lipoprotein [LDL], high density lipoprotein [HDL], triglycerides [Tg], lipoprotein (a), apolipoproteins [apo] A-I, B, B-100, and LDL apo B
- Blood pressure
- Measures of glucose tolerance (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], heart rate variability)
- Tissue levels of fatty acids including plasma or serum phospholipids, platelet phospholipids, erythrocyte membrane phospholipids, granulocyte membrane phospholipids, and monocyte membrane phospholipids.

For most outcomes, we analyzed only the approximately 20 to 30 largest randomized trials. The main findings from our review and analysis are summarized in the next section. While doing the review, we found that several of the key questions and sub-questions posed at the beginning of this report were not addressed by the available studies. For example, most studies that we analyzed evaluated fish or other marine oils and only a few evaluated plant oils. 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. No study incorporated an analysis of how varying dietary omega-6 to omega-3 ratio may alter

the effect of omega-3 fatty acid consumption on outcomes. These and other limitations are addressed in more detail in the Limitations section of this chapter.

Main Findings

Overall, we found 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 the other CVD risk factors or intermediate markers of CVD we analyzed. In addition, there is also no consistent evidence of a detrimental effect of omega-3 fatty acids on glucose tolerance. Details on these and other key findings are summarized below.

As discussed in the accompanying report, *Effects of Omega-3 Fatty Acids on Cardiovascular Disease*, consumption of omega-3 fatty acids from dietary sources or from marine oil or ALA supplements reduces all cause mortality and various CVD outcomes. The cardiovascular benefits of omega-3 fatty acid consumption, though, are not well explained by the fatty acids' effects on the cardiovascular risk factors that we examined. However, the overall cardiovascular benefit may be due to the constellation of effects on lipids, blood pressure, coronary atherosclerosis, and heart rate variability. Reviewing the studies evaluated in this and the accompanying report on cardiovascular outcomes, we found no article that analyzed potential associations between omega-3 fatty acid's effect on cardiovascular risk factors and cardiovascular outcomes.

Effect on Triglycerides and Other Serum Lipids

The strongest, most consistent effect of omega-3 fatty acids was among the 19 studies of Tg. Most of these studies reported a net decrease in Tg of about 10% to 33%. The effect was dose-dependent and generally consistent among healthy subjects and patients with CVD, dyslipidemia, or at elevated risk of CVD. The effect was also greater in studies with higher mean baseline Tg. However, 1 of 2 studies of plant oils (ALA) found a net increase in Tg. Limited data suggest that the effect is not related to sex, age, weight, background diet, or lipid treatment. The effect of duration of intervention is unclear and there were no data regarding sustainment of effect. In addition, no study of diabetic patients had sufficient number of subjects to be analyzed.

The effect of omega-3 fatty acids on other serum lipids was weaker. The 23 studies of total cholesterol and the 19 studies of HDL we analyzed were heterogeneous, but mostly found small (0% to 6%), non-significant net increases in levels of both lipids. The 15 analyzed trials of LDL were fairly uniform in finding small net increases in LDL. The effect of plant oils (ALA) on these lipoproteins was possibly weaker but similar to the effect of marine oils. No differences in effect were seen among different populations, including the diabetic subjects who were evaluated in a sub-analysis. 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 of fish oil reported a steady increase in HDL levels over time beginning at 6 weeks and ending at 12 months. No other studies found an effect of time on lipids and no other covariates were reported to interact with fish oil effects on lipids.

One study compared the effect of purified EPA to purified DHA on these 4 lipids. The results were mixed. EPA lowered total cholesterol significantly (and substantially) more than DHA, DHA increased HDL by a small but significant amount more than EPA, and the effects of the 2 oils were similar in their lack of effect on LDL and their ability to lower Tg.

Effect on Blood Pressure

A recent meta-regression of the effect of fish oils on blood pressure found a small but significant reduction in both systolic and diastolic blood pressure of about 2 mm Hg. The effect was stronger in older and hypertensive populations. Because the meta-regression excluded diabetic populations, we evaluated the 6 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. One study reported no significant difference in blood pressure effect of purified EPA compared to purified DHA.

