World Health Organization

Effects of polyunsaturated fatty acids intake and risk of all-cause mortality, cardiovascular disease, breast cancer, mental health, and type 2 diabetes: a systematic review and meta-analysis of prospective cohort studies

[ABRIDGED VERSION CONTAINING RESULTS FOR n-3 PUFA ONLY]

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ABSTRACT

Objective: To systematically review prospective cohort studies and quantify associations between polyunsaturated fatty acids [PUFA], and all-cause mortality, cardiovascular disease, breast cancer, mental health, inflammatory bowel disease and type 2 diabetes.

Design: Systematic review and meta-analysis of prospective cohort studies.

Data Sources: MEDLINE (from 1946); EMBASE (from 1974); Cochrane Central Registry of Controlled Trials (from 1996), Evidence Based Medicine Reviews (from 1996), and CINAHL (from 1983) were searched through May 26, 2017. Reference lists of retrieved articles and previous systematic and narrative reviews were hand-searched.

Eligibility criteria for selecting studies: Prospective cohort studies reporting associations between PUFA and all-cause mortality, cardiovascular disease, breast cancer, mental health, or type 2 diabetes were eligible.

Data Extraction and Synthesis: Two reviewers independently abstracted design features, participant characteristics, exposures, and outcomes, and assessed risk of bias. Multivariable relative risks were pooled using inverse-variance random effects models. Heterogeneity was assessed (Q statistic) and quantified (I-squared). Potential publication bias was assessed (funnel plots) and subgroup analyses were undertaken (meta-regression).

Results [ABRIDGED]: Total ω-3 fatty acids were associated with a 7% decreased risk of fatal cardiovascular disease (8 studies with 314,876 participants), 26% reduced risk of fatal coronary heart disease (6 studies with 249,756 participants), 32% decreased risk of sudden cardiac death (3 studies with 191,531 participants), 57% decreased risk of myocardial infarction (1 study with 41,578 participants), 18% decreased risk of fatal stroke (1 study with 60,298 participants), and 21% decreased risk of cognitive decline in older age (1 study with 4,809 participants). Higher intakes of long-chain ω-3 fatty acids were associated with a 7% decreased risk of all-cause mortality (9 studies with 485,078 participants), 11% reduced risk of fatal CVD (10 studies with 470,113 participants), 19% reduced risk of fatal CHD (10 studies with 349,586 participants), 57% reduced risk of fatal myocardial infarction (1 study with 18,244 participants), 61% reduced risk of hemorrhagic stroke (2 studies with 20,069 participants), and 28% reduced risk of ulcerative colitis (1 study with 170,805 participants). The confidence in the

estimates for the association between PUFA and all outcomes using the GRADE approach ranged from very low (⊕OOO) to moderate (⊕⊕⊕O).

Conclusions:

[Not included in this version of the report]

INTRODUCTION[1]

[Not included in this version of the report]

METHODS

This review was conducted in accordance with the WHO's guideline development process[37], based on the Cochrane Collaboration approach[38]. Ethical approval was not required for this review.

Data sources

Independent searches were conducted for relevant observational studies assessing the association between polyunsaturated fatty acids and the health outcomes through August 22, 2016. This included searching MEDLINE (from 1946); EMBASE (from 1974); Cochrane Central Registry of Controlled Trials (from 1996), Evidence Based Medicine Reviews (from 1996), and CINAHL (from 1983). Reference lists of retrieved articles and previous systematic and narrative reviews[39] [14 15 40-42] were hand-searched. No language restrictions were imposed.

Study Selection

Prospective cohort studies in humans that reported a measure of association (i.e. hazard ratios or incident rate ratios) between intakes of polyunsaturated fats (total, n-3, LC-n-3, n-6, EPA, DHA, DPA, ARA, LA, αLA, or the polyunsaturated: saturated fat ratio, measured by self-report, and all-cause mortality, cardiovascular diseases (including fatal and non-fatal composite cardiovascular events, fatal and non-fatal coronary events including myocardial infarction, ischemic heart disease, atrial fibrillation, and sudden cardiac death; ischemic or hemorrhagic strokes), breast cancer (pre- and post-menopausal), type 2 diabetes; depression; cognitive decline, or inflammatory bowel disease (Crohn's Disease or ulcerative colitis), measured by self-report, and/or confirmed by medical records or registry linkage , was eligible. One reviewer assessed titles and abstracts of all studies identified through electronic searches. Potentially eligible studies were reviewed independently by a second reviewer, with discrepancies resolved by discussion; when necessary, a senior author was consulted to reach consensus.

[1] ***Commonly used abbreviations:*** CVD = cardiovascular disease; ACM = all-cause mortality; CHD = coronary heart disease; IHD = ischemic heart disease; PUFA = polyunsaturated fatty acids; SFA = saturated fatty acids; MUFA = monounsaturated fatty acids; EPA = eicosapentaenoic acid, DHA = docosahexaenoic acid, DPA = docosapentaenoic acid, ALA = alpha-linolenic acid; %E = percent of energy from this nutrient; g/d = grams per day; mvRR = multivariable-adjusted relative risk; RR= relative risk

Data Extraction

Pairs of authors independently extracted details of the study design, country of conduct, exposure and outcome assessment, participant characteristics, and statistical analyses, including adjustment for confounders, from included studies using pretested instruments, with discrepancies resolved by discussion. Authors were contacted for additional data, where necessary. *Plot Digitizer* (http://plotdigitizer.sourceforge.net/) was used to extract numerical estimates from graphs.

Study Risk of Bias

The Newcastle-Ottawa Scale (NOS)[43] was used to assess the risk of bias of the included studies on the basis of selection of study groups, comparability of groups, and ascertainment of exposure(s) or outcome(s).

Grading of Recommendations Assessment, Development, and Evaluation (GRADE)

The GRADE approach was used to assess the confidence in the effect estimates derived from the body of evidence (quality of evidence) by outcome and produce evidence profiles[34-36]. We limit the presentations of results in the main text to the synthesis of prospective cohort studies, as these are considered the highest level of evidence for observational studies, and thus were used for the GRADE assessments of confidence. Evidence summaries and GRADE assessments were discussed and reviewed by all investigators and reviewed with the WHO Nutrition Guidance Expert Advisory Group (NUGAG) Subgroup on Diet and Health as part of WHO's guideline development process. Confidence in the estimate of each association was categorized into 4 levels, from very low (⊕◯◯◯) to high (⊕⊕⊕⊕).

Outcome definitions (bold are most common)

The included studies provided different degrees of granularity with respect to outcomes assessment. Given the different mechanisms by which polyunsaturated fats are likely to work, notably for cardiovascular diseases, we have provided estimates for the finest degree of outcome specificity as possible. The following outcome definitions were used: <u>All-cause mortality:</u> deaths from any cause during the follow-up period. <u>Total cardiovascular disease:</u> fatal and nonfatal cardiovascular disease or events that occur during the follow-up period are grouped as a single outcome by the study authors, including acute rheumatic fever, chronic rheumatic heart disease, hypertensive disease, **ischemic heart disease**, diseases of pulmonary circulation, **cerebrovascular disease (subarachnoid hemorrhage,**

intracerebral hemorrhage), diseases of arteries, arterioles and capillaries, and other diseases of the circulatory system. Fatal cardiovascular disease: death from cardiovascular disease during the follow-up period, including acute rheumatic fever, chronic rheumatic heart disease, hypertensive disease, **ischemic heart disease**, diseases of pulmonary circulation, **cerebrovascular disease (subarachnoid hemorrhage, intracerebral hemorrhage**), diseases of arteries, arterioles and capillaries, and other diseases of the circulatory system. Fatal coronary heart disease: death from ischemic heart disease, including **myocardial infarction**, angina pectoris, or other forms of chronic ischemic heart disease. Sudden cardiac death: sudden, unexpected death caused by loss of heart function (sudden cardiac arrest). Nonfatal myocardial infarction: a sudden and sometimes fatal occurrence of coronary thrombosis, typically resulting in the death of part of a heart muscle, but that does not result in death. Total CHD: non-fatal myocardial infarction, angina pectoris, and fatal CHD. Total stroke included subarachnoid hemorrhage and intracerebral hemorrhage. Fatal stroke: includes death from any stroke Ischemic stroke: thrombotic and embolic strokes. Atrial fibrillation: an abnormal heart rhythm characterized by rapid and irregular beating. **[ABRIDGED]**

Data Synthesis and Analysis

Statistical synthesis of effect estimates

The principal effect measures were the risk ratio (RR) between extreme levels of intake (highest vs. lowest quantile). For each study, most-adjusted (i.e. the multivariable association measure with the highest number of covariates) estimates and corresponding 95% CIs for each outcome were calculated. Where at least 2 studies provided data, a DerSimonian and Laird random effects meta-analysis were performed, which yields conservative confidence intervals around relative risks in the presence of heterogeneity[44], and when 3 or fewer studies were combined, fixed effect estimates were also considered.

Heterogeneity

Heterogeneity was detected using Cochran's Q test (significant at $P<0.10$), and quantified using the I^2 statistic (ranging from 0% to 100%)[45], and used to assess inconsistency as part of the GRADE assessment of evidence quality. If ≥10 studies were available[46 47] and heterogeneity was substantial ($I^2>60\%$ or $P_Q<0.10$)[45], meta-regression was used to explore heterogeneity.

Dose-Response

The dose-response relation was estimated by using generalized least squares trend estimation, according to the methods developed by Greenland and Longnecker, implemented in STATA (v. 13, College Station, TX) using the "glst"package written by Orsini[48 49]. The goodness-of-fit test was used to assess whether the linearity assumption was tenable. If the P for this test is <0.05, the interpretation is that a straight line is not a good fit to the data; if P>0.05 then a straight line is an adequate fit. If the straight line was not a good fit (i.e. goodness-of-fit test P<0.05), we used a piecewise-polynomial approach, which assumes linear associations across certain ranges of doses ("segments"), the boundaries of which are called knots. We applied restricted cubic splines to create three equally-spaced knots across the distribution to model the association between knots. The procedure described by Orsini and Greenland was finally used to estimate the pooled relative risks for increments of specific exposure values, and scaled to desired units using the "*lincom*" command in STATA. Where these dose-response analyses yielded important findings, they are discussed in the relevant sections of the manuscript, and in the GRADE table footnote corresponding to the "Overall quality of evidence" (column 9) for each relevant analysis.

Sensitivity

Four *a priori* sensitivity analyses were conducted: 1) removing each single study from the meta-analyses, and re-calculating the summary effect (the "leave-one-out" approach[50]); 2) removing studies with NOS scores <7 and re-calculating the pooled effect; and 3) removing risk estimates imputed due to incomplete reporting. A study whose removal either pushed the significance level of the overall effect from <0.05 to ≥0.05 [or vice versa], or altered the nominal effect size by 10% or more was considered an influential outlier. Where these sensitivity analyses yielded important findings, they are discussed in the GRADE table footnotes corresponding to the relevant analysis. If not mentioned, these sensitivity analyses did not influence the association measure.

Publication Bias

If ≥10 studies were available[51], the possibility of publication bias was explored by inspecting funnel plots, and conducting Egger's and Begg's tests (each significant at P<0.10). If publication bias was suspected, results are shown without imputation and with "missing" studies imputed using Duval and Tweedie's *trim-and-fill* method[52].

Software

Primary summary analyses were carried out separately for each outcome using Review Manager, version 5.2 (The Nordic Cochrane Center, the Cochrane Collaboration, and Copenhagen, Denmark). Meta-regression and sensitivity analyses were undertaken using STATA, version 12.1 (StataCorp, College Station, TX).

Role of the funding source

This review was commissioned and partially funded by the WHO as an independent review conducted by researchers from McMaster University (Hamilton, ON).

RESULTS

PUFA and Health Outcomes

Literature Flow

2,636 potentially eligible articles were identified. After duplicates were removed, 2,614 records were screened using title and abstract, and excluded. The full-text versions of 321 full-text papers were considered for inclusion in this synthesis. Two-hundred papers were excluded due to studies with an ineligible population (including children; n=16), ineligible outcome (n=7) or ineligible study type (n=9). Twenty-four of the 321 studies were excluded because they did not present a measure of association suitable for the meta-analysis, 89 did not measure the exposure of interest, 3 were editorials or commentaries, 24 were systematic reviews or a meta-analysis, 16 measured blood fatty acids only, 3 had multiple studies referring to the same cohort; and the full-text of 4 papers were unattainable. Hand-searching of a recent meta-analysis of linoleic acid and CHD[40] identified updated or previously unpublished data from 5 studies (Atherosclerosis Risk in Communities Study, Health Professionals' Follow-up study, Finnish Mobile Health Clinics, Israeli Ischemic Heart Disease Study, and Nurses' Health Study I; n=12 comparisons). Thus in total, 126 primary studies provided estimates that were included in the synthesis **(Figure 1a)**.

The 126 reports of prospective cohort studies of PUFA and health outcomes in the main analysis of primary prevention studies (published between 1981 and 2017) provided 519 comparisons of associations between PUFA and the health outcomes, representing cohorts enrolled from North America (n=52 studies, 205 data points: 1 study providing 2 data points from Canada; 51 studies

providing 203 data points from the United States of America), Europe (54 studies providing 187 data points: 13 studies providing 33 data points from the Netherlands; 9 studies providing 47 data points from Sweden; 7 studies providing 23 data points from Finland; 7 studies providing 30 data points from Denmark; 5 studies providing 13 data points from the United Kingdom; 3 studies providing 10 data points from various pooled European analyses; 5 studies providing 19 data points from Spain; 2 studies providing 9 data points from France; 2 studies providing 2 data points from Italy; and 1 study providing 1 data point from Germany), Asia (17 studies providing 104 data points: 9 studies providing 62 data points from Japan; 3 studies providing 22 data points from Shanghai; 4 studies providing 18 data points from Singapore; and 1 study providing 2 data points from China), and 3 studies (providing 23 data points) from Australia. **(Table 1)**

The distribution of background (i.e. average) PUFA intakes varied across studies, and across types of PUFA. The background total PUFA intake ranged from 1.1% through 9.0% energy (median=5.3; mean =5.3) or 2.9 through 26.7 g/d (median=11.8; mean=12.0). Long-chain n-3 PUFA intake ranged from 0 to 0.7% energy (median=0.15; mean=0.18) or 0 to 1.72 g/d (median=0.283; mean=0.390). ALA intake ranged from 0.2 through 1.1% energy (median=0.6; mean=0.6) or 0.5 through 2.5 g/d (median=1.2; mean=1.3). LA intake ranged from 2.2 through 6.7% energy (median=4.4; mean=4.6) or 5.7 to 14.7 g/d (median=9.4; mean=9.7).

Of the 25 studies that reported the association between long-chain n-3 fatty acids and mortality or cardiovascular outcomes, 24 studies [96.0%] reported associations for total dietary LC-n-3 PUFA only, 14 studies [56.0%] also provided associations for fish and at least one outcome (data not extracted), 1 study [4.0%] looked at supplemental LC-n-3 PUFA separately from diet, 1 study presented both dietary and supplemental LC-n-3 PUFA [4.0%], and one study measured serum LC-n-3 PUFA [4.0%].

Of the 14 studies that reported the association between total n-3 fatty acids and mortality or cardiovascular outcomes, 14 studies [100.0%] reported associations for total dietary n-3 PUFA only, 4 studies [28.6%] also provided associations for fish and at least one outcome (data not extracted), 0 studies [0.0%] looked at supplemental n-3 PUFA separately from diet, and 0 studies [0.0%] presented both dietary and supplemental total n-3 PUFA.

An additional 4 studies of fish or PUFA in secondary prevention were also identified and summarized, as were 4 recent systematic reviews and meta-analyses of fish and CVD and type 2 diabetes; and 1 of walnut consumption and type 2 diabetes.

Study Quality

Of the 126 publications assessed and included in the quantitative synthesis, the median NOS rating was 8 (range: 5 to 9, IQR: 4). A total of 108 studies (85.7%) were rated 7 or higher; 14 studies (11.1%) were rated 6, and 4 studies (3.2%) were rated 5. Overall, 92% of publications scored 4 out of a possible 4 points for unbiased selection of participants, ensuring that the outcome of interest was not present at baseline, and using a validated measure of dietary exposure; 45% scored 2 out of a possible 2 points for ensuring comparability of exposed and unexposed groups with respect to important confounders; and 57% scored 3 out a possible 3 points for rigorous outcome confirmation, and adequate follow-up (>80%). Forty-three studies (34%) documented a measurement of trans-fatty acid intake in the cohort. **(Figure 1b, Table 2)**

Total PUFA and Health Outcomes

[Results not included in this version of the report]

ω-3 PUFA and Health Outcomes

All-Cause Mortality

Four prospective cohort studies (providing 6 comparisons, n=53,734 deaths) examined the association between total ω-3 PUFA intake and all-cause mortality [55 56 102 103]. The summary most-adjusted multivariable risk ratio comparing the highest to the lowest intake of dietary ω-3 PUFA is 0.98 (95% CI: 0.92 to 1.05; P=0.61; I^2=71%; P_{het}=0.004) (**Figure 9**). Subgroup analyses or publication bias tests were not performed (<10 studies). No study was an influential outlier.

Cardiovascular Diseases

Total cardiovascular disease

One prospective cohort study (1 comparison; n=194 events) provided estimates of the association between total dietary ω-3 PUFA and total cardiovascular disease. For total cardiovascular disease, the summary most-adjusted multivariable risk ratio comparing the highest to the lowest intake of dietary PUFA is 1.10 (95% CI: 0.83 to 1.45; *P*=0.50) (**Figure 10-2.2.1**).

Fatal cardiovascular disease

Six studies (8 comparisons; n=19,953 events) provided estimates of the association between total dietary ω-3 PUFA and fatal cardiovascular disease [55 56 102-105]. The summary most-adjusted multivariable risk ratio comparing the highest to the lowest intake of total dietary ω-3 PUFA is 0.93 (95% CI: 0.85 to 1.02; P=0.14; I^2=59%, P_{het}=0.02) (**Figure 10-2.2.2)**. No publication bias was suspected.

Fatal Coronary Heart Disease

Five studies (6 comparisons; n=4,160 events) provided estimates of the association between total dietary ω-3 PUFA and fatal coronary heart disease [102 105-108]. The summary most-adjusted multivariable risk ratio comparing the highest to the lowest intake of dietary PUFA is 0.84 (95% CI: 0.73 to 0.96; P=0.01; I^2=13%, P_{het}=0.33). (**Figure 10-2.2.3)** Assuming linearity, a 2-g increase in n-3 PUFA was associated with a 31% reduced risk of CHD mortality (mvRR: 0.69, 95% CI: 0.44 to 1.08). Assuming linearity, a 0.5% increase in n-3 PUFA was associated with an 18% reduced risk of CHD mortality (mvRR: 0.82, 95% CI: 0.66 to 1.01). (**Appendix 2, Figures 11-12**)

Sudden Cardiac Death

Three studies (3 comparisons; n=529 events) provided estimates of the association between total dietary ω-3 PUFA and sudden cardiac death [65 102 108]. The summary most-adjusted multivariable risk ratio comparing the highest to the lowest intake of total dietary ω-3 PUFA is 0.68 (95% CI: 0.50 to 0.93; P=0.02; I^2=0%; Phet=0.57). (**Figure 10-2.2.4)**

Fatal Myocardial Infarction

One study (1 comparison; n=329 events) provided an estimate of the association between total dietary ω-3 PUFA and fatal myocardial infarction [102]. In this study, the summary most-adjusted multivariable risk ratio comparing the highest to the lowest intake of total dietary ω-3 PUFA is 0.75 (95% CI: 0.47 to 1.19; P=0.22). (**Figure 10-2.2.5)**

Nonfatal Myocardial Infarction

One study (1 comparison; n=1,029 events) provided an estimate of the association between total dietary ω-3 PUFA and nonfatal myocardial infarction[106]. The summary most-adjusted

multivariable risk ratio comparing the highest to the lowest intake of dietary PUFA is 0.73 (95% CI: 0.57 to 0.93; P=0.01). (**Figure 10-2.2.6)**

Total Coronary Heart Disease

Five studies (8 comparisons; n=4,515 events) provided estimates of the association between total dietary ω-3 PUFA and total CHD [69 106 108-110]. The summary most-adjusted multivariable risk ratio comparing the highest to the lowest intake of total dietary ω-3 PUFA is 0.89 (95% CI: 0.74 to 1.08; *P*=0.24; I^2=65%, P_{het}=0.006). (**Figure 10-2.2.7)**

Total myocardial infarction

One study (1 comparison; n=221 events) provided an estimate of the association between total dietary ω-3 PUFA and total myocardial infarction [108]. In this study, the summary most-adjusted multivariable risk ratio comparing the highest to the lowest intake of total dietary ω-3 PUFA is 0.43 (95% CI: 0.24 to 0.78; P=0.005). (**Figure 10-2.2.8)**

Total Stroke

Two studies (2 comparisons; n=815 events) provided estimates of the association between total dietary ω-3 PUFA and total stroke [71 111]. The summary most-adjusted multivariable risk ratio comparing the highest to the lowest intake of dietary PUFA is 0.85 (95% CI: 0.49 to 1.46; *P*=0.80; I^2=38%, P_{het}=0.20). (**Figure 10-2.2.9)**

Fatal Stroke

One study (1 comparison; n=1,298 events) provided an estimate of the association between total dietary ω-3 PUFA and fatal stroke [66]. In this study, the summary most-adjusted multivariable risk ratio comparing the highest to the lowest intake of total dietary ω-3 PUFA is 0.82 (95% CI: 0.66 to 1.01; *P*=0.07). (**Figure 10-2.2.10)**

Fatal Ischemic Stroke

One study (1 comparison; n=319 events) provided an estimate of the association between total dietary ω-3 PUFA and fatal ischemic stroke [66]. In this study, the summary most-adjusted multivariable risk ratio comparing the highest to the lowest intake of total dietary ω-3 PUFA is 1.17 (95% CI: 0.71 to 1.92; *P*=0.54). (**Figure 10-2.2.11)**

Ischemic Stroke

Two studies (3 comparisons; n=1,058 events) provided estimates of the association between total dietary ω-3 PUFA and ischemic stroke [69 111]. The summary most-adjusted multivariable risk ratio comparing the highest to the lowest intake of dietary PUFA is 0.92 (95% CI: 0.73 to 1.15; P=0.45; I^2=19%, P_{het}=0.29). (**Figure 10-2.2.12)**

Hemorrhagic Stroke

One study (1 comparison; n=181 events) provided an estimate of the association between total dietary ω-3 PUFA and hemorrhagic stroke [108]. In this study, the summary most-adjusted multivariable risk ratio comparing the highest to the lowest intake of total dietary ω-3 PUFA is 0.76 (95% CI: 0.43 to 1.36; P=0.35). (**Figure 10-2.2.13)**

Thrombotic Infarction

One study (1 comparison; n=254 events) provided an estimate of the association between total dietary ω-3 PUFA and thrombotic infarction [108]. In this study, the summary most-adjusted multivariable risk ratio comparing the highest to the lowest intake of total dietary ω-3 PUFA is 0.67 (95% CI: 0.42 to 1.07; P=0.09). (**Figure 10-2.2.14)**

Atrial Fibrillation

One study (1 comparison; n=1,441 events) provided an estimate of the association between total dietary ω-3 PUFA and atrial fibrillation [76]. The summary most-adjusted multivariable risk ratio comparing the highest to the lowest intake of dietary PUFA is 1.05 (95% CI: 0.80 to 1.38; P=0.73). (**Figure 10-2.2.15)**

[Other outcomes not included in this version of the report]

GRADE Assessment of Quality of Evidence: For the 22 outcomes assessed, the GRADE assessment of the confidence in the estimates of association between total ω-3 PUFA and health outcomes was moderate for 1 outcome (fatal CHD) [4.5%]; low for 4 outcomes (sudden cardiac death, non-fatal MI, fatal stroke, and cognitive decline) [18.2%] and very low for 17 [77.3%]. Four estimates were at serious risk of bias, 15 had serious inconsistency, and 16 had serious imprecision. **(Table 4)**

Long-Chain ω-3 PUFA and Health Outcomes

All-Cause Mortality

Nine prospective cohort studies (10 comparisons; n=58,799 deaths) examined the association between long-chain ω-3 PUFA intake and all-cause mortality [54 56 116-120]. The summary most-adjusted multivariable risk ratio comparing the highest to the lowest intake of dietary long-chain ω-3 PUFA is 0.93 (95% CI: 0.88 to 0.99; P=0.03; I^2=62%; P_{het}=0.007) (**Figure 17**). Subgroup analyses or publication bias tests were not performed (<10 studies). No study was an influential outlier. The fixed-effect estimate was 0.94 (95% CI: 0.91 to 0.97; P=0.0003). Assuming linearity, a 0.5-g increase in long chain n-3 PUFA was associated with an 8% reduced risk of all-cause mortality (mvRR: 0.92, 95% CI: 0.87 to 0.98). Assuming linearity, a 0.5% increase in long chain n-3 PUFA was associated with an 8% reduced risk of all-cause mortality (mvRR: 0.92, 95% CI: 0.87 to 0.98). (**Appendix 2, Figures 13-14**)

Cardiovascular Diseases

Total cardiovascular disease

Three prospective cohort studies (3 comparisons; n=4,741 events) provided estimates of the association between long-chain ω-3 PUFA intake and total cardiovascular disease events [121-123]. The summary most-adjusted multivariable risk ratio comparing the highest to the lowest intake of dietary long-chain ω-3 PUFA is 0.89 (95% CI: 0.60 to 1.34; P=0.59; I^2=82%; P_{het}=0.004; random-effects) (**Figure 18-3.2.1**). The fixed-effect estimate is 1.00 (95% CI: 0.86 to 1.17).

Fatal cardiovascular disease

Nine studies (10 comparisons; n=17,668 events) provided estimates of the association between long-chain ω-3 PUFA intake and fatal cardiovascular disease[56 105 116 117 120 121 124]. The summary most-adjusted multivariable risk ratio comparing the highest to the lowest intake of total long-chain dietary ω-3 PUFA is 0.89 (95% CI: 0.79 to 1.01; P=0.07; I^2=72%, P_{het}=0.0002; random-effects) (**Figure 18-3.2.2)**. The fixed-effect estimate is 0.93 (95% CI: 0.88 to 0.98; P=0.007). Assuming linearity, a 0.5-g increase in long chain n-3 PUFA was associated with an 11% reduced risk of CVD mortality (mvRR: 0.894, 95% CI: 0.802 to 0.996). Assuming linearity, a 0.5% increase in long chain n-3 PUFA was associated with a 20% reduced risk of CVD mortality (mvRR: 0.80, 95% CI: 0.63 to 1.01). (**Appendix 2, Figures 15-16**) No publication bias was suspected **(Figure PB-Panel C)**.

