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Number 223

Omega–3 Fatty Acids and Cardiovascular Disease: An Updated Systematic Review

Executive Summary

Introduction

Since the first ecological study published in the late 1970s noted a relatively low cardiovascular (CV) mortality in a Greenland Eskimo population with high fish consumption,[1] there have been hundreds of observational studies and clinical trials conducted to evaluate the effect of omega-3 fatty acids (n-3 FA) on CV disease (CVD) and its risk factors and intermediate markers. The n-3 FA (including alpha linolenic acid [ALA], stearidonic acid [SDA], eicosapentaenoic acid [EPA], docosapentaenoic acid [DPA], and docosahexaenoic acid [DHA]) are a group of long-chain and very-long-chain polyunsaturated fatty acids (PUFA) that are substrates for the synthesis of eicosanoids and are important components of cell membranes that impact fluidity. Major dietary sources of ALA include soybean and canola oils, some nuts, and flaxseed. The major dietary sources of EPA and DHA are fish, other marine life, and marine-derived supplements. There is no naturally occurring source of SDA that, per serving, provides amounts of n-3 FA approaching levels (of EPA and DHA) present in oily fish. Naturally occurring sources of SDA—hemp and echium seed oils—are not consumed by the general population.

Since the publication of the original Agency for Healthcare Research and Quality (AHRQ) n-3 FA systematic reviews in 2004[2, 3] the topic of n-3 FA and CVD has remained controversial. This topic has been evaluated by several expert panels considering whether

Evidence-based Practice Program

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. 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.

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.

The full report and this summary are available at www.effectivehealthcare.ahrq.gov/reports/final.cfm.

Agency for Healthcare Research and Quality

recommendations or reference values for intakes of EPA and DHA were warranted, either through naturally occurring sources of n-3 FA (e.g., fish consumption) and/or through the use of dietary supplements and fortified foods.[4-7] In 2002, the Institute of Medicine (IOM) considered the evidence inadequate to establish an estimated average requirement for n-3 FA.[5] For healthy adults, the adequate intake values for ALA are 1.1 g/d for females and 1.6 g/d for males.[5] After evaluating evidence linking the very-long-chain n-3 FA—EPA and DHA—to coronary heart disease (CHD, also known as coronary artery disease) and stroke, the IOM panel suggested that n-3 FA may provide beneficial health effects with respect to CHD and stroke; the acceptable macronutrient distribution range (a range of intakes that is associated with reduced risk of chronic diseases while providing adequate intakes of essential nutrients) for ALA was set at 0.6 to 1.2 percent of energy (roughly equivalent to 1 to 3 g/d), where 10 percent of this range can be consumed as EPA and/or DHA.[5]
For comparison, the mean intake of ALA in the United States has been estimated at 0.6 percent of energy intake (standard deviation 1.0%),[8] equivalent to approximately 1.4 g/d. This intake level is fairly consistent across developed countries (0.3-1.0% of energy). However, estimated EPA and DHA intake in the United States are only 0.05 g/d and 0.08 g/d, respectively.[8] In contrast, mean intake in South Korea is 0.4 g/d of EPA and DHA, combined. Three other expert reports evaluated the potential health benefits of fish and seafood consumption.[4, 6, 7] Based primarily on the availability of observational study data, these panels consistently suggested that regular consumption of fish and seafood is associated with lower risk of CHD and cardiac death. These recommendations were based primarily on assumptions of benefits from EPA and DHA and their content in fish and seafood. However, determination of n-3 FA intake is problematic, both for population recommendations and in regards to research. In practice, all nutrients are quantified using a nutrient database, e.g., the U.S. Department of Agriculture National Nutrient Database for Standard Reference (http://ndb.nal.usda.gov/). The quantity of a nutrient is then estimated by the standard amount of nutrients in foods that are indexed in the nutrient database multiplied by the amount and frequency of the food consumption. However, n-3 FA in foods are not well estimated in the nutrient database and questionnaires commonly do not ask about cooking oils or dressings and may not ask about supplements (so that n-3 FA intake is estimated only from fish consumption); therefore quantification of n-3 FA intake from food frequency questionnaires is poor. Furthermore, some questionnaires do not include portion size, so further estimation or extrapolation of intake is required.

There have been secular trends in the prevention and treatment of CVD over the past several decades, particularly since the 2004 AHRQ reports on n-3 FA and CVD. These trends may have had an important impact on the potential effect or association between n-3 FA intake and CVD outcomes. Important among these trends are the lower rates of cardiac and cerebrovascular disease, concomitant with higher rates of treatment and control of dyslipidemia and hypertension. For at least the past 20 years American adults are increasingly likely to be treated with statins, antihypertensives, and low-dose aspirin. All of these pharmacologic interventions act on metabolic and biochemical pathways that n-3 FA also impact and this confounding may impact the purported CV benefits of n-3 FA, including lipid metabolism, blood pressure (BP) and vascular homeostasis, and inflammatory and coagulation pathways. These treatment trends may have contributed to the lower population-level CV benefit of higher n-3 FA intake because the underlying risk of CVD is now lower, hence, diminishing the potential impact of n-3 FA intake. Furthermore, diagnostic criteria for CVD events (e.g., myocardial infarction [MI]) and CV risk factors (e.g., metabolic syndrome) have been refined over time which may make older studies less applicable in terms of their outcomes and populations.

There are ongoing concerns in the scientific community regarding systematic biases and random errors in the determination of intakes of n-3 FA from dietary and supplement sources, using currently available assessment tools. Nutrient biomarkers can provide an objective measure of dietary status.[9] However, the correspondence between intake and biomarker concentration not only reflects recent intake but also subsequent metabolism. Current biomarkers used to estimate n-3 FA intake include ALA, EPA, DHA, and, less frequently, SDA and DPA, measured in adipose tissue, erythrocytes, plasma, or plasma phospholipids.[9-11] Adipose tissue FA are thought to reflect long-term intake, erythrocyte FA are thought to reflect intake over the previous 120 days, and plasma FA are thought to reflect more recent intake.[10]

Scope of the Review

The National Institutes of Health's Office of Dietary Supplements (ODS) has a long history of commissioning AHRQ-based systematic reviews and research methodology reports for nutrition-related topics (http://ods.od.nih.gov/Research/Evidence-Based_Review_Program.aspx). The purpose of the current ODS-sponsored systematic review is twofold: 1) to update earlier reviews of the state-of-the science on the topic of the effects of n-3 FA on CVD3 and selected CVD risk factors and intermediate

markers of CVD,[2] and 2) to collect additional information that will enhance the usefulness of this report for policy and clinical applications. This review updates the outcomes reported in the previous review and expands the scope to include additional CVD outcomes (peripheral vascular disease, congestive heart failure (CHF), and arrhythmias); it updates BP and plasma lipid outcomes and adds incident hypertension; it adds associations between biomarkers of n-3 FA intake and outcomes. The primary target audience for this report is clinical and nutrition researchers and policymakers, including ODS and panels revising dietary intake recommendations.

Key Questions

The Key Questions address issues of efficacy (i.e., causal relationships from trials), as well as associations (i.e., prospective observational cohort study associations of n-3 FA intake and/or biomarkers with long-term outcomes; or biomarker associations reported in randomized controlled trials [RCTs]). Compared with the Key Questions from the 2004 reports, the current Key Questions expand the scope of the review to include additional CV outcomes (BP, CHF, and arrhythmias), focus on the intermediate outcomes plasma lipids and BP, add the intermediate outcome hypertension, and include associations between biomarkers of intake and outcomes.

1. What is the efficacy or association of n-3 FA (EPA, DHA, EPA+DHA, DPA, SDA, ALA, or total n-3 FA) exposures in reducing CVD outcomes (incident CVD events, including all-cause death, CVD death, nonfatal CVD events, new diagnosis of CVD, peripheral vascular disease, CHF, major arrhythmias, and hypertension diagnosis) and specific CVD risk factors (BP, key plasma lipids)?
    - What is the efficacy or association of n-3 FA in preventing CVD outcomes in people
        - Without known CVD (primary prevention)
        - At high risk for CVD (primary prevention), and
        - With known CVD (secondary prevention)?
    - What is the relative efficacy of different n-3 FA on CVD outcomes and risk factors?
    - Can the CVD outcomes be ordered by strength of intervention effect of n-3 FA?
2. n-3 FA variables and modifiers:
    - How does the efficacy or association of n-3 FA in preventing CVD outcomes and with CVD risk factors differ in subpopulations, including men, premenopausal women, postmenopausal women, and different age or race/ethnicity groups?
    - What are the effects of potential confounders or interacting factors—such as plasma lipids, body mass index, BP, diabetes, kidney disease, other nutrients or supplements, and drugs (e.g., statins, aspirin, diabetes drugs, hormone replacement therapy)?
    - What is the efficacy or association of different ratios of n-3 FA components in dietary supplements or biomarkers on CVD outcomes and risk factors?
    - How does the efficacy or association of n-3 FA on CVD outcomes and risk factors differ by ratios of different n-3 FA—DHA, EPA, and ALA, or other n-3 FA?
    - How does the efficacy or association of n-3 FA on CVD outcomes and risk factors differ by source (e.g., fish and seafood, common plant oils (e.g., soybean, canola), fish oil supplements, fungal-algal supplements, flaxseed oil supplements)?
    - How does the ratio of n-6 FA to n-3 FA intakes or biomarker concentrations affect the efficacy or association of n-3 FA on CVD outcomes and risk factors?
    - Is there a threshold or dose-response relationship between n-3 FA exposures and CVD outcomes and risk factors? Does the study type affect these relationships?
    - How does the duration of intervention or exposure influence the effect of n-3 FA on CVD outcomes and risk factors?
    - What is the effect of baseline n-3 FA status (intake or biomarkers) on the efficacy of n-3 FA intake or supplementation on CVD outcomes and risk factors?
3. Adverse events:
    - What adverse effects are related to n-3 FA intake (in studies of CVD outcomes and risk factors)?
    - What adverse events are reported specifically among people with CVD or diabetes (in studies of CVD outcomes and risk factors)?

Analytic Framework

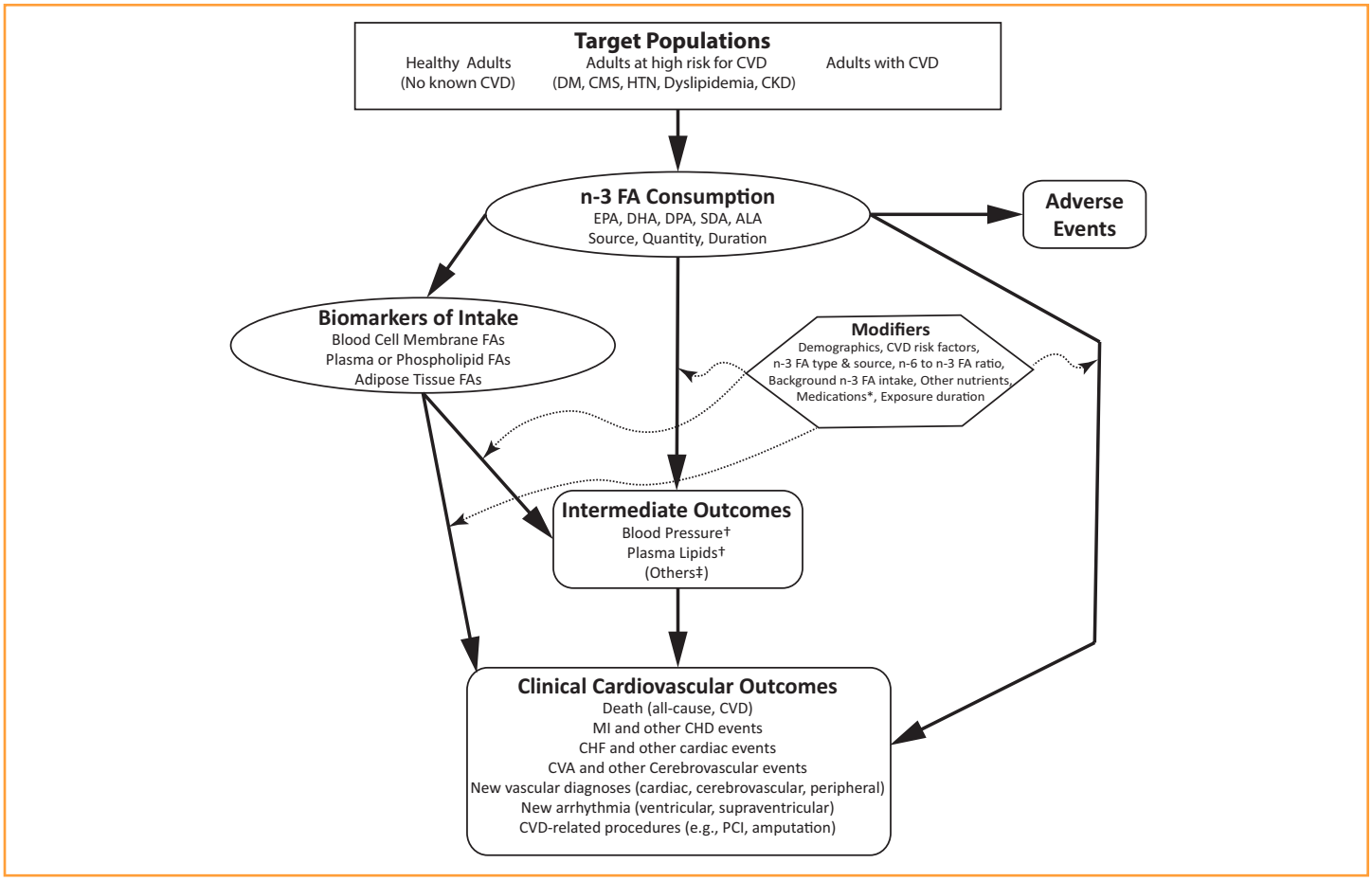
To guide the assessment of studies that examine the association between n-3 FA intake and CV outcomes, the analytic framework maps the specific linkages associating the populations of interest, exposures, modifying factors, and outcomes of interest (Figure A). The framework

graphically presents the key components of well-formulated 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 n-3 FA) to improved health outcomes.

Figure A. Analytic framework for omega–3 fatty acid exposure and cardiovascular disease



Legends: This framework concerns the effect of n-3 FA exposure (as a supplement or from food sources) on CVD and CV risk factors. Populations of interest are noted in the top rectangle, exposure in the oval, outcomes in the rounded rectangles, and effect modifiers in the hexagon.

