Evidence Report/Technology Assessment Disposition of Comments Report

Research Review Title: *Omega-3 Fatty Acids and Cardiovascular Disease: An Updated Systematic Review*

Draft review available for public comment from August 3, 2015, to September 11, 2015.

Research Review Citation: Balk EM, Adam GP, Langberg V, Halladay C, Chung M, Lin L, Robertson S, Yip A, Steele D, Smith BT, Lau J, Lichtenstein AH, Trikalinos TA. Omega−3 Fatty Acids and Cardiovascular Disease: An Updated Systematic Review. Evidence Report/Technology Assessment No. 223. (Prepared by the Brown Evidence-based Practice Center under Contract No. 290−2012−00012−I.) AHRQ Publication No. 16-E002-EF. Rockville, MD: Agency for Healthcare Research and Quality; August 2016. www.effectivehealthcare.ahrq.gov/reports/final.cfm.

Comments to Research Review

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The tables below include the responses by the authors of the review to each comment that was submitted for this draft review. The responses to comments in this disposition report are those of the authors, who are responsible for its contents, and do not necessarily represent the views of the Agency for Healthcare Research and Quality.

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Commentator & Affiliation	Section	Comment	Response
Peer Reviewer #1	General	The report is clinically meaningful and comprehensive. The target population is clear and the key questions are appropriate. The main problems are the limitations of the available RCTs, which the authors explain clearly. I would have liked to see some attention given to additional biomarkers addressed in RCTs, such as effects on glycemia, insulin resistance, inflammatory markers, and markers of thrombosis/coagulation. Perhaps the literature is too limited to address these additional biomarkers. Also did any of the RCTs look at flow-mediated vasodilation or NO-induced endothelial relaxation? I was also particularly interested in the following issues but found the information difficult to access: RCT results after accounting for statin and aspirin use (at least analyses stratfied by use or non-use at baseline), sex-based differences, results of trials using omega-3 acid ethyl esters versus other omega-3 supplements, effects of omega-3s on cognition in RCT settings, effects on type 2 diabetes incidence in RCT settings.	Thank you. Most of the specific outcomes and so forth that the reviewer is interested in were explicitly excluded from the scope of analyses of clinical CVD and specific intermediate outcomes (BP, lipids). A new summary section has been added specific to within-study subgroup analyses in the Results sections. A paragraph within this section is devoted to statins (and the lack of data about aspirin). We have clarified which studies evaluated ethyl esters. We have also added a paragraph in the Summary by n-3 FA (Marine oil, total: EPA+DHA±DPA) about the ethyl ester findings (no different than other studies). The question of whether effects may differ based on formulation (and other factors) was added to the Future Research section of the Discussion.
Peer Reviewer #1	Introduction	The Introduction is well done and lays out the questions and clinical issues clearly. Some attention in the Intro to several of the above issues would have been helpful.	The Introduction has been revised, but only within scope of the review.

Commentator & Affiliation	Section	Comment	Response
Peer Reviewer #1	Methods	The Methods are generally clear and well done. Some of the exclusions of studies on the basis of sample size seem arbitrary: for example, studies of cardiac endoints required at least 10,000 participants, whereas studies of stroke required at least 3000 participants. I do understand, though, that criteria have to be set and may not be fully supportable. Aside from the above suggestions, I think the outcome measures and statistical methods are appropriate.	We have added a small section, under Minimum Sample Sizes, to explain our rationale and to state explicitly that these thresholds are indeed arbitrary.
Peer Reviewer #1	Results	Please see above under general comments. I would have liked to see additional biomarkers, especially of glycemia and inflammation, if a critical mass of research is available. In additional, I think that cognition is an important outcome that is strongly related to vascular health and relevant to omega-3s -- it would be interesting to see the results summarized. I would have liked to see more attention to the issue of effect modification by statin and aspirin use, which may be masking the relatively modest effects of omega-3s in more recent RCTs. Otherwise, the figures, tables, and appendices seem appropriate.	We agree these are of interest, but they are mostly out of scope for the current review. A new summary section has been added specific to within-study subgroup analyses in the Results sections. A paragraph within this section is devoted to statins (and the lack of data about aspirin). In addition, we have added a sentence in this section summarizing that no difference in effects were seen by publication date among RCTs in meta-analyses.
Peer Reviewer #1	Discussion/ Conclusion	I don't thnk the conclusions and future research sections clearly lay out the next steps needed to advance the science. The limitations of the current evidence are well described but it would be helpful for the authors to be clearer about what's needed to fill the gaps. Also, the clinical and public health implications of currently available research could be addressed in more detail.	Thank you. We have made revisions and improvements to the Future Research section. We have more clearly summarized the results for a non-technical audience. As per AHRQ EPC policy, we did not include any clinical or public health guidelines, recommendations, or suggestions.

Commentator & Affiliation	Section	Comment	Response
Peer Reviewer #1	Discussion/ Conclusion	f. Clarity and Usability: Yes, the report is well structured and organized, but I found it challenging to find the specific content areas I was most interested in. The main points are clearly presented, but the conclusions relevant to policy and practice decisions could be improved and addressed in more detail.	A new summary results section has been added which explicitly answers each KQ.
TEP #1	General	This report has important clinical and public health value and is very useful to those developing policy and to the research community. Additionally the nutrition community of practitioners, researchers, and policy makers will derive considerable benefit from the methodology, the broad inclusiveness of studies and the conservative assessment of the evidence available. from the perspective of observational and clinical trials investigators, the review and the discussion (pages 203 and following) afford valuable advice for development of future studies and for measurement of events and outcomes. The key questions were appropriate, clearly stated, complete and were addressed in the study. Parenthetically, I am not unbiased on these latter issues.	Thank you
TEP #1	Introduction	The introduction affords a clear presentation of the scope and rationale for the current review. The authors might also note that there have been remarkable changes in outcome measurements and treatment/prevention behaviors in the period between the original report in 2000 and the current analysis. Diagnostic criteria for ischemic events have changed the outcome landscape for RCTs and for observational studies. The increasingly widespread use of statins and the demonstrated effectiveness and the promotion and use of n-3 supplements could confound the analysis and interpretation. Acknowledgement of these secular changes provides an additional rationale for the current review.	Thank you. This has been added to the Introduction. A new paragraph was added near the start of the Introductions.