Subgroup analyses: We assessed whether the association between long-chain ω-3 PUFA intake and fatal cardiovascular disease differed according to pre-defined study characteristics. Important effect modifiers included: 1) whether or not a study included a measure of trans-fatty acids and adjusted for this in multivariable models, (P=0.10) **(Figure S8)**; 2) the contrast between the highest and lowest PUFA groups (calculated as fold-difference; P=0.07 on ln-transformed values) **(Figure S9)**; and 3) the percentage of current/former smokers in the study (P=0.014) **(Figure S10)**. No effect measure modification was observed by the number of cases (P=0.36), study quality rating (NOS; P=0.37), duration of follow-up (P=0.45), adjustment for dyslipidemia (P=0.36), method of diet assessment (P=0.36), the amount of saturated fat (P=0.95) or polyunsaturated fat (P=0.45) or the P:S ratio (P=0.74), the country of conduct (P=0.74), the sex distribution (P=0.97), adjustment for BP (P=0.60), baseline year of data collection (P=0.88), or mean age of study participants (P=0.55).

Fatal Coronary Heart Disease

Nine studies (10 comparisons; n=5,904 events) provided estimates of the association between total long-chain dietary ω-3 PUFA and fatal coronary heart disease [62 105 116 117 119 120 124-126]. The summary most-adjusted multivariable risk ratio comparing the highest to the lowest intake of total long-chain dietary ω-3 PUFA is 0.81 (95% CI: 0.68 to 0.97; P=0.02; I^2=60%, P_{het}=0.008; random-effects). (**Figure 18-3.2.3**) The fixed-effect estimate is 0.87 (95% CI: 0.79 to 0.96; P=0.004). Assuming linearity, a 0.5-g increase in long chain n-3 PUFA was associated with a 14% reduced risk of CHD mortality (mvRR: 0.86, 95% CI: 0.78 to 0.95). Assuming linearity, a 0.5% increase in long chain n-3 PUFA was associated with a 26% reduced risk of CHD mortality (mvRR: 0.74, 95% CI: 0.60 to 0.90). (**Appendix 2, Figures 17-18**) No publication bias was suspected **(Figure PB-Panel D)**.

Subgroup analyses: We assessed whether the association between long-chain ω-3 PUFA intake and fatal coronary heart disease differed according to pre-defined study characteristics. The association did not differ according to whether or not a study measured trans-fat intake (P=0.37); the contrast between the highest and lowest PUFA groups (calculated as fold-difference; P=0.59 on ln-transformed values); the percentage of current/former smokers in the study (P=0.55); the number of cases (P=0.43); study quality rating (NOS; P=0.87); the duration of follow-up (P=0.73); the method of diet assessment (P=0.42); the amount of saturated (P=0.95) or polyunsaturated fat (P=0.15); the P:S ratio (P=0.39); the country in which studies were

conducted (P=0.66); the sex distribution (P=0.72); whether or not adjustment was done for BP (P=0.48), total energy (P=0.55), or dyslipidemia (P=0.59); the baseline year of data collection (P=0.67); or mean age of study participants (P=0.75).

Sudden Cardiac Death

Three studies (3 comparisons; n=545 events) provided estimates of the association between total long-chain dietary ω-3 PUFA and sudden cardiac death [65 125 127]. The summary most-adjusted multivariable risk ratio comparing the highest to the lowest intake of total dietary long-chain ω-3 PUFA is 0.45 (95% CI: 0.19 to 1.07; P=0.07; I^2=60%; Phet=0.08; random-effects). (**Figure 18-3.2.4)** The fixed-effect estimate is 0.89 (95% CI: 0.77 to 0.95; P=0.004).

Fatal Myocardial Infarction

One study (1 comparison; n=113 events) provided an estimate of the association between total long-chain dietary ω-3 PUFA and fatal myocardial infarction [128]. In this study, the summary most-adjusted multivariable risk ratio comparing the highest to the lowest intake of total dietary long-chain ω-3 PUFA is 0.43 (95% CI: 0.23 to 0.81; P=0.009). (**Figure 18-3.2.5)**

Fatal Arrhythmia

One study (1 comparison, 148 events) provided an estimate of the association between total long-chain dietary ω-3 PUFA and fatal arrhythmia. In this study, the most-adjusted multivariable risk ratio comparing the highest to the lowest intake of total dietary long-chain ω-3 PUFA is 0.45 (95% CI: 0.25 to 0.81; P=0.008). (**Figure 18-3.2.6)**

Nonfatal Myocardial Infarction

Two studies (2 comparisons; n=442 events) provided an estimate of the association between total dietary long-chain ω-3 PUFA and nonfatal myocardial infarction [121 126]. The summary most-adjusted multivariable risk ratio comparing the highest to the lowest intake of total dietary long-chain ω-3 PUFA is 0.73 (95% CI: 0.57 to 0.93; P=0.01). (**Figure 18-3.2.7)**

Total Coronary Heart Disease

Three studies (5 comparisons; n=2,891 events) provided estimates of the association between total dietary long-chain ω-3 PUFA and total CHD [62 69 129]. The summary most-adjusted

multivariable risk ratio comparing the highest to the lowest intake of total dietary long-chain ω-3 PUFA is 0.94 (95% CI: 0.76 to 1.16; *P*=0.55; I^2=60%, P_{het}=0.04; random-effects). (**Figure 18-3.2.8)** The fixed effect estimate is 1.01 (95% CI: 0.90 to 1.14).

Total myocardial infarction

One study (1 comparison; n=281 events) provided an estimate of the association between total dietary long-chain ω-3 PUFA and total myocardial infarction[121]. In this study, the summary most-adjusted multivariable risk ratio comparing the highest to the lowest intake of total dietary long-chain ω-3 PUFA is 1.20 (95% CI: 0.80 to 1.80; P=0.38). (**Figure 18-3.2.9)**

Total Stroke

Two studies (3 comparisons; n=296 events) provided estimates of the association between total dietary long-chain ω-3 PUFA and total stroke [121 130]. The summary most-adjusted multivariable risk ratio comparing the highest to the lowest intake of total dietary long-chain ω-3 PUFA is 0.79 (95% CI: 0.52 to 1.18; *P*=0.25; I^2=45%, P_{het}=0.16). (**Figure 18-3.2.10)**

Fatal Stroke

Four studies (5 comparisons; n=2,414 events) provided an estimate of the association between total dietary long-chain ω-3 PUFA and fatal stroke [105 124 128]. In this study, the summary most-adjusted multivariable risk ratio comparing the highest to the lowest intake of total dietary long-chain ω-3 PUFA is 0.90 (95% CI: 0.79 to 1.04; *P*=0.14; I^2=0%, P_{het}=0.50). (**Figure 18-3.2.11)**

Fatal Ischemic Stroke

One study (1 comparison; n=404 events) provided an estimate of the association between total dietary long-chain ω-3 PUFA and fatal ischemic stroke [117]. In this study, the summary most-adjusted multivariable risk ratio comparing the highest to the lowest intake of total dietary long-chain ω-3 PUFA is 0.53 (95% CI: 0.34 to 0.82; *P*=0.005). (**Figure 18-3.2.12)**

Ischemic Stroke

Two studies (4 comparisons; n=899 events) provided estimates of the association between total dietary long-chain ω-3 PUFA and ischemic stroke [69 130]. The summary most-adjusted

multivariable risk ratio comparing the highest to the lowest intake of total dietary long-chain ω-3 PUFA is 1.00 (95% CI: 0.81 to 1.25; *P*=0.97; I^2=0%, P_{het}=0.53). (**Figure 10-3.2.13)**

Fatal Hemorrhagic Stroke

One study (1 comparison; n=460 events) provided an estimate of the association between total dietary long-chain ω-3 PUFA and fatal hemorrhagic stroke [117]. In this study, the summary most-adjusted multivariable risk ratio comparing the highest to the lowest intake of total dietary long-chain ω-3 PUFA is 0.88 (95% CI: 0.63 to 1.22; P=0.44). (**Figure 10-3.2.14)**

Total Hemorrhagic Stroke

One study (2 comparisons; n=47 events) provided an estimate of the association between total dietary long-chain ω-3 PUFA and total hemorrhagic stroke [130]. In this study, the summary most-adjusted multivariable risk ratio comparing the highest to the lowest intake of total dietary long-chain ω-3 PUFA is 0.39 (95% CI: 0.15 to 1.00; P=0.09; I^2=0%, P_{het}=0.65). (**Figure 10-3.2.15)**

Atrial Fibrillation

Five studies (5 comparisons; n=2,978 events) provided an estimate of the association between total dietary long-chain ω-3 PUFA and atrial fibrillation [131-135]. The summary most-adjusted multivariable risk ratio comparing the highest to the lowest intake of total dietary long-chain ω-3 PUFA is 1.02 (95% CI: 0.82 to 1.28; P=0.83; I^2=71%, P_{het}=0.008; random-effects). (**Figure 18-3.2.16)** The fixed-effect estimate is 1.01 (95% CI: 0.90 to 1.12).

Heart Failure

One study (1 comparison; n=460 events) provided an estimate of the association between total dietary long-chain ω-3 PUFA and incident heart failure [117]. In this study, the summary most-adjusted multivariable risk ratio comparing the highest to the lowest intake of total dietary long-chain ω-3 PUFA is 0.89 (95% CI: 0.69 to 1.14; P=0.36). (**Figure 18-3.2.17)**

[Other outcomes not included in this version of the report]

GRADE Assessment of Quality of Evidence: For the 24 outcomes assessed, the GRADE assessment of the confidence in the estimates of association between total dietary long-chain ω-3 PUFA and health outcomes was moderate for 2 outcomes (all-cause mortality, fatal CHD) [8.3%], low for 1 outcome (fatal

MI) [4.2%] and very low for 18 [75.0%]. Four estimates were at serious risk of bias, 11 had serious inconsistency, and 21 had serious imprecision. **(Table 5)**

Eicosapentaenoic Acid (EPA; 20:5 ω-3) and Health Outcomes

All-Cause Mortality

One prospective cohort study (1 comparison; n=5,836 deaths) examined the association between EPA intake and all-cause mortality [117]. The summary most-adjusted multivariable risk ratio comparing the highest to the lowest intake of EPA is 0.79 (95% CI: 0.72 to 0.87; P<0.00001) (**Figure 25**).

Cardiovascular Diseases

Total cardiovascular disease

No prospective cohort studies were identified that assessed the association between EPA and total cardiovascular disease.

Fatal cardiovascular disease

One study (1 comparison; n=1,789 events) examined the association between EPA intake and cardiovascular mortality [117]. The summary most-adjusted multivariable risk ratio comparing the highest to the lowest intake of EPA is 0.75 (95% CI: 0.63 to 0.90; P=0.002) (**Figure 26-4.2.1**).

Fatal coronary heart disease

Two studies (2 comparisons; n=824 events) examined the association between EPA intake and coronary heart disease mortality [117 125]. The summary most-adjusted multivariable risk ratio comparing the highest to the lowest intake of EPA is 0.78 (95% CI: 0.59 to 1.02; P=0.07; I^2=0%; P_{het}=0.39) (**Figure 26-4.2.2**).

Fatal Myocardial Infarction

No prospective cohort studies were identified that assessed the association between EPA and fatal myocardial infarction.

Total Coronary Heart Disease

Two studies (4 comparisons; n=1,733 events) examined the association between EPA intake and total CHD [109 110]. The summary most-adjusted multivariable risk ratio comparing the highest to the lowest intake of EPA is 0.94 (95% CI: 0.78 to 1.14; P=0.56; I^2=26%; P_{het}=0.26) (**Figure 26-4.2.4**). The summary fixed-effect estimate is 0.94 (95% CI: 0.81 to 1.11; P=0.48).

Fatal Stroke

No prospective cohort studies were identified that assessed the association between EPA and fatal stroke (without distinguishing type of stroke).

Fatal Hemorrhagic Stroke

One study (1 comparison; n=460 events) examined the association between EPA intake and fatal hemorrhagic stroke [117]. The summary most-adjusted multivariable risk ratio comparing the highest to the lowest intake of EPA is 0.81 (95% CI: 0.58 to 1.13; P=0.21) (**Figure 26-4.2.6**).

Fatal Ischemic Stroke

One study (1 comparison; n=404 events) examined the association between EPA intake and fatal ischemic stroke [117]. The summary most-adjusted multivariable risk ratio comparing the highest to the lowest intake of EPA is 0.56 (95% CI: 0.36 to 0.87; P=0.009) (**Figure 26-4.2.7**).

Total stroke

One study (1 comparison; n=404 events) examined the association between EPA intake and total stroke [117]. The summary most-adjusted multivariable risk ratio comparing the highest to the lowest intake of EPA is 1.07 (95% CI: 0.95 to 1.20; P=0.26) (**Figure 26-4.2.9**).

Sudden Cardiac Death

No prospective cohort studies were identified that assessed the association between EPA and sudden cardiac death.

Atrial fibrillation

Three studies (3 comparisons; n=3,285 events) examined the association between EPA intake and atrial fibrillation [76 133 135]. The summary most-adjusted multivariable risk ratio

comparing the highest to the lowest intake of EPA is 0.94 (95% CI: 0.82 to 1.07; P=0.33; I^2=0%; P_{het}=0.72) (**Figure 26-4.2.11**).

[Other outcomes not included in this version of the report]

GRADE Assessment of Quality of Evidence: For the 16 outcomes assessed, the GRADE assessment of the confidence in the estimates of association between total dietary EPA and health outcomes was low for 3 outcomes (all-cause mortality, fatal CVD, and fatal ischemic stroke) [18.8%] and very low for 13 [81.3%]. Two estimates was at serious risk of bias, 11 had serious inconsistency, and 13 had serious imprecision. **(Table 6)**

Docosahexaenoic Acid (DHA; 22-6 ω-3) and Health Outcomes

All-cause mortality

One study (1 comparison; n=5,836 deaths) assessed the association between DHA and all-cause mortality [117]. In this study, the most-adjusted multivariable risk ratio comparing the highest to the lowest intake of DHA is 0.78 (95% CI: 0.71 to 0.86; P<0.00001). **(Figure 33)**

Cardiovascular Diseases

Total CVD

No prospective cohort studies were identified that assessed the association between DHA and total cardiovascular disease.

Fatal CVD

One study (1 comparison; n=1,789 deaths) assessed the association between DHA and CVD deaths [117]. In this study, the most-adjusted multivariable risk ratio comparing the highest to the lowest intake of DHA is 0.76 (95% CI: 0.64 to 0.91; P<0.00001). **(Figure 34-5.2.1)**

Fatal CHD

One study (1 comparison; n=476 deaths) assessed the association between DHA and CHD deaths [117]. In this study, the most-adjusted multivariable risk ratio comparing the highest to the lowest intake of DHA is 0.79 (95% CI: 0.57 to 1.09; P=0.15). **(Figure 34-5.2.2)**

Fatal Myocardial Infarction

No prospective cohort studies were identified that assessed the association between DHA and fatal myocardial infarction.

Sudden Cardiac Death

No prospective cohort studies were identified that assessed the association between DHA and sudden cardiac death.

Total CHD

Two studies (4 comparison; n=1,733 events) assessed the association between DHA and total CHD [109 110]. The summary most-adjusted multivariable risk ratio comparing the highest to the lowest intake of DHA is 0.93 (95% CI: 0.79 to 1.10; P=0.38; I^2=0%; P_{het}=0.45) (**Figure 34-5.2.5**).

Atrial fibrillation

Three studies (3 comparisons, n=3,285 events) assed the association between DHA and atrial fibrillation [76 133 135]. The summary most-adjusted multivariable risk ratio comparing the highest to the lowest intake of DHA is 0.84 (95% CI: 0.63 to 1.13; P=0.25; I^2=62%; P_{het}=0.07). (**Figure 34-5.2.6**) The summary fixed-effect estimate is 0.89 (95% CI: 0.79 to 1.02; P=0.09).

Fatal stroke

No prospective cohort studies were identified that assessed the association between DHA and fatal stroke (total).

Fatal hemorrhagic stroke

One study (1 comparison; n=460 events) assessed the association between DHA and fatal hemorrhagic stroke [117]. In this study, the most-adjusted multivariable risk ratio comparing the highest to the lowest intake of DHA is 0.95 (95% CI: 0.50 to 1.81; P=0.88). **(Figure 34-5.2.8)**

Fatal ischemic stroke

One study (1 comparison; n=404 events) assessed the association between DHA and fatal ischemic stroke [117]. In this study, the most-adjusted multivariable risk ratio comparing the highest to the lowest intake of DHA is 0.55 (95% CI: 0.36 to 0.84; P=0.005). **(Figure 34-5.2.9)**

Total stroke

One study (1 comparison; n=421 events) assessed the association between DHA and total stroke [151]. In this study, the most-adjusted multivariable risk ratio comparing the highest to the lowest intake of DHA is 1.01 (95% CI: 0.91 to 1.12; P=0.85). **(Figure 34-5.2.10)**

[Other outcomes not included in this version of the report]

GRADE Assessment of Quality of Evidence: For the 16 outcomes assessed, the GRADE assessment of the confidence in the estimates of association between DHA and health outcomes was low for 4 outcomes (all-cause mortality, fatal CVD, fatal ischemic stroke, and dementia) [25.0%] and very low for 12 [75.0%]. Two estimates were at serious risk of bias, 12 had serious inconsistency, and 12 had serious imprecision. **(Table 7)**

Docosapentaenoic Acid (DPA; 22:5 ω-3) and Health Outcomes

Cardiovascular Diseases

Total Coronary Heart Disease

One study (2 comparisons; n=1,124 events) provided estimates of the association between DPA and total CHD [109]. The summary most-adjusted multivariable risk ratio comparing the highest to the lowest intake of dietary DPA is 0.91 (95% CI: 0.72 to 1.15; P=0.43; I^2=0%, P_{het}=0.74).

Atrial Fibrillation

One study (1 comparison; n=240 events) provided estimates of the association between DPA and atrial fibrillation [133]. In this study, the most-adjusted multivariable risk ratio comparing the highest to the lowest intake of dietary DPA is 0.95 (95% CI: 0.65 to 1.39; P=0.79).

GRADE Assessment of Quality of Evidence: For the 2 outcomes assessed [100.0%], the GRADE assessment of the confidence in the estimates of association between DPA and health outcomes was

very low. No estimates were at serious risk of bias, none had serious inconsistency, but both had serious imprecision. **(Table 8)**

α-Linolenic Acid (ALA; 18:3 ω-3) and Health Outcomes

All-Cause Mortality

Three studies (4 comparisons; n=35,260 deaths) examined the association between ALA intake and all-cause mortality [56 116 154]. The summary most-adjusted multivariable risk ratio comparing the highest to the lowest intake of ALA is 0.92 (95% CI: 0.82 to 1.03; P=0.14; I^2=76%; P_{het}=0.006; random-effects) (**Figure 41**). The fixed-effect estimate is 0.97 (95% CI: 0.93 to 1.01; P=0.20). The summary RR is 0.99 (95% CI: 0.93 to 1.05; P=0.69; I^2=24%; P_{het}=0.27; random-effects), after removal of Fretts et al.[154], who measured ALA in phospholipids only (not diet). Assuming linearity, a 0.5 g/d increase in ALA was associated with a 5% decreased risk of all-cause mortality (mvRR: 0.95, 95% CI: 0.90 to 0.99). Assuming linearity, a 0.2% increase in energy from ALA was associated with a 2% decreased risk of all-cause mortality (mvRR: 0.98; 95% CI: 0.95 to 1.01). **(Appendix 2, Figures 21-22)**

Cardiovascular Diseases

Total cardiovascular disease

No prospective cohort studies were identified that assessed the association between ALA and total cardiovascular disease.

Fatal cardiovascular disease

Six studies (6 comparisons; n=13,397 events) provided estimates of the association between ALA and fatal cardiovascular disease [56 59 105 116 154]. The summary most-adjusted multivariable risk ratio comparing the highest to lowest intake of dietary ALA is 0.92 (95% CI: 0.82 to 1.03; P=0.14; I^2=76%; P_{het}=0.006; random-effects) **(Figure 42-7.2.1)**. The fixed-effect estimate is 0.88 (95% CI: 0.82 to 0.94; P=0.0001). Assuming linearity, a 0.5 g/d increase in ALA was associated with an 8% decreased risk of CV mortality (mvRR: 0.92, 95% CI: 0.88 to 0.97). Assuming linearity, a 0.5% increase in energy from ALA was associated with a 7% decreased risk of CV mortality (mvRR: 0.93, 95% CI: 0.90 to 0.97). (**Appendix 2, Figures 23-24**)

Fatal Coronary Heart Disease

Eight studies (8 comparisons; n=4,297 events) provided estimates of the association between ALA and fatal CHD [62 105 116 154-157]. The summary most-adjusted multivariable risk ratio comparing the highest to the lowest intake of ALA is 0.84 (95% CI: 0.72 to 0.99; P=0.04; I^2=31%; P_{het}=0.19) (**Figure 42-7.2.2**). The fixed-effect estimate is 0.84 (95% CI: 0.76 to 0.93; P=0.001). Assuming linearity, a 0.5 g/d increase in ALA was associated with an 8% decreased risk of CHD mortality (mvRR: 0.92, 95% CI: 0.87 to 0.97). Assuming linearity, a 0.2 % increase in ALA was associated with an 7% decreased risk of CHD mortality (mvRR: 0.93, 95% CI: 0.88 to 0.97). (**Appendix 2, Figures 25-26**)

Fatal myocardial infarction

No prospective cohort studies were identified that assessed the association between ALA and fatal myocardial infarction.

Sudden Cardiac Death

One study (1 comparison; n=385 events) provided an estimate of the association between ALA and sudden cardiac death [65]. The summary most-adjusted multivariable risk ratio comparing the highest to the lowest intake of dietary ALA is 0.51 (95% CI: 0.33 to 78; P=0.002). (**Figure 42-7.2.4)**

Total Coronary Heart Disease

Six studies (7 comparisons; n=3,360 events) provided estimates of the association between ALA and total CHD[62 129 154 155 157 158]. The summary most-adjusted multivariable risk ratio comparing the highest to the lowest intake of dietary ALA is 0.93 (95% CI: 0.83 to 1.04; *P*=0.21; I^2=0%, P_{het}=0.52). (**Figure 42-7.2.5)** There was no evidence of a dose-response association between ALA and risk of CHD (mvRR: 0.99, 95% CI: 0.96 to 1.03 per 0.5-g and mvRR: 0.99, 95% CI: 0.95 to 1.03 per 0.2%). (**Appendix 2, Figures 27-28**)

Nonfatal Myocardial Infarction

One study (1 comparison; n=597 events) assessed the association between ALA and nonfatal myocardial infarction [156]. The multivariable risk ratio comparing the highest to lowest intake of dietary ALA is 0.85 (95% CI: 0.61 to 1.19; P=0.34). **(Figure 42-7.2.6)**

Fatal Stroke

One study (1 comparison; n=1,298 events) assessed the association between dietary ALA and fatal stroke [105]. In this study the multivariable risk ratio was 0.81 (95% CI: 0.67 to 0.98; P=0.03). **(Figure 42-7.2.7)**

Total Stroke

Three studies (3 comparisons; n=1,000 events) provided estimates of the association between ALA and total stroke. The summary most-adjusted multivariable risk ratio comparing the highest to the lowest category of dietary ALA is 0.90 (95% CI: 0.71 to 1.14; P=0.37; I^2=50%; P_{het}=0.14; random-effects). **(Figure 42-7.2.8)** The fixed effect estimate is 0.98 (95% CI: 0.80 to 1.09).

Ischemic Stroke

Two studies (2 comparisons; n=422 events) provided estimates of the association between ALA and ischemic stroke [154 158]. The summary most-adjusted multivariable risk ratio comparing the highest to lowest intake of ALA is 0.70 (95% CI: 0.50 to 0.97; P=0.03; I^2=0%; P_{het}=1.00). **(Figure 42-7.2.9)**

Hemorrhagic Stroke

One study (1 comparison; n=56 cases) assessed the association between ALA (serum) and hemorrhagic stroke [154]. In this study, the most-adjusted multivariable relative risk is 1.96 (95% CI: 0.73 to 5.27; P=0.18). **(Figure 42-7.2.10)**

Atrial fibrillation

One study (1 comparison; n=1441 events) assessed the association between dietary ALA and atrial fibrillation [76]. In this study, the most-adjusted multivariable relative risk is 0.77 (95% CI: 0.67 to 1.04; P=0.09). **(Figure 42-7.2.11)**

Fatal arrhythmia

One study (1 comparison; n=56 cases) assessed the association between ALA (serum) and fatal arrhythmia [154]. In this study, the most-adjusted multivariable relative risk is 0.68 (95% CI: 0.38 to 1.22; P=0.20). **(Figure 42-7.2.12)**

[Other outcomes not included in this version of the report]

GRADE Assessment of Quality of Evidence: For the 19 outcomes assessed, the GRADE assessment of the confidence in the estimates of association between ALA and health outcomes was moderate for 1 outcome (fatal CVD) [5.3%], low for 4 outcomes (sudden cardiac death, fatal stroke, ischemic stroke, and depression) [21.1%] and very low for 14 [73.7%]. Four estimates were at serious risk of bias, 4 had serious inconsistency, and 13 had serious imprecision. **(Table 9)**

ω-6 PUFA and Health Outcomes

[Results not included in this version of the report]

Linoleic Acid (LA; 18:2 ω-6) and Health Outcomes

[Results not included in this version of the report]

Arachidonic Acid (ARA; 20:4 ω-6) and Health Outcomes

[Results not included in this version of the report]

Polyunsaturated: Saturated Fat Ratio [P: S] and Health Outcomes

[Results not included in this version of the report]

ω-6:ω-3 Ratio and Health Outcomes

[Results not included in this version of the report]

Replacement of saturated fats by polyunsaturated fats

[Results not included in this version of the report]

Meta-analyses of fish, walnuts and health outcomes

Fish and Stroke: Xun et al.[41] meta-analyzed 16 studies (19 cohorts), including 402,127 individuals (10,568 incident cases) with an average 12.8 years of follow-up. Compared with those who never consumed fish or ate fish <1/month, the pooled adjusted HRs of total stroke risk were 0.97 (95% CI, 0.87-1.08), 0.86 (0.80-0.93), 0.91 (0.85-0.98) and 0.87 (0.79-0.96) for those who consumed fish 1-3/month, 1/week, 2-4/week and ~5/week, respectively ($P_{linear\ trend}$) = 0.09; P (nonlinear trend) = 0.02). Study location was a modifier. An inverse association between fish intake and stroke incidence was only

found by studies conducted in North America. The modest inverse associations were more pronounced with ischemic stroke and were attenuated with hemorrhagic stroke.

Fish and type 2 diabetes: ***[Results not included in this version of the report]***

Walnuts and type 2 diabetes: ***[Results not included in this version of the report]***

Fish and CHD Mortality: He at al. [18] meta-analyzed 11 studies (13 cohorts), including 222 364 individuals with an average 11.8 years of follow-up. Compared with those who never consumed fish or ate fish less than once per month, individuals with a higher intake of fish had lower CHD mortality. The pooled multivariate RRs for CHD mortality were 0.89 (95% CI, 0.79 to 1.01) for fish intake 1 to 3 times per month, 0.85 (95% CI, 0.76 to 0.96) for once per week, 0.77 (95% CI, 0.66 to 0.89) for 2 to 4 times per week, and 0.62 (95% CI, 0.46 to 0.82) for 5 or more times per week. Each 20-g/d increase in fish intake was related to a 7% lower risk of CHD mortality (P for trend=0.03).