* Specifically, CV medications, statins, antihypertensives, diabetes medications, hormone replacement regimens.

† Systolic blood pressure, diastolic blood pressure, mean arterial pressure, high density lipoprotein cholesterol (HDL-c), low density lipoprotein cholesterol (LDL-c), total/HDL-c ratio, LDL c/HDL-c ratio, triglycerides.

‡ Many other intermediate outcomes are likely in the causal pathway between n-3 FA intake and CV outcome, but only blood pressure and plasma lipids were included in the review.

Abbreviations: ALA = alpha linolenic acid, CHD = coronary heart disease, CHF = congestive heart failure, CKD = nondialysis-dependent chronic kidney disease, CMS = cardiometabolic syndrome, CVA = cerebrovascular accident (stroke), CVD = cardiovascular disease, DHA = docosahexaenoic acid, DM = diabetes mellitus, DPA = docosapentaenoic acid, EPA = eicosapentaenoic acid, FA = fatty acid, HTN = hypertension, MI = myocardial infarction, n-3 = omega−3, n-6 = omega−6, PCI = percutaneous coronary intervention, SDA = stearidonic acid.

Methods

The present review evaluates the effects of, and the associations between, n-3 FA (EPA, DPA, ALA and n-3 FA biomarkers) and CVD outcomes. The Brown Evidence-based Practice Center (EPC) conducted the review based on a systematic review of the published scientific literature using established methodologies as outlined in the AHRQ Methods Guide for Effectiveness and Comparative Effectiveness Reviews (Methods Guide).[12]

The review was conducted in parallel with a systematic review of n-3 FA and child and maternal health, conducted by another EPC. Several aspects of the review were coordinated, including eligibility criteria and search strategies regarding interventions and exposures structure of the reviews, as well as assessments of the studies' risk of bias, strength of the bodies of evidence, and extraction of study characteristics needed to assess causality.

We convened a Technical Expert Panel (TEP) to help refine the research questions and protocol, including the Key Questions, analytic framework, study eligibility criteria, literature search, and analysis plans.

Literature Search

Search Strategy

We conducted literature searches of studies in MEDLINE®, both the Cochrane Central Trials Registry® and Cochrane Database of Systematic Reviews®, Embase®, and CAB Abstracts® from 2002 to 8 June 2015 (to overlap with the last search run for the 2004 reviews). We searched publications back to 2000 for the newly added outcomes and for biomarkers of n-3 FA intake. We also rescreened and included all studies from the original reviews that met current eligibility criteria. Titles and abstracts were independently double-screened to identify articles relevant to each Key Question. We also reviewed reference lists of related systematic reviews for other potentially eligible studies.

Inclusion and Exclusion Criteria

For all Key Questions, the eligibility criteria are:

Populations

- Healthy adults (≥18 years) without CVD or with low to intermediate risk for CVD
- Adults at high risk for CVD (e.g., with diabetes, cardiometabolic syndrome, hypertension, dyslipidemia, nondialysis chronic kidney disease)
-
- Adults with clinical CVD (e.g., history of myocardial infarction [MI], angina, stroke, arrhythmia)
- Exclude populations chosen for having a non-CVD or nondiabetes-related disease (e.g., cancer, gastrointestinal disease, rheumatic disease, dialysis)

Interventions/Exposures

- n-3 FA supplements
- n-3 FA supplemented foods (e.g., eggs)
- n-3 FA content in diet
- Biomarkers of n-3 FA intake
- n-3 FA content of food or supplements must have been explicitly quantified (by any method). Therefore, studies, such as those of fish diet where only servings per week were defined or Mediterranean diet studies without quantified n-3 FA, were excluded. The n-3 FA quantification could be of total n-3 FA, of a specific n-3 FA (e.g., ALA, purified DHA) or of combined long-chain n-3 FA (EPA, DHA, and DPA, regardless of source; hereafter referred to as marine oils).
- Exclude mixed interventions of n-3 FA and other dietary or supplement differences (e.g., n-3 FA and vitamin E versus placebo; n-3 FA as part of a low-fat diet versus usual diet). However, factorial design (and other) studies that compared (for example) n-3 FA versus control, with or without another intervention (e.g., statins) were included.
- Exclude n-3 FA dose ≥6 g/d
- Exclude weight-loss interventions

Comparators

- Placebo or no n-3 FA intervention
- Different n-3 FA source intervention
- Different n-3 FA concentration intervention
- Different n-3 FA dietary exposure (e.g., comparison of quantiles)
- Different n-3 FA biomarker levels (e.g., comparison of quantiles)

Outcomes

- All-cause death
- Cardiovascular (CV), cerebrovascular, and peripheral vascular events:
  - Fatal vascular events (e.g., due to MI, stroke)

- Total incident vascular events (e.g., MI, stroke, transient ischemic attack, unstable angina, major adverse CV events [MACE]; total events include fatal and nonfatal events; total stroke includes ischemic and hemorrhagic stroke)
- Coronary heart disease (CHD, also known as coronary artery disease), new diagnosis
- Congestive heart failure (CHF), new diagnosis
- Cerebrovascular disease, new diagnosis
- Peripheral vascular disease, new diagnosis
- Ventricular arrhythmia, new diagnosis, including sudden cardiac death [SCD]
- Supraventricular arrhythmia (including atrial fibrillation [AFib]), new diagnosis
- Major vascular interventions/procedures (e.g., revascularization, thrombolysis, lower extremity amputation, defibrillator placement)

- Major CVD risk factors (intermediate outcomes):
  - Blood pressure (BP) (new-onset hypertension, systolic, diastolic, and mean arterial pressure [MAP])
  - Key plasma lipids (i.e., high density lipoprotein cholesterol [HDL-c], low density lipoprotein cholesterol [LDL-c], total/HDL-c ratio, LDL-c/HDL-c ratio, triglycerides [Tg])
- Adverse events (e.g., bleeding, major gastrointestinal disturbance), only from intervention studies of supplements

Timing

- Clinical outcomes, including new-onset hypertension (all study designs): ≥1 year followup (and intervention duration, as applicable)
- Intermediate outcomes (BP and plasma lipids) (all study designs): ≥1 month followup
- Adverse events (all study designs): no minimum followup

Setting

- Community-dwelling (noninstitutionalized) individuals

Study Design

- RCTs (all outcomes)
- Randomized cross-over studies (BP and plasma lipids, adverse events)
-
- Prospective nonrandomized comparative studies (clinical outcomes, adverse events)
- Prospective cohort (single group) studies, where groups were compared based on n-3 FA intake or intake biomarker values (clinical outcomes). Observational studies must have reported multivariate analyses.
- Exclude: Retrospective or case control studies or cross-sectional studies (but include prospective nested case control studies). Studies must have had measures of intake prior to outcome.
- Minimum sample sizes
  Due to the very large number of potentially eligible studies (more than 400), we applied arbitrary thresholds based on sample size, followup duration, and whether subgroup or interaction analyses were reported. These were designed to give preference to larger studies with longer followup duration or that reported interaction analyses of interest.
  - RCTs
    - We aimed for a minimum of about 25 RCTs for each of the BP and plasma lipid outcomes. We preferentially included RCTs that reported relevant subgroup, interaction, or factorial analyses.
      - For RCTs with BP or lipid outcomes with subgroup, interaction, or factorial analyses, we included parallel design RCTs with a minimum of 30 participants per arm, factorial RCTs with a minimum of 30 participants per n-3 FA intervention, and crossover trials with a minimum of 20 participants.
      - For RCTs with lipid outcomes without subgroup analyses, we included parallel design RCTs with a minimum of 200 participants per arm, factorial RCTs with a minimum of 200 participants per n-3 FA intervention, and crossover trials with a minimum of 100 participants.
      - For RCTs with BP outcomes without subgroup analyses, if followup was ≥6 months, we included all RCTs; if followup was <6 months (≥1 month), we included parallel design RCTs with

a minimum of 80 participants per arm, factorial RCTs with a minimum of 80 participants per n-3 FA intervention, and crossover trials with a minimum of 40 participants.

- For RCTs with CVD event outcomes, we included all RCTs with at least 10 participants per arm.

- Longitudinal observational studies
  - We aimed for a minimum of about 10 observational studies for each broad clinical outcome (see bullets below) and also for dietary marine oils, dietary ALA, marine oil biomarkers, and ALA biomarkers.
    - For cardiac event outcomes, we included observational studies with at least 10,000 participants.
    - For death outcomes, we included observational studies with at least 10,000 participants.
    - For stroke event outcomes, we included observational studies with at least 3000 participants.
    - For arrhythmia event outcomes, we included observational studies with at least 2000 participants.
    - For CHF event outcomes, we included observational studies with at least 700 participants.
    - For peripheral vascular disease event, incident hypertension, MACE, and revascularization outcomes, we included observational studies with at least 500 participants.
    - We screened smaller sample size observational studies (starting with the largest studies) to include additional studies of ALA biomarkers, regardless of the outcomes analyzed.
- In all instances, if a study met eligibility criteria for any outcome, we extracted all outcomes of interest from that study; therefore, there are multiple instances of studies being included for an outcome even though the study might not have met study size criteria for that specific outcome.

- English language publications
- Peer reviewed publications

Quality (Risk of Bias) Assessment of Individual Studies

We assessed the methodological quality of each study based on predefined criteria. For RCTs, we used the Cochrane risk of bias tool[13] and for observational studies we used relevant questions from the Newcastle Ottawa Scale.[14] Additionally, we included nutrition study specific risk of bias questions (e.g., related to uncertainty of dietary assessment measurements).[15-17]

Data Synthesis

Statistical analyses were conducted in Stata version 13.1 (StataCorp, College Station, Texas). We conducted random effects model meta-analyses of comparative studies (i.e., RCTs) if, for each set of studies with the same outcome and intervention and comparator pair, there were at least six studies. We meta-analyzed multivariate observational cohorts when at least four cohorts analyzed the same n-3 FA, measure, and outcome.

Strength of the Body of Evidence

We graded the strength of the body of evidence per the AHRQ Methods Guide on assessing the strength of evidence for each outcome.[18] The strength-of-evidence dimensional ratings are summarized in Evidence Profile tables that detail our reasoning behind the overall strength of evidence rating.

Peer Review and Public Commentary

A draft version of this report was reviewed by a panel of expert reviewers and the general public. The reviewers were either directly invited by the EPC or offered comments through a public review process. Revisions of the draft were made, where appropriate, based on their comments. The draft and final reports were also reviewed by the Task Order Officer and an Associate Editor from another EPC. However, the findings and conclusions are those of the authors, who are responsible for the contents of the report.

Results

The literature searches yielded 11,440 citations. Reference lists from existing systematic reviews yielded 203 additional citations (which mostly represented articles published before 2002). Of these, 829 abstracts met basic eligibility criteria. As described in the Methods chapter of

the full report (under *Study Selection*), using an evidence map process, we selected 463 articles for full text review, of which 147 articles met eligibility criteria, representing 61 RCTs (in 82 articles) and 37 longitudinal observational studies (in 65 articles).

Across RCTs, the studies generally had few risk of bias concerns. Among the 61 RCTs, 23 (38%) had no high risk of bias / study quality limitations; an additional 26 RCTs (43%) had one risk of bias limitation and 6 (10%) had two risk of bias limitations. None of the remaining 6 RCTs (10%) had more than four study limitations (of 10 explicitly assessed potential limitations). The most common risk of bias limitation was a lack of intention-to-treat analyses; 12 RCTs (20%) clearly did not conduct intention-to-treat analyses (one of these conducted an intention-to-treat analysis for the outcome death, but not for lipid outcomes); 12 additional RCTs (20%) were unclear whether intention-to-treat analyses were conducted. Ten RCTs (16%) did not blind study participants (and 4 additional, 7%, were unclear whether they blinded participants), often because the intervention was dietary and could not be blinded. However, only 7 RCTs (11%) clearly did not blind outcome assessors (nine additional RCTs, 14%, were unclear regarding outcome assessor blinding). Attrition bias, primarily due to dropout rates greater than 20 percent, was present in 9 RCTs (15%). Other potential biases were less common.

Across the observational studies, there were fairly few risk of bias concerns. Nine of 37 studies (24%) had no high risk of bias concerns; 20 (54%) had only a single high risk of bias concern (of 7 explicitly assessed potential limitations) and 6 (16%) had two risk of bias concerns. The 2 remaining studies (5%) had three risk of bias concerns. No study was deemed to have high risk of selection bias (regarding whether the outcome was present at baseline) and all adequately adjusted for confounders. The majority of studies used a dietary assessment tool that did not include dietary supplements (18 of 29 applicable studies; 62%); an additional 4 studies (14%) were unclear whether dietary supplements were used. Sixteen studies (43%) did not adequately reported baseline nutrient exposures. Bias due to lack of outcome assessor blinding was infrequent (3 studies [8%]; 4 studies [11%] were unclear), as was attrition bias (1 study [3%]; 4 studies [11%] were unclear). All observational studies reported multivariate analyses (this was an eligibility criterion).

The trials of clinical outcomes were almost all conducted in populations at increased risk of CVD, largely related to dyslipidemia, or with CVD. The trials that reported intermediate outcomes (BP and lipoproteins), were conducted in generally healthy, at-risk, and CVD populations. The observational studies, in contrast, were almost all conducted in general (unrestricted by CVD or risk factors) or healthy populations. One observational study evaluated BP; none evaluated lipids.

In this Executive Summary, we present the results by n-3 FA, first summarizing the strength of evidence across studies, then separately summarizing the clinical CV event outcomes from RCTs, the intermediate CV outcomes from RCTs, the observational study associations with n-3 FA intake, and the observational study associations with n-3 FA biomarkers. We also include the findings regarding adverse events and a summary directly addressing each Key Question. For the interested reader, the main report primarily summarizes the study results first by outcome, then by n-3 FA, then by study design. A listing of effects or associations of n-3 FA and outcomes by the strength of evidence supporting the findings is included at the start of the Discussion section.

Summary by n-3 FA

The trials of clinical outcomes were almost all conducted in populations at increased risk of CVD, largely related to dyslipidemia, or with CVD. The trials that reported intermediate outcomes (BP and lipoproteins), were conducted in generally healthy, at-risk, and CVD populations. The observational studies, in contrast, were almost all conducted in general (unrestricted by CVD or risk factors) or healthy populations. One observational study evaluated BP; none evaluated lipids.