Commentator & Affiliation	Section	Comment	Response
TEP #1	Methods	The inclusion/exclusion criteria are appropriate and the search strategy logical and clearly stated. The definitions of the outcome measures could be improved. The abbreviations are defined when first used and in the footnote but the diagnoses included within several categories needs clearer specification in the text. Specifically, MACE is a composite measure as is CVD. What entities are included in each? It is recognized that diagnostic measures are evolving rapidly and the studies may include different assessments depending on the study timing, but it is important to elaborate those that are included in this analysis. This is covered in the appendices but should be provided up front especially as MACE conveys more statistically relevant information. The statistical methods are appropriate.	The outcomes MACE and CVD have been clarified, made more distinct from each other, and described more explicitly. We have two separate outcome sections for these, MACE and CVD death. MACE is more inclusive than CVD death (including nonfatal CVD events). Otherwise, CVD, per se, is not an outcome. There is no standard definition of MACE. The second paragraph of the RCT section of the MACE results describes study's definitions.
TEP #1	Results	The results are clearly stated and the tabular and graphical presentations are complete and clear. The studies included are consistent with the stated intention and all inclusive.	Thank you.
TEP #1	Discussion/ Conclusion	The discussion provides conservative inferences of the analysis and explains the consistencies with earlier studies while discussing the apparent contradictions among observational and trial findings. I believe that further attention might be given to the potential secular influences of outcome assessment and treatment. The declining rates of CVD clinical events and death as well as increasing incidence of CHF (with its vague diagnostic criteria) mean that the studies are initiated and conducted against an evolving background. Further, the widespread use of statins which have a profound effect on vascular outcomes and the decline in smoking with a similar profound effect confound the findings. The importance of dietary inclusion of more foods containing n-3 fatty acids receives appropriate attention for current interpretation and planning of future studies. But I believe these other issues should receive attention as well. The design of new trials and decisions about new followup measurements in observational studies would benefit from these insights.	We have added smoking, aspirin, and changes in CVD rates over time to our discussion about possible shifts over time (which already included statins). We also added the comment that none of the meta-regressions were significant for publication date, but these were underpowered.

Commentator & Affiliation	Section	Comment	Response
TEP #1	Clarity and Usability	The report is well structured and the findings clearly presented. The seemingly contradictory findings from observational and trial studies are addressed. The rather inexplicable contrasts among the vascular outcomes might be clarified by explanation of composite and single outcomes. the evolution in assessment and the limitations of statistical inference when sample sizes of endpoints are limited in studies of fixed size but diminishing endpoints.	It isn't clear that there is a pattern across clinical outcomes regarding which show evidence of effect of or association with n-3 FA. Differences between observational and trial data are discussed in the Limitations.
TEP #2	General	Most readers will not have an understanding of the complexity of interpreting studies of omega-3 fatty acids since they are not sufficiently knowledgeable of other factors influencing metabolism and the subsequent phsiologocal impact. Questions posed are reasonable, but even some of the study designs of publications used did not take into account the interactions among fatty acids and the factors that may impact physiological response. (More comments on attached comment document.)	We agree with your general comment and address your more specific comments where they are presented in this document.
TEP #2	Methods	Better identificaion of how quantitation of fatty acid intake was conducted would be useful.	None of the observational studies provided complete information about how fatty acid intake was quantified beyond "FFQ". Some said they measured "direct intake". This deficiency was added to the limitations. Also, we have added to the eligibility criteria that n-3 FA content of food or supplements must be explicitly quantified (by any method).
TEP #2	Clarity and Usability	Unfortunately, the results of the update do not make significant changes from past report due to the nature of the data available in the literature.	We agree there are not major differences.

Commentator & Affiliation	Section	Comment	Response
TEP #2	Miscellaneous	• It is not explicitly stated how the reviewers handled the references from the earlier systematic effort. Did the reviewers undertake another primary review or did they accept the summary completed back in about 2004? Perhaps the section on page 46, lines 16-32 could be clearer.	We have made it more explicit in the Methods sections that we used existing systematic reviews only for their references and did a de novo review. There is no indication in the Methods or Results that we used any prior summary results.
TEP #2	Miscellaneous	• Page 15 (and page 44) – energy balance should be recognized and identified among the modifiers in the figure and its potential influence should be discussed in the text. Although weight loss studies were apparently excluded, it would be of scientific interest if any comment about weight change was included in the assessment of subjects within studies included.	As the reviewer notes, we excluded weight loss studies. We have added a short description of FFQ and their deficiencies regarding n-3 FA to the introduction. We would have captured weight changes subgroup analyses, if reported. We have no data to report.

Commentator & Affiliation	Section	Comment	Response
TEP #2	Miscellaneous	• For the longer-term trials, some comment or discussion should occur with regard to weight change since weight change could relate to many of the outcomes being examined. Unfortunately, I suspect many studies did not make specific mention of weight since it was not considered a variable. OIn such cases, this fact should be a limitation of the cited study.	Weight change was not an outcome of interest. Weight loss intervention studies were excluded. Weight change was not reported in eligible studies that we saw. We did not include post hoc subgroups (defined by status at study end; e.g., by weight loss). For all these reasons, we have nothing evidence-based to say about weight change and its relationship with n-3 FA. We have added a few sentences to this effect in the Limitations section of the Discussion in the main report. We did not include lack of reporting or adjustment for weight change as a risk of bias determination method.
TEP #2	Miscellaneous	• For reports of observational studies, there should be some discussion of the potential for other factors (besides omega-3 fatty acids) that may be different. Did authors of included studies assess whether there may have been associated factors that were also different between study groups?	We have clarified that observational studies had to have multivariate analyses (in the Design part of PICO-D) to control for other differences between analyzed cohorts. We re-iterated this eligibility criterion in the risk of bias section of the Results.