Prospective Cohort Studies of PUFA and Fish in Secondary Prevention

Total PUFA

[Results not included in this version of the report]

Fish

Two prospective cohort studies (3 comparisons; Figure 79)[172 173] assessed the association between high intakes of fish in secondary prevention of CHD death in patients with established CHD. The pooled estimate from these 3 studies (92 events) is 0.49 (95% CI: 0.29 to 0.81; P=0.005; I^2=0%; P_{het}=0.76),

Long-chain ω-3 fatty acids

Two prospective cohort studies[174 175] assessed the impact of long-chain ω-3 fatty acids in secondary prevention. In the first study, Benedetto et al. followed 2,100 patients undergoing coronary artery bypass grafting in a tertiary care hospital[174]. At discharge, the decision to prescribe n-3 PUFA therapy (900 mg EPA+DHA) was at the discretion of the referring cardiologist. The treatment reduced all-cause mortality by 44% (mvRR: 0.56; 95% CI: 0.31 to 1.00). Greene et al. performed a retrospective observational cohort study including patients from 5 Italian Local Health Units who were discharged from the hospital with a primary diagnosis of AMI[175]. Of the 11,269 patients enrolled, 2,425 patients (21.5%) were prescribed n-3 PUFA during follow-up. After adjusting for patient characteristics and

concurrent therapies, n-3 PUFA treatment was associated with reduced all-cause mortality (mvHR: 0.75, 95% CI 0.59 to 0.97) and recurrent AMI (mvHR: 0.65, 95% CI 0.49 to 0.87) through 12-month follow-up. The pooled estimate of these two studies for all-cause mortality (n=1,339 events) is 0.72 (95% CI: 0.57 to 0.91; I^2=0%, P=0.005).

DISCUSSION [ABRIDGED]

This systematic review and meta-analysis of prospective cohort studies of polyunsaturated fatty acids and health outcome in adults find that data for health outcomes other than cardiovascular disease and diabetes are not well-studied. The confidence rating for all of the reviewed associations ranged from very low [81.9%; n=145 assessments] to low [18.1%; n=32 assessments] **(Figure 80)**. The methodological quality of the included cohort studies was generally good, with >85% of included studies rating 7 out of 9 or higher on the Newcastle-Ottawa Scale **(Figure 1b)**

The most common reason for downgrading the confidence in the body of evidence for associations was imprecision, as the confidence intervals for most summary estimates were wide and could not exclude a null association. This was not always simply attributable to low sample size, as many associations with over 500 events were imprecise and included an RR of 1.0. Differences in results across studies are likely due in no small part, to differences in comprehensiveness of dietary assessment methods—though our meta-regression was likely underpowered to detect this. For example, some studies measured diet with a single 24-hour recall; others used multiple, updated semi-quantitative food frequency questionnaires validated against biomarkers and other dietary instruments. When assumed effect sizes are small, as they typically are for dietary associations (RR usually from 0.7 to 1.3), power to detect these in the presence of measurement error is typically low.

Inconsistency across studies was generally present in most analyses. Explanations of inconsistency were not easy to find, but univariate meta-regression approaches identified study size, study quality (for total PUFA and all-cause mortality; and linoleic acid and total and fatal CHD), duration of follow-up (for total PUFA and all-cause mortality), measurement and appropriate adjustment of trans-fatty acids (total PUFA and all-cause mortality; and long-chain n-3 and fatal CVD and type 2 diabetes), measurement for causal intermediates (such as dyslipidemia for long-chain PUFA and fatal CVD; and hypertension for long-chain n-3 PUFA and type 2 diabetes), and method of dietary assessment (for total PUFA and all-cause mortality), dose range (for long-chain n-3 PUFA and fatal CVD and type 2 diabetes), percent of smokers in the study (for long-chain n-3 and fatal CVD), and country of conduct (for long-chain n-3 and

type 2 diabetes) were common predictors, which explained 38-91% of the between-study variance across outcomes. Heterogeneity across studies for long-chain n-3 PUFA and fatal CHD was not explained by any study-level attributes.

This review has several strengths. First, the confidence in the estimates was assessed using *GRADE*, to facilitate guideline development. Second, studies were identified via a systematic search of the literature, augmented with manual searches of reference lists of published papers and systematic reviews. Third, the quantitative synthesis was focused on studies measuring comparable outcomes using similar designs, reducing methodological heterogeneity.

This review has important limitations related to evidence synthesis and quality. First, meta-analytic techniques depend on the availability of conceptually similar and combinable effect estimates across studies. If such estimates are not available, the ability to pool all available and relevant data in a meaningful way is compromised, and the pooled estimate of effect may be suboptimal.

Second, observational studies cannot provide causal evidence of an effect of polyunsaturated fatty acids on the development of health outcomes addressed; they can only describe associations. Measurement error is often serious in epidemiologic studies of diet and disease, which may bias such associations towards the null. Major limitations of the included studies appear in (Newcastle-Ottawa evaluations) and in the footnotes to the GRADE tables. These include unrepresentative cohorts or a vaguely defined cohort sampling frame, misclassification of exposure due to inaccurate measurement tools (selection and exposure measurement biases); failure to account for major confounders such as age, socioeconomic status, smoking, total energy, or family history (non-comparability biases); lack of validated outcome measures or insufficient study duration to observe a high number of events (outcome assessment biases). Additionally, random error or the inability to distinguish polyunsaturated from trans-unsaturated fats may attenuate the observed associations with health outcomes—suggesting the true effect of consuming these nutrients is likely larger than reported. This error may arise from several sources, including residual confounding, recall bias, and exposure misclassification. The reviewed studies typically relied food frequency questionnaires, 24-hour recalls, or 7-day food records, each of which have serious limitations in their ability to accurately capture long-term dietary fat intake.

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PRISMA Flow Diagram

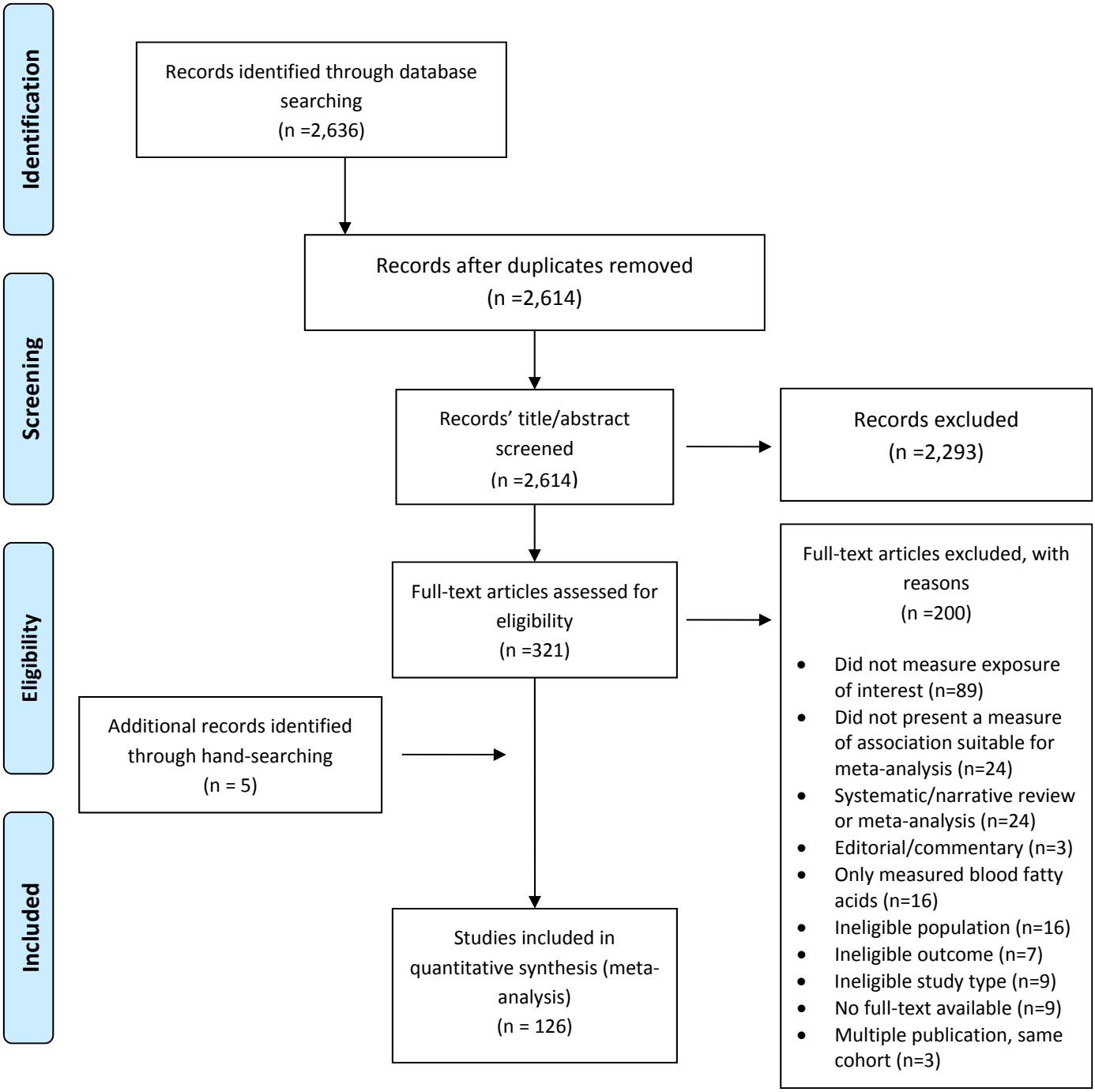


Figure 1a. PRISMA 2009 Flow Diagram (Moher et al., 2009)

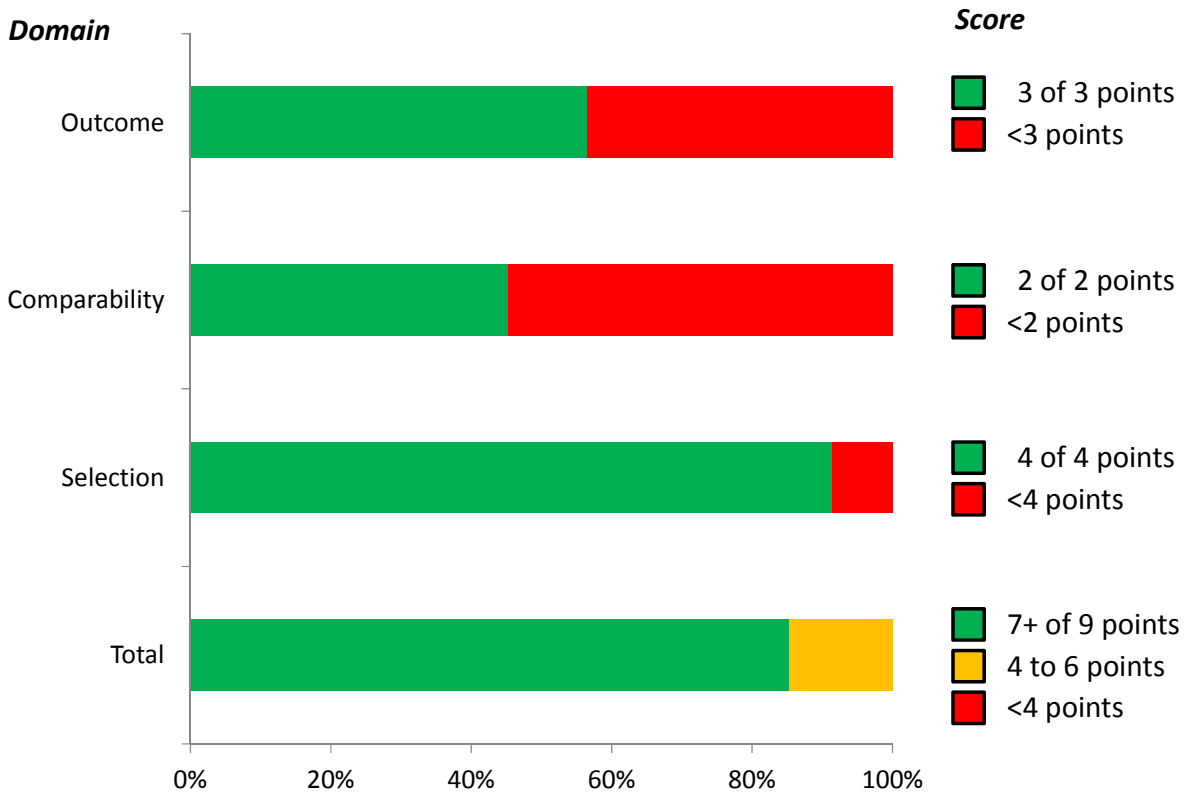


Figure 1b. Summary of NOS Ratings on each domain and summary score.

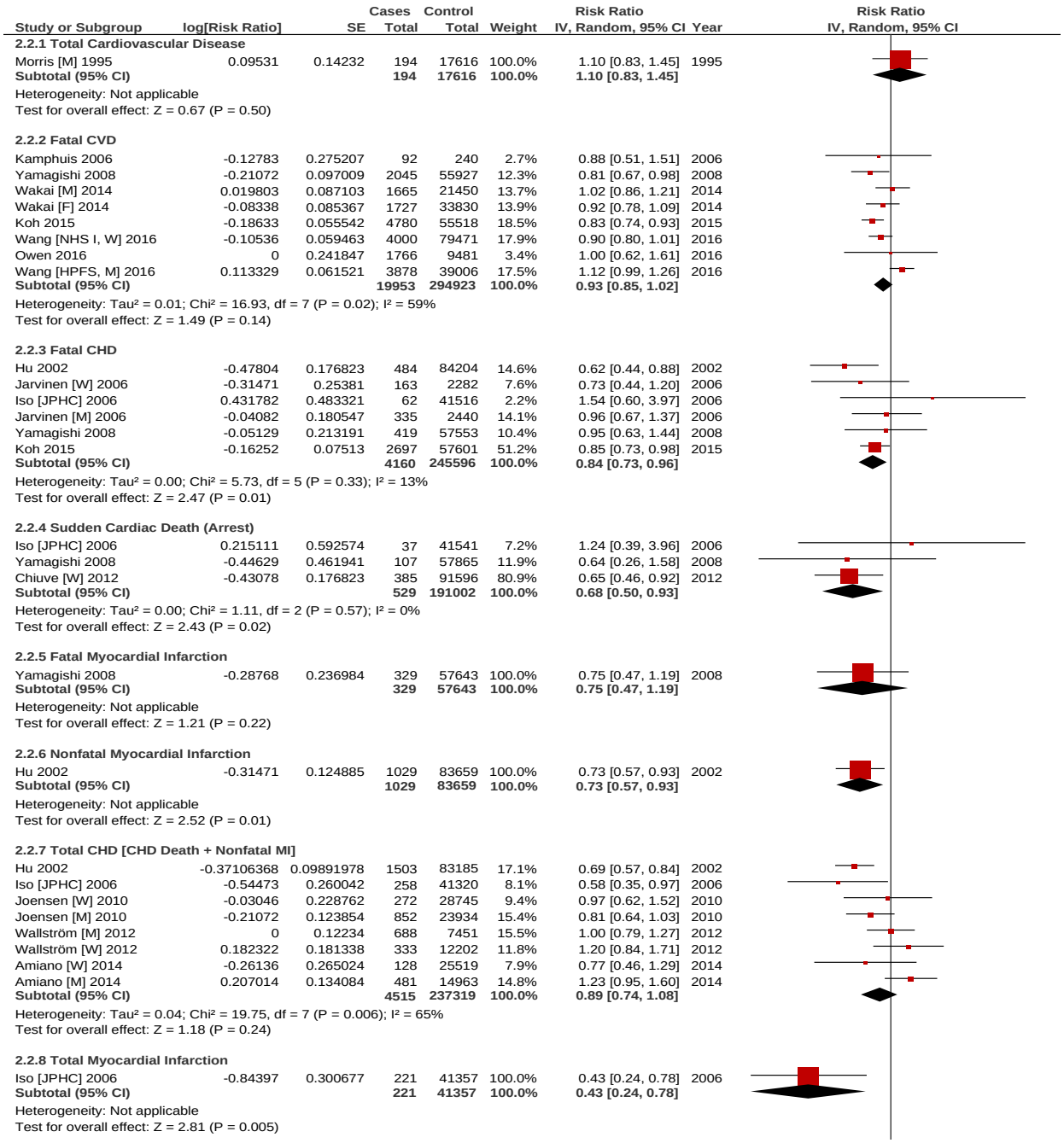


Figure 10. Pooled most-adjusted risk ratio of ω-3 polyunsaturated fatty acids and cardiovascular diseases (n=23 studies)

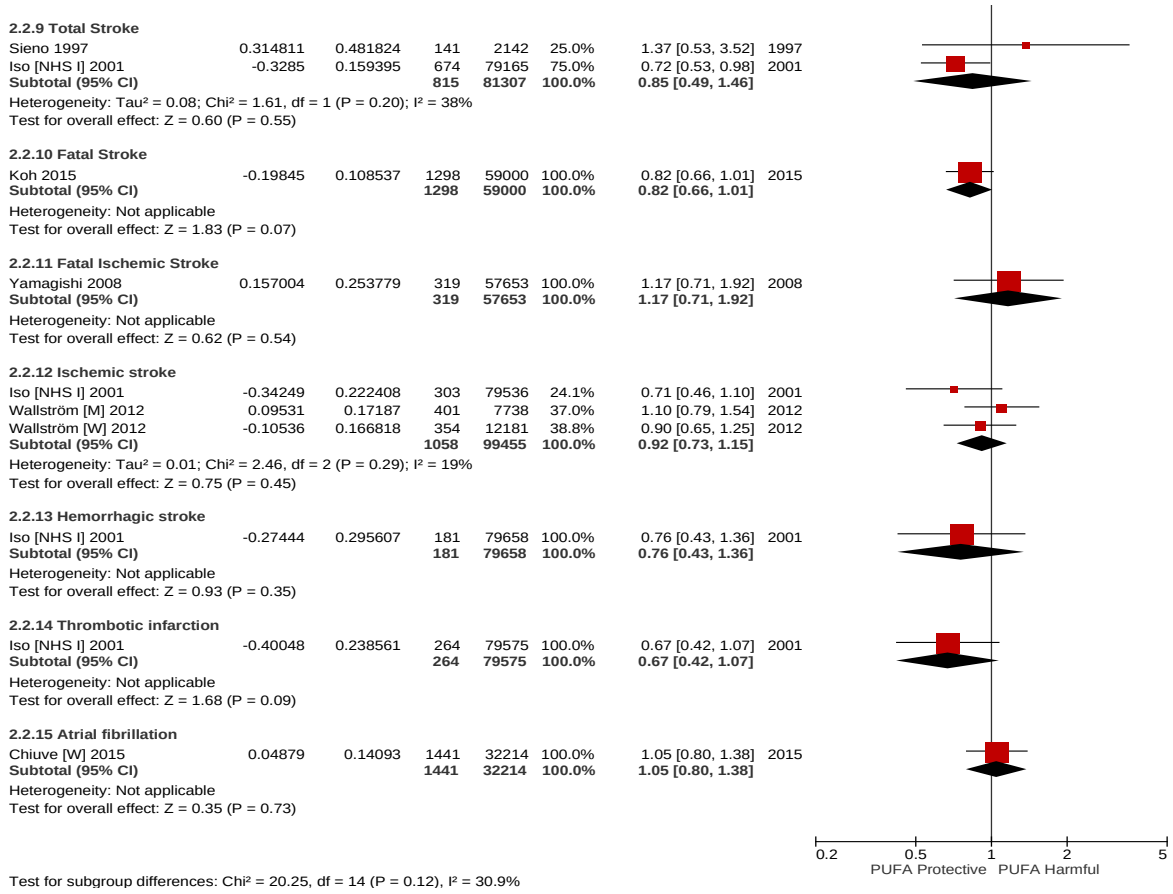


Figure 10 (cont'd). Pooled most-adjusted risk ratio of ω-3 polyunsaturated fatty acids and cardiovascular diseases (n=23 studies)

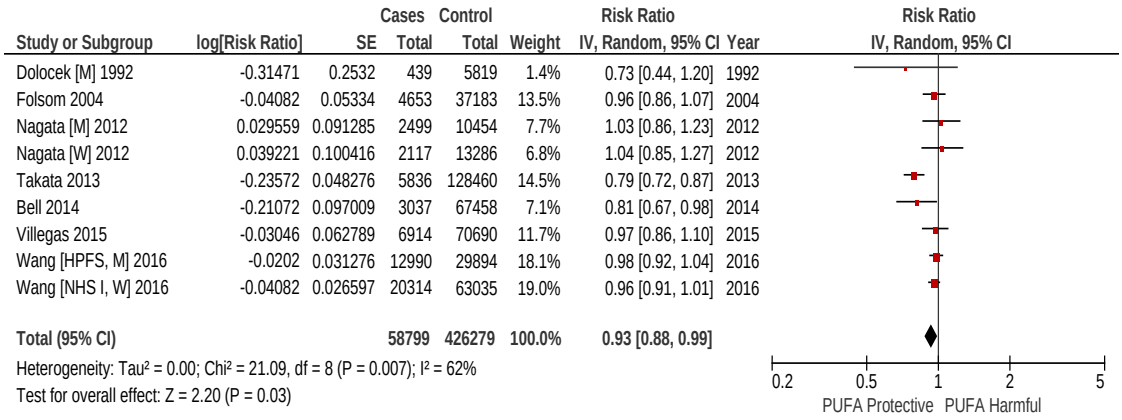


Figure 17. Pooled most-adjusted risk ratio of long-chain ω-3 polyunsaturated fatty acids and all-cause mortality (n=6 studies)

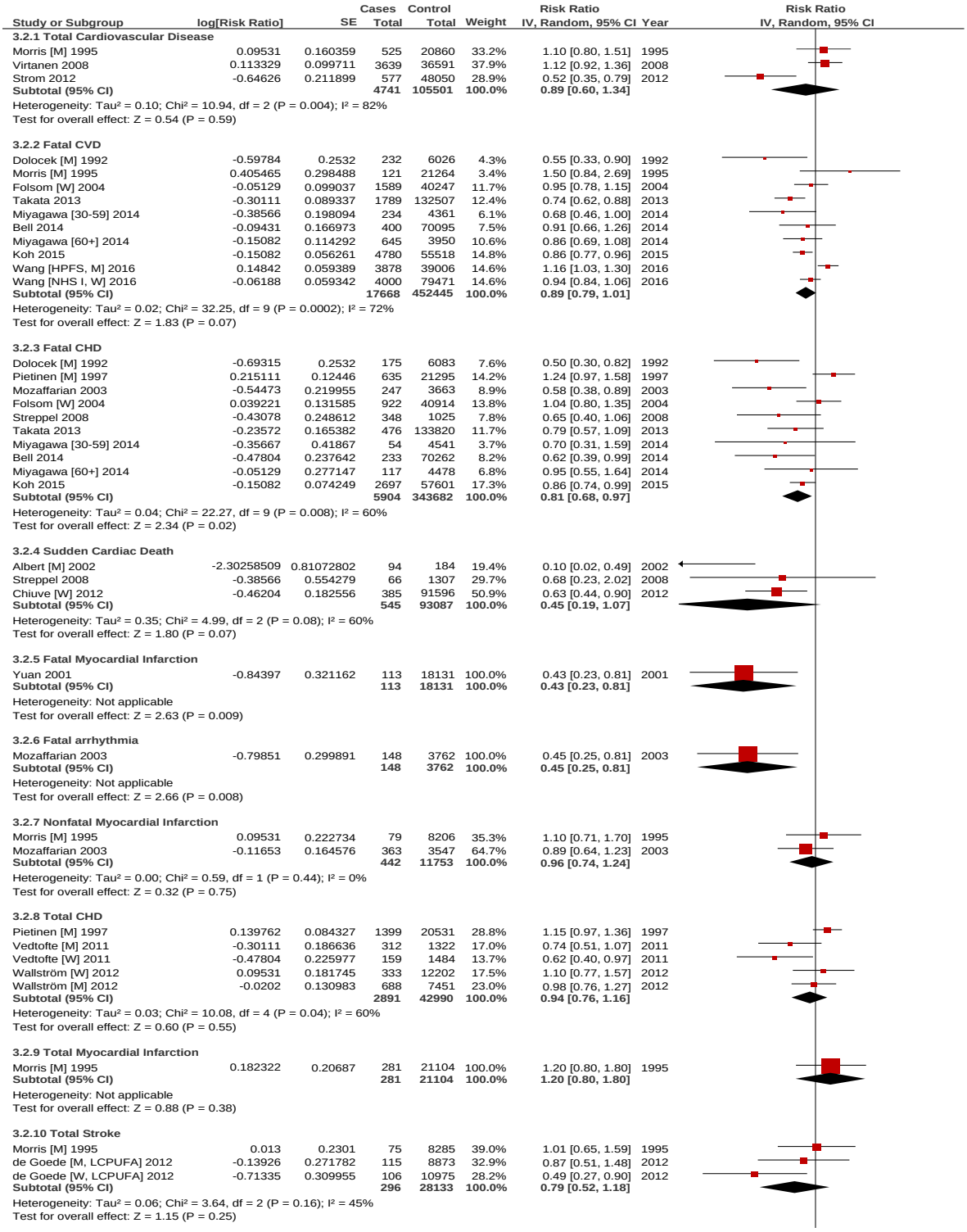


Figure 18. Pooled most-adjusted risk ratio of long-chain ω-3 polyunsaturated fatty acids and cardiovascular diseases (n=29 studies)

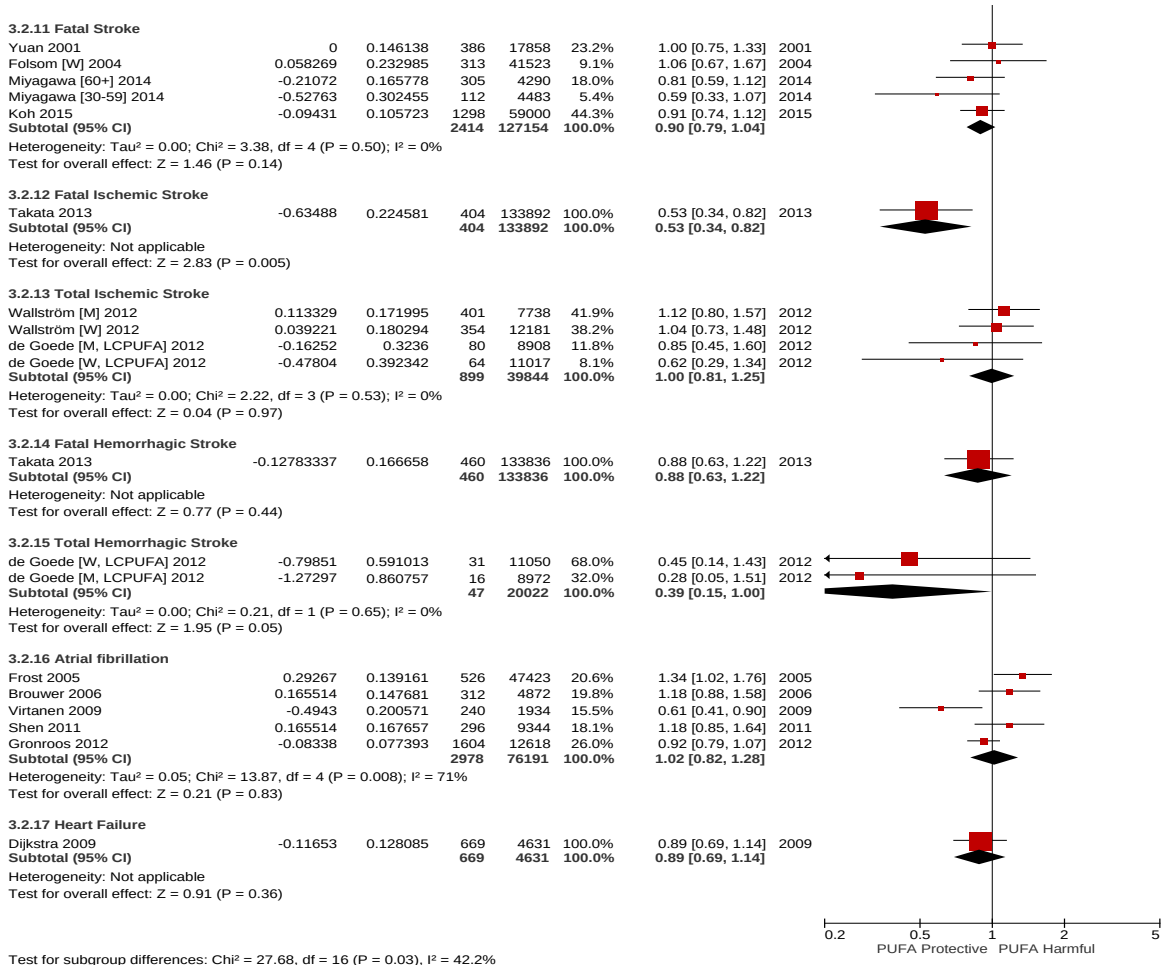


Figure 18 (cont'd). Pooled most-adjusted risk ratio of long-chain ω-3 polyunsaturated fatty acids and cardiovascular diseases (n=29 studies)

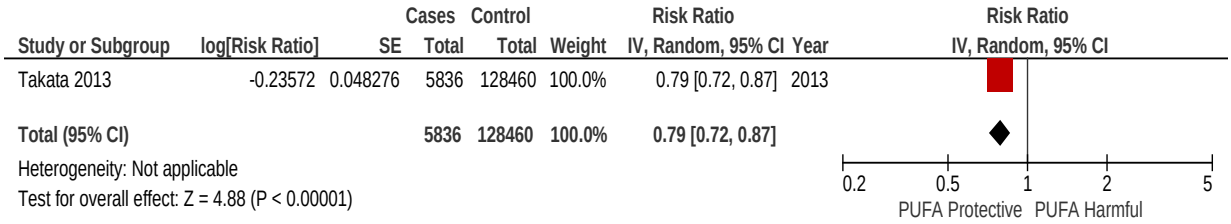


Figure 25. Pooled most-adjusted risk ratio of eicosapentaenoic acid (EPA) and all-cause mortality (n=1 study).