Total n-3 FA (ALA+EPA+DHA)

Overall, there is insufficient evidence regarding the effect of or association between total n-3 FA (combined ALA and marine oils) and clinical or intermediate outcomes. There is low strength of evidence of no association between total n-3 FA intake and stroke death, and total (fatal and nonfatal) MI (each association based on longitudinal observational studies of dietary intake). For both outcomes, the strength of evidence was rated low because of a lack of confirmatory RCT data.

Clinical Event Outcomes, RCTs

No RCTs reported clinical event outcomes for comparisons of total n-3 FA versus placebo.

Intermediate Outcomes, RCTs

Two RCTs that evaluated BP compared combined ALA and marine oil (ALA 1.2 g/d [canola oil] or 2 g ["plant oil"] and 3.6 or 0.4 g EPA+DHA) versus placebo reported

on intermediate outcomes. Neither trial found significant effects on BP, LDL-c, HDL-c, Tg, or Total:HDL-c ratio.

Observational Studies, Intake

Seven studies evaluated total n-3 FA intake. For each outcome there was no consistent (and replicated) significant association between total n-3 FA intake and risk reduction. One of three studies found a significant association between higher total n-3 FA intake and higher risk of MACE. In contrast, one of three studies found an association of higher intake with reduced risk of CVD death; one of two studies found a significant association of higher intake with reduced risk of MI death; one study each found significant associations of higher intake with lower risk of death from ischemic stroke or CHF. The other studies found no significant associations. No studies found significant associations with all-cause death (1 study), CHD death (2 studies), total (ischemic and hemorrhagic) stroke death (3 studies), total MI (1 study), total stroke (fatal and nonfatal) (1 study), SCD (1 study), or incident hypertension (1 study).

One study found no significant difference in association of total n-3 FA with total CVD death between men and women. Another study found no significant differences in association by different baseline Total:HDL-c ratios between total n-3 FA intake and risk of MI death, total stroke death, or ischemic stroke death.

Observational Studies, Biomarkers

Three studies evaluated biomarkers for total n-3 FA (combined; plasma, blood, or erythrocyte). One study evaluated numerous outcomes and found significant associations between higher biomarker level and reduced risk of most outcomes (CVD death, CHD death, all-cause death, CHD, ischemic stroke, SCD, AFib, and CHF), but not stroke death, total stroke, or hemorrhagic stroke. In contrast, a second study found no significant association with CHD. The third study found no significant association overall with incident hypertension, but did find a significant association in between higher total n-3 FA biomarker levels and lower risk of hypertension in younger women (<55 years old) but not in older women.

Marine Oil, Total: EPA+DHA±DPA

Overall, there is low, moderate, or high strength of evidence of no effect (or association) of marine oils and most clinical CVD outcomes and BP, and high strength of evidence of significant effects of higher marine oil intake on lipoproteins and Tg. There is insufficient evidence for many outcomes of interest. Specifically, there is high strength of evidence of that marine oils statistically significantly lower Tg—possibly with greater effects with higher doses and in people with higher baseline Tg—and statistically significantly raise HDL-c and LDL-c by similar amounts. There is also high strength of evidence that marine oil significantly lowers Total:HDL-c ratio and low strength of evidence that marine oil significantly lowers risk of ischemic stroke (for which no RCTs confirmed the observational study finding). There is a high strength of evidence of no effect of marine oil on risk of MACE, all-cause death, SCD, revascularization, and BP, moderate strength of evidence of no effect of marine oil on risk of AFib, and low strength of evidence of no effect of marine oil on risk of CVD death, CHD death, total CHD, MI, CHF, total stroke, and hemorrhagic stroke. Strength of evidence was rated as low for CHD and hemorrhagic stroke due to a lack of confirmatory RCT data; and for CVD death, CHF, and total stroke because RCTs and observational studies yielded conflicting conclusions (RCTs found no effect, observational studies found statistically significant associations). Strength of evidence was rated low for CHD death primarily because RCTs and observational studies both yielded imprecise estimates suggesting no effect/association. For MI, the strength of evidence was rated low primarily because the summary effect size estimate was relatively strong (HR = 0.88), but the 95% CI only minimally crossed the significance threshold (95% CI 0.77 to 1.02); this scenario yielded low confidence that the conclusion would remain stable with future RCTs and subsequent greater statistical power. This issue was also pertinent for CVD death where summary HR = 0.92 (95% CI 0.82 to 1.02).There is insufficient evidence for other outcomes.

Four RCTs explicitly evaluated (purified) EPA and/or DHA ethyl esters; all other trials explicitly or implicitly evaluated marine oil preparations. No study directly compared formulations. The effects on clinical and intermediate outcomes found among the ethyl ester trials were all statistically or qualitatively similar to the effects found in other studies.

Clinical Event Outcomes, RCTs

Regarding clinical event outcomes, 19 trials in populations at increased risk for CVD (3 RCTs) and CVD populations (17 RCTs) mostly found no significant effects of marine oil (EPA+DHA±DPA) versus placebo on specific clinical event outcomes. Across RCTs, EPA+DHA doses ranged from 0.34 to 6 g/d (median 0.866 g/d). Followup ranged from 1 to over 10 years (median 3.9 years).

Two of 17 trials found significantly lower risk of all-cause death with EPA+DHA (both 0.866 g/d; HR = 0.79 and 0.91), however, the meta-analyzed HR was nonsignificant at 0.97 (95% CI 0.92 to 1.03) with no differences across trials by marine oil dose, followup time, or population (CVD, at risk, healthy). Four trials also found no within-study subgroup differences in effect on death for multiple subgroup comparisons.

Ten RCTs reported on MACE, only two of which found significant reductions in outcome with 0.866 g/d EPA+DHA at 3.9 year followup and with 1.8 g/d EPA at 5 year followup (in an at-risk population, but not in a parallel CVD population). Meta-analysis of MACE found a no effect (HR=0.96; 95% CI 0.91 to 1.02) with no significant differences across studies by marine oil dose (range 0.4−2 g/d), followup time (range 1−5 y), or population category. Within-study subgroup analyses found a significant effect in women but not men in one trial, but no significant difference in effect between sexes in a second trial, and no differences between multiple subgroups in three trials.

None of the 11 trials that reported on total MI found a significant effect. Meta-analysis, however, found a nonsignificant effect size (HR=0.88; 95% CI 0.77 to 1.02), with no significant differences across studies by marine oil dose, followup time, or population category. In one trial, no significant difference in effect was found based on cointervention with B vitamins.

Two of seven RCTs found significant effects of 0.866 g/d marine oil (EPA+DHA) on risk of CVD death in populations of people with existing CVD. Meta-analysis found a nonsignificant effect size (HR=0.92; 95% CI 0.82 to 1.02), with no significant differences across studies by marine oil dose, followup time, or population.

Nine RCTs all found no significant effect of EPA+DHA with SCD; by meta-analysis (with the EPA trial), summary HR=1.04 (95% CI 0.92 to 1.17). Seven RCTs also found no significant effect of marine oils with total stroke; by meta-analysis, summary HR=0.98 (95% CI 0.88 to 1.09).

Six RCTs evaluated angina pectoris, three stable angina, one hospitalization for angina, and three unstable angina. One trial found that 1.8 g/d of purified EPA ethyl ester had an additive effect on statin to reduce unstable angina incidence after 5 years in people with dyslipidemia; however the five trials in people with existing CVD found no significant effects of 0.84 to 6 g/d marine oils. The six RCTs evaluating CHF had a similar pattern. The one trial of 0.85 g/d marine oil in people with multiple risk factors for CHF found a significant risk reduction in CHF hospitalization with n-3 FA supplementation, but the five studies in people with existing CVD found no significant effects of 0.84 to 6 g/d marine oils.

All EPA+DHA RCTs that evaluated revascularization (6 trials), CHD death (4 trials), total stroke death (3 trials), AFib (3 trials), and CHF death (1 trial) found no significant effect of marine oils. One trial found an effect in participants with diabetes that was not seen in those without diabetes, but no test of interaction was reported. Two trials compared effect of marine oils on AFib in multiple subgroups, finding no significant differences.

Four EPA+DHA RCTs found inconsistent effects on cardiac death, with effect sizes ranging from 0.45 to 1.45. One trial found a statistically significant *reduction* in cardiac death with 0.866 g/d EPA+DHA at 3.5 years (RR=0.65; 95% CI 0.51 to 0.82); one trial found a statistically significant *increase* in cardiac death with a fish diet with EPA+DHA supplements (0.855 g/d EPA+DHA; HR=1.45; 95% CI 1.05 to 1.99), but no significant effect on cardiac death among people only given advice to increase fish intake (by 0.45 g/d EPA+DHA) or in two other trials of 0.96 and 2.6 g/d EPA+DHA. The trial that found increased risk with combined fish diet and EPA+DHA supplementation found no significant difference in effect between multiple sets of subgroups based on drug cointervention.

Intermediate Outcomes, RCTs

Twenty-nine RCTs that compared EPA+DHA to placebo evaluated systolic BP, of which 28 also reported on diastolic BP. Ten RCTs were in healthy populations, 13 in those at risk for CVD, and six in those with CVD. All trials found no significant difference in BP across EPA+DHA doses of 0.30 to 6 g/d and followup durations of 1 month to 6 years. By meta-analysis, no significant effects on systolic (summary net difference = 0.10 mmHg; 95% CI −0.20 to 0.40) or diastolic (summary net difference = −0.19 mmHg; 95% CI −0.43 to 0.05) BP were found. Four of the trials also found no effect on MAP. By meta-regression, no differences in effect across studies were found by marine oil dose, followup duration or population. Three trials directly compared different EPA+DHA doses and found no differences in effect (1.7 vs. 0.8 g/d; 1.8 vs. 0.9 or 0.45 g/d; 3.4 vs. 1.7 g/d). One trial found no difference in effect between people with normal BP or prehypertension.

Numerous included RCTs compared the effect of marine oils and placebo (or equivalent) on blood lipids. Thirty-nine RCTs evaluated LDL-c and 34 evaluated HDL-c. Marine oil doses ranged from 0.3 to 6 g/d (median 2.4 g/d) and study followup times ranged from 1 month to

6 years (median 3 months). Meta-analysis of the effect of marine oils on LDL-c found a statistically significant, but small effect *increasing* LDL-c (1.98 mg/dL; 95% CI 0.38 to 3.58). Marine oils increased HDL-c also by a statistically significant, but small effect (0.92 mg/dL; 95% CI 0.18 to 1.66). For both lipoprotein fractions, no significant differences in effect across studies were found by marine oil dose, followup duration or population. Seven studies found no significant differences in effect within study by EPA+DHA dose. For HDL-c, three trials found no significant difference in effect between people using statins or not; one or two trials, each, found no significant differences between subgroups based on sex or age. One trial found a larger HDL-c effect in a subgroup also randomized to an exercise regimen; one of two trials found a larger HDL-c effect in people with impaired glucose tolerance compared to those with normoglycemia. Eight trials mostly found no significant effects of marine oil (0.4−5 g/d for 1 month to 3 years) on Total:HDL-c ratio, but with a statistically significant summary effect of −0.17 (95% CI −0.26 to −0.09). One trial of 2.8 g/d EPA+DHA found no significant effect on LDL:HDL-c ratio; another trial found no significant difference in change in ratio between 3.4 and 1.7 g/d EPA+DHA.

Forty-one included RCTs mostly found significant effects of marine oils (0.3−6 g/d; median 2.4 g/d for 1 month to 6 years; median 3 months) on Tg levels. Meta-analysis found a summary net change of −24 mg/dL (95% CI −31 to−18), with no significant difference in effect based on population or followup time across studies. By metaregression, each increase in mean baseline Tg concentration by 1 mg/dL was associated with a greater net decrease in Tg concentration of −0.15 mg/dL (95% CI −0.22 to−0.08; P<0.0001); each increase of EPA+DHA dose by 1 g/d was also associated with a greater net decrease in Tg concentration of −5.9 mg/dL (95% CI −9.9 to−2.0; P=0.003). No clear inflection point was found at any dose. Five of six trials found no significant difference in Tg change by EPA+DHA dose, but across trials all doses of 3.4 and 4 g/d lowered Tg concentration by at least 30 mg/dL more than lower doses (1−2 g/d), while all pairwise comparisons of lower doses (1.7−3 g/d) to even lower doses (0.7−2.25 g/d) found much smaller differences between doses (−17 to 6 mg/dL). Two trials both found significantly larger Tg concentration lowering effects of EPA (3.6 or 3.3 g/d) than DHA (3.8 or 3.7 g/d). No significant differences were found based on statin use (4 trials), vitamin C use (1 trial), concurrent high or low linoleic acid diet (1 trial), concurrent general dietary advice (1 trial), or age (1 trial). One trial found a significantly larger effect on Tg among people also taking a multivitamin. One trial found a larger effect of higher dose EPA+DHA (1.8 g/d) in men than women, but no significant difference between sexes at 0.8 g/d. One trial found no significant difference in effect between people with impaired glucose tolerance and those with noninsulin dependent diabetes, but among those with diabetes, a larger effect was found in those with baseline HDL-c ≤35 mg/dL compared to higher levels.

Observational Studies, Intake

Twenty-one observational studies evaluated associations between total EPA+DHA±DPA intake (regardless of source) and numerous clinical outcomes. Only eight (38%) of these found significant associations with any clinical outcome.

By meta-analysis, overall there is a statistically significant association between marine oil intake and CVD death across a median dose range of 0.066 to 1.58 g/d (effect size per g/d = 0.88; 95% CI 0.82 to 0.95). Meta-analyses with the addition of a spline knot point at different dose thresholds (from 0.1 to 1.2 g/d) consistently found a stronger association (of higher dose being associated with lower risk) at lower doses than at higher doses (ES below knot less than 1; ES above knot closer to 1). This implies the possibility of a ceiling effect (where intake above a certain level adds no further benefit). However, at no dose threshold was there a statistically significant difference between the ES below the dose threshold (knot) and above the threshold. The best fit curve was found with a knot at 0.3 g/d. The lowest P value between lower-dose and higher-dose ES estimates was found at 0.2 g/d (P=0.26).