Commentator & Affiliation	Section	Comment	Response
TEP #2	Miscellaneous	• Page 35:line 29 – In the brief discussion in this paragraph, it might be educational here or in relevant sections of the full report text to indicate that levels of omega-3 intake in most studies conducted within the US are approximate to the normal dietary intake of omega-3 fatty acids in countries such as Japan. It is recognized that the review criteria limited the types of studies that could be used, but some comment on the relative level of intake in different populations may be useful.	We have added to the Introduction information on US and other country ALA and EPA/DHA intake from a recent SR.
TEP #2	Miscellaneous	• A point that is not clear to this reader is whether the studies used in the report made any adjustment of dietary intake of omega-3 fatty acids that were not specifically quantitated or attempted to assess whether intake of fish and other fats differed amongst the study groups. Since all studies needed to have quantitation of omega-3 intake, how was the consumption of fish or other foods containing omega-3 fatty acids handled? Perhaps a brief description of the methodology to quantitate omega-3 levels for the included studies might be considered to be included in the methodology section.	None of the observational studies provided complete information about how fatty acid intake was quantified beyond "FFQ". Some said they measured "direct intake". It is not clear how studies could make "any adjustment of dietary intake of omega-3 fatty acids that were not specifically quantitated." Regardless, we included reported multivariate adjusted models. We mention the reporting deficiency of quantification of n-3 FA in the Limitations. We have added to the eligibility criteria that n-3 FA content of food or supplements must be explicitly quantified *(by any method)*.

Commentator & Affiliation	Section	Comment	Response
TEP #2	Miscellaneous	• In the analyses, it was not clear if there was an attempt to assess an interaction between levels of omega-3 fatty acid intake and the total fat intake or the ratio of omega-3 to omega-6 intake. It is possible that outcomes may be influenced by relative intake levels and not just absolute intake levels. It may not be possible to conduct these types of analyses within the framework of the conducted review; however, perhaps some comment could be made to indicate this inability as another functional limitation within the report.	The new summary of results by KQ now makes it explicit that no analysis of the n-3 to n-6 FA ratio could be made, thus, including interactions between n-3 FA and n-3 to n-6 FA ratio; this is also true for total fat intake analyses. This deficiency is mentioned in the Limitations section.
TEP #2	Miscellaneous	• One of the questions identified in the charge to the development of the report (one location is page 43, lines 12-14) relates to the efficacy/association based on the source of the omega-3 fatty acids. Since this question may be of interest to a reasonable proportion of the readers, it would, be good to have a brief summary under its own heading as to the nature of any observations made. Within the report, it is difficult to tease out the source since the generic use of marine oils is generally used. This reviewer anticipates that there would be insufficient evidence to draw any conclusions, but even this type of comment would be worthy of its own paragraph under its own heading. Sometimes the heading "Marine oil comparisons" is used, but these comparisons typically relate to the different omega-3 fatty acids found in marine sources and not a comparison of the actual source of the omega-3 fatty acids (such as from fungal or algae). The other type of comparison that is provided is marine oil versus ALA (or plant source).	We have updated the sources of n-3 FA intake as possible. The new results summary by KQ now explicitly states that we were not able to come to any conclusions regarding n-3 FA source.
TEP #2	Miscellaneous	• Recognizing the following comment is more of an editorial decision, for the reader, there would be a significant value for inserting a table in the text of the report summarizing the strength of evidence grading used as cited in the following reference: Berkman ND, Lohr KN, Ansari MT, et al. Grading the strength of a body of evidence when assessing health care interventions: an EPC update. Journal of clinical epidemiology. 2014 Dec 20. PMID: 25721570.	The Berkman, Lohr, Ansari, Balk, et al. article is the source for the system used to grade SoE, and is referenced. We believe our summary is sufficient.
Peer Reviewer #2	General	The purpose of the report is well stated, as are (1) the methods used to gather and analyze the data obtained, (2) state the results found, and (3) support the conclusions and recommendations made.	Thank you.

Commentator & Affiliation	Section	Comment	Response
Peer Reviewer #2	General	I believe that a good job was done in identifying the three major study populations: healthy adults without cardiovascular disease (CVD), those at risk for CVD, and those with clinical CVD. A good job was done in identifying these populations, which are both clinically meaningful and important to understanding and improving public health.	Thank you
Peer Reviewer #2	General	The key questions addressed were appropriate and explicitly stated, and I believe that the results obtained will help drive future research in this area and also possibly drive future clinical practice guidelines and affect policy decisions by health providers and by research funding and regulatory agencies.	Thank you
Peer Reviewer #2	General	Given popular claims of clear antiarrhythmic benefits of n3 FAs, it is particularly good to see that this update includes RCTs and observational studies examining the effects of n3 FAs on atrial fibrillation, ventricular arrhythmias, and sudden cardiac death.	Thank you
Peer Reviewer #2	General	Table a (pp. ES21-22) provides an excellent summary of the main findings.	Thank you
Peer Reviewer #2	Introduction	The brief description of the metabolic pathway of conversion from ALA to DHA (p1, Figure 1) is adequate but does not indicate the amount of each n3 FA required to produce the subsequent, longer chain n3 FA. Some might infer from the figure that ingestion of 1g of ALA would result in an equal amount of DHA and thus perhaps be pharmacologically equivalent, which is at best misleading. There is also little indication that ingestion of different ratios of n3 and other FAs may affect conversion rates and plasma levels of specific n3 FAs.	Thank you for pointing this out. We have made additions to the text about rates of conversion.
Peer Reviewer #2	Introduction	The description of biomarkers used to assess n3 FA levels is appropriate and adequate.	Thank you
Peer Reviewer #2	Introduction	The rationale for why it is of interest to compare RCT "results across different exposure/intervention products" might be expanded a bit to assure the reader that apples and oranges are not being compared.	We have rephrased and added to the sentence to minimize any interpretation that “apples and oranges” are being compared.
Peer Reviewer #2	Introduction	The key questions asked and the analytical framework utilized to gather data and obtain results are well stated and clear.	Thank you
Peer Reviewer #2	Methods	The methods described are appropriate and consistent with those routinely utilized by EPCs and accepted by AHRQ and reviewers.	Thank you
Peer Reviewer #2	Methods	Page 7, line 29 -- the abbreviation "TEP" does not appear to have been defined in the list of abbreviations provided on pp. vi and vii; although, I know that it likely to "Technical Expert Panel."	Thank you. This has been fixed.