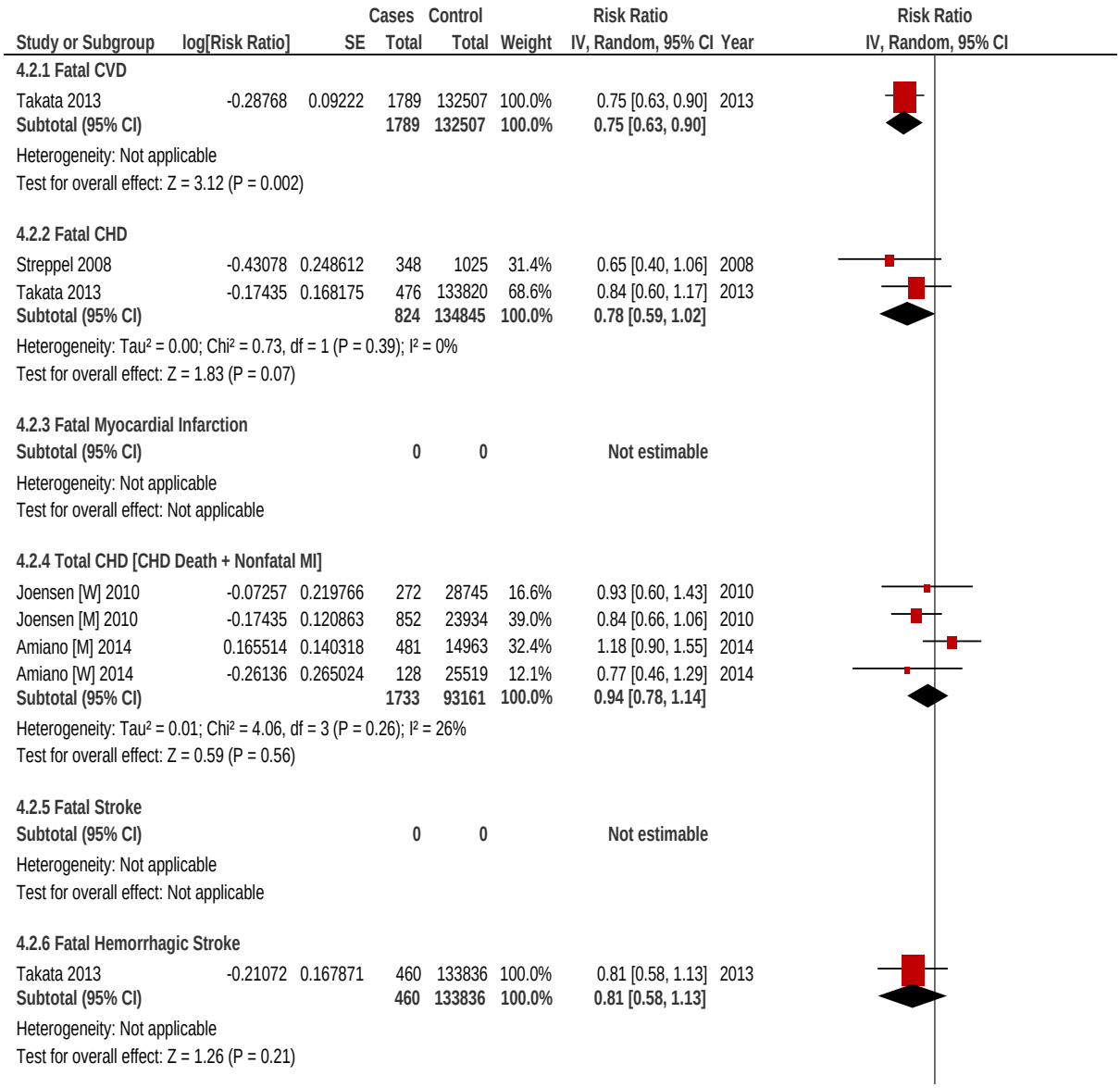


Figure 26. Pooled most-adjusted risk ratio of eicosapentaenoic acid (EPA) and cardiovascular diseases (n=10 studies).

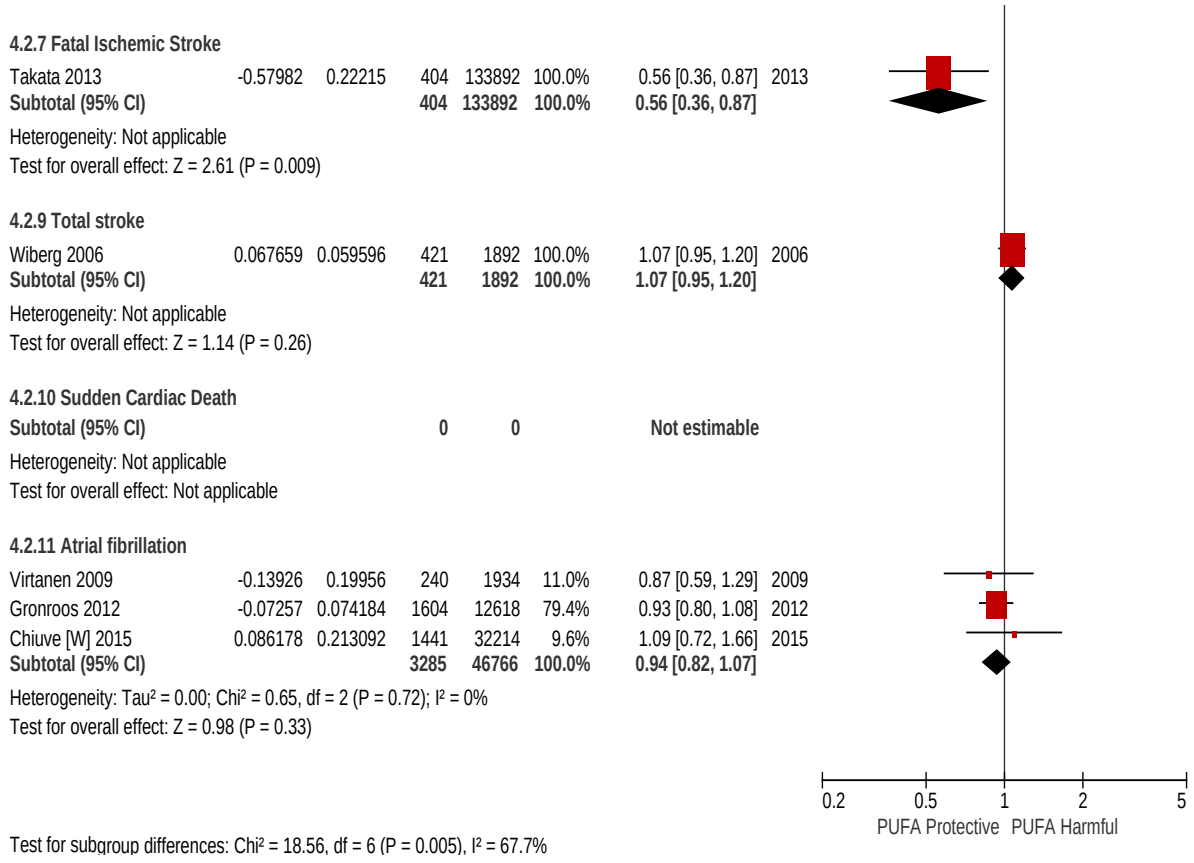


Figure 26 (cont'd). Pooled most-adjusted risk ratio of eicosapentaenoic acid (EPA) and cardiovascular diseases (n=10 studies).

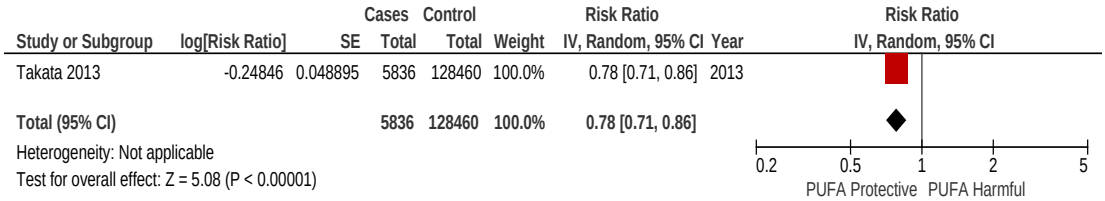


Figure 33. Most-adjusted risk ratio of docosahexaenoic acid (DHA) and all-cause mortality (n=1 study).

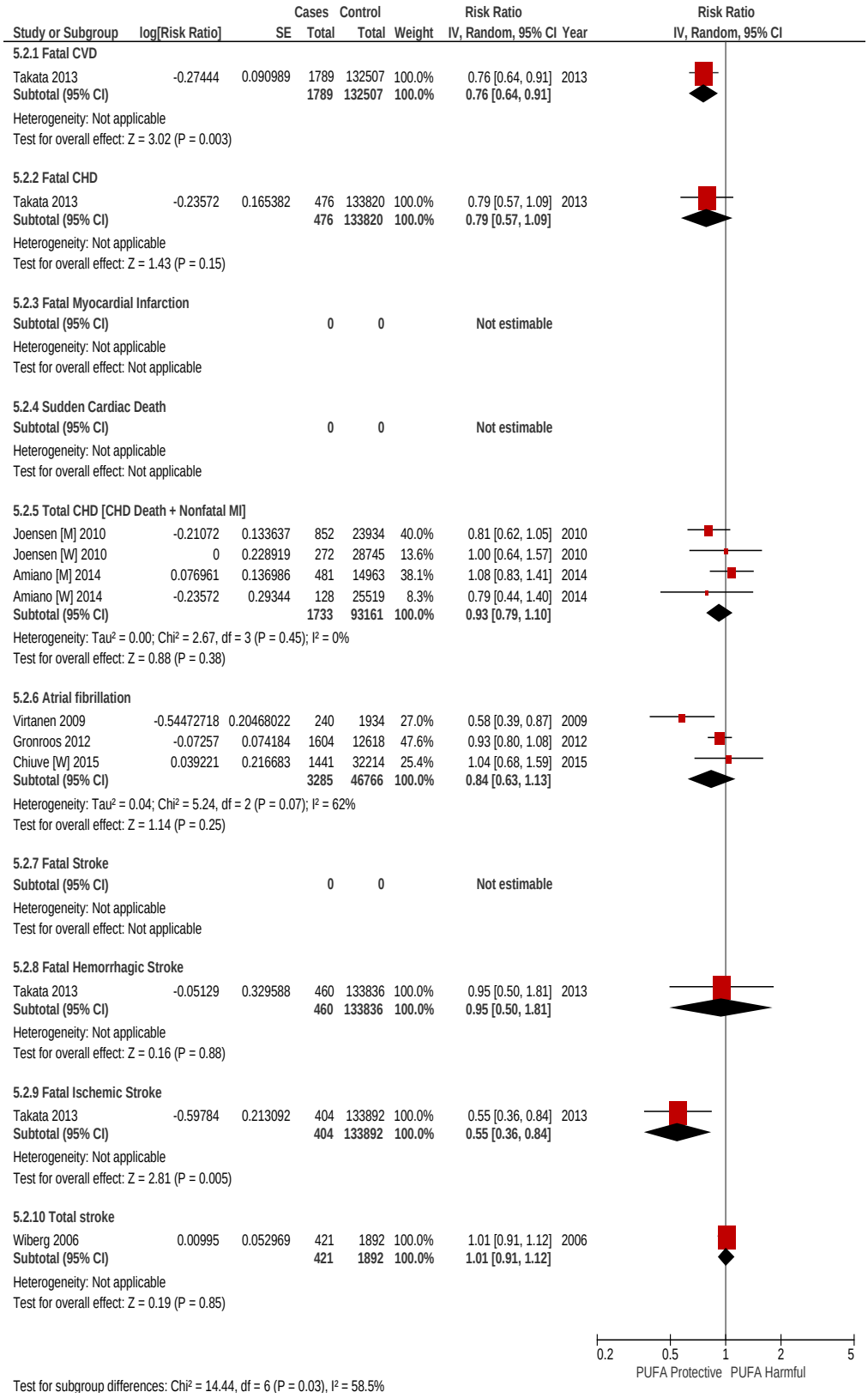


Figure 34. Pooled most-adjusted risk ratio of DHA and cardiovascular diseases (n=9 studies)

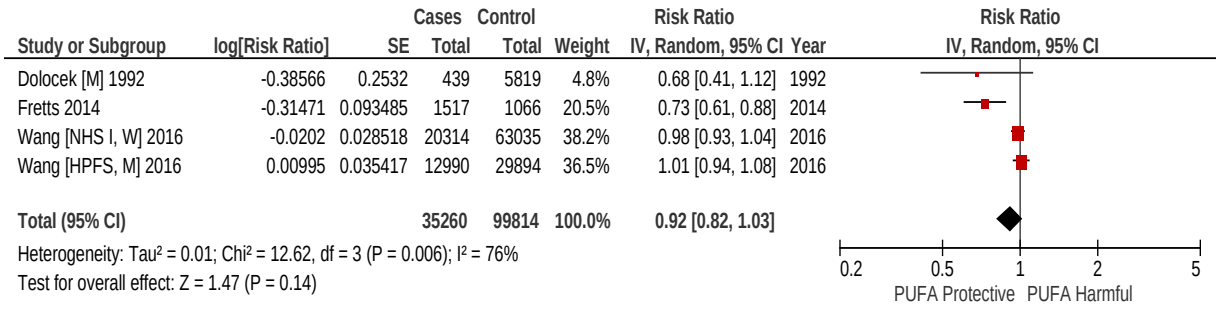


Figure 41. Pooled most-adjusted risk ratio of alpha-linolenic acid (ALA) and all-cause mortality (n=4 studies).

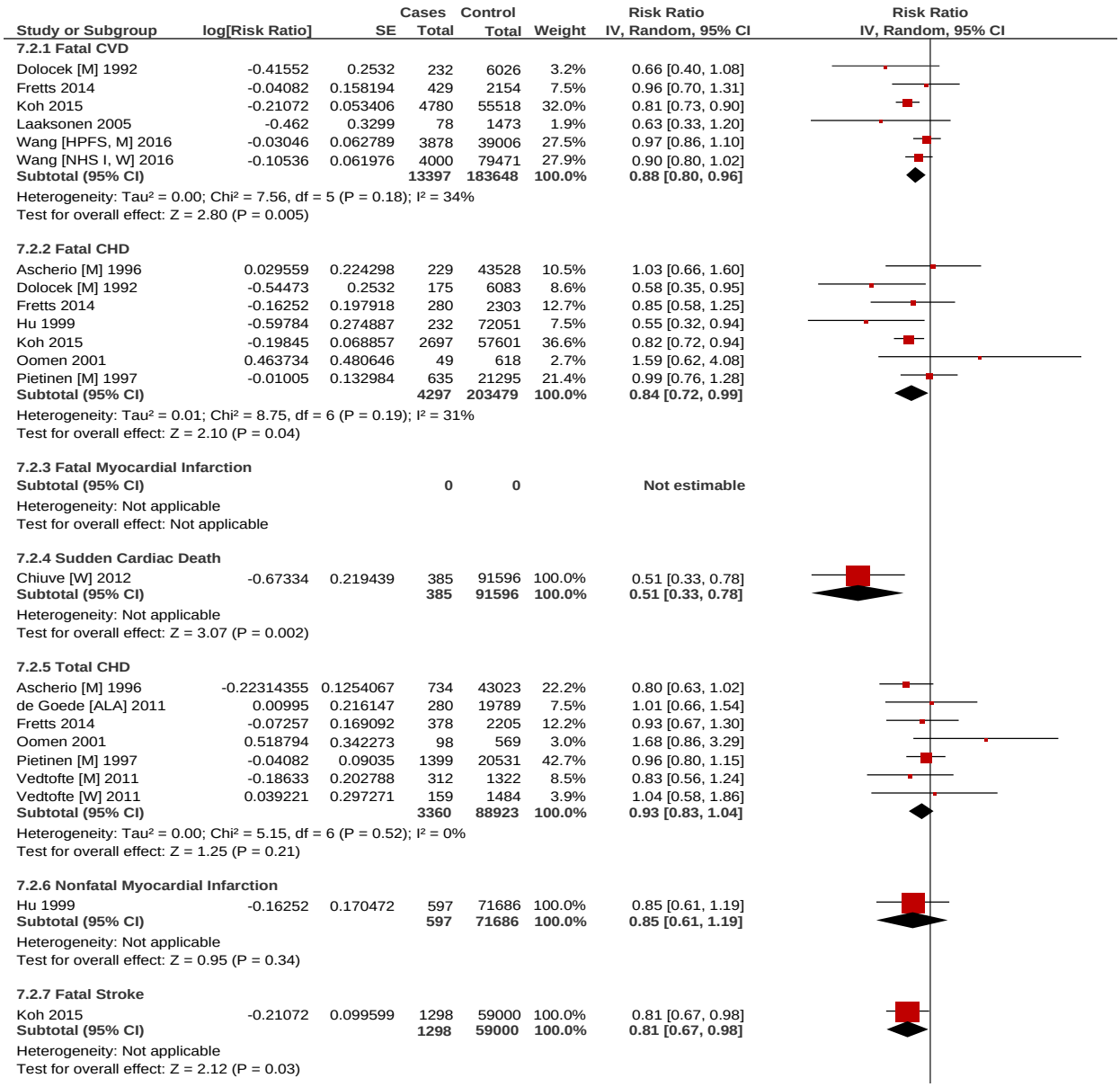


Figure 42. Pooled most-adjusted risk ratio of alpha-linolenic acid (ALA) and cardiovascular outcomes (n=16 studies).

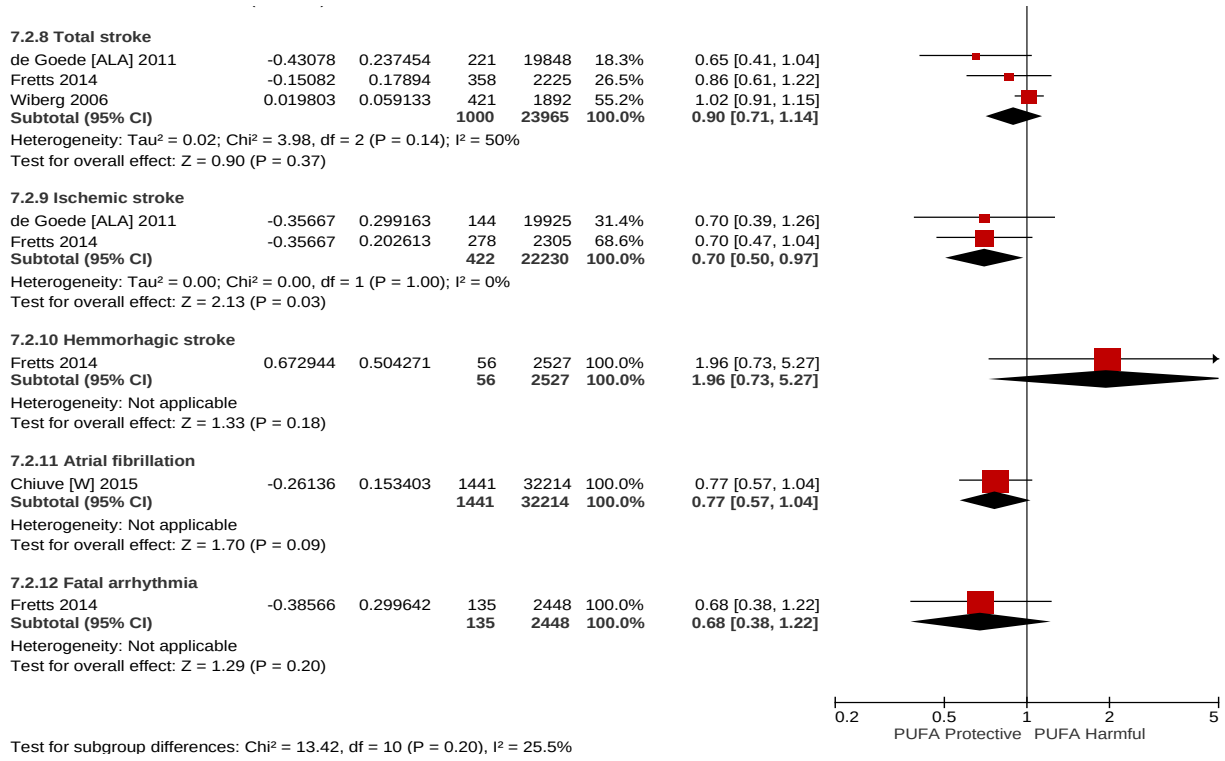


Figure 42 (cont'd). Pooled most-adjusted risk ratio of alpha-linolenic acid (ALA) and cardiovascular outcomes (n=16 studies).

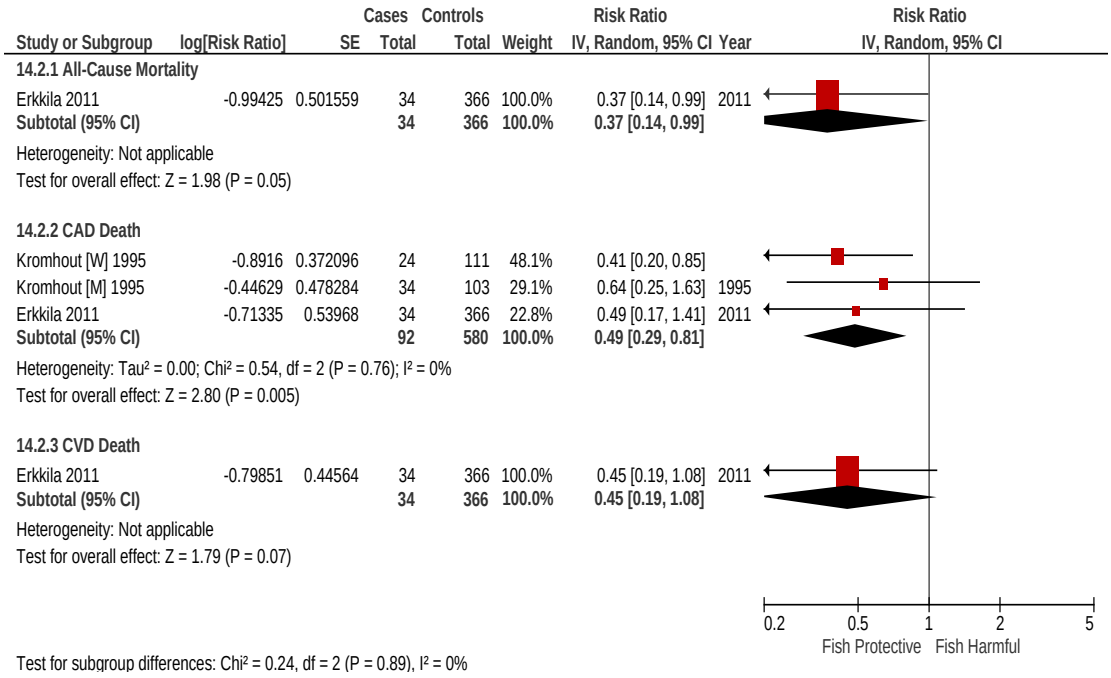


Figure 78. Most-adjusted risk ratio of fish in secondary prevention (n=2 studies).