By meta-analysis, overall there no significant association between marine oil intake and CHD death across a median dose range of 0.04 to 2.1 g/d (effect size per g/d = 1.09; 95% CI 0.76 to 1.57). Meta-analyses with the addition of a spline knot point at different dose thresholds (from 0.1 to 1.2 g/d) found stronger associations (of higher dose being associated with lower risk) at lower doses than at higher doses (ES below knot less than 1; ES above knot closer to 1) for knots below 0.7 g/d, but stronger associations at higher doses above 0.7 g/d. However, the differences in effect size between lower and higher doses were always highly nonsignificant, implying no difference in association. The best fit curve was found with a knot at 0.5 g/d. The lowest P value between lower-dose and higher-dose ES estimates was found at the lowest tested threshold, 0.1 g/d (P=0.46).

By meta-analysis, overall there no significant association between marine oil intake and all-cause death across a median dose range of 0.066 to 1.58 g/d (effect size per

g/d = 0.62; 95% CI 0.31 to 1.25). However, meta-analyses with the addition of a spline knot point at different dose thresholds (from 0.1 to 1.2 g/d) consistently found stronger associations (of higher dose being associated with lower risk) at *lower* doses than at higher doses (ES below knot less than 1; ES above knot closer to 1). This implies the possibility of a ceiling effect (where intake above a certain level adds no further benefit). For thresholds ≤0.4 g/d the associations are statistically significant at lower doses, but not statistically significant at higher doses. The difference between low- and high-dose associations is statistically significantly different at a threshold of 0.2 g/d (P=0.047). The best fit curve was found with a knot at 0.3 g/d. This analysis may suggest that marine oil intake above about 0.2 to 0.4 g/d may not further strengthen any association between higher marine oil intake and lower rate of all-cause death.

By meta-analysis, overall there no significant association between marine oil intake and CHD across a median dose range of 0.038 to 3.47 g/d (effect size per g/d = 0.94; 95% CI 0.81 to 1.10). Meta-analyses with the addition of a spline knot point at different dose thresholds (from 0.1 to 1.4 g/d) consistently found a stronger association (of higher dose being associated with lower risk) at lower doses than at higher doses (ES below knot less than 1, ES above know about 1). At all knot points the differences were nonsignificant. This weakly suggests the possibility of a ceiling effect (where intake above a certain level adds no further benefit). The best fit curve was found with a knot at 0.4 g/d. The P values for differences between lower- and higher-dose knots were between 0.12 and 0.14 at all knots ≥0.3 g/d.

By meta-analysis, overall there is a statistically significant association between marine oil intake and total stroke across a median dosage range of 0.025 to 0.6 g/d (effect size per g/d = 0.68; 95% CI 0.53 to 0.87). Meta-analyses with the addition of a spline knot point at different dose thresholds (from 0.1 to 0.5 g/d) consistently found a much stronger association (of higher dose being associated with lower risk) at *lower* doses than at higher doses (ES below know less than 1; ES above know greater than 1); although, the difference in effect sizes above and below the knots were never statistically significant This implies a possible ceiling effect ceiling effect (where intake above a certain level adds no further benefit). However, given that the differences between lower and higher dose ES remained large across the range of testable dose thresholds, the actual ceiling dose threshold may be above the analyzable range (i.e., >0.5 g/d). The best fit curve was found with the lowest knot at 0.1 g/d. The P values for differences between lower- and higher-dose effect sizes ranged from 0.14 to 0.20.

By meta-analysis, overall there is a statistically significant association between higher marine oil intake and lower risk of ischemic stroke across a median dosage range of 0.025 to 0.6 g/d (effect size per g/d = 0.51; 95% CI 0.29 to 0.89). Meta-analyses with the addition of a spline knot point at different dose thresholds (from 0.1 to 0.5 g/d) consistently found a much stronger association (of higher dose being associated with lower risk) at *lower* doses than at higher doses (ES below knot less than 1; ES above knot near or greater than 1). All effect sizes below the knots were statistically significant and all above the knots were nonsignificant. The differences between lower- and higher-dose effect sizes were all statistically significant (P=0.03-0.049). This implies a ceiling effect (where intake above a certain level adds no further benefit). However, it is unclear what the threshold may be, as it may be greater than the highest threshold tested (0.4 g/d). The best fit curve was found with a knot at either 0.3 or 0.4 g/d. The difference between lower-dose and higher-dose ES estimates was statistically significant with a knot at 0.1 g/d.

By meta-analysis, overall there is no significant association between marine oil intake and hemorrhagic stroke across a median dosage range of 0.025 to 0.6 g/d (effect size per g/d = 0.61; 95% CI 0.34 to 1.11). Meta-analyses with the addition of a spline knot point at different dose thresholds (from 0.1 to 0.5 g/d) consistently found similar associations above and below the knots. At no threshold was the difference in effect sizes statistically significant. The best fit curve was found with a knot at 0.1 g/d. The lowest P value between lower-dose and higher-dose ES estimates was found at 0.5 g/d (P=0.78).

By meta-analysis, overall there is a just-significant association between higher marine oil intake and decreased risk of CHF across a median dosage range of 0.014 to 0.71 g/d (effect size per g/d = 0.76; 95% CI 0.58 to 1.00). Meta-analyses with the addition of a spline knot point at different dose thresholds (from 0.1 to 0.5 g/d) consistently found a stronger association (of higher dose being associated with lower risk) at *lower* doses than at higher doses (ES below knot less than 1; ES above knot closer to 1). This implies the possibility of a ceiling effect (where intake above a certain level adds no further benefit). However, given that the differences between lower and higher dose ES remained large across the range of testable dose thresholds, the actual ceiling dose threshold may be above the analyzable range (i.e., >0.5 g/d). At thresholds of 0.1 and 0.2 g/d, the difference in effect size at lower and higher doses were statistically significant (P values 0.04 and 0.03, respectively). But the most significant difference was found at the highest threshold tested, 0.5 g/d (P=0.02). The best fit curve was found with the lowest knot tested, 0.1 g/d.

A minority of studies found significant associations of decreased risk of other outcomes with increasing intake of EPA+DHA±DPA: MACE (1 of 2 studies), all-cause death (1 of 3 studies), CVD death (1 of 4 studies), CHD death (3 of 7 studies), MI (1 of 2 studies), incident CHF (1 of 5 studies), and AFib (1 of 3 studies). No studies found significant associations with cardiac death (1 study), total stroke death (1 study), ischemic stroke death (1 study), coronary revascularization (1 study), ventricular arrhythmia (1 study), SCD (2 studies), and incident hypertension (1 study). One study each analyzed MI death and ischemic stroke death and found a significant association.

Observational Studies, Biomarkers

Five studies evaluated combined EPA+DHA±DPA biomarkers, including adipose tissue, cholesteryl ester, erythrocyte, phospholipid, and plasma n-3 FA levels. Of the outcomes evaluated, none was analyzed by more than two studies. One study each found no significant association between various biomarker levels and MI, hemorrhagic stroke, total stroke (with a P value of 0.07), or cardiac death. One study found a significant association between higher phospholipid EPA+DHA+DPA and incident CHD. Another found a significant association between higher adipose EPA+DHA+DPA and acute coronary syndrome in men, but not in women. Two studies each evaluated CHF, ischemic stroke, and MACE. For each outcome only one of the studies found significant associations with EPA+DHA±DPA biomarker levels. In one of the studies of CHF, phospholipid EPA+DHA+DPA level was associated with the outcome in women only but cholesteryl ester EPA+DHA+DPA levels were not associated in either sex.

EPA

For the most part, there is insufficient evidence regarding the effect of or association with EPA (specifically) and CVD clinical and intermediate outcomes. There is low strength of evidence of no association between EPA intake and CHD and between EPA biomarkers and AFib; no RCTs evaluated these outcomes.

Clinical Event Outcomes, RCTs

Regarding clinical event outcomes, one trial in an at risk population (dyslipidemia), found that after 5 years, compared with placebo, people taking purified EPA 1.8 g/d had significantly lower risk of MACE and angina, but no significant difference in all-cause death, CHD death, coronary revascularization, SCD, or MI (also in the subgroup of people with prior CVD). Subgroup analysis for CHD death found no clear difference between those who also had CVD versus those without CVD.

Intermediate Outcomes, RCTs

Two RCTs evaluated BP or lipid outcomes. One trial of purified EPA 3.8 g/d versus placebo found no significant effect of EPA supplementation on systolic BP, diastolic BP, or MAP. This trial and another of EPA 3.3 g/d found no significant effect of EPA supplementation on LDL-c or HDL-c. Both trials, however, found significant net reductions in Tg concentration (−42 and −23 mg/dL). The trial of EPA 3.8 g/d also found a significant reduction in Total:HDL-c ratio (−0.2).

Observational Studies, Intake

Eight studies evaluated associations between estimated total EPA intake and clinical outcomes. No outcome was evaluated by more than two studies. One study each found no significant association between EPA intake and acute coronary syndrome, ischemic stroke, or total stroke death. One study found a significant association between higher EPA intake and lower ischemic stroke death in healthy adults (in quantiles with median EPA intake >0.07 g/d in men and >0.06 g/d in women), but no association with hemorrhagic stroke death. One study found a significant association between higher EPA intake and lower risk of all-cause death (>0.01 g/d) in healthy adults; another study found a significant association with lower risk of MACE in healthy adults (>0.09 g/d). Two studies, each, found no significant associations between EPA intake and incident CHD (although P=0.06 in one) or CHD death. For both incident hypertension and CVD death, one of two studies found significant associations between higher EPA (0.02 g/d for hypertension and 0.01 g/d for CVD death) intake and lower risk of hypertension and CVD death; the other studies found no such associations.

Observational Studies, Biomarkers

Ten studies evaluated associations between various EPA biomarkers and clinical outcomes. Three studies of healthy adults evaluated incident CHD. Two of these studies found that increased plasma or phospholipid EPA levels were associated with reduced risk of CHD; the third study found no significant association between blood EPA levels and CHD risk. Three studies (two in healthy adults, one in people with hypercholesterolemia) evaluated MACE; the study of people with hypercholesterolemia found an association of reduced MACE risk with higher plasma EPA, as did one study of phospholipid EPA in healthy

adults. The third study found no significant association between erythrocyte EPA and MACE in healthy adults. Three studies, two in healthy adults and one in adults with a history of MI, evaluated CHF; in one study of healthy adults, higher plasma EPA was associated with reduced CHF risk, but the other study of healthy adults found no association with phospholipid or cholesteryl ester EPA and CHF risk. The study in people with a history of MI also found an association between higher blood EPA level and lower CHF risk. In this latter study, significant interactions were found for sex (no association was seen in women, in contrast with a significant association in men), statin use (those on statins had no association, in contrast with those not on statins), and baseline HDL-c level (those with higher HDL-c, ≥40 mg/dL, had no association, in contrast with those with lower HDL-c, <40 mg/dL). No interactions were found for age, use of angiotensin receptor blocker drugs, use of beta blocker drugs, diabetes, dyslipidemia, baseline LDL-c, hypertension, glomerular filtration function, or hypertriglyceridemia.

One of three studies found a significant association between higher EPA biomarkers (plasma EPA) and lower risk of death in healthy adults, but a second study of plasma EPA in healthy adults found no such association; nor did a study of blood EPA in people with a history of MI. One of two studies of plasma EPA in healthy adults found a significant association of higher plasma EPA with lower risk of CVD death. Two studies found no significant association between EPA biomarkers and ischemic stroke. One study found a significant association between erythrocyte EPA and incident hypertension. One study each found no associations between EPA biomarker levels and acute coronary syndrome, AFib, SCD, MI, hemorrhagic stroke, total stroke, cardiac death, CHD death, or total stroke death.

DHA

For the most part, there is insufficient evidence regarding the effect of or association with DHA and CVD clinical and intermediate outcomes. There is moderate strength of evidence of no effect of purified DHA supplementation on BP or LDL-c and low strength of evidence of no association between DHA intake and incident CHD (from observational studies only).

Clinical Event Outcomes, RCTs

No trial that reported clinical event outcomes evaluated DHA alone.

Intermediate Outcomes, RCTs

Two trials compared purified DHA (3.6 and 2 g/d) to placebo and found no significant effects on systolic or diastolic BP. One of the trials also found no significant effect on MAP. Three trials of DHA (3.7, 3.6, or 2 g/d) also found no significant effect compared to placebo on LDL-c or HDL-c. Two trials (3.7 and 3.6 g/d) reported on Tg concentration changes and both found significant net reductions compared to placebo with DHA supplementation (−27 and −29 mg/dL). The trial of DHA 3.6 g/d also found a significant reduction in Total:HDL-c ratio (−0.3) compared to placebo.

Observational Studies, Intake

Eight studies evaluated the association between estimated total DHA intake (specifically) and risk of clinical outcomes. No outcome was reported in more than two studies. Two studies found significant associations between higher DHA intake and lower risk of incident hypertension in healthy young adults (18−30 years old in one study; 39−54 year old women in a subgroup of one study), but not in an older subgroup (55−89 years old in one study). In the study of young adults, a significant association was found in quartiles with DHA intake >0.06 g/d compared to quartiles with lower intake. One of two studies of healthy adults found an association of lower CVD death with DHA intake >0.15 g/d. Two studies each found no association with CHD death or incident CHD (in populations with a broad range of ages, from 20−69 to 45−84 years old). One study each found significant associations of higher DHA intake with increased incidence of MACE (>0.15 g/d DHA), ischemic stroke death (>0.15 g/d), and all-cause death (>0.02 g/d). In one study each, no associations were found with acute coronary syndrome, ischemic stroke, hemorrhagic stroke death, or total stroke death.

Observational Studies, Biomarkers

Eleven studies evaluated various DHA biomarkers and their associations with clinical outcomes. Overall, a high proportion of observational studies found statistically significant associations between higher DHA biomarker levels and decreased risk of outcomes. Four studies evaluated MACE (with various definitions); two found significant associations between higher DHA biomarker levels (phospholipid and adipose DHA) and lower risk of MACE in healthy adults. The other two studies found no association, one in hypercholesterolemic adults on statins (plasma DHA) and one in healthy adults (erythrocyte DHA). Two of three studies in healthy adults found significant associations between higher plasma or

phospholipid DHA and lower CHD risk; the third study, also in healthy adults, found no association with blood DHA. Three studies evaluated CHF. One found associations between higher cholesteryl ester and phospholipid DHA and lower risk of incident CHF in healthy women, but not healthy men (whether the associations were significantly different between women and men was not reported). One study found that overall, there was no significant association of CHF with blood DHA in adults with a history of MI, but that there were significant associations in subgroups of people, such that significant association between higher blood DHA and lower risk of CHF were found in a population with a history of MI not taking a statin (P interaction with statin use = 0.003), ≥65 years old (P interaction = 0.051), with LDL-c ≥100 mg/dL (P interaction = 0.068), and with HDL-c ≤40 mg/dL (P interaction = 0.096). Three studies also evaluated all-cause death, two of which found significantly lower risk of death with higher plasma DHA (healthy adults) and blood DHA (in people with a history of MI who were not taking statins); another study of healthy adults found no association with plasma DHA.