Commentator & Affiliation	Section	Comment	Response
Peer Reviewer #2	Methods	Page 13, line 6 -- the abbreviation "SoE", used in the abstract and several tables, could be used here for consistency and ease of reading.	Although, the phrase (strength of evidence) is used repeatedly, we think using SoE in text is generally awkward and prefer to keep it spelled out in the main text and in the Main Findings tables (A & B in the ES) where the phrase is used in sentences.
Peer Reviewer #2	Methods	Page 13, line 42 -- "EPC" not defined in the list of abbreviations on page vi and vii.	Added to list and used appropriately throughout
Peer Reviewer #2	Results	The Results section is tedious to read by nature of the amount of information included. The authors have done an excellent job to diminish this criticism by carefully providing an overview of the results and organizing them by category.	Thank you
Peer Reviewer #2	Results	Because of the relatively few number of RCTs (55) and observational studies (33) included, the x-axis in figure 4 (p. 15, line 39-53) and figure 5 (p. 16, lines 15-29) could be expressed in absolute numbers, rather than percentages.	We have changed to absolute numbers.
Peer Reviewer #2	Results	Table X (pp. 17-18) does a nice job summarizing study outcomes; although the meaning of font colors and shading are difficult to discern--e.g., no table cells are noted with an orange font. In addition, the abbreviation "Rd" is defined in the table legend, but does not appear in the table.	We chose to have a consistent legend for similar tables to avoid errors and provide a complete list of possible relevant situations.
Peer Reviewer #2	Results	Figure A.2 is extremely useful, but lacks a legend.	All figure legends have been updated.
Peer Reviewer #2	Results	Table A.3 (referenced on p. 20, line 49) is not found.	Call-outs to appendixes have been fixed. All figure and table numbers have been standardized.

Commentator & Affiliation	Section	Comment	Response
Peer Reviewer #2	Results	Sections on Sudden Cardiac Death (SCD, pp. 85-19) and Atrial Fibrillation (AF, pp. 91-97) appear fine. The relation between ventricular and supraventricular arrhythmias to SCD and AF are not clearly delineated in these and the previous sections. The studies in patients with implantable cardiac defibrillators (ICDs) detect the occurrence of a shockable ventricular arrhythmia, which if not treated would be expected to result in SCD; these studies have been included as studies on the effects of n3 FAs on SCD (appropriately). As always, it is unfortunate that some RCTs (e.g., Darghosian L, Free M, Li J, Gebretsadik T, Bian A, Shintani A, McBride BF, Solus J, Milne G, Crossley GH, Thompson D, Vidaillet H, Okafor H, Darbar D, Murray KT, Stein CM. Effect of omega-three polyunsaturated fatty acids on inflammation, oxidative stress, and recurrence of atrial fibrillation. Am J Cardiol. 2015 Jan 15;115(2):196-201. doi: 10.1016/j.amjcard.2014.10.022. Epub 2014 Oct 29.) have been published since the date for inclusion. Inclusion of this and other more recent studies may have altered outcomes and SoE for the various effects. This is a recognized limitation/weakness of a systematic review such as this and emphasizes the need for periodic updated systematic reviews like this.	We believe that sudden cardiac death, ventricular tachycardia, and atrial fibrillation are clearly distinct; although, clearly there is an overlap between SCD and ventricular arrhythmias. We categorized outcomes bases on how they were described and reported in the studies. We have clarified in the atrial fibrillation sections that, in theory, we included supraventricular arrhythmias, but that all studies evaluated atrial fibrillation. Darghosian was rejected because they had only a 6 month follow-up, not because of publication date. We required a minimum 12 month follow-up.
Peer Reviewer #2	Discussion/ Conclusion	Tables Disc. 1 and Disc. 2 provide nice, clear summaries of the main findings of both significant and non-significant effects of n3 FAs on cardiovascular outcomes. Afib is not included in either of these tables, yet more than a page (pp. 91-92) is devoted to this condition in the results section. And later on page 209, the authors indicate that "there is moderate to high strength of evidence of no effect of (or association between) marine oil and <>, <>, AFib, <>, ..." Rationale for exclusion of Afib should be provided.	This was an oversight. Atrial fibrillation has been added. Thank you.

Commentator & Affiliation	Section	Comment	Response
Peer Reviewer #2	Discussion/ Conclusion	In the section on future research recommendations, the authors may also wish to indicate that the total FA status of study participants should be evaluated at the beginning and conclusion of the study. This would better permit future evaluation of how 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-- one of the specific key questions indicated on pp. ES-2 and 4 of the manuscript. Also, possible differences in the effects of various specific n3 FAs, as well, as their possible concentration dependent effects, are important to better identify in future research studies, as indicated.	These points have been further clarified and made more explicit in the revised Future Research section.
Peer Reviewer #2	Clarity and Usability	See above comments by section. In addition, there are a few instances (a very few) instance where jargon/abbreviations are use that are not defined in the table of abbreviations on pp. vi and vii. For instance: p. v, ln 37, "SoE"; p. ES5, ln. 7, "EPC", and p.7, ln. 29, "TEP". In general the manuscript is well written and clear. Do the authors wish to speculate on the need for a future update systematic review on this topic and its timing?	Thank you. We have rechecked the use of abbreviations throughout and have used them consistently and ensured that they are included in the list of abbreviations. We cannot speculate on the future timing of an SR update. However, this review may undergo an AHRQ surveillance for possible updating, based on normal AHRQ processes.