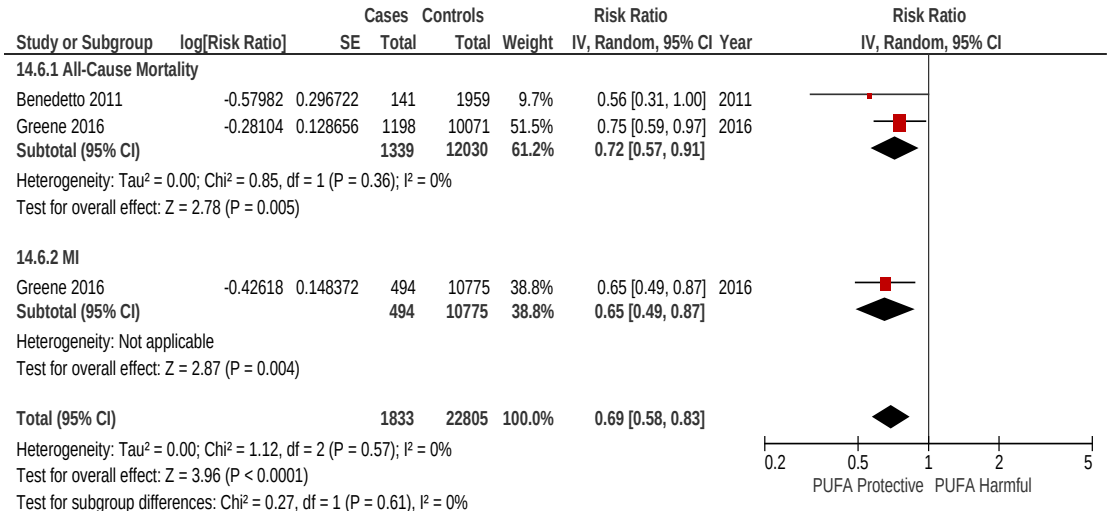


Figure 79. Most-adjusted risk ratio of LC n-3 fatty acids in secondary prevention (n=2 studies)

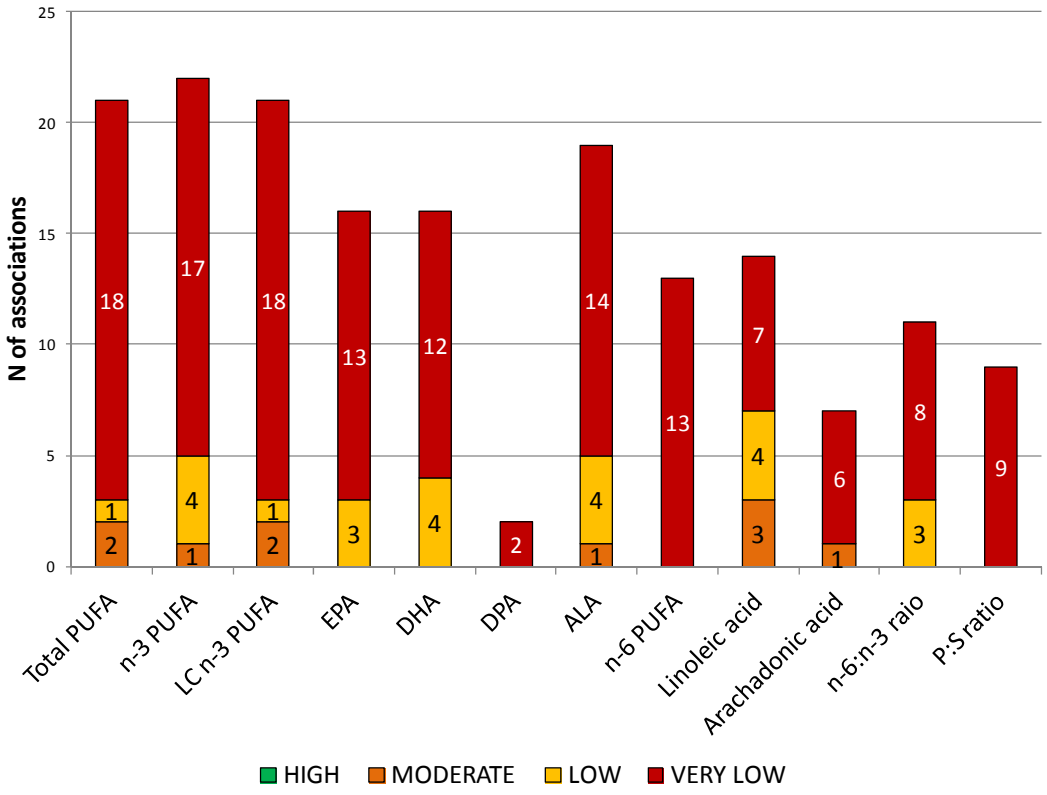
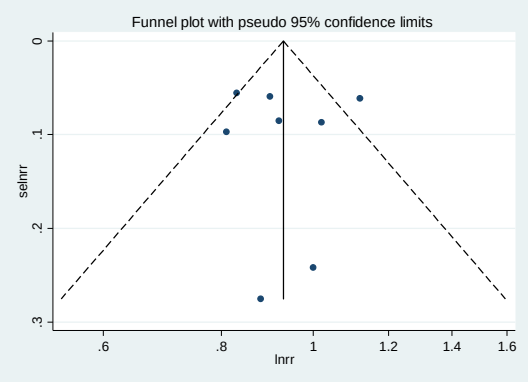
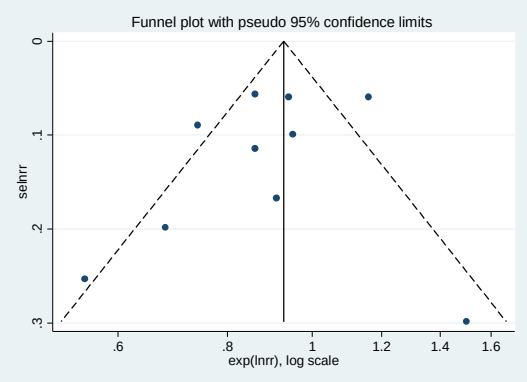


Figure 80. Summary of GRADE assessments of confidence in the body of evidence for each fatty acid (or class of fatty acids) and health outcomes.

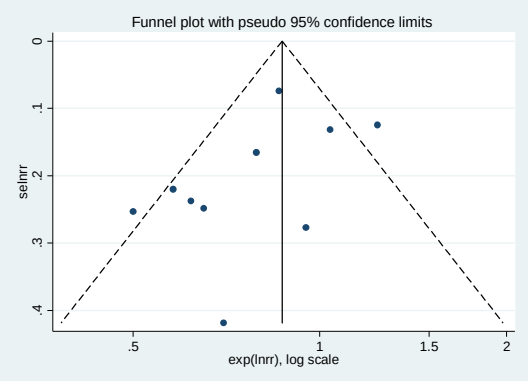
Figure PB. ***Publication Bias plots for comparisons with >=10 studies.***



Panel B: ω-3 PUFA and fatal CVD.
No publication bias suspected.



Panel C: Long chain ω-3 PUFA and fatal CVD.
No publication bias suspected.



Panel D: Long chain ω-3 PUFA and fatal CHD.
No publication bias suspected.

Figure S8. ***Meta-regression of long-chain ω-3 PUFA and fatal CVD (TFA)***
Panel A: Subgroup analysis by whether or not TFA were measured in the study (P=0.10)

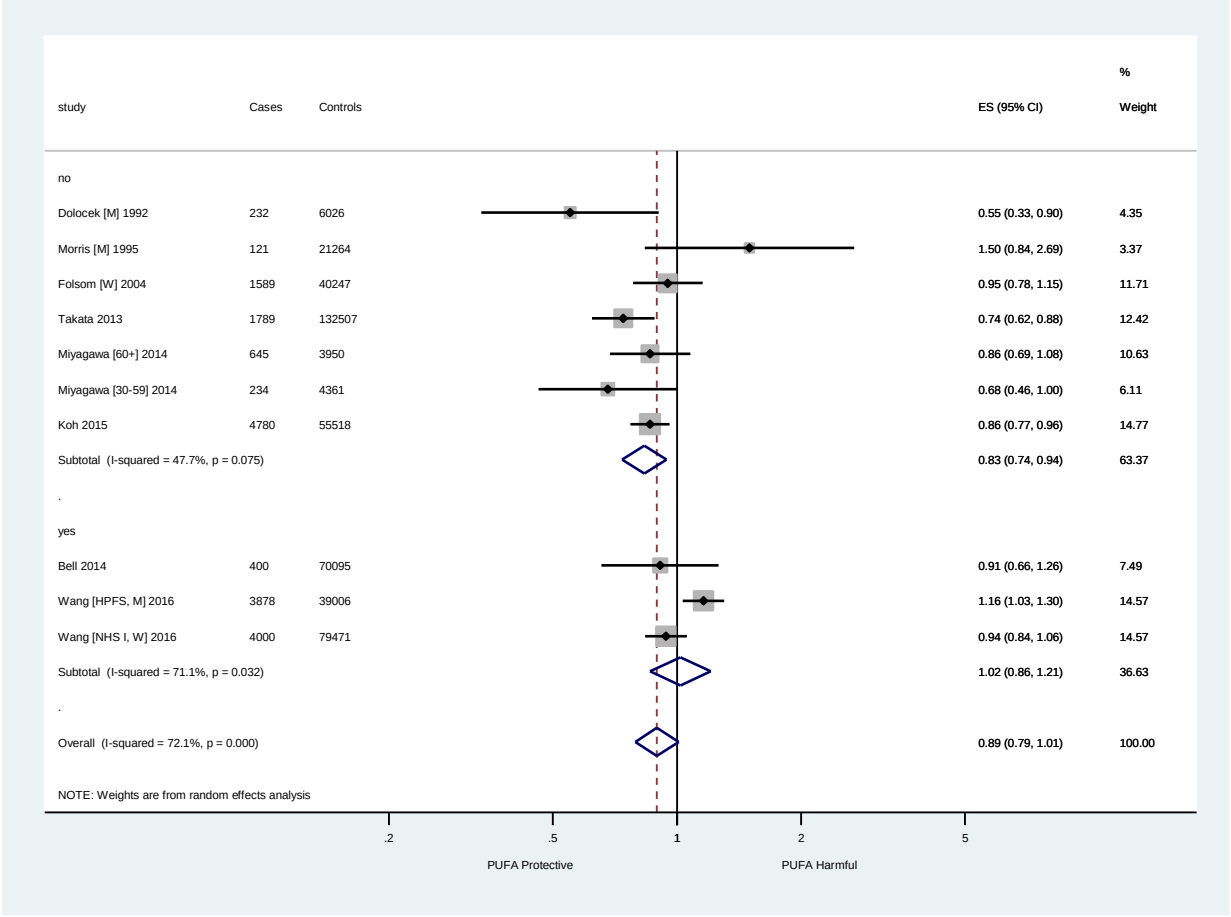
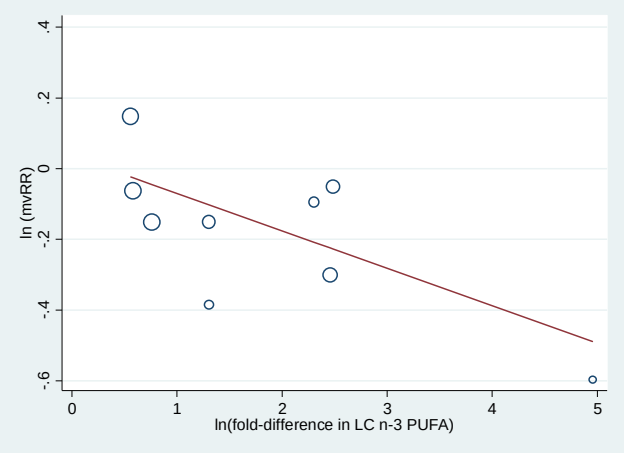


Figure S9. ***Meta-regression of long-chain n-3 PUFA and fatal CVD (log-fold difference)***

Panel A: Effect size increases as ln (fold-difference) increases (P=0.07). Each 1-unit increase in ln (fold-difference) was associated with a 10% relative risk increase (mvRR: 0.90; 95% CI: 0.80 to 1.01).



Panel B: Subgroup analysis by log-fold change (split by median).

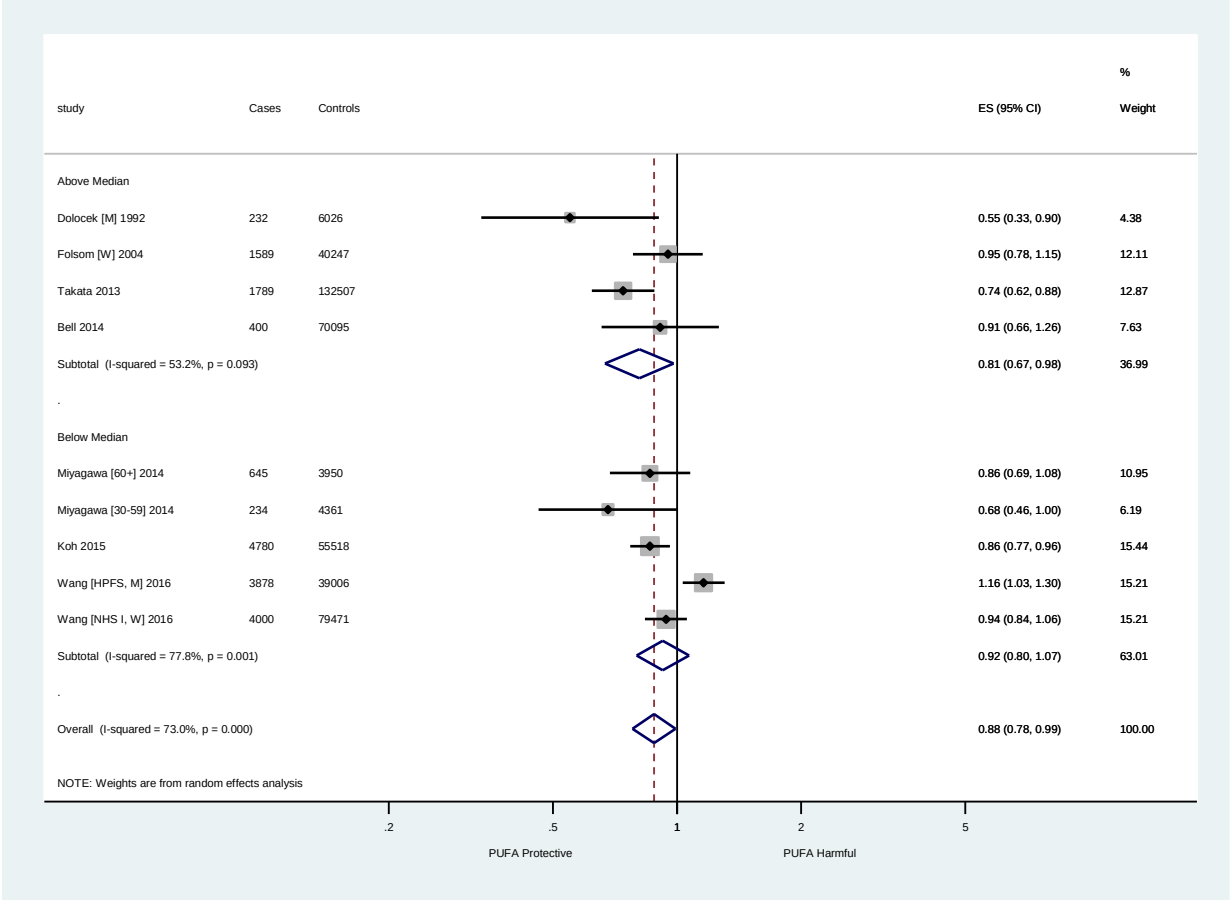


Figure S10. ***Meta-regression of long-chain n-3 PUFA and fatal CVD (current/former smokers)***

Panel A: Effect size increases as % enrolled current/former smokers (P=0.014). A 10% increase in enrolled current/former smokers was associated with a 7% relative risk increase (mvRR: 0.93; 95% CI: 0.89 to 0.98).

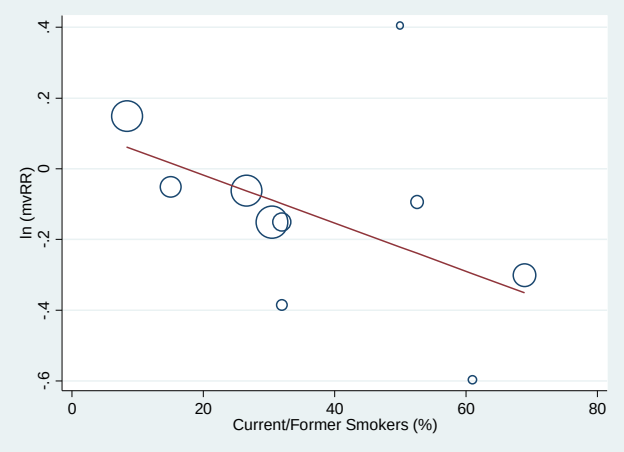


Table 1. Characteristics of included prospective cohort studies.

Source (Country)	Age (years)	Sex (% Men)	Number of Participants	Number of Events	Follow-up (years)	Exposures Assessed	Exposure Contrast	Outcome validation	Adjustment for confounders	NOS Score	Funding
Albert et al. 2002 USA Physicians' Health Study	49-68	100	278	201 sudden death from cardiac causes	17	Long chain n-3, n-6 polyunsaturated, short chain n-3, α-Linolenic, *(serum sample)*	Q4 vs. Q1 (6.87 vs. 3.58 E %)	Events reported by postal authorities or next of kin, validated by review of medical records by blinded physicians	Age, assignment to aspirin and beta carotene treatment or placebo, BMI, history of diabetes, history of hypertension, history of hypercholesterolemia, alcohol consumption, frequency of vigorous exercise, parental history of MI before the age of 60 years, trans unsaturated fatty acid levels and monounsaturated fatty acid levels.		National Institutes of Health (USA), National Heart, Lung, and Blood Institute
Alhazmi et al. 2013 Australia Australian Longitudinal Study on Women's Health	45-50	100	8370	311 Incident T2DM	6	Total PUFA, n3 PUFA, ALA, EPA, DHA, EPA+DHA, n-6 PUFA, n6:n3 *(validated FFQ)*	Q5 vs. Q1 (g/day)	Events were self reported and validated by random sample whose medical and pharmaceutical records were reviewed.	Area of residence, education, current smoker status, physical activity, self-rated health as good, menopausal status, BMI, alcohol consumption, total energy intake, fibre and specific types of fat.		Australian Government Department of Health and Ageing
Atkinson et al. 2011 United Kingdom Caerphilly Prospective Study (CaPS)	45-59	100	3 265	225 Strokes (209 ischemic and 19 hemorrhagic)	22	Oily Fish Intake (*validated* SQFFQ)	Q5 vs. Q1	Events were self reported, and supplemented by inspection of hospital and general practitioner notes, radiology records and post-mortem reports, and by further questioning of study participants of close relatives. Two independent experts confirmed the final diagnosis.	Age, total energy, smoking status, adult social class, marital status, alcohol intake, vitamin C intake, vegetable fibre intake, blood pressure, cholesterol, BMI, fasting glucose, diabetes, atrial fibrillation, childhood social class and existing ischemic heart disease.	8	National Health Service Executive (UK)

Bassett et al. 2016 Australia Melbourne Collaborative Cohort Study	54.8	**0**	2491	470 breast cancer	12	Total PUFA, n-3 PUFA, n-6 PUFA, n-6:n-3 PUFA(FFQ+ Blood)	Q5 vs. Q1 (% Phospholipids, diet)	Cases were identified from notifications of first diagnoses of breast cancer to the Victorian Cancer Registry (VCR).	Country of birth, menopausal status, age at menarche, parity and lactation, OC use, HT use, physical activity, alcohol consumption, smoking status, education, family history of cancer, total energy intake from food and BMI.		-
Bork et al. 2016 Denmark Danish cohort study Diet, Cancer and Health	50-64	**48**	57,053	3089 MI	17	ALA (validated sqFFQ + adipose tissue analysis)	Continuous (g/day and % content in Adipose Tissue)	Events were reported using Danish nationwide registers.	BMI, waist circumference, smoking, physical activity, alcohol consumption, and length of education, menopausal status, use of hormone replacement therapy, self-reported history of hypercholesterolemia or use of lipid-lowering medication, self-reported history of hypertension or use of antihypertensive medication, and self-reported history of diabetes mellitus		None
Brostow et al. 2011 Singapore The Singapore Chinese Health Study	45-74	**check**	43,176	2252 Incident type 2 diabetes	11	Total n-3, n-6, n6:n3, EPA+DHA (marine), non-marine (ALA n-3) *(Validated FFQ)*	Q5 vs. Q1 (g/day)	Events were self reported and were validated by reviewing hospital records and by participant completion of a supplementary questionnaire. A random sample who did not report diabetes were tested for blood sugar levels to ensure accurate self report of no event.	Age, sex, dialect, year of interview, educational level, BMI, physical activity, smoking status, alcohol use, hypertension, intakes of omega-6 or omega-3, monounsaturated fat, saturated fat, dietary fiber, protein, and total energy.		NIH
Brouwer et al. 2006 Netherlands Rotterdam Study	59-76	41	5184	312 Atrial Fibrillation	8	EPA and DHA Intake *(Self report and dietician interview with validated FFQ)*	Q3 vs. Q1 (≥144 vs. ≤ 43 mg/day)	Events were reported by ECG readings by blinded physicians, GP reports and hospital discharge summaries.	Age, sex, energy intake, diabetes mellitus, alcohol intake, systolic blood pressure, HDL and total cholesterol levels, intake of saturated fatty acids, smoking status, and previous myocardial infarction.		Dutch Government
Byrne et al. 2002	50	0	44,697	1,071 breast cancer	14	Linoleic Acid Intake *(FFQ)*	Q5 vs. Q1	Events were self reported, confirmed by review of medical	Age in months; height; age at menarche; combined age at menopause and use of postmenopausal		NIH Grant **"The costs of publication**

USA NHS				cases		****Oleic acid as well, not included in analysis (not listed in spreadsheet but can add if requested)**		records	hormones, combined parity and age at first birth; body mass index at age 18; weight change since age 18; intake of total energy; alcohol intake; family history of breast cancer; vitamin A and other fat types.		**of this article were defrayed in part by the payment of page charges. This article must therefore be hereby marked *advertisement* in accordance with 18 U.S.C. Section 1734 solely to indicate this fact."**
Chiuve et al. 2015 USA WHS	≥45	0	33,665	1441 cases of incident AF (929 paroxysmal and 467 persistent/chronic)	20	Total PUFA Intake *(validated FFQ)*	Q5 vs. Q1 (7.6 vs. 4.1 median %E)	Events were self reported and confirmed by medical records	Age, protein, total calories, smoking, BMI, height, alcohol, exercise, education, race, randomization group (b-carotene, vitamin E, and aspirin) systolic blood pressure, and diagnosis of hypertension, high cholesterol and diabetes, cardiovascular disease, or congestive heart failure during follow-up, SFAs, MUFAs, total PUFAs, and trans fat.		Watkins Discovery Award, National Heart Lung Blood Institute, National Institute of Health (US)
De Goede et al. 2013 Netherlands Monitoring Project on Risk Factors for Chronic Diseases (MORGEN)	20-65	53	358	179 incident cases of stroke (93 ischemic strokes, 50 hemorrhagic strokes, and 36 unspecified strokes)	13	Plasma n-3 PUFAs: Linoleic acid Arachidonic acid α-linolenic acid EPA:DHA *(no fasting blood sample - mass percentages of total fatty acid methyl esters)*	Case vs. controls	Events were ascertained using the national hospital discharge register and causes of death on Statistics Netherlands.	Age, gender, enrollment date, smoking, BMI, education level, alcohol intake, diabetes, hypertension and hypercholesterolemia.		Alpro Foundation (Belgium), Ministry of Health, Welfare and Sport of the Netherlands, the National Institute for Public Health and the Environment, Bilthoven, The Netherlands, and the Europe Against Cancer Program of the European Union
Djoussé et al. 2011 USA WHS	45-	0	36,328	2370 incident T2DM	16	EPA, DHA, α-linolenic acid and fish intakes *(validated FFQ)*	Q5 vs. Q1 (g/day)	Events were self-reported and validated primarily through the collection of supplementary information from participants.	Age, BMI, parental history of diabetes, smoking, exercise, alcohol intake, menopausal status, red-meat intake, and quintiles of energy intake, linoleic acid, α-linolenic acid, dietary magnesium, trans fat, saturated fat, cereal fiber, and glycemic index.		National Cancer Institute, National Heart, Lung, and Blood Institute
Dominiguez et al. 2015	23- 55 **(from**	39-43 **(from**	17,292	143 Incident	15	Diabetes Dietary Score *(validated*	Q5 vs. Q1 (Diabete	Events were self-reported with data involving diagnoses and	Age, year of recruitment, sex, total energy intake, following a special diet, snacking between meals, BMI, physical		Spanish Ministry of Health and European Regional Development Fund

Spain SUN Project	**table)**	**table)**		T2DM		*FFQ)*	s Dietary Score high vs. low)	prescriptions. Reports from physicians with the detailed diagnosis were requested to supplement this information.	activity, hours of television watching, hours sitting down, smoking, marital status, personal history of hypertension, and family history of diabetes.		(FEDER), Navarra Regional Government and FPU fellowship from the Spanish Government
Dow et al. 2016 France E3N Cohort	52.9	0	71334	2610 Incident T2DM	18	Total PUFA, n-3 PUFA, n-6 PUFA (validated dietary questionnaire)	Q3 vs. Q1 (g/day)	Events were self-reported using follow up questionnaires or through diabetes drug reimbursement from health insurance records.	Daily energy intake, alcohol consumption, level of education, family history of diabetes, physical activity, hypertension, hypercholesterolaemia, smoking status and tertile groups of remaining fatty acid groups.		Cardiovasculaire, Obésité, Rein, Diabète (CORDDIM) Program
Engeset et al. 2006 Europe EPIC	25-70	0	310,671	4,776 invasive incident breast cancers	12 max (6.4 median)	Total Fish Intake *(FFQ and 24h food recall)*	Q5 vs. Q1 (g/day)	Events were reported using country specific population cancer registries and on a combination of methods including health insurance, cancer and pathology registries, and active follow-up through study subjects and their next-of-kin in 3 countries	Centre location, adjusted for time of follow-up, energy intake from fat, alcohol intake, height, weight, age at menarche, number of full-term pregnancies and age at first full term pregnancy, therapy, current use of oral contraceptives and menopausal status.		Europe Against Cancer Program of the European Commission (SANCO), Deutsche Krebshilfe, Deutsches Krebsforschungszen- trum, German Federal Ministry of Education and Research, Danish Cancer Society, Health Research Fund (FIS) of the Spanish Ministry of Health, Spanish Regional Governments of Andalucia, Asturia, Basque Country, The ISCIII Network RCESP, Cancer Re- search UK, Medical Research Council, Stroke Association (United Kingdom), British Heart Foundation, Department of Health (United Kingdom), Food Standards Agency (United Kingdom), Welcome Trust, (United Kingdom), Italian Association for Research on Cancer (AIRC), Compagnia di San Paolo (Italy), Dutch Ministry of Public Health, Welfare and Sports, World Cancer Research Fund, Norwegian Cancer Society,

											Research Council of Norway, French League against Cancer (LNCC), National Institute for Health and Medical Research (INSERM) (France), Mutuelle G en erale de l'Education Nationale (MGEN) (France), 3M Company, France, Greek Ministry of Health, Greek Ministry of Education, Swedish Cancer Society, Swedish Research Council, Regional Government of Skane.
Engeset et al. 2015 Europe EPIC	35–70	28	509,308	32,587 deaths	18	Total fish consumption *(validated FFQ),* analyzed by gender	Q5 vs. Q1 (g/day) and continuous (10 g increment)	Events were recorded using cancer registries, boards of health, and death indices.	Energy from fat, energy from carbohydrates and proteins, dietary fibres, red meat, processed meat, vegetables, fruit, alcohol intake, body mass index, physical activity, smoking, education, lean and fatty fish.		European Commission (DG-SANCO) and the International Agency for Research on Cancer, Danish Cancer Society (Denmark); Ligue Contre le Cancer, Institut Gustave Roussy, Mutuelle Ge ́ne ́rale de l'Education Nationale, Institut National de la Sante ́ et de la Recherche Me ́dicale (INSERM) (France); Deutsche Krebshilfe, Deutsches Krebsforschungszentrum and Federal Ministry of Education and Research (Germany); Ministry of Health and Social Solidarity, Stavros Niarchos Foundation and Hellenic Health Foundation (Greece); Italian Association for Research on Cancer (AIRC), Compagnia di San Paolo, and National Research Council (Italy); Dutch Ministry of Public