Two studies found nonsignificant associations between higher cholesteryl ester DHA (P=0.07), phospholipid DHA (P=0.08), and plasma DHA (P=0.052) and lower risk of ischemic stroke in healthy adults. One study of healthy adults found an association between higher plasma DHA and lower risk of CVD death (both studies evaluated plasma DHA). One study each found significant associations between higher DHA biomarker levels and lower incidence of AFib, SCD, and CHD death (all plasma DHA in healthy adults). One study found a significant association between higher adipose DHA and lower risk of acute coronary syndrome in healthy men, but not healthy women. Another study found a significant association between higher erythrocyte DHA and lower risk of incident hypertension in healthy women aged 39 to 54 years, but not in women older than 54 years. One study found no significant associations between plasma DHA and both total stroke and total stroke death in healthy adults. One study, each, found no significant associations with MI, hemorrhagic stroke, or cardiac death.

DPA

Overall, there is insufficient evidence regarding effect of or association between DPA (specifically) and CVD clinical and intermediate outcomes. There is low strength of evidence of no association between DPA biomarker levels and risk of AFib (from observational studies only).

RCTs

No eligible RCTs compared purified DPA formulations versus placebo.

Observational Studies, Intake

Two observational studies evaluated estimated total DPA intake (specifically). One study found no significant association between DPA intake and acute coronary syndrome in either healthy men or women. The other found significant associations between higher DPA intake and both incident CHD and MACE in healthy adults, in both instances with a significant association in the quartile with DPA intake >0.04 g/d.

Observational Studies, Biomarkers

Seven studies evaluated the association of various DPA biomarkers with clinical outcomes, all in healthy adults. No outcome was evaluated by more than three studies. One study in adults age ≥65 years evaluated several clinical outcomes. It found significant associations between higher plasma DPA and lower risks of all-cause and CVD death, nonsignificant associations with incident CHF (P=0.057) and total stroke death (P=0.056), but no significant associations with AFib, SCD, hemorrhagic, ischemic, or total stroke, or CHD death. For both CHD and MACE, one study found a significant association between higher blood DPA and lower incident CHD, but two studies found no association with plasma or phospholipid DPA. Similarly, one study found a significant association between higher adipose tissue DPA and lower MACE risk, but two found no association with phospholipid or erythrocyte DPA. One study evaluated acute coronary syndrome and found a significantly lower risk in men with higher adipose tissue DPA, but no significant association in women. One study evaluated incident hypertension and found a significant association of higher erythrocyte DPA and lower hypertension risk in younger women (39−54 years old), but not older women (55−89 years old). One study found no significant association with cardiac death.

SDA

Overall, there is insufficient evidence regarding effect of or association between SDA (specifically) and CVD clinical and intermediate outcomes.

RCTs

A single study compared 1.2 g/d SDA to placebo in patients at risk for CVD and found no significant differences in change in systolic or diastolic BP, or LDL-c, HDL-c, or Tg at 6 weeks.

Observational Studies

A single eligible observational study in healthy men evaluated baseline erythrocyte SDA and clinical outcomes. Erythrocyte SDA was not significantly associated with either MACE or cardiac death.

Marine Oil FA Comparisons

There is insufficient evidence regarding comparisons of specific marine oil FA (e.g., EPA vs. DHA).

Clinical Event Outcomes, RCTs

No trial that reported clinical event outcomes compared marine oil FA.

Intermediate Outcomes, RCTs

Two trials that compared marine oil FA (EPA 3.8 g/d vs. DHA 3.6 g/d; EPA+DHA 3.4 and 1.7 g/d vs. EPA 1.8 g/d) found no significant differences in effect on BP, LDL-c, HDL-c, Tg, or Total:HDL-c ratio.

One trial compared 2.0 g/d SDA and 1.9 g/d EPA+DHA+DPA in healthy people. At 2 month followup, no significant differences in change in systolic or diastolic BP, or LDL-c, HDL-c, Tg, Total:HDL-c, or LDL:HDL-c ratios were found.

ALA

There is moderate strength of evidence of no significant effect of ALA intake on BP, LDL-c, HDL-c, or Tg. There is low strength of evidence of no association between ALA intake or biomarker level and CHD or CHD death, AFib, and CHF, each based primarily on observational studies; there was only a single or no RCTs evaluating these outcomes. There is insufficient evidence regarding other outcomes.

Clinical Event Outcomes, RCTs

Two RCTs that evaluated ALA supplementation versus placebo reported clinical event outcomes, one in participants with CVD and one in healthy participants. All analyses were nonsignificant, for all-cause death (2 trials) and from one trial each, MACE, CVD death, cardiac death, CHD death, CHF death, total MI, incident angina, total stroke, ventricular arrhythmia, and SCD. Within-study subgroup analyses revealed no significant differences in effect for various subgroups for MACE (1 trial) or with or without diabetes for CHD death (1 trial).

Intermediate Outcomes, RCTs

Five ALA RCTs evaluated BP, with doses ranging from 1.4 to 5.9 g/d for 1 to 3.4 years. All found no significant effect on systolic or diastolic BP, mostly with wide confidence intervals. One of the trials found no significant difference in effect of ALA on BP between a subgroup with hypertension and the study population as a whole. Another trial found no significant difference in effect between 1.4 and 5.9 g/d ALA. No trial reported on MAP.

Five trials reported no significant effects of ALA on LDL-c, HDL-c, Tg, or Total:HDL-c ratio (3 trials). No differences in effect were found in the one trial that compared 1.4 and 5.9 g/d ALA. No trial reported on LDL:HDL-c ratio.

Observational Studies, Intake

Thirteen observational studies evaluated ALA intake. One of these was a pooling of 11 prior studies (the pooled studies were not included in duplicate for the outcomes evaluated by the pooling study). The large majority of analyses found no significant associations; only two studies found any significant associations between higher ALA intake and clinical outcomes.

By meta-analysis, overall there is no statistically significant association between ALA intake and CHD death across a median dose range of 0.59 to 2.5 g/d (effect size per g/d = 0.94; 95% CI 0.85 to 1.03). Meta-analyses with the addition of a spline knot point at different dose thresholds (from 0.6 to 1.2 g/d) consistently found a stronger association (of higher dose being associated with lower risk) at higher doses than at lower doses (ES above knot < ES below knot); although the differences were generally small and all were nonsignificant. The best fit curve was found with a knot at 0.9 g/d. The lowest P value between lower-dose and higher-dose ES estimates was found at 1.2 g/d (P=0.44), the highest dose threshold that could be tested.

By meta-analysis, overall there is no association between ALA intake and CHD across a median dosage range of 0.2 to 2.5 g/d (effect size per g/d = 0.97; 95% CI 0.92 to 1.03). Meta-analyses with the addition of a spline knot point at different dose thresholds (from 0.5 to 1.4 g/d) consistently found marginally smaller ES at lower doses than at higher doses. At no dose threshold was there a statistically significant difference between the ES below the dose

threshold (knot) and above the threshold. The best fit curve was found with a knot at 0.7 g/d, the threshold that also had the lowest P value (P=0.34).

Two studies both found significant associations between higher ALA intake and reduced all-cause death (>2.2 g/d in healthy adults; also in healthy men but insufficient data were reported regarding a dose threshold). One of two studies found a significant association between higher ALA intake (>0.6 g/d) and lower risk of SCD in healthy women but not in a subset of women with CVD; the second study found no significant association in healthy adults. One of two studies found a significant association between higher ALA intake (unclear threshold) and lower risk of CVD death in younger men (35−57 years old), but another study found no association in older men (≥65 years old). For all other analyzed clinical outcomes, no significant associations were found with ALA intake, including CHF (4 studies), CVD (3 studies), MACE (2 studies), hemorrhagic and ischemic stroke (2 studies each), AFib (1 study), and hypertension (1 study).

Observational Studies, Biomarkers

Eight studies evaluated various ALA biomarkers. Almost all analyses found no significant associations between ALA biomarkers and clinical outcomes. No outcome was evaluated by more than three studies. For CHF, one study found a significant association between higher plasma ALA and CHF in healthy men, but two other studies found no significant associations in healthy adults across levels of plasma, cholesteryl ester, or phospholipid ALA. One of two studies found a significant association between higher plasma ALA and lower risk of CVD death, but the other study found no significant association with plasma ALA in healthy adults. No significant associations were found for ischemic stroke (3 studies), incident CHD, hemorrhagic and total stroke (2 studies each), MACE (2 studies), all-cause death (2 studies), or AFib, SCD, incident hypertension, cardiac death, or CHD death (1 study each).

Marine Oil Versus ALA

There is insufficient evidence of direct comparisons between marine oil and ALA intake on CVD outcomes. Across studies, the indirect comparison between marine oil and ALA is unclear, largely because there are insufficient studies that evaluated ALA. However, for Tg and HDL-c, where there is high strength of evidence of significant effects of higher dose of marine oil improving Tg and HDL-c, there is moderate strength of evidence of no effect of ALA intake on these intermediate outcomes.

Clinical Event Outcomes, RCTs

No trial that reported clinical event outcomes directly compared marine oils and ALA.

Intermediate Outcomes, RCTs

One trial that compared two doses of EPA+DHA (1.7 and 0.8 g/d) with ALA 4.5 g/d found no differences in systolic or diastolic BP at 4 months. Across trials, there was no evidence that intake of any type of n-3 FA had an effect on BP; no difference in effect was apparent between marine oil and ALA trials.

Two trials that compared EPA+DHA (0.8 and 1.7 g/d in one trial, 0.4 g/d in the other) to ALA (4.5 g/d [rapeseed oil margarine] and 2 g/d ["plant oil" margarine], respectively) for 6 months and 3.4 years found no differences between intake of n-3 FA and LDL-c, HDL-c, or Tg levels. Neither trial reported on lipid ratios. No evident differences were found across trials between marine oils and ALA for their (nonsignificant) effects on LDL-c and HDL-c. In contrast with the two trials that directly compared EPA+DHA and ALA, 32 marine oil (versus placebo) trials fairly consistently found significant effect on Tg reduction in contrast with the four ALA (versus placebo) trials, which mostly had imprecise estimates of effects on Tg.

Subgroup Analyses Summary

Overall, 24 RCTs and 9 observational studies reported on subgroup (or factorial) analyses. For most outcomes, there is insufficient evidence regarding differential effects (or associations) in different subgroups of study participants evaluated within studies. Metaregression results across studies are summarized in the summary by n-3 FA, above. (In brief, only for the effect of marine oil on Tg was there an indication across studies of interactions by dose and baseline Tg, with larger effects with higher dose and higher baseline Tg.) Among outcomes with sufficient RCT data to allow meta-analysis, no discernable difference in effect was found across trials based on publication year.

Twenty-two subgroup analyses by sex were reported (10 with ALA, 11 with marine oil, 1 with total n-3 FA). One of three RCTs of marine oil on MACE found a greater beneficial effect of n-3 FA in women (HR [supplement vs. placebo] =0.82 in women vs. 1.04 in men; P interaction = 0.04). One of three observational studies of CHF found a stronger association with between higher blood EPA and lower risk of CHF in men than women (HR [lower intake vs. higher intake] = 5.82 in men vs. 0.69 in women; P interaction = 0.008), but no interaction with blood DHA. One RCT found a stronger effect on lowering Tg of

supplementation with higher-dose marine oil (1.8 g/d) in men than in women (difference not reported; P interaction = 0.038), but this interaction was not found with lower-dose marine oil (0.7 g/d). All 19 other analyses were not statistically significant (or no statistical difference was reported).

Twenty subgroup analyses by statin use were reported (1 with ALA, 19 with marine oil). All but one study found difference in effect or association based on statin use. One study found a stronger association between higher blood DHA and, separately, higher blood EPA, and lower risk of CHF in those not using statins; DHA: HR [lower intake vs. higher intake] = 6.65 (without statins) vs. 0.74 (with statins), P interaction = 0.003; EPA: HR [lower intake vs. higher intake] = 6.40 (without statins) vs. 1.45 (with statins), P interaction = 0.048. A relatively small number of RCTs of lipoproteins (LDL-c and HDL-c) and Tg analyzed interactions between n-3 FA and statins and found no interaction between statin use and the effect of marine oil supplementation on lipids (LDL-c 5 RCTs, Tg 4 RCTs, HDL-c 3 RCTs). No studies explicitly compared the interaction of n-3 FA intake (or biomarker level) with aspirin intake on outcomes.

Sixteen subgroup analyses comparing those with and without diabetes were reported (6 with ALA, 10 with marine oil). Two RCT analyses reported only that a statistically significant effect of n-3 FA was found among participants with diabetes but no significant effect was found those without diabetes (marine oil and CHD death, ALA and ventricular arrhythmia). All other analyses reported no difference in effect or association based on diabetes status.