Commentator & Affiliation	Section	Comment	Response
Peer Reviewer #3	General	In general this is very thorough, elaborate review. My only major point is that in my opinion the possible role of dose and what doses are used are not made clear enough. They are mentioned in the tables, but in the figures it remains unclear what doses we are talking about and e.g. for the clinically non relevant effect on HDL cholesterol it would also be useful to mention on what average dose this is based. Furthermore, exclusion of small trials with lipid and BP outcomes are in my opinion not a good decision.	The figures explicitly include the n-3 FA dose of each study. The final format (as opposed to the draft format) of the figures is cleaner and probably easier to read. While excluding smaller BP and lipid studies might not be ideal, it was necessary. Unless one believes there is a major bias among the larger studies and those with subgroup analyses, etc., one wouldn't expect different results with these studies added.
Peer Reviewer #3	Introduction	Page 40 of 791: line 32: refs are missing.	Thank you. This has been fixed.
Peer Reviewer #3	Introduction	You may want to refer to Hodson, Skeaff and Fielding, 2008.	Thank you. We have added this to the introduction.
Peer Reviewer #3	Introduction	Numbering of key questions is incorrect.	Thank you. This has been fixed.

Commentator & Affiliation	Section	Comment	Response
Peer Reviewer #3	Methods	Page 48 of 791: I have my doubst about the minimum sample size. I think that inclusion of RCT's with CVD events outcomes with 10 participants per arm is ridiculously low... In practice it is not big problem because there are no such trials. In contrast, the minimum sample sizes for RCTs with lipid and BP outcomes may direct your results in inclusion of less controlled and thereby more imprecise results. Very good, small trials are no excluded, I have my doubts about that.	We strongly believe that the clinical outcomes are paramount. We also believe that BP and lipid results are well-known and stable over the past 10-15 years, at least. We chose to be as inclusive as possible for clinical outcomes, since these are critical. We had to restrict BP and lipids given the vast number of these studies that continue to be produced. It is unclear why exclusion of small studies would lead to inclusion of less controlled and more imprecise results.
Peer Reviewer #3	Methods	Page 53 of 791: with in should be within	Thank you. This has been fixed.
Peer Reviewer #3	Results	The presentation of the results is fine, sufficient detail is provided on all studies. Raitt et al. seems to be missing from some analyses. See minor comments below.	The omission of Raitt has been corrected.
Peer Reviewer #3	Results	Page 64 of 791: Kromhout 2010 is not from Scandinavia.	Thank you. This has been fixed.
Peer Reviewer #3	Results	Page 74 of 791: Why is Raitt et al. 2005 not included in this analysis? They do provide data on cardiac death	This omission was corrected. Raitt 2005 is in the current meta-analysis.
Peer Reviewer #3	Results	Page 91 of 791: Again: why is Raitt et al. not included?	This omission was corrected. Raitt 2005 is in the current meta-analysis.

Commentator & Affiliation	Section	Comment	Response
Peer Reviewer #3	Results	Page 124 of 791 lines 56-60: The text does not match with figure U.2. It says 8 trials, but only sic are included in the figure. The HR also does not match with the HR in the figure (Raitt and Brouwer are missing).	We clarified throughout that not all studies included in tables (and text) can be included in meta-analyses because of incomplete reporting by several studies. All tables, figures, and meta-analyses have been updated.
Peer Reviewer #3	Results	Page 205, 206 and 207of 791: Kromhout is not from Scandinavia.	This has been fixed throughout.
Peer Reviewer #3	Results	Page 210 of 791: :iu is not from Scandinavia.	This has been fixed throughout.
Peer Reviewer #3	Discussion/ Conclusion	Discussion and conclusion is fine, except that I would like to see more emphasis on the possible role of dose.	We added a sentence in the paragraph about dose about Tg, which is the only outcome for which there is evidence of a dose effect.
Peer Reviewer #3	Clarity and Usability	The report is well structured and organized. The main points are clearly presented, but I would like to see more emphasis on the role of the possible dose (this is done well for triglycerides, but can be improved for other outcome measures). The conclusions are relevant	There is no evidence of a statistically significant dose effect for other outcomes.
Peer Reviewer #3	Abstract	The use and explanation of abbreviations in the abstract is inconsistent. Sometimes they are only explained in the text, sometimes in the text and the list of abbreviations, sometimes only in the list.	We have revised our approach to abbreviations.
Peer Reviewer #3	Executive summary	Page 12 of 791 line 22: References are missing.	Thank you. This has been fixed.
Peer Reviewer #3	Executive summary	Page 12 of 791 line 47: You may want to refer to Hodson, Skeaff & Fielding Prog Lipid Res 2008	Thank you. We have added this citation.
TEP #3	General	The report has high clinical value. The key questions are appropriate and explicitly stated. The target audience of this report can be more clearly defined.	We have added a statement in the Introduction paragraph about the purpose of the review.
TEP #3	Introduction	Background of the research question is comprehensively reviewed, purpose and scope of the current work is clearly described, and the key questions are explicitly stated.	Thank you