											Health, Welfare and Sports (VWS), Netherlands Cancer Registry (NKR), LK Research Funds, Dutch Prevention Funds, Dutch ZON (Zorg Onderzoek Nederland), World Cancer Research Fund (WCRF), Statistics Netherlands (The Netherlands); ERC-2009-AdG 232997 and Nordforsk, Nordic Centre of Excellence programme on Food, Nutrition and Health (Norway); Health Research Fund (FIS), Regional Governments of Andalućıa, Asturias, Basque Country, Murcia and Navarra, ISCIII RETIC (Spain); Swedish Cancer Society, Swedish Scientific Council and Regional Government of Skåne and Va¨sterbotten (Sweden); Cancer Research UK, Medical Research Council (United Kingdom).
Fehily et al. 1994 UK Caerphilly IHD Study	45-59	100	665	21 new onset IHD events	5	PUFA Intake *(blood sample after overnight fast and 7 day weighted food intake records)*	Q3 vs. Q1 (%E), case vs. control (g/100g total fatty acids)	Events were retrieved in three ways: comparing baseline and follow up ECGs, hospital records and deaths **(not indicated how deaths were reported)**	Age, BMI and smoking.		**Not reported**
Gammelmark et al. 2016 Denmark Danish cohort study Diet, Cancer and	50-64	**48**	57053	3089 incident MI	17	n-3 PUFA Intake (Validated sqFFQ)	Q5 vs. Q1 (>28 g/day vs. 0-8 g/day)	Events were retrieved using the Danish National Patient Registry and/or the Danish Causes of Death Registry	Smoking, BMI, waist circumference, physical activity, alcohol intake, educational level, menopausal status, history of diabetes mellitus, hypertension and hypercholesterolaemia, total energy intake, intake of fruits and vegetables		The Danish Heart Association, Hertha Christensen Foundation, Danish Cancer Society

Health									and intake of nuts		
Gau et al. 2011 Singapore Singapore Longitudinal Aging Studies (SLAS)	≥55	**35/36(from table)**	1,475	Cognitive decline	2	Daily long chain omega-3 polyunsaturated fatty acid supplement intake *(self reported single question)*	Intake vs. no intake of PUFA supplement	Events were recorded by comparing baseline MMSE scores to follow up MMSE score. A drop of at least two MMSE points was defined as cognitive decline.	Age, gender, education, number of medical comorbidity, the presence of vascular risk factors/diseases, smoking, alcohol drinking, depression, APOE status (e4 allele carriers vs. non-carriers), nutritional status, level of leisure activities, baseline MMSE and length of follow-up.		Biomedical Research Council, Agency for Science, Technology and Research in Singapore.
Gronroos et al. 2012 USA Atherosclerosis Risk in Communities (ARIC) cohort	45–64	**48-55 (from table)**	14,222 – fish analysis, 3817 – plasma DHA and EPA	1,604 Atrial Fibrillation events	17.6 (average)	Intake of fish and fish-derived EPA and DHA *(FFQ and plasma samples)*	Q4 vs. Q1 (>2 vs. 0 Servings of fish per week), Q4 vs. Q1 (% total fatty acid – DHA+EPA)	Events were reported using three sources: hospital discharge notes, ECGs performed during follow up exams and death certificates	Center, age, race, sex, energy intake, body mass index, education, exercise levels, smoking status and amount, alcohol intake, LDL cholesterol, HDL cholesterol, use of cholesterol lowering medications, systolic blood pressure, use of antihypertensive medications, diabetes, coronary heart disease, and ECG-defined left ventricular hypertrophy.		National Heart, Lung, and Blood Institute, American Heart Association
González et al. 2000 Spain EPIC - Spain	29 -69	44	11,883	Association between BMI and fat intake accounted for <1% of variance	0	PUFA Intake *(validated diet history questionnaire)*	Obese vs. Overweight vs. Normal vs. Underweight (%E – multi linear regression model)	Events were recorded at follow up appointments (height and weight measurements).	Age, housework, sport, other leisure activities, work activity, smoking, education level, parity, menopause and diet adjusted.		Europe Against Cancer Programme of the European Union, Health Research Fund, Spanish Ministry of Health
Haraldsdottir et al. 2017	54	0	9,340	744 incidence breast	27.3	Fish consumption (validated FFQ)	Q3 vs. Q1 (>4 vs. <2 portions	Events were ascertained using the Icelandic Cancer Registry and the	Age upon entry, education, family history of breast cancer, BMI in midlife, age at first child, age at menarche, intake of milk, rye, meat, fish liver oil,		NIH (Iceland), Intramural Research Program of the National Institute on Aging, the Icelandic Heart

Iceland Age, Gene/Environment Susceptibility (AGES)-Reykjavik Study and Reykjavik Study				cancer			per week)	Directorate of Health.	salted/smoked fish in adolescence and fish and alcohol in midlife.		Association, and the Icelandic Parliament, Icelandic Centre for Research, Public Health Fund of the Icelandic Directorate of Health.
Harding et al. 2004 UK EPIC-Norfolk	40–78	45	21,472	414 incident cases of diabetes	7	Polyunsaturated fat: Saturated fat ratio *(SQFFQ)*	**check**	Events were reported by self-report of diabetes from the first and second follow-up health and lifestyle questionnaires, self-report of diabetes-specific medication, diabetes medication brought to a follow up visit and an HbA_{1c} level of greater than 7 percent at either the baseline or follow-up health check Hospital admissions data for EPIC-Norfolk participants were screened to identify those who were admitted to a hospital for a diabetes-related condition. Office of National Statistics death certificate data with coding for diabetes was also used.	Total energy intake, age, sex, family history of diabetes, smoking, physical activity, total fat, total protein, alcohol consumption, BMI and waist: hip ratio.		Cancer Research Campaign, the Medical Research Council, the Stroke Association, the British Heart Foundation, the Department of Health, the Commission of the European Union's Europe against Cancer Programme, and the Department for Environment, Food, and Rural Affairs
Hu et al. 2003 USA NHS	30-55	0	5103	362 incident cases of CHD (141 CHD deaths and 221	16	Average w-3 Fatty acids, Average Frequency of Fish Intake *(SQFFQ)*	Q5 vs. Q1 (g/day) and (≥ 5/week vs.	Events were self reported and confirmed by medical records. Deaths were identified from state vital records and the National Death	Age, smoking status, body mass index, alcohol intake, parental history of myocardial infarction, menopausal status and postmenopausal hormone use, moderate to vigorous activities, usual aspirin use, multivitamin		National Institutes of Health, American Heart Association Established Investigator Award

				nonfatal myocardial infarctions), 468 deaths from all causes (161 from CHD or stroke, 172 from cancer, and 135 from other causes)			<1/month)	Index or reported by next of kin and the postal system.	supplement use, vitamin E supplement use, history of hypertension, hypercholesterolemia, duration of diabetes, hypoglycemic medication, *trans* fat, the ratio of polyunsaturated fat to saturated fat, and dietary fiber.		
Hu et al. 1998 USA NHS	34-59	0	86016	1255 major coronary disease events (861 cases of non-fatal myocardial infarction and 394 cases of fatal coronary heart disease	14	Frequency of Nut Consumption *(61 Item Dietary Questionnaire)*	Q3. vs Q1 (≥ 2-4 vs. almost never, consumption of nuts)	Events were reported through review of medical records by physicians blinded to risk factors. Deaths were reported by families and postal officials and through the National Death Index.	Age, time period, BMI, cigarette smoking,? history of HTN, history of diabetes and hypercholesterolemia; menopausal status, parental history of myocardial infarction before 60 years of age; use of multivitamins; use of vitamin E supplements; alcohol consumption, aspirin use, vigorous exercise >1/week; and total energy intake.		United States National Institute of Health
Jakobsen et al. 2014 Denmark Diet, Cancer and Health follow-up study	55	44	29152?	**check**	6	Intake of n-6 PUFA intake at different levels of intake of carbohydrates *(validated SQFFQ)*	Q5 vs. Q1 (n-6 PUFA Intake 6.9 vs. 3.4 %E)	Events were reported through a self-administered questionnaire. The method was verified within the cohort.	Sex, age, BMI at recruitment, waist circumference at recruitment, education, smoking status, leisure-time physical activity, alcohol consumption, and intakes of proteins, long-chain n-3 PUFA and energy.		Danish Council for Strategic Research, European Commission as an Integrated Project under the 6th Framework Programme
Jakobsen et al. 2009 11 American and European	37-76 (from table)	Not listed	344,696	5249 coronary events and 2155 coronary	10	PUFA, n-3 and n-6 fatty acids substitution for SFAs *(validated FFQ or dietary history*	Q2 vs. Q1 (Per 5 E% increase) -	Events were ascertained using standardized criteria.	Intake of MUFAs, PUFAs, trans fatty acids, protein, and CHs expressed as percentages of total energy intake and total energy intake, age at baseline, the calendar year in which the baseline questionnaire, smoking, BMI, physical		National Heart, Lung, and Blood Institute, National Institutes of Health, the Danish Heart Foundation, Female Researchers in Joint Action program from the

cohort studies				deaths		*interview)*	confirm		activity, highest attained education level, alcohol intake, history of HTN, energy-adjusted quintiles of fiber intake and cholesterol intake.		Danish Medical Research Council
Kromhout et al. 1995 Netherlands Longitudinal health survey by CHB (one of the authors)	64-87	50	272	58 CHD, 67 cancer and 187 all cause mortality	17	Consumption of Fish (Dietitian estimations based on self report, postal area and groceries)	Fish vs. No fish	Events reported through review of death certificates.	Age and smoking.		Netherlands Nutrition Council, Netherlands Prevention Foundation
Kushi et al., 1985; U.S.A.-Ireland (Ireland-Boston Heart Study)	40-60	100	1,001	110 CHD deaths	23	Total PUFA *(Burke diet history)*	Top 3rd vs. Bottom 3rd	Death certificates for all decedents reviewed and adjudicated according to ICD-9	Age, cohort, SBP, serum cholesterol, LVH, smoking, alcohol and cohort.	6	NIH, Irish Heart Foundation, Harvard School of Public Health
Kyrozis et al. 2009 Greece EPIC-Greece cohort	≥60	38	610	**Check**	13	PUFA Intake *(validated SQFFQ)*	Q9 vs. Q1 (g/day)	Events were retrieved using the Geriatric Depression Scale, as well as assessments cognitive function (MMSE) and medical variables (diagnosed with cancer, myocardial infarction or stroke).	Gender, age, marital status, years of education, height, BMI, physical activity, smoking, alcohol intake, coffee intake, energy daily intake, hypertension at baseline, diabetes at baseline, MMSE score follow up, cancer at follow up, cardiac disease at follow up, cerebrovascular disease at follow up, total lipids, PUFAs, Monounsaturated lipids, saturated lipids, fish and seafood, seed oils and olive oil.		Europe against cancer Program of the european Commission, the Greek Ministry of Health, the Greek Ministry of Education, an unrestricted grant to the University of Athens in honor of ‘‘Vasilios and Nafsika Tricha,” the Hellenic Health Foundation, European Social Fund and National Resources.
Larsson et al. 2017 Sweden Cohort of Swedish Men and the Swedish	45-83	53	72,984	6095 incident Atrial Fibrillation	12	Fish Consumption, n-3 PUFA (Validated FFQ)	Q5 vs. Q1 (>0.567 g/day vs. <0.243 g/day)	Events were identified using the Swedish National Patient Register.	Age, sex, education, smoking, history of hypertension, diabetes, body mass index, walking/bicycling, family history of myocardial infarction, alcohol consumption, and total energy intake		Swedish Research Council, Karolinska Institutet's Strategic Research Program

Mammography Cohort											
Larsson et al. 2012 Sweden Swedish Mammog-raphy Cohort	49–83	0	34,670	1680 stroke events (1310 cerebral infarctions, 233 hemorrhagic strokes, and 137 unspecified strokes)	Mean: 10.4	Intake of PUFAs , αLinolenic acid, long chain n-3 PUFA, n-6 PUFA *(validated FFQ)*	Q5 vs. Q1 (g/day)	Events were retrieved using the Swedish Hospital Discharge Registry. Information on dates of death for deceased participants was obtained from the Swedish Death Registry.	Age, smoking status and pack-years of smoking, education, BMI, total physical activity, history of hypertension, history of diabetes, aspirin use, family history of myocardial infarction, and intakes of alcohol, protein, and dietary fiber.		Swedish Council for Working Life and Social Research and Karolinska Institutet
Li et al. 2011 SA National Health and Nutrition Examination Survey	25–74	40	5068	237 women and 562 men, severely depressed mood	11	Frequency of Fish Consumption *(3 month FFQ)*	Q3 vs. Q1 (>1 serving per week vs. <1 serving per week)	Events were reported at follow up using the Center for Epidemiologic Studies Depression Scale (CES-D) questionnaire. Severe depressed mood was defined as CES-D score≥22 and/or taking anti-depression medicine	Race/ethnicity, education attainment, family income level, marital status, types of residence area, occupation, employment status, body mass index, alcohol drinking, cigarette smoking, serum total cholesterol, total dietary energy intake, saturated fatty intake, frequency of eating fruit and vegetables assessed at the baseline survey, and self-evaluate health status and the history of major physical diseases (cancer, diabetes, stroke and heart attack).		National Center for Health Statistics; National Institute on Aging; National Cancer Institute; National Center for Chronic Disease Prevention and Health Promotion; National Institute of Child Health and Human Development; National Heart, Lung, and Blood Institute; National Institute on Alcohol Abuse and Alcoholism; National Institute of Mental Health; National Institute of Diabetes and Digestive and Kidney Diseases; National Institute of Arthritis and Musculoskeletal and Skin Diseases; National Institute of Allergy and Infectious Diseases; National Institute of Neurological and Communicative Disorders and Stroke; and U.S. Department of Agriculture
Lopez et al. 2011	67-100	**44-57 (depending on**	266	42 had dementia and 30 had	5	Plasma DHA *(non-fasting blood sample)* and dietary	Q3 vs. Q1	Failure of three baseline assessments determined participants at risk of	Age, sex, apolipoprotein E status, education, and history of stroke.		National Institute of Diabetes and Digestive and Kidney Diseases, National

USA Rancho Bernardo Cohort		**tertile)**		possible or probable Alzheimer's Disease		DHA *(validated FFQ)*		developing dementia. Test included: MMSE, Heaton Visual Reproduction Test, Category Fluency Assessment, Buschke-Fuld Selective Reminding Test. These participants underwent extensive neuropsychological testing, neurological and general medical examinations, laboratory tests, and computerized tomography evaluation of the brain, results aided neurologist in categorizing participants in diagnostic groups.			Institute on Aging, National Institutes of Health/National Institute of Aging
Meyer et al. 2001 USA Iowa Women's Health Study	55-69	0	35,988	1,890 Incident Diabetes	11	Polyunsaturated Fats, Long chain w-3 fatty acids *(validated FFQ)*	Q5 vs. Q1 (g/day)	Events were self reported and validated by a physician.	Age, smoking, alcohol consumption, BMI, waist-to-hip ratio, physical activity, demographic factors, and dietary magnesium and cereal fiber.		National Cancer Institute (USA)
Morris et al. 2005 USA Chicago Health and Aging Project (CHAP)	≥65	36-40 (fromtable)	3718	Rate of decline was reduced by 10% to 13% per year among persons who consumed 1 or more fish meals per week compared with those with less than weekly consumptio	6	Weekly frequency of fish consumption, n-3 fatty acids, DHA, EPA, α-linolenic acid *(Harvard FFQ)*	Q4 vs. Q1 (g/day)	Events were reported by comparing baseline results to follow up from: the East Boston Tests of Immediate and Delayed Recall, the Mini-Mental State Examination, and the Symbol Digit Modalities Test	Age, age^2, energy intake, sex, race, education, education X race, sex X age, time, 1 fish meal per week, 2 fish meals or more per week, and time interactions with each of age, energy intake, education, sex, race, education X race, 1 fish meal per week, 2 or more fish meals per week, cognitive activity, physical activity, alcohol consumption, food intake of vitamin E, total vitamin C, food intake of niacin, saturated fat, trans fat, and quartiles of -3 fatty acid intake **– may want to confirm this**		National Institute on Aging

				n.							
Mozaffarian et al. 2003 USA Cardiovascular Health Study (CHS)	≥65	41.5 (average from table- **check**	3910	247 Ischemic Heart Disease deaths (48 primary and 100 secondary arrhythmic deaths) and 363 incident nonfatal MIs	9.3 (mean)	Amount and Type Fish Consumed *(validated FFQ)*	Q4 vs. Q1(≥3 servings per week vs. <1 servings per month)	Events identified by annual follow up and telephone interviews validated by centralized committee using interviews, medical records, death certificates, medical examiner forms, hospitalization paperwork	Age, gender, education, diabetes, current smoking, pack years of smoking, tuna/other fish, fried fish/fish sandwich consumption, BMI systolic BP LDL cholesterol, HDL cholesterol, triglycerides, C-reactive protein, and intake of saturated fat, alcohol, beef/pork, fruits, and vegetables.		National Heart, Lung, and Blood Institute PI Salary Support: VA Health Services Research and Development fellowship at the VA Puget Sound Health Care Center
Nimptsch et al. 2010 Germany EPIC-Heidelberg	35-64	46	20,049	**check**	6.5 mean	n-6, LA, ARA, ALA, DHA+EPA (FFQ)	**check**	Events self reported and confirmed by a calibration method - **check**	Other PUFA, MUFA and SFA intakes, protein, alcohol intake, total energy intake, baseline weight and height, age at baseline, physical activity, education, smoking, follow-up-time and menopausal status at baseline		Kurt- Eberhard-Bode-Stiftung, Deutsche Forschungsgemeinschaft, Graduiertenkolleg 793 scholarship
Owen et al. 2016 Australia Australian Diabetes, Obesity and Lifestyle Study	51	45	11,247		13	n-3 PUFA, n-6 PUFA, Fish intake (Validated sqFFQ)	Q5 vs. Q1 (g)	Events were recorded using the Australian National Death Index.	Age, sex, previous CVD, education, exercise, diabetes, total dietary energy and smoking		Australian National Health and Medical Research Council, Australian Government Department of Health and Ageing, Abbott Australasia Pty Ltd, Alphapharm Pty Ltd, Amgen Australia, AstraZeneca, Bristol-Myers Squibb, City Health Centre-Diabetes Service-Canberra, Department of Health and Community Services—Northern Territory, Department of Health and Human Services—Tasmania, Department of Health—New South Wales, Department of Health—

											Western Australia, Department of Health—South Australia, Department of Human Services—Victoria, Diabetes Australia, Diabetes Australia Northern Territory, Eli Lilly Australia, Estate of the Late Edward Wilson, GlaxoSmithKline, Jack Brockhoff Foundation, Janssen-Cilag, Kidney Health Australia, Marian & FH Flack Trust, Menzies Research Institute, Merck Sharp & Dohme, Novartis Pharmaceuticals, Novo Nordisk Pharmaceuticals, P zer Pty Ltd, Pratt Foundation, Queensland Health, Roche Diagnostics Australia, Royal Prince Alfred Hospital, Sydney, Sano Aventis, sanosynthelabo and the Victorian Government's OIS Program
Owen et al. 2016 Australia Australian Diabetes, Obesity and Lifestyle Study (AusDiab)	25-84	45	11,247	1265 deaths, 277 CVD deaths	12.6 median	n-3 and n-6 Median PUFA intake *(SQFFQ)*	Q5 vs. Q1 (g)	Vital status and causes of death were collected by death registry linkage.	Age, sex, previous CVD, education, exercise, diabetes, total dietary energy and smoking.		Australian National Health and Medical Research Council, Australian Government Department of Health and Ageing, Abbott Australasia Pty Ltd, αpharm Pty Ltd, Amgen Australia, AstraZeneca, Bristol-Myers Squibb, City Health Centre-Diabetes Service-Canberra, Department of Health and Community Services—Northern Territory, Department of Health and Human Services—Tasmania, Department, of Health—New South Wales, Department of Health—Western Australia,

											Department of Health—South Australia, Department of Human Services—Victoria, Diabetes Australia, Diabetes Australia Northern Territory, Eli Lilly Australia, Estate of the Late Edward Wilson, GlaxoSmithKline, Jack Brockhoff Foundation, Janssen-Cilag, Kidney Health Australia, Marian & FH Flack Trust, Menzies Research Institute, Merck Sharp & Dohme, Novartis Pharmaceuticals, Novo Nordisk Pharmaceuticals, Pfizer Pty Ltd, Pratt Foundation, Queensland Health, Roche Diagnostics Australia, Royal Prince Alfred Hospital, Sydney, Sanofi Aventis, sanofi-synthelabo, the Victorian Government's OIS Program, National Health and Medical Research Council Fellowship
Patel et al. 2010 England European Prospective Investigation into Cancer and Nutrition–Norfolk study	40–79	53	383	199 T2DM	11 - **confirm**	Polyunsaturated fatty acids: n-3, α-Linolenic acid (18:3n–3) Eicosapentaenoic acid (20:5n–3) Docosapentaenoic acid (22:5n–3) Docosahexaenoic acid (22:6n–3) n-6, Linoleic acid (18:2n–6) c-Linolenic acid (18:3n–6) Eicosadienoic acid	Q3 vs. Q1	Events were ascertained by using multiple sources: self-report of a physician's diagnosis of diabetes or diabetes medication on any of the follow-up health and lifestyle questionnaires or diabetes medication brought to the follow-up health check visit, record linkage used to trace each participant for diabetes diagnosis, diabetes-related deaths	Age, sex, family history of diabetes, BMI, smoking status, physical activity, and alcohol intake.		Medical Re- search Council UK and Cancer Research UK, European Union; the Stroke Association; the British Heart Foundation; the Department of Health; the Food Standards Agency; Ministry of Agriculture, Fisheries and Food; the Wellcome Trust; InterAct project

						(20:2n–6) Dihomo-c-linolenic acid (20:3n–6) Arachidonic acid (20:4n–6) Adrenic acid (22:4n–6) *(validated FFQ)*		were flagged by linkage to the National Death Registry.			
Pietinen et al. 1997 Finland Finish α-Tocopherol, Beta-Carotene Cancer Prevention Study	50-69	100	21 930	1399 major coronary events (first non-fatal MI), 635 coronary deaths	6	PUFA, linoleic, linolenic, n-3 fish FA (*validated* FFQ)	Q5 vs Q1 (g)	National hospital discharge registered diagnosis and obtained hospital and pathology reports; deaths confirmed through national population register	Age, treatment group, smoking, BMI, blood pressure, energy, alcohol, fibre, education and physical activity.	8	National Cancer Institute (USA), Academy of Finland
Rhee et al. 2017 USA Women's Health Study	**54**	**0**	39,876	1,941 CVD	11	Intakes of tuna and dark fish, ALA and EPA+DHA (validated sqFFQ)	Q5 vs. Q1 (%E)	Events were self-reported on incident physician diagnoses of cardiovascular events. Medical records were obtained to verify all cardiovascular events. Deaths were confirmed by review of autopsy reports, death certificates, medical records, or information obtained from next of kin or family members.	Randomized treatment, age, BMI, smoking, alcohol intake, physical activity, oral contraceptive use, use of hormones as defined under HRT, multivitamin use, family history of MI, and baseline history of hypertension, high cholesterol, diabetes, intakes of dietary fiber, fruits and vegetables, trans fat, ratio of polyunsaturated to saturated fat, and sodium.		NIH (USA)
Sala – Vila et al. 2016 Spain PREDIMED trial	**67**	**42.5**	7202	431 deaths occurred (104 cardiovascular disease, 55 coronary heart disease, 32 sudden cardiac death, 25 stroke)	5.9	LC -3 PUFA, ALA (Validated sqFFQ)	Control vs. MedDiet +nets vs. MedDiet + EVOO (assigned intervention)	Endpoints were ascertained using yearly questionnaires and examinations for all participants, contact with primary care physicians, yearly review of medical records, and linkage to the National Death Index. Medical records of deceased participants were requested.	Age, sex, body mass index, current smoking status, physical activity, total energy intake, history of diabetes, history of hyperlipidemia, history of hypertension, alcohol intake, fiber, vegetables, fruits, red meat and meeting the ISSFAL recommendation of eicosapentaenoic acid and docosahexaenoic acid consumption for primary cardiovascular prevention		Instituto de Salud Carlos III (ISCIII) (Spanish Ministry of Economy), Centro Nacional de Investigaciones Cardiovasculares CNIC, the Spanish Ministry of Science and Innovation (MICINN), the California Walnut Commission

Sanchez-Villegas et al. 2007 Spain SUN Cohort	**Not indicated**	**Not indicated**	7,903	173 cases of depression, 335 cases of anxiety, and 4 cases of stress	2	Mean consumption of w-3 fatty acids and fish consumption *(validated FFQ)*	Q5 vs. Q1 (g/day)	Events were self reported physician diagnosis and medication prescription.	Age, sex, incapacitating disease, energy intake, physical activity during leisure time, and change in physical activity since baseline.		Spanish Ministry of Health, Navarra Regional Government
Sanchez-Villegas et al. 2011 Spain SUN Cohort	37.5 (SD: 11.5)	42	12,059	657 new cases of depression	10	Polyunsaturated Fat intake *(validated FFQ)*	Q5 vs. Q1 (19.0 vs. 9.3 g/day)	Events were self reported diagnosis by a doctor or use of antidepressant medication.	Sex, age, smoking, leisure time physical activity, total energy intake, BMI and adherence to the Mediterranean Dietary Pattern.		Spanish Government Instituto de Salud Carlos III, Fondo de Investigaciones Sanitarias and the Navarra Regional Government
Shen et al. 2011 USA Framingham Heart Study	62 +/- 10 yrs	44	4526	296 incident Afib (177 men, 119 women)	4	Mean n-3 PUFA intake and Weekly Total fish and Dark Fish Intake *(validated FFQ)*	Q4 vs. Q1 (460 vs. 80 mg/d) and Q3 vs. Q1 (>4 servings /week vs. never or <1 serving/ week)	Events were recorded during interim medical evaluations at hospitals and by external clinicians. All cases were reviewed by a Framingham Health Study cardiologists. Review included ECGs and outside records.	Age, sex, BMI, systolic blood pressure, hypertension treatment, electrocardiogram, PR interval, significant heart murmur, and heart failure.		National Heart, Lung, and Blood Institute, the National Institute of Diabetes and Digestive and Kidney Diseases, the US Department of Agriculture Research, the American Heart Association and the National Institutes of Health
Smith-Warner et al. 2001 USA Pooling Project	28-90 (widest range), varied depending on study.	0	351,821	7,329 incident invasive breast cancer cases		PUFA Intake *(validated FFQ)*	Q4 vs. Q1 (Pooled Analysis), per 5% increases in energy from total fat and specific fat subtype	Event reporting varied depending on the study included.	Percent energy from protein, percent energy from alcohol, age at menarche, parity, age at birth of first child, menopausal status at diagnosis, postmenopausal hormone use, oral contraceptive use, history of benign breast disease, family history of breast cancer, smoking status, education, body mass index, body mass index-menopausal status at diagnosis interaction term, height, fiber intake and energy intake.		National Institutes of Health, Cancer Research Foundation of America/American Society of Preventive Oncology, American Cancer Society