Adverse Events

Of 61 RCTs included in this systematic review, only 4 RCTs of EPA/DHA ethyl ester, 19 RCTs of marine oils (EPA+DHA), 1 RCT of ALA, and 1 RCT comparing total n-3 FA, marine oil, ALA, and placebo reported information on adverse events that may or may not be associated with the interventions. There were no serious adverse events that were considered related to the study interventions in these 25 RCTs. Four of the 20 marine oil RCTs and one of the two ALA trials reported no adverse events. Most of the reported adverse events were mild and transient, such as gastrointestinal discomforts, nausea, skin abnormalities, eczema, pain, allergic reactions, fishy taste, headache, and infection. The most common adverse events related to n-3 FA supplements (that occurred more frequently among those taking supplements) were mild gastrointestinal effects such as belching (0.4-58% [marine oil] vs. 1.7-4% [placebo]; 2 studies), nausea (3.6-8.9% vs. 1.0-5.6%, 2 studies), diarrhea (5.1-8.9% vs. 2.0%, 1 study), or fishy taste (5.3-67% vs. 0-3%, 2 studies), or combined gastrointestinal symptoms (e.g., nausea, diarrhea, or epigastric discomfort) (marine oil: 1.5.0-6% vs. 0.8-4.5%, 7 studies; total n-3 FA: 1.3% vs. 0.8%, 1 study; ALA: 0.8% vs. 0.8%). Only one study explicitly reported on bleeding (hemorrhages such as cerebral and fundal bleedings, epistaxis, and subcutaneous bleeding), finding a higher rate with EPA ethyl ester and statin (1.1%) versus statin alone (0.6%, P<0.0001). This study was one of only two trials that reported statistically significantly more adverse events with marine oils than placebo. No study reported statistically significant higher rates of serious or severe adverse events between study arms, and no serious or severe adverse event was attributed to n-3 FA. Six of the marine oil trials explicit stated that most or all adverse events were mild. Three studies reported on the rate of adverse events leading to discontinuation, none of which were reported as statistically significantly different between groups (1.4-17% vs. 0.9-26%).

Summary by Key Question

Key Question 1

What is the efficacy or association of n-3 FA (EPA, DHA, EPA+DHA, DPA, SDA, ALA, or total n-3 FA) exposures in reducing CVD outcomes (incident CVD events, including all-cause death, CVD death, nonfatal CVD events, new diagnosis of CVD, peripheral vascular disease, CHF, major arrhythmias, and hypertension diagnosis) and specific CVD risk factors (BP, key plasma lipids)?

- Total n-3 FA
  - Overall, there is insufficient evidence regarding the effect of or association between total n-3 FA (combined ALA and marine oils) and clinical or intermediate outcomes. There is low strength of evidence of no association between total n-3 FA intake and stroke death, and total (fatal and nonfatal) MI (each association based on longitudinal observational studies of dietary intake).
  - For each outcome there was no consistent (and replicated) significant association between total n-3 FA intake and risk reduction.
- Marine oils
  - There is high strength of evidence of that marine oils statistically significantly lower Tg—possibly with greater effects with higher doses and in people with higher baseline Tg—and statistically significantly raise HDL-c

and LDL-c by similar amounts. There is also high strength of evidence that marine oil significantly lowers Total:HDL-c ratio.

- There is low strength of evidence that marine oil significantly lowers risk of ischemic stroke.
- There is a high strength of evidence of no effect of marine oil on risk of MACE, all-cause death, SCD, revascularization, and BP; moderate strength of evidence of no effect of marine oil on risk of AFib; and low strength of evidence of no effect of marine oil on risk of CVD death, CHD death, total CHD, MI, angina pectoris, CHF, total stroke, and hemorrhagic stroke. There is insufficient evidence for other outcomes.

- Marine oil, EPA
  - There is insufficient evidence regarding the effect of or association with EPA (specifically) and most CVD clinical and intermediate outcomes. There is low strength of evidence of no association between EPA intake and CHD and between EPA biomarkers and AFib.
- Marine oil, DHA
  - For the most part, there is insufficient evidence regarding the effect of or association with DHA and CVD clinical and intermediate outcomes. There is moderate strength of evidence of no effect of purified DHA supplementation on BP or LDL-c and low strength of evidence of no association between DHA intake and incident CHD (from observational studies).
- Marine oil, DPA
  - Overall, there is insufficient evidence regarding effect of or association between DPA (specifically) and most CVD clinical and intermediate outcomes. There is low strength of evidence of no association between DPA biomarker levels and risk of AFib.
- SDA
  - Overall, there is insufficient evidence regarding effect of or association between SDA (specifically) and CVD clinical and intermediate outcomes.
- ALA
  - There is moderate strength of evidence of no significant effect of ALA intake on BP, LDL-c, HDL-c, or Tg. There is low strength of evidence of no association between ALA intake or biomarker level and CHD or CHD death, AFib, and CHF, each based on observational studies. There is insufficient evidence regarding other outcomes.

Key Question 1, Subquestions

1.1.1. What is the efficacy or association of n-3 FA in preventing CVD outcomes in people without known CVD (primary prevention)?

- There was insufficient evidence for cardiac death, CHF death, ischemic stroke death, hemorrhagic stroke death, revascularization, acute coronary syndrome, angina pectoris, ventricular arrhythmia, incident hypertension, TC/HDL-c ratio, and LDL-c/HDL-c ratio.
- There was insufficient RCT evidence and inconsistent observational evidence for CHD death, MI death, all-cause death, total MI, and SCD.
- There was insufficient RCT evidence but observational evidence of no association for MACE, CVD death, total stroke death, incident CHD, total stroke, ischemic stroke, hemorrhagic stroke, AFib, and CHF.
- There was strong RCT evidence for no effect for BP (systolic and diastolic), MAP (only 3 trials), LDL-c, and HDL-c.
- There was strong RCT evidence for a significant protective effect for Tg.

1.1.2. What is the efficacy or association of n-3 FA in preventing CVD outcomes in people at high risk for CVD (primary prevention)?

- There was insufficient evidence for CVD death, cardiac death, CHD death, MI death, CHF death, total stroke death, ischemic stroke death, hemorrhagic stroke death, incident CHD, revascularization, acute coronary syndrome, angina pectoris, total stroke, ischemic stroke, hemorrhagic stroke, SCD, AFib, ventricular arrhythmia, CHF, incident hypertension and MAP.
- There was inconsistent RCT evidence for total MI.
- There was strong RCT evidence for no effect for MACE, all-cause death, BP (systolic and diastolic), LDL-c, HDL-c, TC/HDL-c ratio, and LDL-c/HDL-c ratio.
- There was strong RCT evidence for a significant protective effect for Tg.

1.1.3. What is the efficacy or association of n-3 FA in preventing CVD outcomes in people with known CVD (secondary prevention)?

- There was insufficient evidence for MI death, CHF death, total stroke death, ischemic stroke death, hemorrhagic stroke death, CHD, acute coronary syndrome, angina pectoris, ischemic stroke, hemorrhagic stroke, ventricular arrhythmia, incident hypertension, MAP, TC/HDL-c ratio, and LDL-c/HDL-c ratio.
- There was inconsistent RCT evidence for CVD death and cardiac death. There was RCT evidence of no effect for MACE, CHD death, all-cause death, total MI, revascularization, total stroke, SCD, AFib, and CHF.
- There was strong RCT evidence of no effect for BP (systolic and diastolic) and LDL-c.
- There was strong RCT evidence of a protective effect for HDL-c and Tg.

1.2. What is the relative efficacy of different n-3 FA on CVD outcomes and risk factors?

- There is low strength of evidence of no difference between EPA+DHA and its individual components.
- There is low strength of evidence of greater efficacy of marine oils over ALA.

1.3. Can the CVD outcomes be ordered by strength of intervention effect of n-3 FA ?

- Based on the summary effect sizes of meta-analyzed RCTs, marine oils had no significant effect on CVD outcomes. The order of effect sizes of CVD outcomes with sufficient data to allow meta-analysis, was MI (ES=0.88), CVD death (ES=0.92), MACE (ES=0.96), all-cause death (ES=0.97), total stroke (ES=0.98), and SCD (ES=1.04).

Key Question 2

n-3 FA variables and modifiers
2.1. How does the efficacy or association of n-3 FA in preventing CVD outcomes and with CVD risk factors differ in subpopulations, including men, premenopausal women, postmenopausal women, and different age or race/ethnicity groups?

- There was insufficient evidence to assess the efficacy or association of n-3 FA in preventing CVD outcomes and with CVD risk factors in subgroups based on race/ethnicity and whether women were pre- or postmenopausal.
- 5 studies (mostly observational) found no significant differences in association based on age, with cutoffs for subgroups ranging between 60 and 70 years of age.
- Two studies found no interaction with age as a continuous variable. One trial found a significant difference in favor of women, two observational studies found a significant difference in favor of men, and 9 studies (mix of RCT and observational) found no difference between men and women.

2.2 What are the effects of potential confounders or interacting factors—such as plasma lipids, body mass index, BP, diabetes, kidney disease, other nutrients or supplements, and drugs (e.g., statins, aspirin, diabetes drugs, hormone replacement therapy)?

- There was insufficient evidence to assess the following potential confounders or interacting factors: beta-blocker use, baseline HDL-c, glargine use, nitrate use, digoxin use, diuretic use, eGFR, ACEi use, anticoagulant use, total cholesterol levels, or use of fish oil supplements.
- There was inconsistent evidence for the following potential confounders or interacting factors: Tg levels, statin use, b-vitamin use, and baseline LDL-c.
- There was evidence of no interactions with body mass index, hypertension status, diabetes status, and baseline TC/HDL-c ratio.

2.3 What is the efficacy or association of different ratios of n-3 FA components in dietary supplements or biomarkers on CVD outcomes and risk factors?

- No study directly compared efficacy or association of different ratios of n-3 FA components on outcomes. Across studies, there were insufficient data to make these assessments.

2.4 How does the efficacy or association of n-3 FA on CVD outcomes and risk factors differ by ratios of different n-3 FA—DHA, EPA, and ALA, or other n-3 FA?

- No study directly compared efficacy or association of different ratios of n-3 FA components on outcomes. Across studies, there were insufficient data to make these assessments.

2.5 How does the efficacy or association of n-3 FA on CVD outcomes and risk factors differ by source (e.g., fish and vs. seafood, common plant oils (e.g., soybean vs,, canola), fish oil supplements, fungal-algal supplements, flaxseed oil supplements)?

- No study directly compared efficacy or association of different sources of n-3 FA on outcomes. Across

studies, there were insufficient data to make these assessments.

2.6 How does the ratio of n-6 FA to n-3 FA intakes or biomarker concentrations affect the efficacy or association of n-3 FA on CVD outcomes and risk factors?

- No trial or observational studies evaluated n-6 FA to n-3 FA intake concentrations and no differences across studies by this ratio was evident.

2.7 Is there a threshold or dose-response relationship between n-3 FA exposures and CVD outcomes and risk factors? Does the study type affect these relationships?

- Among trials, for all clinical CVD outcomes there is insufficient evidence regarding a dose-response relationship within or between trials.
- For BP, LDL-c, and HDL-c, trials do not find significant differences in effect by marine oil dose either within or between trials.
- Trials comparing marine oil doses mostly found no significant difference between higher and lower dose marine oils. However, a possible pattern could be discerned such that higher doses of 3.4 or 4 g/d reduced Tg by at least 30 mg/dL more than lower doses of 1 to 2 g/d. Higher doses ≤3 g/d (1.7−3 g/d) yielded much smaller relative differences in Tg change compared to lower doses (0.7−2.25 g/d). By metaregression, each increase of EPA+DHA dose by 1 g/d was associated with a greater net change Tg of −5.9 mg/dL (95% CI −9.9 to−2.0; P=0.003); no inflection point was found above which the association plateaued.
- Metaregressions of observational studies yielded the following conclusions:
    - For all-cause death, there may be a ceiling effect at about 0.2 g/d, such that increasing marine oil intake up to this level may be associated with lower all-cause death, but increasing intake above this level may not be associated with further decreased risk.
    - For total stroke, ischemic stroke, and CHF, at lower ranges of intake there were statistically significant associations between higher marine oil intake level and lower risk of outcome, in contrast to associations found at higher ranges of intake. However, the associations at lower and higher doses were not statistically significant from each other. For ischemic stroke, associations between higher doses and risk of stroke were stronger and statistically significant across lower doses than at higher doses (with thresholds between lower and higher doses from 0.1 and 0.4 g/d) and the differences in associations between lower and higher doses were statistically significant. Any dose inflection point that may exist is likely to be beyond the range of testable thresholds (i.e., >0.4 g/d). Similarly, for CHF significant associations were found at lower doses, in contrast to at higher doses, with thresholds ranging from 0.1 to 0.5 g/d, and the differences were statistically significant at most thresholds. Any dose inflection point that may exist is likely to be beyond the range of testable thresholds (i.e., >0.5 g/d).
    - For CVD death, CHD death, total CHD, and hemorrhagic stroke, there were no apparent differences in association between marine oil intake dose and outcome at lower or higher dose ranges.
    - For CHD death and CHD, there were no apparent differences in association between ALA intake dose and outcome at lower or higher dose ranges.

2.8 How does the duration of intervention or exposure influence the effect of n-3 FA on CVD outcomes and risk factors?

- None of the meta-regressions found a significant interaction for follow-up time. No difference in effect was found within studies at different durations of intervention. Observational studies did not evaluate differences in duration of exposure.

2.9 What is the effect of baseline n-3 FA status (intake or biomarkers) on the efficacy of n-3 FA intake or supplementation on CVD outcomes and risk factors?

- No study found a significant difference in subgroups based on baseline fish or n-3 FA intake.

Key Question 3

Adverse events
3.1 What adverse effects are related to n-3 FA intake (in studies of CVD outcomes and risk factors)?

- No serious or severe adverse events were related to n-3 FA intake (supplementation). Most reported adverse events were mild and gastrointestinal in nature; however, only 2 of 25 trials reported statistically significant differences in adverse events between n-3 FA supplements and placebo.

3.2 What adverse events are reported specifically among people with CVD or diabetes (in studies of CVD outcomes and risk factors)?

- Among 10 trials of patients with CVD (9 with marine oil, 1 with total n-3 FA, 2 with ALA), either no adverse events or no significant difference between n-3 FA and placebo were reported.
- A single study reported adverse events from a trial of people with diabetes, finding no significant differences in serious or nonserious adverse events between marine oil and placebo.

Discussion

Overall Summary of Key Findings

In this systematic review we identified 61 eligible RCTs (in 82 publications) and 37 eligible prospective longitudinal studies (in 65 publications) for inclusion, based on prespecified eligibility criteria. Most of the RCTs evaluated the effects of marine oil supplements (EPA+DHA) compared with placebo on clinical CVD outcomes in populations at risk for CVD or with CVD, while most of the observational studies examined the associations between intake of various individual n-3 FA, alone and in combination with each other, in relation to long-term CVD events in generally healthy populations. The RCTs of intermediate CVD outcomes (BP and lipids) were conducted in all three populations of interest (generally healthy, at risk for CVD—primarily due to dyslipidemia, or with CVD). However, none of the observational studies evaluated BP or lipids.