Commentator & Affiliation	Section	Comment	Response
TEP #3	Methods	The inclusion / exclusion criteria are well justified, except that the exclusion of weight-loss intervention is debatable. Major CVD risk factors can also consider diabetes. The definition of each outcome could be further expanded and specified when needed. The statistical methods for meta-analysis, study quality evaluation, and strength of evidence assessment are appropriate and clearly presented with reference.	We have added a section to the methods section regarding the rationale for not included weight loss intervention studies. And we have added this decision (and the lack of reporting regarding weight loss) as potential limitations in the discussion. Diabetes was considered as an at-risk population. But diabetes is not a CVD outcome, nor was it considered to be a standard CVD intermediate outcome of interest. While a worthy outcome for evaluation, it did not fall within the purview of this review. We have expanded some definitions of outcomes that do not have a specific definition. Most importantly, we have the range of definitions of major adverse cardiovascular events used by studies.
TEP #3	Results	Details in the Results are appropriate, characteristics of the included studies are clearly described, key data are well presented. Figures and Tables are comprehensive and adequate.	Thank you
TEP #3	Discussion/ Conclusion	Summary of main findings and overall results are appropriate. Discussion on implications are reasonable. The limitations as considered are adequate. Future research section are helpful and compelling.	Thank you

Commentator & Affiliation	Section	Comment	Response
TEP #3	Clarity and Usability	Given the large number of studies and large amount of data, the analysis is very well-structured and report well-organized. The conclusions are clearly stated, the results are relevant to clinical practice and public health guidance. The comparison to the earlier review demonstrates additional information contributed by this updated report.	Thank you
Peer Reviewer #4	General	This is a very careful and detailed description and evaluation of the pertinent literature, based on a convincing workplan and giving clear answers to logically separated questions. It requires, however, verty careful reading in order to appreciate the reasoning behind the answers, even the summary answers	Thank you
Peer Reviewer #4	Introduction	The introduction is concise and appropriate to explain the need for this review	Thank you
Peer Reviewer #4	Methods	yes, to all questions	Thank you
Peer Reviewer #4	Results	The amount of detail presented is impressive and extremely useful. that implies that the studies are clearly described when putting together the information contained in the appendices. The tables and figures, particularly, are clear and informative. I cannot think of any study that should have been included.	Thank you
Peer Reviewer #4	Discussion/ Conclusion	The implications of the findings are clearly stated. The limitations both of the evaluated studies and of the review are clearly stated and the consequently formulated future research recommendations should be very useful.	Thank you
Peer Reviewer #4	Clarity and Usability	The Report is extremely well structured and organised. The conclusions should be useful for policy and practice decisions provided the limitations of the Review are appropriately taken into account. New are the increased precision of the relationship between omega-3 fatty acids intake and cardiovascular health outcomes.	Thank you
Peer Reviewer #4	Minor comments (editorial mostly)	page ES-1, line 18/19: no source of SDA mentioned Figure A: Clinical Cardiovascular outcomes: "CV" should be CVD page ES-7: the two last bullet points are the same page ES-9, line 3: (will) also be reviewed page ES-11, line 12/13: associated between page ES-14, line 26: risk of what? page ES-15, line 54: MACE; the page ES-17, line 32: found in page ES-20, line 46: no differences in page 12, line 1: within	We added a sentence about current and potentially future sources of SDA. Other typos have been fixed. Thank you.

Commentator & Affiliation	Section	Comment	Response
Peer Reviewer #4	Minor comments (editorial mostly)	page 24, table: Tavazzi 2008, columns on numbers: the numbers of the cases and the total number are reversed page 25 and 26, Table: Kromhout 2010: Netherlands page 29, line 25: Figure B.2 (not A.2) page47, line 36: stroke page 52, line 43: studies that page 54, lines 13 and 24: association page78, lines 27 and 28: stroke (not CHD) page 84, lines 21 and 22: stroke (not CHD)	Typos have been fixed. Thank you.
Peer Reviewer #4	Minor comments (editorial mostly)	page 90, figure U.4; page 94, figure V.4: explanation of symbols is missing page 92, line 1-4: incomplete sentence, makes no sense page 94, line 48: two RCTs that compared page 95, line 6: CHF page 96, line 54: delete "were not associated with risk of CHF" page 100, table W.6, title: I presume this is about marine oil and CHF page 101, line 53: incomplete sentence page 153, line 6: 95% CI -45, -22 page 177, line 11: evidence that page 180, line 8: decreasing risk with page 198, line 6: differences in page 205, line 523: one "consistently find" can be deleted	Typos have been fixed. Thank you.
Peer Reviewer #4	Additional material	In case the text related to the figures are to be transferred to the final report, many of them need some editorial changes; for figure 1 the omega-6 fatty acids should be deleted.	Text in figures and throughout has been copyedited and will go through another round of copyediting before posting (publication). Omega-6 FA ("n-6") are not in Figure 1.