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Solfrizzi et al. 2006 Italy Italian Longitudinal Study on Aging	65-84	55	**95 (completed final survey), 578 met inclusion criteria**	MMSE Score Decline **(cannot find number)**	8.5	Polyunsaturated Fatty Acid Intake *(validated FFQ)*	Change in cognitive function vs. baseline	Repeated testing with MMSE – **check**	Sex, age, education, Charlson comorbidity index, BMI, MMSE baseline score, and total energy intake at baseline.		Italian Longitudinal Study on Aging, AFORIGE
Sonestedt et al. 2007 Sweden Malmo¨ Diet and Cancer	50-73	0	12 781	428 postmenopausal incident breast cancer	9.5 (average)	n-6 fatty acids *(validated FFQ)*	Q5 vs. Q1 14.1 vs. 6.6 g/day)	Events retrieved from Swedish Cancer Registry and the Southern Swedish Regional Cancer Registry	Total energy, age, method version, diet interviewer and season.		Swedish Cancer Society, the Swedish Medical Research Council, the European Commission and the City of Malmo¨
Sonestedt et al. 2008 Sweden Malmo¨ Diet and Cancer	≥50	0	11,699	430 incident invasive breast cancer	13	n-6 PUFA and Heterocyclic amine intake *(7 day diet history, FFQ and interview)*	Q3 vs. Q1	Events were retrieved from the Swedish Cancer Registry.	Age, method, season, total energy intake, weight, height, smoking habits, alcohol consumption, leisure time physical activity, hours of household work, educational level, current use of hormone replacement therapy and age at menopause.		**Could not find**
Strom et al. 2011 Denmark Danish National Birth Cohort	15.7–46.9	100	48 627	577 total cardiovascular events (328 hypertensive disease, 146 cerebrovascular disease, and 103 ischemic heart disease)	12	Fish intake and LCn3FA intake *(FFQ)*	>30g/day vs. 0-3 g/day Q5 vs. Q1 (0.73 vs. 0.06 median g/day)	Events were retrieved through the Danish National Patient Registry and the Danish Register of Causes of Death	Physical activity, pre-pregnant body mass index, smoking, school, cohabitant status, parity, occupation, pre-pregnant alcohol intake, total energy intake, intake of saturated fat, dietary fiber, and *trans*-fatty acids		Faroese Research Council, the Fisheries Research Fund of the Faroe Islands, the European Union sixth framework programme Integrated Research Project SEAFOOD- plus, the European Union sixth frame- work programme EARNEST, and the Nordic Working Group on Fishery Research.

van Dam et al. 2002 USA Health Professionals Follow-Up Study	40-75	100	42504	1321 Incident T2DM	12	Linoleic Acid, α – linoleic acids, oleic acid, Long Chain w-3 Fat *(validated FFQ)*	Q5 vs. Q1 (E %)	Events self reported, validated with sample of medical records	Age, total energy intake, time period, physical activity, cigarette smoking, alcohol consumption. Hypercholesterolemia, hypertension, family history of T2DM, intake of cereal fiber, intake of magnesium and BMI.		National Institutes of Health (USA), American Diabetes Association
van Gelder et al. 2007 Netherlands Zutphen Elderly Study	70-89	100	210	Men who did not consume fish had a cognitive decline of 1.2 points, 4 times the decline in men who consumed fish	5	Fish Consumption, DHA+ EPA Intake (Cross-check dietary history method)	Q3 vs. Q1 (g/day)	MMSE was used to identify cognitive decline throughout follow up period.	Age, education, alcohol consumption, smoking status, physical activity, energy intake and baseline cognitive functioning.		European Union (to DK) for the Healthy Ageing: Longitudinal study in Europe
Velie et al. 2000 United States Breast Cancer Detection Demonstration Project (BCDDP)	41-91	0	40022	996 Breast Cancer	5.3 (average), 14 max	Oleic acid, Linoleic acid *(validated FFQ)*	Q5 vs. Q1	Event status obtained from self-report, death certificates, pathology reports and relatives.	Total energy, body mass index, height, family history of breast□birth, educational level, alcohol use, age at menarche, and history of benign breast disease.		National Cancer Institute (USA)
Vercambre et al.	76-82	0	4809	518 DECO score <33 716 4-IADL score 0, 268 both declines	16 – **check**	PUFA□, n-6 Fatty Acids, n-3 fatty acids, α linolenic acid, long chain n-3, n-6:n-3 Fatty acids ratio *(validated FFQ)*	Q3 vs. Q1 (g)	Events were self reported and were confirmed using a 4-IADL score telephone interview.	Age, Education level, BMI, physical activity, daily energy intake, smoking status, supplement of Vitamin D, supplement of other vitamins, use of postmenopausal hormones, history of depression, history of cancer, history of CHD, history of stroke, history of DM, history of HTN and history of		French League against Cancer, the European Community, 3M Company, Mutuelle Gé né rale de l'Education Nationale (MGEN), French Institute of Health and Medical Research, Gustave Roussy Institute, several general

									hypercholesterolemia.		councils in France, Statlife Company and the 'Association Nationale de la Recherche Technique
Virtanen et al. 2008 USA Health Professionals Follow-Up Study (HPFS)	40-75	100	40230	9715 major chronic disease events occurred, including 3639 cardiovascular disease events, 4690 cancers, and 1386 deaths from non-traumatic causes.	18	EPA +DHA *(validated FFQ)*	Q5 vs. Q1 (≥ 0.6 vs. <.05 g/day)	Events were self reported, confirmed with medical records by blinded physicians. Deaths were ascertained from relatives, postal authorities, or the National Death Index, and cause of death was classified according to medical records, death certificates, and autopsy findings.	Age, BMI, smoking, physical activity, history of diabetes, hypertension, or hypercholesterolemia, first-degree family history of myocardial infarction before age 60 y, first-degree family history of colon cancer; aspirin use, multivitamin supplement use, glycemic load, and intakes of protein, fiber, *trans* fat, saturated fat, n-6 fatty acids, α-linolenic acid, red meat, total calories, and alcohol.		National Institutes of Health, Finnish Cultural Foundation, Helsingin Sanomat Centennial Foundation, Finnish Foundation for Cardiovascular Research, Yrjö Jahnsson Foundation, and University of Kuopio.
Virtanen et al. 2014 USA Kuopio Ischemic Heart Disease Risk Factor study	42-60	100	2212	422 Incident T2DM	20	EPA, DPA, ALA, EPA+DHA+DPA *(Serum PUFA)*	Q4 vs. Q1 (%)	Events were assessed by self-administered questionnaires and fasting and 2-h oral glucose tolerance test blood glucose measurement at re-examination after the baseline and by record linkage to hospital discharge registry and reimbursement register on diabetes medication expenses	Age, examination year, BMI, family history of type 2 diabetes, smoking, education years, leisure-time physical activity and intake of alcohol and serum linoleic acid.		**Check**
Wiberg et al. 2006 Sweden Uppsala Longitudinal	50-83	100	2313	421 stroke or TIA	32 (average 29.3)	Linoleic, g-linolenic, α-linolenic, arachidonic acid, DHA *(serum cholesterol ester levels)*	Stroke outcome vs. baseline	Events identified via hospital discharge records and cause of death registries	Antihypertensive, antidiabetic, lipid-lowering drugs, hypertension, diabetes, atrial fibrillation, cardiovascular disease, the metabolic syndrome, serum cholesterol, smoking, and physical activity.		Medical Faculty at Uppsala University, the Uppsala Geriatric Fund, and the Swedish Heart Lung Foundation

Study of Adult Men											
Yaemsiri et al. 2013 USA Women's Health Initiative Observational Study	50-79	0	1,928 (964 matched pairs)	964 cases of incident ischemic stroke (96 atherothrombotic strokes, 250 lacunar strokes, 209 cardio embolic strokes, 42 ischemic strokes of other determined cause, 366 ischemic strokes of undetermined cause, and 1 was missing subtype information)	10	18:3n3,20:4n3,20:5n3, 22:5n3,22:6n3,18:2n6,18:3n6,20:2n6,20:3n6,20:4n6,22:4n6,22:5n6 *(serum Fatty Acids)*	1 SD increment in serum Fatty acids	Events were self reported, confirmed by physician through medical charts, brain imaging, or death certificates	Age, race, time to follow up, BMI, smoking, DM, aspirin use, total cholesterol to HDL-C ratio, normalized-triglycerides, systolic blood pressure and antihypertensive medication use.		National Heart, Lung, and Blood Institute, National Institutes of Health, US Department of Health and Human Services, National Institute of Neurological Disorders and Stroke, American Heart Association
Yary et al. 2017 Finland Kuopio Ischaemic Heart Disease Risk Factor Study	42–60	100	2179	58 hospital discharge diagnosis of depression	21.5	Dihomo-γ-linolenic acid (serum fatty acids)	-	Events were identified at baseline using the 18-item Human Population Laboratory (HPL) Depression Scale and were obtained during follow up using linkage to the National Hospital Discharge Register.	Age, examination year, smoking status, marital status, education, alcohol intake, leisure-time physical activity, body mass index, total energy intake, history of cardiovascular diseases, history of diabetes, history of mental illness, inolenic acid (%), eicosapentaenoic acid (%), docosahexaenoic acid (%), alpha-linolenic acid (%), gamma-linolenic acid (%), arachidonic acid (%), CRP (mg/L) and depressive symptoms at baseline.		Not reported

Table 2. Summary of Newcastle-Ottawa ratings of included prospective cohort studies.

References	Selection (4)	Comparability (2)	Outcome (3)	Total (9)	Comments
Akbaraly 2011	4	2	2	8	Whitehall; registry deaths only
Alhazmi et al. 2014	4	1	1	6	Did not control for family history; self-report of diabetes only with a 70% confirmation in a subset; attrition rate unclear
Albert 2002	4	1	3	8	blood levels; PHS; no adjustment for total energy
Amiano 2014	4	2	3	9	Spanish EPIC (55-60% participation); 10.4 y follow-up; validated cases by study team review
Ananthakrishnan 2014	4	2	3	9	NHS I and II; adjusted for aspirin and NSAID
Ascherio et al. 1996	4	1	2	7	Health Professionals' Follow-up Study; no socioeconomic status adjustment
Bell 2014	4	2	3	9	VITAL cohort; FFQ validated; excluded people with malabsorption of supplements *FOOD + SUPPLEMENTS*; registry cases adjudicated by study team; 4-6 y f/u
Brouwer 2006	4	1	3	8	Rotterdam study; response rate 78% (from all 10,275 inhabitants of Omoord, suburb of Rotterdam); adjusted age, sex, education, energy, vitamin E; mean age 68 y; followed for 6 y
Brostow 2011	4	2	3	9	Singapore Chinese Health Study, population recruitment; validated FFQ; some had CHD at baseline (about 3-5%) but estimates for those without dx at baseline; registry for deaths but "virtually complete"
Byrne 2002	4	2	3	9	NHS I

Chan 2014	4	S	3	7	EPIC- Europe; 11 countries; all FFQ's validated country-specific; did not adjust for NSAID, ethnicity, aspirin; 6-13 y f/u
Chiuve, 2012	4	2	3	9	NHS; selecting one profession helps homogenize SES
Chiuve, 2015	4	2	3	9	NHS; selecting one profession helps homogenize SES
Cho et al. 2004	4	2	3	9	NHS II; 8 y follow-up; validated FFQ
Colangelo 2009	4	1	2	7	CARDIA study; validated and reliable FFQ; adjusted for some but not all important confounders; CES-D scale for depression; large proportion excluded (1798/5500) because of no diet at year 10; 20 y follow up
De Goede et al 2011 (ala/la)	4	2	3	9	MORGEN Study; validated FFQ published; validated probabilistic determinant model for cause of death (+1); 8-13 y /fu
De Goede et al 2012 (lcpufa)	4	2	3	9	MORGEN Study; validated FFQ published; validated probabilistic determinant model for cause of death (+1); 8-13 y /fu
Dijkstra et al 2009	4	1	3	8	Rotterdam study; response rate 78% (from all 10,275 inhabitants of Omoord, suburb of Rotterdam); adjusted age, sex, education, energy, vitamin E; mean age 68 y; followed for 6 y
Djousse 2011	4	2	2	8	WHS; 12.4 y f/u ; good adjustment for covariates, validation of cases by interview; no reports on attrition
Dolocek et al 1992	4	1	2	7	24-h recall (multiple x 4); 10.5 y follow-up; no description of dropouts; independent coding of deaths; no adjustment for total energy
Englehart 2012 (Rotterdam)	4	1	3	8	Rotterdam study; response rate 78% (from all 10,275 inhabitants of Omoord, suburb of Rotterdam); adjusted age, sex, education, energy, vitamin E; mean age 68 y; followed for 6 y
Esrey et al. 1996	3	0	2	5	No socioeconomic status adjustment; no family history; attrition

Fehily et al. 1993	4	0	3	7	Had ischemic heart disease at baseline; no socioeconomic status adjustment; no family history adjustment; no energy adjustment; 5.6% died before 5 year visit or attrition
Folsom 2004	4	2	1	7	confirmed cases with interview/survey; unclear dropout
Fretts 2014	4	1	3	8	did not adjust for total energy intake; 9.3 y f/u
Gago-Dominguez 2003	4	1	2	7	no adjustment for total energy; cases from registry only (no confirmation)
Gao, 2011	4	0	2	6	Singapore Longitudinal Aging Studies (SLAS) (78% response); supplement users (validated by producing supplements); not much In the way of adjustment; cog decline over 1.5 y; high LTFU (n=889 lost/1475)!
Gillman et al. 1997	4	0	3	7	24h recall, but validated; no adjustment for family history; <2% attrition
Gronroos et al.	4	2	3	9	ARIC; hospital discharge codes validated (PPV=89%); 17.6 y f/u; valid FFQ
Harding et al. 2004	4	1	3	8	Did not adjust for socioeconomic status
Hart 2008	4	0	2	6	country-specific validated FFQ; MD confirmed dx of registry-identified cases; no description of follow-up; no matching for smoking, SES; no adjustment for energy, aspirin/NSAIDs
He et al. 2003	4	1	2	7	Health Professionals' Follow-up Study cohort; no adjustment for family history
Holmes et al 1999	4	2	3	9	NHS I ; adjusted for PMH use, height, weight
Hu et al. 1999	4	1	2	7	Nurses' Health Study cohort; no socioeconomic status adjustment
Hu et al. 2002	4	2	3	9	Nurses' Health Study cohort; no socioeconomic status adjustment;

					16 y f/u
IBD in EPIC 2009	4	1	3	8	IBD in EPIC; validated FFQ; not full adjustment
Iso - JPHC, et al 2006	4	2	2	8	JPHC (pop based sample; 80% participation rate); FFQ V and R; 11 y f/u; no details on LTFU
Jakobsen et al. 2004	4	2	2	8	Did exclude people with diabetes; socioeconomic status by record linkage only
Jarvinen et al. 2006	4	1	2	7	20 y of follow up; random sample of M/W; registry deaths but also those who died abroad; validated diet interview with 26 fish questions!; no family history adjustment; dropouts unclear
John et al 2010	4	1	2	7	7-day food diaries, validated w/16-d weighed records, biomarkers ; registry cases reviewed by study gastroenterologist; age-matched (prospective Case-control); adjustment for energy, smoking, other fats; 4.2 y follow-up (short)
Joensen 2010	4	1	3	8	Diet, Cancer, and Health; validated FFQ; no adjustment for total energy; 7.6 y
Kalmijn [ZES] 1997	3	2	1	6	Detailed dietary interview; ZES (74% response rate to initial invitation); 32% cognitively impaired in 1990- but this study measured cognitive decline; cog stat assessed by trained RA; 3 y f/u in very old men; 342/476 had follow-up info (29%)
Kalmijn [Rotterdam] 1997	4	1	3	8	Rotterdam study; response rate 78% (from all 10,275 inhabitants of Omoord, suburb of Rotterdam); adjusted age, sex, education, energy, vitamin E; mean age 68 y; followed for 6 y
Kamphuis 2006	4	2	3	9	10 y follow-up; ZES_ population based PC of healthy elderly men; no LTFU (0%)
Kaushik 2009	4	2	3	9	NHS I, NHS II, HPFS

Kim et al 2006	4	2	3	9	20 y of follow up
Koh 2015	4	2	3	9	Singapore Chinese Health Study, population recruitment; validated FFQ; some had CHD at baseline (about 3-5%) but estimates for those without dx at baseline; registry for deaths but "virtually complete"
Knekt 1990	4	1	2	7	enrolled women had no previous cancer; diet history method good, with reproducibility study; only adjusted for age; 20 y f/u and histological conf. of registry-ID cases; no dropout description
Kushi et al. 1985	2	0	3	5	Diet history; unclear if free from CHD at baseline; no adjustment for socioeconomic status, energy
Kushi et al. 1992	4	1	3	8	IWHS; detailed LTFU
Laaksonen et al. 2005	4	2	3	9	Random, age-stratified sample Eastern Finland; coefficient of variation 5%, 4-day diet records; validated deaths; no attrition
Leosdottir et al. 2005	4	1	2	7	no family history adjustment
Leosdottir et al. 2007	4	1	2	7	no family history adjustment
Li et al, 2015	4	2	3	9	NHS/HPFS
Linos 2010	4	2	3	9	NHS
Lof 2007	4	2	2	8	no description of loss-to-follow up
Lopez 2011	4	1	3	8	Rancho Bernardo; adjusted for age, sex and history of stroke, education
Lucas 2011	4	2	3	9	NHS; 10 y follow-up

Meyer et al. 2001	4	1	1	6	Iowa Women's Health Study; self-reports of diabetes and validation sub-study showed 36% over-reported diabetes (poor specificity a threat to validity); no family history adjustment
Miyagawa 2014	4	1	2	7	Japan - NIPPON; 24 y f/u; 3d weighted food records; did not adjust for total energy; record linkage only; described LTFU
Morris et al 1995	4	1	2	7	PHS; validated and reproducible for fish specifically; did not adjust for total energy; all endpoints reviewed by study physician; 4 y follow-up (>99.7%)
Morris et al 2003	4	1	3	8	Chicago Health and Aging Project; (74% participation); no Alzheimer's at baseline; south side Chicago residents; 3.9 y f/u in aged 65-94 is ok; f/u complete; adjusted for age, education but not baseline score or total energy
Mozaffarian 2003	4	1	3	8	did not adjust for total energy intake; 9.3 y f/u
Murff 2011	4	2	3	9	Shanghai WHS; 92% response; high follow-up (>97%); 8.9 y. cases found in registry verified by hospital chart review
Nagata et al., 2012	4	2	3	9	Takayama study; validated SQ-FFQ (Japanese tables of Food Composition)
Oh et al. 2005	4	1	2	7	Nurses' Health Study cohort; no socioeconomic status adjustment
Oomen 2001	3	2	1	6	10 y follow-up; ZES_ population based PC of healthy elderly men; no LTFU (0%)
Owen et al. 2016	4	2	2	8	12.6 y follow-up; for mortality, adjusted for age, sex, and total energy- which is probably ok; registry only; validated FFQ
Patel et al 2010	4	1	1	6	adjusted for age, family history, smoking; data on participation rate/dropout not clear

Pietinen et al. 1997	4	1	3	8	Finnish Alpha-Tocopherol, Beta-Carotene Cancer Prevention Study; no family history data collected, even acknowledged as a limitation
Posner et al. 1991	4	1	3	8	24h recall but validated; no adjustment for family history <2% attrition
Poudel-Tandukar 2011	4	2	2	8	JPHC (population-based sample with 80% participation rate); FFQ V and R; 11 y f/u; no details on LTFU
Salmeron et al. 2001	4	1	3	8	Nurses' Health Study cohort; no socioeconomic status adjustment
Sanchez-Villegas 2007	4	0	3	7	excluded depression at baseline; self-reported depression but self-report has been validated against MD diagnosis in subsample; university graduates; no adjustment for SES, family history of depression
Sauvaget et al. 2004	1	2	2	5	A-bomb survivors; used only 1-day diary (but well-trained); unclear of all free of cardiovascular disease at baseline; death certificates only
Schaefer 2006	4	1	2	7	Framingham HS; dementia diagnosed according to DSM IV; validated FFQ; 4 y follow-up; excluded dementia before 20th exam
Sczaniecka et al 2012	4	2	3	9	excluded BCA at baseline and pre- or peri-menopausal; LTFU <1%
Sanchez-Villegas 2011	4	0	3	7	excluded depression at baseline; self-reported depression but self-report has been validated against MD diagnosis in subsample; university graduates; no adjustment for SES, family history of depression
Schulze et al., 2008	4	1	3	8	EPIC-Potsdam; validated FFQ; response >90%; self-report confirmed by family MD; no adjustment for family history

Seino et al. 1997	4	0	3	7	Validated FFQ; all Japanese >40 y. eligible; no adjustment for smoking, socioeconomic status, family history
Shekelle et al, 1981	3	0	3	6	Diet history not validated; did not control for family history, socioeconomic status, or total energy intake
Shen 2011	4	1	2	7	Framingham HS; AF was validated by FHS cardiologists who reviewed and classified all available electrocardiograms from FHS clinic and outside records; validated FFQ; 4 y follow-up
Simila et al. 2012	4	1	3	8	Finnish Alpha-Tocopherol, Beta-Carotene Cancer Prevention Study; no family history data collected
Solfrizzi et al 2005	4	0	3	7	elderly cohort; no adjustment for energy nor trans fat; ILSA
Solfrizzi et al 2006	4	0	3	7	elderly cohort; no adjustment for energy nor trans fat; ILSA
Sonestedt 2007	4	1	1	6	MDC; validated SQFFQ; adjustment for total energy and age only; 9.5 y follow-up; no description of attrition; registry link only
Song et al. 2004	4	2	3	9	Representative sample; long follow-up; validated exposures and outcomes
Streppel 2008	4	2	2	8	dietary cross-check; validated; 40 y of follow-up; no details on follow-up
Strom 2012	4	1	1	6	DNBC; pregnant women only; registry deaths only; 8 y follow up; no description of dropouts; did not control for family history
Takata et al. 2013	4	2	2	8	death certificates only; 92-94% follow-up and 12 y f/u; SHANGHAI MHS + SHANGHAI WHS
Thiebaut et al 2007	4	1	1	6	AARP Diet and health study; 4.4 y follow-up; valid and reproducible FFQ; cases through registry linkage only; didn't adjust for height (alone- as BMI ok), or family history

Van Dam et al. 2002	4	1	2	7	Health Professionals' Follow-up Study cohort; no family history adjustment
Van den brandt et al 1993	4	1	1	6	case-cohort study (3,500 of 120,700 M+W aged 55-69) in Netherlands (population); registry cases only; did not adjust for PMH use; 3.3 y of f/u only
Van Woundenbergh 2009	4	1	3	8	Rotterdam study; response rate 78% (from all 10,275 inhabitants of Omoord, suburb of Rotterdam); adjusted for age, sex, education, energy, vitamin E; mean age 68 y; followed for 6 y
Vedtofte et al. 2011	4	2	3	9	Glostrup Population Studies (1914, MONICA-I, MONICA-III); those with previous CHD excluded; validations studies of events published- and good; 23 y f-u; no adjustment for trans fat
Velie 2000	4	2	3	9	BCDDP; validated Block FFQ; 5.3 y f/u
Vercambre et al. 2010	4	2	3	9	no trans fats; E3N
Virtanen 2008 AJCN	4	2	3	9	NHS I and II
Virtanen 2009 AJCN	3	1	3	7	Kuopio Ischemic Heart Disease Study; serum measures only; no adjustment for diet; linked data reviewed by study physician; 17.7 y; only n=3 people LTFU!
Virtanen 2014	4	2	2	8	Kuopio Ischemic Heart Disease Study; serum and diet measures; no adjustment for total energy linked data reviewed by study physician; 19.3 y; only n=3 people LTFU
Villegas 2011	4	2	2	8	Shanghai WHS; Shanghai MHS; 92% response W; 75% for M; high follow-up (>97%); cases self-report and confirmed by (33% not confirmed; but results similar); MHS = 4.1 y; WHS = 8.9 y

Villegas 2015	4	2	2	8	southern community cohort study; 5.5 y follow-up; lower SES; validated for antioxidants; link to National Death Index only
Vooripps et al 2002	4	2	2	8	Netherlands cohort study; validated FFQ; excluded prevalent cancers; no adjustment for PMH use (but age of menarche, menopause, etc.); registry cases only; no LTFU
Wakai et al, 2005	4	2	3	9	documented follow up; FFQ validated
Wakai et al., 2014	4	2	2	8	Response rate was 83%
Wallstrom et al., 2012	4	2	2	8	population based (men and women living In Malmo born 1923-1950) and living there in 1991-1996; validated FFQ; <1% LTFU; medical record review for cases
Wang et al. 2016	4	2	3	9	NHS and HPFS; selecting one profession helps homogenize SES
Wiberg et al. 2006	3	0	2	5	All men residing in Uppsala County eligible (82% participation); no reliability measures for fatty acids no adjustment for socioeconomic status, family history, energy; no independent adjudication
Wirfalt et al 2002	4	1	1	6	case-control study (but prospective exposure); did not control for family history; registry cases only; follow-up may be as low as 3 y for those recruited in 1996 (through 1999)
Wolfe et al 2009	3	1	3	7	NHEFS; 10 y; 1 single 24-h recall; incomplete adjustment
Wolk et al 1998	4	2	2	8	Validated FFQ; histologically confirmed cases; no description of dropouts
Xu et al. 2006	3	1	3	7	Cohort - Strong Heart Study; single 24-hr dietary recall; no socioeconomic status info
Yaemsiri et al. 2012	4	1	3	8	No family history adjustment

Yamagishi et al. 2008	4	1	2	7	JACC; 12.7 y of follow-up- FFQ validated- all death certificates reviewed (mandatory report) but what if death occurs outside Japan
Yuan et al, 2001	4	2	3	9	Shanghai; FFQ validated well; no history of cancer; 99% complete follow up and described LTFU; no adjustment for FU but did do risk factors; 12 y f/u

Table 4. GRADE Evidence Profile for prospective cohort studies of ω-3 PUFA and health outcomes.