The main findings of the studies, regarding effect or association of higher n-3 FA intake or biomarker level and outcomes are summarized in the following tables. Table A includes analyses of n-3 FA and outcome pairs for which there is evidence to support an effect or association of higher n-3 FA intake and risk of a CVD outcome or on a CV risk factor. These include high strength of evidence that higher marine oil intake statistically significantly raises HDL-c, lowers Tg concentration and Total:HDL-c ratio, but also raises LDL-c. There is low strength of evidence that higher marine oil intake is associated with lower risk of ischemic stroke.

Table B includes analyses of n-3 FA and outcome pairs for which there is evidence supporting no effect or association of n-3 FA intake (or biomarker level) and outcomes. These include high strength of evidence for no effect of or association between marine oil intake and MACE, all-cause mortality, SCD, coronary revascularization, or BP; moderate strength of evidence of no association between marine oil intake and AFib, and between DHA intake and BP or LDL-c, and between ALA and BP, LDL-c, HDL-c, or Tg; and low strength of evidence of no association between total n-3 FA intake and stroke death or MI; between marine oil intake and CVD death, CHD death, total CHD, MI, angina pectoris, CHF, total stroke or hemorrhagic stroke; between EPA intake and CHD; between EPA biomarkers and AFib; between DHA intake and CHD; between DPA biomarkers and AFib; and between ALA intake and CHD, CHD death, AFib, or CHF. Analyses of n-3 FA and outcome pairs not included in the table provided insufficient evidence.

In brief, 61 RCTs and 37 longitudinal observational studies were included. Most RCTs and observational studies had few risk of bias concerns.

- **Total n-3 FA** (EPA+DHA+ALA):
  - There is low strength of evidence of no association between total n-3 FA intake and stroke death or MI.
  - There is insufficient evidence for other outcomes.
- **Marine oils, total** (primarily EPA+DHA):
  - There is high strength of evidence that higher marine oil intake lowers Tg, raises HDL-c, lowers Total:HDL-c ratio, but raises LDL-c; also moderate or high strength of evidence that higher marine oil intake does not affect MACE, all-cause death, SCD, coronary revascularization, AFib, or BP.
  - There is low strength of evidence of associations between higher marine oil intake and decreased risk of ischemic stroke. There is low strength of evidence of no association with CVD death, CHD death, total CHD, MI, angina pectoris, CHF, total stroke, or hemorrhagic stroke.
  - There is insufficient evidence for other outcomes.
- **Marine oil FA** (EPA, DHA, DPA), individually:
  - There is moderate strength of evidence of no effect of purified DHA supplementation (intake) and altering BP or LDL-c.
  - There is low strength of evidence of no associations between EPA or DHA intake (separately) and CHD, and between EPA or DPA biomarkers and AFib.

- There is insufficient evidence for other specific marine oil FA (EPA, DHA, DPA, or SDA) and outcomes.

- **ALA**:
  - There is moderate strength of evidence of no effect of ALA intake on BP, LDL-c, HDL-c, or Tg.
  - There is low strength of evidence of no association between ALA intake or biomarker level and CHD, CHD death, AFib, and CHF.
  - There is insufficient evidence for other outcomes.

- **Other n-3 FA analyses**:
  - There is insufficient evidence comparing n-3 FA to each other.

- **Subgroup analyses**:
  - 19 of 22 studies found no interaction of sex on any effect of n-3 FA.
  - 19 of 20 studies found no differential effect by statin co-use.
  - Within 16 studies evaluating diabetes subgroups, 2 found statistically significant beneficial effects of n-3 FA in those with diabetes, but not in those without diabetes, but no test of interaction was reported.

The 61 RCTs mostly compared marine oil supplements to placebo on CVD outcomes in populations at risk for CVD or with CVD, while the 37 observational studies mostly examined associations between various individual n-3 FA and long-term CVD events in generally healthy populations. Compared to the prior report on n-3 FA and CVD, there is more robust RCT evidence on ALA and on clinical CV outcomes; also, by design there are newly added data on associations between n-3 FA biomarkers and CV outcomes. However, conclusions regarding the effect of n-3 FA intake on CV outcomes or associations with outcomes remain substantially unchanged. Future RCTs would be needed to establish adequate evidence of the effect of n-3 FA on CVD outcomes or to clarify differential effects in different groups of people.

Studies within each category of analysis (by study design and by n-3 FA) were diverse, due to differences in outcomes evaluated, definitions of specific outcomes, as well as the n-3 FA intervention doses or compositions (for RCTs) or the dietary/biomarker n-3 FA exposure assessments and quantifications (for observational studies). Overall we found a lack of conclusive or consistent findings for CVD events within RCTs, mostly due to sparse data and underpowered trials as indicated by wide confidence intervals. The majority of the individual RCTs did not find statistically significant effects of marine oil supplements (EPA+DHA, various doses) on CVD outcomes. Pooled meta-analyses suggest that people with CVD or at risk for CVD who received marine oil supplements may have a small risk reduction in CVD death (pooled HR 0.92; 95% CI 0.82 to 1.02) compared with those who received placebo. Across outcomes, the effects of marine oil supplements were often larger in earlier RCTs than in more recent RCTs. These data may be confounded by shifts over time in concomitant therapy to reduce CVD risk (e.g., statins, aspirin), decreasing smoking rates, and overall declining rates of CVD events. No meta-regression across studies found significant changes in effect sizes by publication year; however, it is likely that all such meta-regressions of clinical outcomes were underpowered due to relatively small numbers of trials.

Observational studies were mixed regarding the associations between n-3 FA intake or biomarkers and risk of MACE (where each study used its own combination of specific CVD outcomes). The strength of associations between higher levels of n-3 FA and lower risk of CVD outcomes, when found, were often larger than those in RCTs. While all observational studies adjusted associations for potentially confounding variables, the specific variables included in models varied greatly across observational studies. Furthermore, all observational studies compared higher intake levels of n-3 FA with lowest intake level, which included people who may have other nutrition deficiencies that may affect chronic disease risks but often cannot be “controlled for” in the analyses (resulting in residual, uncontrolled confounding).

Table A. Main findings of high, moderate, or low strength of evidence of significant effects or associations between omega-3 fatty acids and outcomes

There is **high** strength of evidence for the following effects or associations of *higher* n-3 FA intake or biomarker levels and *lower* CVD risks or events:

- Marine oil* supplementation (or increased intake) and an increase in HDL-c
  - RCTs (of mostly supplements)
  - Summary net change in HDL-c: 0.9 mg/dL (95% CI 0.2, 1.6)
- Marine oil supplementation (or increased intake) and a decrease in Tg
  - RCTs (of mostly supplements)
  - Summary net change in Tg: −24 mg/dL (95% CI −31, −18)
- Marine oil supplementation (or increased intake) and a decrease in total cholesterol to HDL-c ratio
  - RCTs (of mostly supplements)
  - Summary net change in Total:HDL-c ratio: −0.17 (95% CI −0.26, −0.09)

There is **high** strength of evidence for the following effects or associations of *higher* n-3 FA intake or biomarker levels and *higher* CVD risk:

- Marine oil supplementation (or increased intake) and an increase in LDL-c
  - RCTs (of mostly supplements)
  - Summary net change in LDL-c: 2.0 mg/dL (95% CI 0.4, 3.6)

There is **low** strength of evidence for the following effects or associations of higher n-3 FA intake and lower CVD risks or events:

- Marine oil increased intake and a lower risk of ischemic stroke
  - Observational studies (of total dietary intake), significant by metaregression: 0.51 (95% CI 0.29, 0.89) per g/d

* Statements about "marine oil" are based on all evidence of analyses of EPA+DHA+DPA, EPA+DHA, EPA, DHA, and DPA.

Abbreviations: CHD = coronary heart disease (also known as coronary artery disease), CHF = congestive heart failure, CI = confidence interval, CVD = cardiovascular disease, DHA = docosahexaenoic acid, DPA = docosapentaenoic acid, EPA = eicosapentaenoic acid, HDL-c = high density lipoprotein cholesterol, HR = hazard ratio, LDL-c = low density lipoprotein cholesterol, n-3 FA = omega-3 fatty acids, RCT = randomized controlled trial, Tg = triglycerides.

Table B. Main findings of high, moderate, or low strength of evidence of no significant effects or associations between omega-3 fatty acids and outcomes

There is **high** strength of evidence of no *effect* or association of n-3 FA intake or biomarker level and the following outcomes:

- Marine oil supplementation (or increased intake) and risk of MACE
  - RCTs (of mostly supplements); observational studies also found no significant associations
  - Summary effect size (RCTs): 0.96 (95% CI 0.91, 1.02)
- Marine oil* intake and all-cause death
  - RCTs (of mostly supplements) supported by observational studies (of total dietary intake)
  - Summary effect size (RCTs): 0.97 (95% CI 0.92, 1.03)
  - Observational studies (of total dietary intake): 0.62 (95% CI 0.31, 1.25) per g/d
- Marine oil intake and SCD
- RCTs (of mostly supplements) supported by an observational study (of total dietary intake)
  Summary effect size (RCTs): 1.04 (95% CI 0.92, 1.17)
- Marine oil intake and coronary revascularization
  - RCTs (of mostly supplements) supported by an observational study (of total dietary intake)
- Marine oil intake and systolic or diastolic blood pressure
  - RCTs (of mostly supplements)
  - Summary net change in systolic blood pressure: 0.1 mg/dL (95% CI −0.2, 0.4)
  - Summary net change in diastolic blood pressure: −0.2 mg/dL (95% CI −0.4, 0.5)

Table B. Main findings of high, moderate, or low strength of evidence of no significant effects or associations between omega-3 fatty acids and outcomes (continued)

There is **moderate** strength of evidence of no effect or association of n-3 FA intake or biomarker level and the following outcomes:

- Marine oil intake and atrial fibrillation
  - RCTs (of mostly supplements); observational studies of intake were inconsistent
- Purified DHA supplementation and systolic or diastolic blood pressure
  - RCTs
- Purified DHA supplementation and LDL-c
  - RCTs
- ALA intake and systolic or diastolic blood pressure
  - RCTs (of mostly supplements)
- ALA intake and LDL-c, HDL-c, and Tg
  - RCTs (of mostly supplements)

There is **low** strength of evidence of no effect or association of n-3 FA intake or biomarker level and the following outcomes:

- Total n-3 FA intake and stroke death
  - Observational studies (of total dietary intake and biomarkers)
- Total n-3 FA intake and myocardial infarction
  - Observational studies (of total dietary intake)
- Marine oil intake and CVD death
  - Summary effect size (RCTs): 0.92 (95% CI 0.82, 1.02)†
  - Observational studies (of total dietary intake): 0.88 (95% CI 0.82, 0.95) per g/d
- Marine oil intake and CHD death
  - RCTs (of mostly supplements) imprecise
  - Observational studies (of total dietary intake): 1.09 (95% CI 0.76, 1.57) per g/d
- Marine oil intake and CHD
  - Observational studies (of total dietary intake), supported by a single study of n-3 FA biomarkers
  - Observational studies (of total dietary intake): 0.94 (95% CI 0.81, 1.10) per g/d
- Marine oil intake and myocardial infarction
  - Summary effect size (RCTs): 0.88 (95% CI 0.77, 1.02)†
- Marine oil intake and angina pectoris
  - RCTs (of supplements) with heterogeneous outcomes (definitions of angina pectoris)
- Marine oil intake and CHF
  - RCTs (of mostly supplements) imprecise and could not be meta-analyzed, all nonsignificant
  - Observational studies (of total dietary intake) significant by metaregression: 0.76 (95% CI 0.58, 1.00) per g/d ($P<0.05$)
- Marine oil intake and total stroke (fatal and nonfatal ischemic and hemorrhagic stroke)
  - Summary effect size (RCTs): 0.97 (95% CI 0.83, 1.13)
  - Observational studies (of total dietary intake): 0.68 (95% CI 0.53, 0.87) per g/d
- Marine oil intake and hemorrhagic stroke
  - Observational studies (of total dietary intake): 0.61 (95% CI 0.34, 1.11) per g/d
- EPA intake and CHD
  - Observational studies (of total dietary intake)
- EPA biomarkers and atrial fibrillation
  - Observational studies (of biomarkers)

Table B. Main findings of high, moderate, or low strength of evidence of no significant effects or associations between omega-3 fatty acids and outcomes (continued)

There is **low** strength of evidence of no effect or association of n-3 FA intake or biomarker level and the following outcomes (continued):

- DHA intake and CHD
  - Observational studies (of total dietary intake and biomarkers)
- DPA biomarkers and atrial fibrillation
  - Observational studies (of biomarkers)
- ALA intake and CHD death and, separately, total CHD
  - Observational studies (of total dietary intake); CHD death finding supported by one RCT (of supplementation) and one observational study of biomarkers
  - Observational studies (of total dietary intake): CHD death 0.94 (95% CI 0.85, 1.03) per g/d
  - Observational studies (of total dietary intake): CHD 0.97 (95% CI 0.92, 1.03) per g/d
- ALA intake and atrial fibrillation
  - Observational studies (of total dietary intake and biomarkers)
- ALA intake and CHF
  - Observational studies (of total dietary intake and biomarkers), supported by one RCT (of supplementation)

* Statements about "marine oil" are based on all evidence of analyses of EPA+DHA+DPA, EPA+DHA, EPA, DHA, and DPA.
† There is low confidence that this summary estimate would remain suggestive of no effect with the addition of future trial data (and greater statistical power).

Abbreviations: ALA = alphalinolenic acid, CHD = coronary heart disease, CHF = congestive heart failure, DHA = docosahexaenoic acid, DPA = docosapentaenoic acid, EPA = eicosapentaenoic acid, HDL-c = high density lipoprotein cholesterol, LDL-c = low density lipoprotein cholesterol, MACE = major adverse cardiovascular event (including cardiac and stroke events and death; variously defined by studies), n-3 FA = omega-3 fatty acids, RCT = randomized controlled trial, SCD = sudden cardiac death, Tg = triglycerides.