Commentator & Affiliation	Section	Comment	Response
Public Reviewer #1 Kathleen Gans Brangs PhD/AstraZeneca	Introduction	AstraZeneca thanks you for the opportunity to comment on the draft report. The attached information is supplied in response to an open public comment period. These materials may include information not found in the currently approved prescribing information for EPANOVA. The enclosed information is intended to provide pertinent data as part of the public comment opportunity and should in no way be construed as a recommendation for the use of this product in any manner other than as approved by the Food and Drug Administration and as described in the prescribing information for EPANOVA. Prescribing information for EPANOVA may be obtained from www.astrazenecaus.com or by calling the Information Center at AstraZeneca at 18002369933.	Thank you
Public Reviewer #1 Kathleen Gans Brangs PhD/AstraZeneca	Tables	Page 142 TAble AE.1 High density lipoprotein cholesterol RCTs. Add HDL values from Maki 2013 publication Figure 3 page 1405. Maki et al does provide HDL values that are located in the notes section of the figure	Data from Maki 2013 have been added and corrected. Thank you.
Public Reviewer #1 Kathleen Gans Brangs PhD/AstraZeneca	Tables	Pg 155 Tble AF.1 Triglycerides RCT. Add TG values from Maki 2013 publication Figure 3 page 1405. Maki et al does provide TG values that are located in the notes section of the figure	Data from Maki 2013 have been added and corrected. Thank you.
Public Reviewer #1 Kathleen Gans Brangs PhD/AstraZeneca	Tables	Pg 167 Table AG.1 Total Chol to HDLc ratio RCT. Last row on table Maki 2013Column Int n3 FA ALA alpha linolenic acid is not mentioned in the study. Based on the values ALA is referring to Arachidonic acid change to Arachidonic acid or remove the wording ALA.	This was a typo in the table (adding in ALA to the intervention), not a confusion with AA. This has been corrected.
Public Reviewer #1 Kathleen Gans Brangs PhD/AstraZeneca	Appendixes	Appendix D Page D8 Baseline values. Placebo arm Change HDL levels to 38.3 9.0 Placebo arm Insert TG levels 280 70.72 gram arm Change HDL levels to 38.7 9.92 gram arm Insert TG levels to 284 76.74 gram arm Change HDL level to 38.8 10.94 gram arm Insert TG levels to 287 82.8Ref Data found in Maki 2013 notes section of Figure 3 page 1405	Data from Maki 2013 have been added or corrected. Thank you.
Public Reviewer #1 Kathleen Gans Brangs PhD/AstraZeneca	Appendixes	App E pg E13 Study Design. Eligibility Criteria is cut and paste from publication however not in its entirety its cut off at 2 there is more information after that. See Kastelein 2014 page 96. Consider adding the rest of the eligibility criteria or summarizing the criteria attached is possible summarization.Patients 18 years old average body mass index 20 m2 with an average TG concentration of at least 500 mgdL to less than 2000 mgdL who were either untreated for dyslipidemia or were using a stable dosage of a statin cholesterol absorption inhibitor or combination were included in the study.	The formatting has been fixed so “omitted” text is now visible.

Commentator & Affiliation	Section	Comment	Response
Public Reviewer #1 Kathleen Gans Brangs PhD/AstraZeneca	Appendixes	App G.1 pg 420 row 206213 Comparative Studies. ALA values not available the values for ALA is Arachidonic acid values. Change word ALA to Arachidonic acid or remove the ALA values listed for this section.	This was a typo in the table (adding in ALA to the intervention), not a confusion with AA. This has been corrected.
Public Reviewer #1 Kathleen Gans Brangs PhD/AstraZeneca	Appendixes	App G.1 pg 439 row 298301 LIPIDS COLUMN Comparative Studies. Lipids column Change HDL value to 38.3 9.0 Add TG value 280 70.7 Should be 174 29.591.7 27.338.3 9.0280 70.7 Ref Data found in Maki 2013 notes section of Figure 3 page 1405	Data from Maki 2013 have been added or corrected. Thank you.
Public Reviewer #2 Harry B. Rice, PhD/ Global Organization for EPA and DHA Omega-3s (GOED)	General	The Global Organization for EPA and DHA Omega-3s (GOED) is an association of processors, refiners, manufacturers, distributors, marketers, retailers and supporters of products containing eicosapentaenoic acid (EPA) and docosahexaenoic acid (DHA) omega-3 fatty acids. GOED's membership represents a broad range of businesses, from small entrepreneurs to multinational food companies. The Organization's objectives are to educate consumers about the health benefits of EPA/DHA and to collaborate with government groups, the healthcare community and the industry on issues related to omega-3s, while setting high standards for our business sector. As such, our members have a profound interest in ensuring that valuable information regarding EPA and DHA is communicated to consumers in a meaningful and timely way. Thus said, we appreciate the opportunity to provide comments on the draft report "Omega-3 Fatty Acids and Cardiovascular Disease: An Updated Systematic Review."	Thank you.
Public Reviewer #2 Harry B. Rice, PhD/ Global Organization for EPA and DHA Omega-3s (GOED)	Appendices	• Appendix B, Excluded Studies, includes an entry for Garbagnati et al., 2009 (PMID 19276620) with the rejection reason of "Not n3 specifically". Group 2 received n-3 PUFAs; therefore, the study should not have been excluded.	The sample size was too small (N<30) in each arm at 1 year followup. This rejection reason has been changed in the Appendix.
Public Reviewer #2 Harry B. Rice, PhD/ Global Organization for EPA and DHA Omega-3s (GOED)	Blood Pressure, Systolic and Diastolic (pages 101-123)	GOED is pleased that the scope of the updated review has been expanded to include additional key outcomes, including blood pressure (BP), but we are confused by the references to n-3 FAs or marine oils not affecting BP. For example, on page 5, in the conclusions of the abstract, it states, "Marine oils have no significant effect on BP." Also, on page 204, it states that there is a high strength of evidence of no effect or association of n-3 FA intake or biomarker level on either systolic (SBP) or diastolic blood pressure (DBP).	Our conclusions are consistent with our findings.

Commentator & Affiliation	Section	Comment	Response
Public Reviewer #2 Harry B. Rice, PhD/ Global Organization for EPA and DHA Omega-3s (GOED)	BP, continued	The confusion is due to the results being drastically different from those found in a GOED-sponsored meta-analysis published in the American Journal of Hypertension (http://www.ncbi.nlm.nih.gov/pmc/articles/PMC4054797/) and serving as the basis for GOED's health claim petition submission to the FDA for EPA and DHA and reduction of blood pressure in the general population. The petition is currently under review and GOED expects a decision by November 2, 2015.	Apparently, eligibility criteria differed between the two reviews. Different meta-analytic techniques were also used.