Effect on Restenosis after Coronary Angioplasty

We performed a meta-analysis of the 12 randomized trials that reported restenosis rates after coronary angioplasty. All evaluated fish oils. We found heterogeneity of results across studies but an overall trend toward a net reduction of relative risk of 14% with fish oil intake. Two studies reported no significant difference in effect between men and women.

Effect on Exercise Capacity and Heart Rate Variability

The 6 available studies examining exercise tolerance testing suggest that fish oil consumption may benefit exercise capacity among patients with coronary artery disease, although the effect may be small. Three analyses of heart rate variability in 2 study populations concluded that fish oil supplementation among patients with recent myocardial infarction, and dietary fish consumption in healthy people, improves heart rate variability, which may, in turn, reduce the incidence of ventricular arrhythmias. However, fish oil supplementation did not improve heart rate variability in the same healthy population.

Effect on Other Cardiovascular Risk Factors and Intermediate Markers

The effects of omega-3 fatty acids on the other outcomes that we evaluated were either small or inconsistent across studies.

Apolipoproteins. No consistent effect was found across 14 studies of Lp(a), although one study reported a small but significant net decrease in subjects with elevated baseline Lp(a) levels compared to those with lower baseline levels. There were insufficient studies to compare different omega-3 fatty acids. The 27 studies of apo A-I that we analyzed generally found no effect or either a small increase or decrease in level with omega-3 fatty acid consumption. Limited evidence suggested that purified EPA may decrease apo A-I levels while DHA has no effect, and that there is no difference in effect between fish oils and ALA. There was little consistency of effect in the 25 studies of total apo B. The 4 available studies of apo B-100 found

a range of effects from a 5% decrease to a 15% increase in level. Most of the 6 studies of LDL apo B found large, significant net increases in LDL apo B with omega-3 fatty acid consumption.

C-reactive protein. The 5 available studies of CRP found no effect with fish oil supplementation or dietary fish.

Measures of hemostasis. No consistent effect was found among the 24 analyzed studies of fibrinogen, the 19 analyzed studies of factor VII, or the 5 available randomized trials of factor VIII. The 9 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, generally no effect was found with omega-3 fatty acid intake. The few studies that compared types of omega-3 fatty acids found no difference in effect on these measures of hemostasis, with the exception that 2 studies came to opposite conclusions regarding whether fish oil prolonged platelet aggregation by a greater degree than ALA, and 1 study concluded that DHA may be less potent at prolonging platelet aggregation than EPA.

Carotid intima-media thickness. The 4 available studies of carotid IMT were heterogeneous. The randomized trial found no effect of fish oil but 2 cross-sectional studies found that dietary omega-3 fatty acid was correlated with thinner IMT; the cohort study of plant oil margarine was inconclusive.

Glucose tolerance. Overall, the studies of markers of glucose tolerance found no consistent effect of omega-3 fatty acids. There was a wide range of net effects of omega-3 fatty acids on fasting blood sugar across the 17 analyzed studies. Heterogeneity was present regardless of the make-up of the study population, although the range of effect was widest among diabetic patients. Within studies there were no apparent differences in effect of different omega-3 fatty acids on fasting blood sugar. Among the 18 analyzed studies of Hgb A_{1c} there was no substantial significant effect of omega-3 fatty acid consumption, regardless of study population. A single study found no difference in effect of purified EPA and purified DHA on Hgb A_{1c}. The 15 randomized trials of fasting insulin levels were very heterogeneous. Similar heterogeneity existed among the 9 studies of generally euglycemic populations as among the studies of diabetics and obese subjects. Within studies there were no apparent differences in effect of different omega-3 fatty acids on fasting insulin levels.