The overall findings for the effects of marine oil supplements on intermediate CVD outcomes remain largely unchanged since the original report. In this update, there were no significant effects found in 22 RCTs that compared marine oils (0.3−6 g/d) on systolic or diastolic BP compared with placebo. Thirty-nine RCTs evaluated LDL-c and HDL-c. Meta-analyses of the effect of marine oils on HDL-c and LDL-c found small, but statistically significant amounts (summary net change HDL-c = 0.9 mg/dL [95% CI 0.2 to 1.7]; LDL-c = 2.0 mg/dL [95% CI 0.4 to 3.6]). The clinical significance of these small increases in both HDL-c and LDL-c on CVD outcomes, particularly in combination, is unclear. For both lipid outcomes, no differences in effect across studies were found by marine oil dose, followup duration or population. The strongest effect of marine oils (0.3−6 g/d) was found among the 41 RCTs of Tg. Meta-analysis found a summary net change of −24 mg/dL (95% CI −31 to −18), with no significant difference in effect based on population or followup time across studies. However, across trials, the effect was dose-dependent and also dependent on the studies' mean baseline Tg values. By metaregression, each increase of EPA+DHA dose by 1 g/d was also associated with a greater net change Tg of −5.9 mg/dL (95% CI −9.9 to −2.0) and each increase in mean baseline Tg level by 1 mg/dL was associated with a greater net change Tg of −0.15 mg/dL (95% CI −0.22 to −0.08). However, the few trials that directly compared marine oil doses did not consistently find consistently find a dose effect; although, marine oil doses ≥3 g/d all resulted in larger reductions in Tg compared to lower doses, in contrast to doses <3 g/d which had smaller reductions in Tg compared to even lower doses. There were no observational studies evaluating these intermediate CVD outcomes.

In the original report, there was only one RCT of ALA (linseed oil) versus control oil (sunflower seed oil), conducted in the 1960s, that evaluated clinical event outcomes. In this update we identified only one additional RCT of ALA (plant source not reported) versus placebo (oleic acid) in participants with a history of MI that reported clinical outcomes. Given the sparseness of trials of the effect on clinical CVD outcomes of higher ALA intake and the differences between the two trials, no conclusion can be drawn regarding effect of ALA on CVD outcomes. For intermediate outcomes, five ALA RCTs (with doses ranging from 1.4 to 5.9 g/d) evaluated BP outcomes, and

four of the five RCTs also evaluated LDL-c, HDL-c, Tg, or Total:HDL-c ratio (2 trials) outcomes. All found no significant differences in these outcomes between ALA and placebo. Thirteen observational studies evaluated ALA intake. The large majority of analyses found no significant associations; only two studies found any significant associations between higher ALA intake and clinical outcomes (reduced all-cause death, SCD, and CHD death risks).

The potential intake threshold-effects of n-3 FA on CVD events could not be determined from the RCTs because there were limited number of RCTs for many outcomes and most RCTs did not find significant effects. Using data from observational studies, the linear dose-response and potential threshold effects of n-3 FA on several CVD events were tested by meta-analytical techniques. There was a significant association between higher EPA and DHA intake and lower risk of ischemic stroke across a median dosage range of 0.025 to 0.6 g/d (effect size per g/d = 0.51; 95% CI 0.29 to 0.89), but no dose-response relationships found between EPA and DHA intake and both CHD and hemorrhagic stroke. The interpretations of the threshold-effects (in observational studies) were limited because differences in associations at lower doses (statistically significant associations between higher intake and lower risk) and associations at higher doses (no significant associations between intake and outcome) were generally similar regardless of the cut point chosen between lower and higher dose analyses.

No differences in effects or associations were found between different populations (healthy or general population, at increased risk for CVD—largely due to dyslipidemia, or with CVD). However, this conclusion is weak given that few studies compared populations, few RCTs were conducted in healthy populations and few observational studies were conducted in at risk or CVD populations.

Limitations

Overall, both RCTs and observational studies (i.e., lngitudinal observational and nested case-control studies) included in this systematic review generally had few risk of bias concerns. For clinical CVD outcomes, all but one of the RCTs was conducted in either high risk individuals or people with existing CVD. In contrast, most observational studies examining the associations between dietary n-3 FA intake or biomarkers of n-3 FA intake and clinical outcomes were conducted in generally healthy populations. Few trials compared n-3 FA dose, formulation, or source. No trial compared different n-3 to n-6 FA ratios of supplements or intake. None of the observational studies attempted to determine a threshold effect of any associations between n-3 FA and the outcome of interest.

There are numerous differences between RCTs and observational studies, making the comparisons across the two study designs difficult to make. Of note, the doses of marine oil supplements (EPA+DHA) in RCTs were often much higher than the highest intake reported for observational studies. Furthermore, not all observational studies explicitly included n-3 FA supplements in their assessment of intake and very few of the RCTs attempted to account for background fish or n-3 FA intake as an effect modifier.

While this report represents a complete systematic review, it does not encompass all trials or longitudinal observational studies that report on CVD and intermediate outcomes. Particularly, if one includes small studies (trials with <30 participants per study group or observational studies with <100 participants, several hundred more studies could potentially have met eligibility criteria. Due to time and resource limitations, we restricted the review to the approximately 100 studies that are most likely to have adequately addressed the primary research questions of interest.

Future Research Recommendations

Future RCTs should fully characterize both the preparations of n-3 FA interventions and placebos used for the intervention in terms of the FA composition and molecular form of the FA (e.g., ethyl esters, Tg), as well as indicating their sources. The placebo foods and oils should have the same caloric density and to the extent possible similar food or oil types as the source of n-3 FA. The composition of the background diet should also be reported, as should FA composition, macronutrient content and whether the participants were weight-stable. Researchers are encouraged to use standard, common CVD outcomes to allow comparison across studies. Assessment of n-3 FA status and intake should be evaluated at study entry and post-intervention in all study participants using to better understand potential changes in n-3 FA intake in populations with different background diets (e.g., whether the effect of supplementation differs in people with high- or low-fish diets). If trials include participants with a broad range of n-3 FA status or intake (e.g., with both high- and low-fish diets), subgroup analyses should be conducted to evaluate possible differential effects based on n-3 these variables. The effects (or lack thereof) of marine oils (EPA+DHA) on BP, LDL-c, HDL-c, and Tg are well established so additional RCTs on these intermediate outcomes alone are unlikely to add any new knowledge, and therefore are not recommended.

There is an ongoing need to improve self-reported dietary assessment methods and food databases for all nutrients including n-3 FA. As national dietary patterns shift and new processed foods are introduced into the marketplace, food composition tables used to analyze food frequency questionnaire data need to be updated to ensure accurate estimation of n-3 FA (and other nutrient) intake. Similar to trial registries, a data repository for raw observational study data would greatly improve the transparency of data analyses (potentially reduce both reporting and publication biases) and the appropriateness and methodology of meta-analytical techniques for pooling observational studies. An individual participant-level meta-analysis of observational studies of marine oils could address limitations of the study-level meta-analyses that are currently feasible.

Conclusions

Results from the RCTs of clinical event outcomes are applicable only to at-risk-of-CVD and CVD populations because there is insufficient trial evidence of the effect of n-3 FA on clinical CVD outcomes in healthy populations. Results from the RCTs of intermediate outcomes; however, are applicable to all populations (healthy, at risk, and with CVD) since the trials included a range of people from the different populations. In contrast, results from observational studies (which did not evaluate intermediate outcomes) are applicable only to generally healthy populations. We graded the strength of the body of evidence for each intervention/exposure and comparison of intervention, and for each outcome by assessing the number of studies, their study designs, the study limitations (i.e., risk of bias and overall methodological quality), the directness of the evidence to the Key Questions, the consistency of study results, the precision of any estimates of effect, the likelihood of reporting bias, and the overall findings across studies. We concluded that there is insufficient evidence regarding the effect of or association between total n-3 FA (ALA + marine oils [EPA+DHA±DPA]) and clinical or intermediate outcomes. There is low strength of evidence of no association between total n-3 FA intake and stroke death, and total MI (each association based on longitudinal observational studies). For marine oil (EPA+DHA±DPA), there is insufficient evidence for most outcomes of interest but there is low to high strength of evidence of a beneficial effect of higher marine oil intake for selected CVD and intermediate outcomes. Specifically, there is high strength of evidence that marine oils clinically and statistically significantly lower Tg—possibly with greater effects with higher doses and in people with higher baseline Tg. There is also high strength of evidence that marine oils statistically, but arguably not clinically, significantly raise HDL-c. There is also high strength of evidence that marine oil significantly lowers Total:HDL-c ratio. There is moderate strength of evidence that marine oil supplementation lowers risk of MACE and CVD death. There is a high strength of evidence of no effect of marine oil on risk of total stroke, but low strength of evidence of no associations of marine oil intake and ischemic or hemorrhagic stroke. There is low strength of evidence for associations between higher EPA+DHA intake and decreased risk of CHD and CHF, based on observational studies. However, there is moderate to high strength of evidence of no effect of (or association between) marine oil and all-cause death, MI, AFib, CHF, SCD, revascularization, BP, LDL-c, or LDL:HDL-c ratio. There is also low strength of evidence of no effect of marine oil intake and CHD death.

For individual n-3 FA, there is insufficient evidence regarding the effect of or association with EPA, DHA, DPA, SDA, or ALA (specifically) and most CVD clinical outcomes. For EPA, there is low strength of evidence of no association between EPA intake and CHD and between EPA biomarkers and AFib. For DHA, there is moderate strength of evidence of no effect of purified DHA supplementation on BP or LDL-c and low strength of evidence of no association between DHA intake and incident CHD (from observational studies). For DPA (no RCT was identified), there is low strength of evidence of an association between higher DPA biomarker levels and lower risk of AFib. For ALA, there is moderate strength of evidence of no significant effect of ALA intake on BP, LDL-c, HDL-c, or Tg. There is low strength of evidence of no association between ALA intake or biomarker level and CHD or CHD death, AFib, CHF, total and ischemic stroke, based on observational studies.

There is insufficient evidence of direct comparisons between marine oil and ALA intake on CVD outcomes. Across studies, the indirect comparison between marine oil and ALA is unclear, largely because there is insufficient evidence regarding the effect or association of ALA with clinical CVD outcomes. However, where there is high strength of evidence of significant effects of marine oil on improving Tg and HDL-c, there is moderate strength of evidence of no effect of ALA intake on these intermediate outcomes. No RCTs examined the additive effects of n-3 FA versus the effects of individual n-3 FA.

References

1. Bang HO, Dyerberg J, Hjoorne N. The composition of food consumed by Greenland Eskimos. Acta medica Scandinavica. 1976;200(1-2):69-73. PMID: 961471.
2. Balk E, Chung M, Lichtenstein A, et al. Effects of omega-3 fatty acids on cardiovascular risk factors and intermediate markers of cardiovascular disease. Evid Rep Technol Assess (Summ). 2004 Mar(93):1-6. PMID: 15133887.
3. Wang C, Chung M, Lichtenstein A, et al. Effects of omega-3 fatty acids on cardiovascular disease. Evid Rep Technol Assess (Summ). 2004 Mar(94):1-8. PMID: 15133888.
4. Report of the Joint FAO/WHO Expert Consultation on the Risks and Benefits of Fish Consumption. Food and Agriculture Organization of the United Nations; Geneva: World Health Organization Rome; 2011.
5. Institute of Medicine, Panel on Micronutrients. Dietary Reference Intakes for Energy, Carbohydrate, Fiber, Fat, Fatty Acids, Cholesterol, Protein and Amino Acids. Panel on Macronutrients Panel on the Definition of Dietary Fiber, Subcommittee on Upper Reference Levels of Nutrients, Subcommittee on Interpretation and Uses of Dietary Reference Intakes, and the Standing Committee on the Scientific Evaluation of Dietary Reference Intakes, Food and Nutrition Board: National Academies Press; 2005.
6. McGuire S. US Department of Agriculture and US Department of Health and Human Services, Dietary Guidelines for Americans, 2010. Washington, DC: US Government Printing Office, January 2011. Adv Nutr. 2011;2(3):293-4.
7. Institute of Medicine. Seafood Choices: Balancing Benefits and Risks. Nesheim MC, Yaktine AL, editors. Washington, DC: The National Academies Press; 2007.
8. Harika RK, Eilander A, Alssema M, et al. Intake of fatty acids in general populations worldwide does not meet dietary recommendations to prevent coronary heart disease: a systematic review of data from 40 countries. Ann Nutr Metab. 2013;63(3):229-38. PMID: 24192557.
9. Hodson L, Skeaff CM, Fielding BA. Fatty acid composition of adipose tissue and blood in humans and its use as a biomarker of dietary intake. Prog Lipid Res. 2008 Sep;47(5):348-80. PMID: 18435934.
10. Serra-Majem L, Nissensohn M, Overby NC, et al. Dietary methods and biomarkers of omega 3 fatty acids: a systematic review. Br J Nutr. 2012 Jun;107 Suppl 2:S64-76. PMID: 22591904.
11. Fekete K, Marosvolgyi T, Jakobik V, et al. Methods of assessment of n-3 long-chain polyunsaturated fatty acid status in humans: a systematic review. Am J Clin Nutr. 2009 Jun;89(6):2070S-84S. PMID: 19420097.
12. Methods guide for effectiveness and comparative effectiveness reviews: Agency for Healthcare Research and Quality; 2011.
13. Higgins JP, Altman DG, Gotzsche PC, et al. The Cochrane Collaboration's tool for assessing risk of bias in randomised trials. BMJ (Clinical research ed). 2011;343:d5928. PMID: 22008217.
14. Wells GA SB, O'Connell D, Peterson J, Welch V, Losos M, Tugwell P. The Newcastle-Ottawa Scale (NOS) for assessing the quality of nonrandomised studies in meta-analyses. [9/25/2014]; Available from: http://www.ohri.ca/programs/clinical_epidemiology/oxford.asp, .
15. Chung M, Balk EM, Brendel M, et al. Vitamin D and calcium: a systematic review of health outcomes. Evidence report/technology assessment. 2009 Aug(183):1-420. PMID: 20629479.
16. Chung M, Balk EM, Ip S, et al. Reporting of systematic reviews of micronutrients and health: a critical appraisal. Am J Clin Nutr. 2009 Apr;89(4):1099-113. PMID: 19244363.
17. Lichtenstein AH, Yetley EA, Lau J. Application of systematic review methodology to the field of nutrition. J. Nutr. 2008 Dec;138(12):2297-306. PMID: 19022948.
18. Berkman ND, Lohr KN, Ansari MT, et al. Grading the strength of a body of evidence when assessing health care interventions: an EPC update. J Clin Epidemiol. 2014 Dec 20. PMID: 25721570.

Full Report

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