Commentator & Affiliation	Section	Comment	Response
Public Reviewer #2 Harry B. Rice, PhD/ Global Organization for EPA and DHA Omega-3s (GOED)	BP, continued	In that meta-analysis, 70 RCTs were included. Compared with placebo, EPA+DHA provision reduced systolic blood pressure (−1.52mm Hg; 95% CI = −2.25 to −0.79) and diastolic blood pressure (−0.99mm Hg; 95% CI = −1.54 to −0.44) in the meta-analyses of all studies combined. The strongest effects of EPA+DHA were observed among untreated hypertensive subjects (systolic blood pressure =−4.51mm Hg, 95% CI = −6.12 to −2.83; diastolic blood pressure = −3.05mm Hg, 95% CI = −4.35 to−1.74), although blood pressure also was lowered among normotensive subjects (systolic blood pressure =−1.25mm Hg, 95% CI = −2.05 to −0.46; diastolic blood pressure = −0.62mm Hg, 95% CI = −1.22 to−0.02). These results are not only statistically significant, but clinically significant.	We were unable to meaningfully analyze the subset of studies that evaluated untreated hypertensives. We did not include an adequate number of studies that explicitly or specifically reported on untreated hypertensives. Almost all such studies in the Miller analysis did not meet our eligibility criteria based on sample size or short duration of follow-up. As we note in the Limitations section of the Discussion, there are limitations to the approach that we had to take because of the vast scope of the review. We focused on the largest studies and those with longer follow-up duration (which are potentially most generalizable) and those most likely to provide information about subgroup differences. By some criteria, based on their larger size (than omitted studies), they are likely to have been of greater methodological rigor. *Continued in next row.*

Commentator & Affiliation	Section	Comment	Response
Public Reviewer #2 Harry B. Rice, PhD/ Global Organization for EPA and DHA Omega-3s (GOED)	BP, continued	(Prior comment, response continued)	*Continued from previous row.* Another important difference, is that we used the restricted maximum likelihood method for meta-analysis, a more conservative method (resulting in wider CI and higher P value) that is among methods currently recommended. “The DL method [which implicitly was used in Miller et al.; although the article describes a fixed effect model] produces confidence bounds that are too narrow (and P values that are typically too small) when the number of studies is small or when there are substantive differences among study estimates.” (Cornell et al. Ann Intern Med. 2014;160:267–270.)

Commentator & Affiliation	Section	Comment	Response
Public Reviewer # 2 Harry B. Rice, PhD/ Global Organization for EPA and DHA Omega-3s (GOED)	BP, continued	The AHRQ meta-analysis included approximately two thirds fewer RCTs compared to the GOED-sponsored meta-analysis. Since BP is a newly added outcome, the search strategy started in 2000 and went through November 19, 2014 and also included "eligible studies from the original reports and relevant existing systematic reviews." It appears that the older studies are only from the original CVD report where BP was discussed. What's not clear is why there is such a discrepancy in the number of included RCTs between the AHRQ meta-analysis compared to the GOED-sponsored meta-analysis. GOED believes a large number of studies that should have been included in the meta-analysis were inadvertently missed. GOED requests you rerun your literature search and confirm the inclusion of all applicable older studies by checking all relevant existing systematic reviews, including the GOED-sponsored meta-analysis published online March 6, 2014.	Well over 100 RCTs were potentially eligible (for SBP and DBP alone). As noted, it was necessary to restrict the number of studies for analysis. We do not believe we introduced bias by choosing the largest trials with longer follow-up duration and those that were most pertinent to our sub-questions about interactions. It is important to note that about half the studies included in our review were excluded from the GOED-sponsored meta-analysis. BP was not a newly added outcome. We included (at the screening stage) all studies included in the original AHRQ review (and other existing systematic reviews), including the SR included in the original AHRQ review. All studies in the Miller review were checked for eligibility. All those that were not included had already been rejected (mostly based on sample size, too-short duration, or mixed interventions).

Commentator & Affiliation	Section	Comment	Response
Public Reviewer # 2 Harry B. Rice, PhD/ Global Organization for EPA and DHA Omega-3s (GOED)	BP, continued	As an aside, there is a discrepancy in the number of included RCTs comparing marine oils on SBP and DBP. According to page 102 of the report, for systolic and diastolic blood pressure, data on the effect of marine oil (EPA+DHA) from 25 and 24 RCTs, respectively, was used. On page 205, there is reference to 22 RCTs comparing marine oils on SBP or DBP. Based on the extracted data for SBP (pages 105-113) and DBP (pages 115-122), the reference on page 102 to 25 and 24 RCTs for SBP and DBP, respectively, appears to be correct.	All numbers have been updated and revised. Thank you.
Public Reviewer #2 Harry B. Rice, PhD/ Global Organization for EPA and DHA Omega-3s (GOED)	Adverse Events	Curiously, while there are key questions listed that are related to adverse events, there is no mention of the findings. GOED would like to bring your attention to two government reports that have been published concerning the safety of EPA and DHA. Both reports concluded that there was insufficient data to establish a tolerable upper intake level (UL) which indicates an absence of safety concerns. The first report, from the Norwegian Scientific Committee for Food Safety (VKM), concluded that supplemental intakes of EPA+DHA at doses up to 6.9 g/day do not raise safety concerns. (Norwegian Scientific Committee for Food Safety (VKM) (2011). Evaluation of negative and positive health effects of n-3 fatty acids as constituents of food supplements and fortified foods. Norwegian Scientific Committee for Food Safety. Available online at http://www.vkm.no/dav/c7a41adb79.pdf..) The second report, from the European Food Safety Authority (EFSA), concluded that supplemental intakes of EPA+DHA at doses up to 5.0 g/day do not raise safety concerns. (EFSA Panel on Dietetic Products, Nutrition and Allergies (2012). Scientific Opinion related to the Tolerable Upper Intake Level of eicosapentaenoic acid (EPA), docosahexaenoic acid (DHA) and docosapentaenoic acid (DPA). EFSA J 10(7):2815. Available online at http://www.efsa.europa.eu/en/efsajournal/doc/2815.pdf.) Finally, in 2012, GOED commissioned a safety assessment on EPA and DHA from Spherix, Inc. The results are commensurate with the reports from VKM and EFSA. GOED would be happy to share a copy with you.	The accidental omission of the adverse events section has been corrected.