Tissue Levels of Fatty Acids

Meta-regression of 30 studies revealed direct relationships between dose of omega-3 fatty acids consumed and changes in measured levels of eicosapentaenoic acid (EPA, 20:5 n-3) and docosahexaenoic acid (DHA, 22:6 n-3), either as plasma or serum phospholipids, platelet phospholipids, or erythrocyte membranes. 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. Granulocyte and monocyte membrane phospholipid levels also increased after omega-3 fatty acid supplementation in individual studies.

Limitations

We identified about 60 potential CVD risk factors and intermediate markers of CVD and evaluated 23 of these in this evidence report. While some of these outcomes have been demonstrated to be important risk factors for CVD or markers of CVD, it is unclear whether this is true for all. The measurement techniques for a number of the outcomes we evaluated also have not been standardized, which complicated our interpretation of individual study findings and limited our ability to compare studies. Thus, the effects of omega-3 fatty acids on various putative risk factors and intermediate markers, and the implications for risk of CVD events, are uncertain.

While we endeavored to do a complete, systematic review of the literature on the effect of omega-3 fatty acids on CVD risk factors and intermediate markers of CVD, we were unable to critically evaluate all 350 potentially eligible studies due to time and resource limitations. Nevertheless, our findings regarding the main effects of omega-3 fatty acids on the outcomes we evaluated should be valid since we analyzed the largest randomized trials. Thus, studies not included were either non-randomized studies, which would provide more biased effect estimates, or smaller trials, which, by definition, are generally less powered than the larger studies. However, excluding non-randomized studies and small trials may have affected the availability of evidence regarding many of the secondary questions related to the effect of covariates, dosage, duration, and the like. In particular, few of the studies we analyzed evaluated plant oils. However, since few of the excluded studies evaluated plant oils, broadening our inclusion criteria may not have been helpful to this area of inquiry. In addition, for several outcomes, we analyzed a minority of the potentially available studies of diabetic patients. This was particularly the case for studies of lipid outcomes.

Although several studies performed multivariate analyses to adjust for potential confounders, few studies explicitly evaluated the effects of omega-3 fatty acids on specific subgroups as identified in the key questions. Thus, conclusions regarding these questions 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. Furthermore, only one study evaluated only women, limiting conclusions that could be made across studies based on sex.

Most conclusions that we were able to draw, particularly for different populations, were based on across-study comparisons, which cannot account for confounders.

Many studies evaluated multiple risk factors. Thus, many of the outcomes we analyzed were secondary outcomes that were often inadequately powered and reported. Many studies simply reported that the results were not significant without quantifying their results; these studies were not included in our analyses. Non-significant results would still be useful in a systematic review and meta-analysis.

Finally, the ratio of omega-6 to omega-3 fatty acids was so rarely reported that no analyses could be performed on this metric.

Future research

We offer the following recommendations for future research on omega-3 fatty acids and their effect on CVD risk factors and intermediate markers of CVD:

- Future studies on CVD risk factors and intermediate markers of CVD should address the question of possible differences in the effect of omega-3 fatty acids in different sub-populations and as related to different covariates, including dose and duration of intake.

- The potential effect of alpha linolenic acid (ALA, 18:3 n-3) is unknown. More multi-center trials are needed to assess the effect of ALA, separate from the effect of EPA+DHA, on CVD risk factors.

- Additional research is needed to clarify the effect of omega-3 fatty acids on markers of glucose tolerance. Specifically, sufficiently large trials are needed that perform appropriate sub-analyses to determine the cause of heterogeneity in effect across studies.

- The total dietary omega-6 to omega-3 fatty acid ratio should be estimated, reported, and analyzed in terms of its effect on outcomes and its association with any effect of omega-3 fatty acid treatment.

- Future research should attempt to determine the effect of higher fish intake on the consumption of other foods in the diet, specifically sources of saturated fat such as meat and cheese.

- Future prospective cohort studies and diet trials on fish consumption should place special emphasis to collecting data regarding the quantity and type of fish consumed and the method of preparation.