Quality Assessment									Summary of Findings			
Exposure	**Outcome**	**Participants (# studies)**	**Risk of bias**	**Inconsistency**	**Indirectness**	**Imprecision**	**Publication bias**	**Overall quality of evidence**	**Study event rate (%)**	**Absolute -adjusted (per 10,000)[2]**	**Most-adjusted MV RR**	**Importance**
ω-3 PUFA	All-cause mortality	254,124 (6 studies)	Serious risk of bias[3]	Serious inconsistency[4]	No serious indirectness	Serious imprecision[5]	Unreliable to assess[6]	⊕OOO **VERY LOW[7]**	53,374/200,390 (21.1%)	28 fewer (from 113 fewer to 71 more)	0.98 (0.92 to 1.05)	CRITICAL
	CVD Total	17,810 (1 study)	Serious risk of bias[8]	Not assessed	No serious indirectness	Serious imprecision[9]	Not assessed	⊕OOO **VERY LOW[10]**	194/17,810 (1.1%)	517 more (from 879 fewer to 2,330 more)	1.10 (0.83 to 1.45)	CRITICAL
	CVD Fatal	314,876 (8 studies)	No serious risk of bias[11]	No serious inconsistency[12]	No serious indirectness	No serious imprecision[13]	No serious publication bias[14]	⊕OOO **VERY LOW[15]**	19,953/314,876 (6.3%)	28 fewer (from 60 fewer to 8 more)	0.93 (0.85 to 1.02)	CRITICAL

[2] See APPENDIX for sources of absolute event rates

[3] Median NOS=8 (Range: 7-9). Two studies that adjusted for trans-fat intake mvRR: 0.95 (95% CI: 0.92 to 0.99). Studies that did not, mvRR: 1.02 (95% CI: 0.90 to 1.16).

[4] I-squared=71%; 4 of 6 estimates consistent with protection; one study (Owen, 2016) shows statistically significant risk of harm (mvRR: 1.39)

[5] 95% CI crosses 1. Pooled estimate consistent with 8% decreased risk through 5% increased risk.

[6] Due to small number of studies (n<10) risk of publication bias not formally assessed.

[7] Prospective cohort studies start with GRADE of LOW. Downgraded for risk of bias, inconsistency, imprecision.

[8] NOS = 7 for this study. Did not adjust for total energy, and only 4 y. of follow-up (but >99% complete).

[9] 95% CI consistent with 17% decreased risk through 45% increased risk; <500 events.

[10] Prospective cohort studies start with GRADE of LOW. Downgraded for risk of bias, imprecision.

[11] Median NOS= 8.5 (range: 7 to 9). 2 studies measured trans-fats. In these studies, mvRR: 1.00 (0.81 to 1.24); in remaining 6 studies, mvRR: 0.88 (95% CI: 0.82 to 0.95)

[12] I-squared = 59%; removal of Harvard HPFS cohort (Wang 2016; 17.5% of total weight) results in significant mvRR estimate (0.89; 95% CI: 0.83 to 0.94). All 6 remaining comparisons have point estimates <1.0

[13] 95% CI crosses of RE estimate crosses 1 (consistent with 15% reduced risk through 2% increased risk). Removal of Wang (HPFS) or use of fixed-effect model results in narrow CI (consistent with between 17 and 6% reduced risk without Wang HPFS or between 12 and 2% reduced risk using all studies, fixed-effect)

[14] Egger's test P=0.973; Begg's test P=0.711

	CHD Fatal	249,756 (6 studies)	No serious risk of bias[16]	No serious inconsistency[17]	No serious indirectness	No serious imprecision[18]	Unable to reliably assess[19]	⊕⊕⊕◯ **MODERATE**[20]	4,160/249,756 (1.7%)	45 fewer (from 76 fewer to 11 fewer)	0.84 (0.73 to 0.96)	CRITICAL
	Sudden cardiac death	191,531 (3 studies)	No serious risk of bias[21]	No serious inconsistency[22]	No serious indirectness	No serious imprecision[23]	Unable to reliably assess[24]	⊕⊕◯◯ **LOW**[25]	529/191,531 (0.28%)	24 fewer (between 39 fewer and 5 fewer)	0.68 (0.50 to 0.93)	IMPORTANT
	Myocardial infarction total[26]	41,578 (1 study)	No serious risk of bias[27]	Not assessed	No serious indirectness	Serious imprecision[28]	Not assessed	⊕◯◯◯ **VERY LOW**[29]	221/41,578 (0.5%)	48 fewer (from 65 fewer to 18 fewer)	0.43 (0.24 to 0.78)	IMPORTANT
	Fatal myocardial infarction	57,972 (1 study)	No serious risk of bias[30]	Not assessed	No serious indirectness	Serious imprecision[31]	Unable to reliably assess[32]	⊕◯◯◯ **VERY LOW**[33]	329/57,972 (0.6%)	1 fewer (from 4 fewer to 0)	0.75 (0.57 to 0.93)	IMPORTANT

[15] Prospective cohort studies start with GRADE of LOW. Downgraded for risk of bias, imprecision.
[16] Median NOS=7.5 (range: 7 to 9). One study measured trans-fats (Hu 2002). Its removal does not alter the pooled estimate.
[17] I-squared = 13%. All point estimates consistent with protection.
[18] 95% CI consistent with between 27% and 4% reduced risk of fatal CVD; >500 events.
[19] Due to small number of studies (n<10) risk of publication bias not formally assessed.
[20] Prospective cohort studies start with GRADE of LOW. Not downgraded. Upgraded to MODERATE for dose-response. Assuming linearity, a 0.5% increase in n-3 PUFA was associated with an 18% reduced risk of CHD mortality (mvRR: 0.82, 95% CI: 0.66 to 1.01). Assuming linearity, a 2-g increase in n-3 PUFA was associated with a 31% reduced risk of CHD mortality (mvRR: 0.69, 95% CI: 0.44 to 1.08).
[21] Median NOS=8 (range: 7 to 9). Similar results in study that did measure trans fats (Chiuve) and that which did not (Yamagishi 2008).
[22] I-squared=0%. One study (Iso; weight=7.2%) shows non-significant increased harm (mvRR=1.24); while Chiuve and Yamagishi show significant protection (mvRR=0.65)
[23] 95% CI consistent with between 50% and 7% reduced risk of sudden cardiac death; >500 events.
[24] Due to small number of studies (n<10), risk of publication bias not formally assessed.
[25] Prospective cohort studies start with GRADE of LOW. Not downgraded.
[26] Study did not distinguish fatal from non-fatal (Iso, 2006, JPHC)
[27] NOS=8; no measurement of trans-fats
[28] 95% CI is consistent with a 76% through 22% decreased risk; <500 events.
[29] Prospective cohort studies start with GRADE of LOW. Downgraded for risk of imprecision.
[30] NOS=7; study failed to adjust for family history of CVD.
[31] 95% CI of estimate from this study consistent with 53% decreased risk through 19% increased risk; <500 cases.
[32] Not likely to be operating; several other major cohorts has published this finding, but typically as part of a composite endpoint (CVD or IHD or CHD) such that it cannot be assessed on its own.

	Nonfatal myocardial infarction	84,688 (1 study)	No serious risk of bias[34]	Not assessed	No serious indirectness	No serious imprecision[35]	Not assessed	⊕⊕OO **LOW**[36]	1,029/84,688 (1.2%)	14 fewer (from 25 fewer to 4 fewer)	0.73 (0.57 to 0.93)	IMPORTANT
	CHD Total	241,384 (8 studies)	No serious risk of bias[37]	Serious inconsistency[38]	No serious indirectness	Serious imprecision[39]	No serious publication bias[40]	⊕OOO **VERY LOW**[41]	4,515/241,834 (1.9%)	90 fewer (from 213 fewer to 65 more)	0.89 (0.74 to 1.08)	CRITICAL
	Stroke total	82,122 (2 studies)	Serious risk of bias[42]	Serious inconsistency[43]	No serious indirectness	Serious imprecision[44]	Unable to reliably assess	⊕OOO **VERY LOW**[45]	815/81,307 (0.99%)	7 fewer (from 24 fewer to 22 more)	0.85 (0.49 to 1.46)	IMPORTANT
	Stroke fatal	60,298 (1 study)	No serious risk of bias[46]	Not assessed	No serious indirectness	No serious imprecision[47]	Unable to reliably assess	⊕⊕OO **LOW**[48]	1,298/60,298 (2.2%)	72 fewer (from 136 fewer to 4 more)	0.82 (0.66 to 1.01)	IMPORTANT
	Stroke ischemic	100,513 (3 studies)	No serious	No serious inconsistency[50]	No serious indirectness	Serious imprecision[51]	Unable to reliably	⊕OOO **VERY**	1,058/100,513 (1.1%)	22 fewer (from 73	0.92 (0.73 to 1.15)	IMPORTANT

[33] Prospective cohort studies start with GRADE of LOW. Downgraded for risk of imprecision.
[34] NOS=9; 1 study in women (NHS)
[35] 95% CI ranges from 53% decreased risk to 7% decreased risk; >500 events
[36] Prospective cohort studies start with GRADE of LOW. Not downgraded.
[37] Median NOS=9 (range: 8 to 9); one study that measured trans fats (Hu) shows statistically significant mvRR: 0.69 (0.57 to 0.84); other do not: pooled mvRR: 0.95 (0.80 to 1.13)
[38] I-squared = 65%; 5 studies (58% weight) point estimated are protective; 3 are harmful (42% weight)
[39] 95% CI ranges from 26% decreased risk through 8% increased risk; >500 events. Fixed-effect estimate ranges from 11% decreased risk through 3% decreased risk
[40] Egger's test P=0.807; Begg's test P=1.00
[41] Prospective cohort studies start with GRADE of LOW. Downgraded for serious imprecision, inconsistency.
[42] 2 studies (Sieno; NOS=7 and Iso [2001]; NOS=8). Neither study reported or adjusted for trans-fats.
[43] I-squared = 38%; one study (Iso, 2001) shows statistically significant RR=0.72 and the other (Sieno, 1997) shows non-significant RR 1.37.
[44] 95% CI of pooled estimate consistent with 51% decrease through 46% increased risk; >500 events.
[45] Prospective cohort studies start with GRADE of LOW. Downgraded for imprecision and inconsistency.
[46] One study (NOS=9); no trans fat reporting.
[47] 95% CI crosses 1, but consistent with a 34% decreased to a 1% increased risk of event. Not downgraded; >1000 events.
[48] Prospective cohort studies start with GRADE of LOW. Not downgraded.

			risk of bias[49]				assess	**LOW**[52]		fewer to 41 more)		
	Stroke hemorrhagic	79,839 (1 study)	No serious risk of bias[53]	Not assessed	No serious indirectness	Serious imprecision[54]	Unable to reliably assess	⊕OOO **VERY LOW**[55]	181/79,839 (0.23%)	8 fewer (from 19 fewer to 15 more)	0.76 (0.43 to 1.36)	IMPORTANT
	Stroke thrombotic	79,839 (1 study)	No serious risk of bias[56]	Not assessed	No serious indirectness	Serious imprecision[57]	Unable to reliably assess	⊕OOO **VERY LOW**[58]	264/79,839 (0.33%)	10 fewer (from 20 fewer to 2 more)	0.67 (0.42 to 1.07)	IMPORTANT
	Atrial fibrillation	32,214 (1 study)	No serious risk of bias[59]	Not assessed	No serious indirectness	Serious imprecision[60]	Unreliable to assess[61]	⊕OOO **VERY LOW**[62]	1,441/32,214 (4.3%)	2 more (from 7 fewer to 14 more)	1.05 (0.80 to 1.38)	IMPORTANT
	Type 2 diabetes	51,928 (3 studies)	No serious risk of bias[63]	Serious inconsistency[64]	No serious indirectness	Serious imprecision[65]	Unable to reliably assess[66]	⊕OOO **VERY LOW**[67]	2,762/51,928 (5.3%)	11 fewer (213 fewer to 308 more)	0.98 (0.62 to 1.55)	CRITICAL

[50] I-squared = 19%
[51] 95% CI crosses 1, consistent with a 27% decreased through 15% increased risk; no study CI excluded 1.
[49] Median NOS = 9 (range: 8-9). No studies quantified trans fats.
[52] Prospective cohort studies start with GRADE of LOW. Downgraded for imprecision.
[53] 1 study, NOS = 9. Trans fats measured.
[54] 95% CI crosses 1; consistent with a 57% decreased risk through 36% increased risk; <500 events.
[55] Prospective cohort studies start with GRADE of LOW. Downgraded for imprecision.
[56] 1 study, NOS = 9. Trans fats measured.
[57] 95% CI crosses 1; consistent with a 56% decreased risk through 7% increased risk; <500 events.
[58] Prospective cohort studies start with GRADE of LOW. Downgraded for imprecision.
[59] 1 study, NOS=9 with adjustment for trans-fats
[60] 95% CI from this study ranges from 20% decreased risk to 38% increased risk.
[61] Due to small number of studies (n<10) risk of publication bias not formally assessed.
[62] Prospective cohort studies start with GRADE of LOW. Downgraded for imprecision.
[63] All 3 studies NOS = 8; none adjusted for Trans –fats.
[64] I-squared = 78%; one study (Broslow) finds significant protection (mvRR: 0.78); one study (Alhazmi) finds significant harm.
[65] 95% CI consistent with 38% decreased risk through 55% increased risk; >2000 cases
[66] Due to small number of studies (n<10) risk of publication bias not formally assessed.
[67] Prospective cohort studies start with GRADE of LOW. Downgraded for imprecision, inconsistency.

	Dementia	6,522 (3 studies)	No serious risk of bias[68]	No serious inconsistency[69]	No serious indirectness	Serious imprecision[70]	Unable to assess reliably[71]	⊕OOO **VERY LOW**[72]	379/6,522 (5.8%)	88 fewer (from 307 fewer to 253 more)	0.86 (0.54 to 1.39)	IMPORTANT
	Cognitive decline	4,809 (1 study)	No serious risk of bias[73]	Not assessed	No serious indirectness	No serious imprecision[74]	Unable to assess reliably[75]	⊕⊕OO **LOW**[76]	598/4,809 (12.4%)	277 fewer (from 494 fewer to 13 fewer)	0.79 (0.63 to 0.99)	IMPORTANT
	Depression	7,903 (1 study)	No serious risk of bias[77]	Not assessed	No serious indirectness	Serious imprecision[78]	Not assessed	⊕OOO **VERY LOW**[79]	406/7,497 (5.1%)	280 more (from 1,715 fewer to 4,551 more)	1.08 (0.51 to 2.30)	IMPORTANT
	Crohn's Disease	0 (0 studies)	-	-	-	-	-	-	-	-	-	IMPORTANT
	Ulcerative colitis	170,918 (2 studies)	No serious risk of bias[80]	No serious inconsistency[81]	No serious indirectness	Serious imprecision[82]	Unable to assess reliably[83]	⊕OOO **VERY LOW**[84]	360/170,918 (0.21%)	2 fewer (from 8 fewer to 6 more)	0.67 (0.27 to 1.65)	IMPORTANT

[68] Median NOS = 8 (range: 6 to 8)
[69] I-squared = 44%
[70] 95% CI includes 54% decreased risk through 39% increased risk; <500 studies
[71] Due to small number of studies (n<10) risk of publication bias not formally assessed.
[72] Prospective cohort studies start with GRADE of LOW. Downgraded for imprecision.
[73] 1 study; NOS =9
[74] 95% CI consistent with from 37% to 1% decrease; >500 events.
[75] Due to small number of studies (n<10) risk of publication bias not formally assessed.
[76] Prospective cohort studies start with GRADE of LOW. Not downgraded.
[77] NOS=7; measured trans-fats.
[78] 95% CI includes 49% decreased risk through 130% increased risk; <500 cases.
[79] Prospective cohort studies start with GRADE of LOW. Downgraded for imprecision.
[80] 2 studies; NOS = 9 (Ananthakrishnahan; adjusted for trans-fats) and 7 (John; no adjustment for trans fats).
[81] I-squared = 47%; both point estimates < 1.0

	All breast cancer	61,903 (2 studies)	No serious risk of bias[85]	No serious inconsistency[86]	No serious indirectness	Serious imprecision[87]	Unable to assess reliably[88]	⊕◯◯◯ **VERY LOW**[89]	443/61,903 (0.72%)	1 fewer (from 3 fewer to 0)	0.82 (0.63 to 1.07)	CRITICAL
	Premenopausal breast cancer	0 (0 studies)	-	-	-	-	-	-	-	-	-	CRITICAL
	Postmenopausal breast cancer	18,524 (2 studies)	Serious risk of bias[90]	Serious inconsistency[91]	No serious indirectness	Serious imprecision[92]	Unable to assess reliably[93]	⊕◯◯◯ **VERY LOW**[94]	313/18,524 (1.7%)	63 more (from 46 fewer to 271 more)	1.37 (0.73 to 2.59)	CRITICAL

[82] 95% CI crosses 1; consistent with 73% reduced risk through 65% increased risk. Fixed-Effect estimate: 0.83 (95% CI: 0.60 to 1.16)
[83] Due to small number of studies (n<10) risk of publication bias not formally assessed.
[84] Prospective cohort studies start with GRADE of LOW. Downgraded for imprecision.
[85] 2 studies; NOS = 7-9; Gago-Dominguez did not adjust for trans-fats
[86] I-squared = 0%; both point estimates consistent with protection.
[87] 95% CI consistent with 37% decreased risk through 7% increased risk; N<500 events
[88] Due to small number of studies (n<10) risk of publication bias not formally assessed.
[89] Prospective cohort studies start with GRADE of LOW. Downgraded for imprecision.
[90] 2 studies; NOS=9 for Wakai (2005), but 6 for Wirfalt (2002); Wirfalt did not adjust for some key covariates, and cases were identified through registry link only (no review by study team)
[91] I-squared = 54%; one study consistent with 81% increased risk (95% CI: 9 to 300%); the other finds non-significant protection (6% decreased risk; from 54% reduced risk through 92% increased risk)
[92] 95% CI consistent with 27% decreased risk through 159% increased risk.
[93] Due to small number of studies (n<10) risk of publication bias not formally assessed.
[94] Prospective cohort studies start with GRADE of LOW. Downgraded for imprecision, inconsistency.

Table 5. GRADE Evidence Profile for prospective cohort studies of long-chain ω-3 PUFA and health outcomes.

Quality Assessment									Summary of Findings			
Exposure	**Outcome**	**Participants (# studies)**	**Risk of bias**	**Inconsistency**	**Indirectness**	**Imprecision**	**Publication bias**	**Overall quality of evidence**	**Study event rate (%)**	**Absolute-adjusted (per 10,000)[95]**	**Most-adjusted MV RR**	**Importance**
Long chain ω-3 PUFA	All-cause mortality	485,078 (9 studies)	No serious risk of bias[96]	No serious inconsistency[97]	No serious indirectness	No serious imprecision[98]	Not detected[99]	⊕⊕⊕◯ **MODERATE[100]**	58,799/485,078 (12.1%)	99 fewer (from 169 fewer to 14 fewer)	0.93 (0.88 to 0.99)	CRITICAL
	CVD Total	110,242 (3 studies)	Serious risk of bias[101]	No serious inconsistency [102]	No serious indirectness	Serious imprecision [103]	Unable to assess reliably[104]	⊕◯◯◯ **VERY LOW[105]**	4,741/110,242 (4.3%)	500 fewer (from 1,821 fewer to 1,547 more)	0.89 (0.60 to 1.34)	CRITICAL

[95] See APPENDIX for sources of absolute event rates

[96] Median NOS=9 (Range: 7-9). Three studies that adjusted for trans-fat intake mvRR: 0.95 (95% CI: 0.90 to 1.01). Studies that did not, mvRR: 0.93 (95% CI: 0.84 to 1.04).

[97] I-squared=62%; 7 of 9 estimates consistent with protection; two studies (2 arms from Nagata, 2012; 14.5% weight) shows small, non-significant risk of harm (mvRR: 1.04; P=0.62)

[98] Pooled estimate consistent with 12% decreased risk through 1% decreased risk.

[99] P-value = 0.917 (Begg's test); 0.521 (Egger's)

[100] Prospective cohort studies start with GRADE of LOW. Not downgraded. Upgraded to MODERATE for dose-response. Assuming linearity, a 0.5-g increase in long chain n-3 PUFA was associated with an 8% reduced risk of all-cause mortality (mvRR: 0.92, 95% CI: 0.87 to 0.98). Assuming linearity, a 0.5% increase in long chain n-3 PUFA was associated with an 8% reduced risk of all-cause mortality (mvRR: 0.92, 95% CI: 0.87 to 0.98).

[101] Median NOS=7 (range: 6 to 9). One study (Strom) which showed significant protection (mvRR: 0.52) was at risk of bias due to recruitment of pregnant women only, failure to adjust for family history, and for a poor description of follow-up

[102] Removal of Strom reduces heterogeneity to 0%

[103] 95% CI of pooled estimate consistent with 40% reduced risk through 34% increased risk; n>4000 cases.

[104] Due to small number of studies (n<10), publication bias not formally assessed.

[105] Prospective cohort studies begin with GRADE of LOW. Downgraded for imprecision, risk of bias. Assuming linearity, a 0.5-g increase in long chain n-3 PUFA was associated with an 11% reduced risk of CVD mortality (mvRR: 0.894, 95% CI: 0.802 to 0.996). Assuming linearity, a 0.5% increase in long chain n-3 PUFA was associated with a 20% reduced risk of CVD mortality (mvRR: 0.80, 95% CI: 0.63 to 1.01).

	CVD Fatal	470,113 (10 studies)	Serious risk of bias[106]	Serious inconsistency [107]	No serious indirectness	Serious imprecision [108]	No evidence of publication bias[109]	⊕○○○ **VERY LOW**[110]	17,668/470,113 (3.8%)	44 fewer (from 84 fewer to 4 more)	0.89 (0.79 to 1.01)	CRITICAL
	CHD Fatal	349,586 (10 studies)	No serious risk of bias[111]	No serious inconsistency [112]	No serious indirectness	No serious imprecision [113]	No evidence of publication bias[114]	⊕⊕⊕○ **MODERATE**[115]	5,904/349,586 (1.7%)	53 fewer (from 90 fewer to 8 fewer)	0.81 (0.68 to 0.97)	CRITICAL
	Sudden cardiac death	93,632 (3 studies)	No serious risk of bias[116]	No serious inconsistency [117]	No serious indirectness	Serious imprecision [118]	Unable to assess reliably[119]	⊕○○○ **VERY LOW**[120]	545/93,632 (0.58%)	41 fewer (from 64 fewer to 5 more)	0.45 (0.19 to 1.07)	IMPORTANT
	Myocardial infarction total	21,385 (1 study)	Serious risk of bias[121]	Not assessed	No serious indirectness	Not assessed	Unable to assess reliably[122]	⊕○○○ **VERY LOW**[123]	281/21,385 (1.3%)	17 more (from 17 fewer to	1.20 (0.80 to 1.80)	IMPORTANT

[106] Median NOS = 7.5 (range: 7 to 9); 3 studies (Bell, Wang HPFS, Wang NHS) adjusted for trans-fats. In these studies, mvRR: 1.02 (0.86 to 1.21); in remaining: 0.83 (0.74 to 0.94)—overall pooled association still protective.

[107] I-squared = 72%; 7 of 10 studies point estimates protective but only 3 significant.

[108] 95% CI of pooled estimate consistent with 21% decreased through 1% increased risk of fatal CVD.

[109] Begg's test P= 0.721; Egger's test P=0.411

[110] Prospective cohort studies begin with GRADE of LOW. Downgraded for risk of bias, inconsistency, and imprecision.

[111] Median NOS = 8 (range: 7 to 9); 2 studies measured trans-fats (Pietinen, Bell). In these studies, mvRR=0.90 (0.19 to 1.07); in the remaining 8, mvRR=0.79 (0.67 to 0.93)

[112] I-squared=77%; 8 of 10 studies have point estimates consistent with benefit (4 significant).

[113] 95% CI consistent with 32 to 3% decreased risk; >5000 events

[114] Begg's test P=0.283; Egger's test P=0.164

[115] Prospective cohort studies begin with GRADE of LOW. Not downgraded. Upgraded to MODERATE for dose-response. Assuming linearity, a 0.5-g increase in long chain n-3 PUFA was associated with a 14% reduced risk of CHD mortality (mvRR: 0.86, 95% CI: 0.78 to 0.95). Assuming linearity, a 0.5% increase in long chain n-3 PUFA was associated with a 26% reduced risk of CHD mortality (mvRR: 0.74, 95% CI: 0.60 to 0.90).

[116] Median NOS = 8 (range: 8 to 9); study that did not adjust for trans-fats also had the fewest events. Its point estimate in the same direction as the other 2 studies.

[117] I-squared = 60%; all studies have point estimates consistent with >30% reduced risk.

[118] 95% CI of pooled estimate consistent with 81% reduced risk through 7% increased risk (random-effects); fixed-effect consistent with 58% to 18% reduced risk.

[119] Due to small number of studies (n<10), publication bias not formally assessed.

[120] Prospective cohort studies begin with GRADE of LOW. Downgraded for imprecision.

[121] NOS=7; failed to adjust for total energy

[122] Due to small number of studies (n<10), publication bias not formally assessed.

										68 more)		
	Fatal myocardial infarction	18,244 (1 study)	No serious risk of bias[124]	Not assessed	No serious indirectness	No serious imprecision [125]	Unable to assess reliably[126]	⊕⊕OO **LOW[127]**	113/18,244 (0.62%)	2 fewer (from 8 fewer to 0)	0.43 (0.23 to 0.81)	IMPORTANT
	Nonfatal myocardial infarction	12,195 (2 studies)	No serious risk of bias[128]	Not assessed	No serious indirectness	Serious imprecision [129]	Unable to assess reliably	⊕OOO **VERY LOW[130]**	442/12,195 (3.6%)	3 fewer (from 21 fewer to 19 more)	0.96 (0.74 to 1.24)	IMPORTANT
	CHD Total	45,881 (5 studies)	No serious risk of bias[131]	No serious inconsistency [132]	No serious indirectness	Serious imprecision [133]	Unable to assess reliably[134]	⊕OOO **VERY LOW[135]**	2,891/45,881 (6.3%)	36 fewer (from 144 fewer to 96 more)	0.94 (0.76 to 1.16)	CRITICAL
	Stroke total	28,429 (3 studies)	No serious risk of bias[136]	Serious inconsistency [137]	No serious indirectness	Serious imprecision [138]	Unable to assess reliably[139]	⊕OOO **VERY LOW[140]**	296/28,429 (1.0%)	10 fewer (from 23 fewer to 9 more)	0.79 (0.52 to 1.18)	IMPORTANT

[123] Prospective cohort studies begin with GRADE of LOW. Downgraded for risk of bias.
[124] NOS = 9, but did not adjust for trans fats.
[125] 95% CI consistent with 77% through 19% decreased risk; n=113 events. Not downgraded because 19% decreased risk still important.
[126] Due to small number of studies (n<10), publication bias not formally assessed.
[127] Prospective cohort studies begin with GRADE of LOW. Not downgraded.
[128] 2 studies; NOS = 7 and 8; neither study adjusted for trans-fats
[129] 95% CI consistent with 26% decreased risk through 24% increased risk; <500 events.
[130] Prospective cohort studies begin with GRADE of LOW. Downgraded for imprecision.
[131] All studies NOS =9; all measured trans-fats.
[132] 5 studies; 3 point estimates below 1.0; 2 above. None statistically significant.
[133] 95% CI consistent with 24% reduced risk through 16% increased risk.
[134] Due to small number of studies (n<10), publication bias not formally assessed.
[135] Prospective cohort studies begin with GRADE of LOW. Downgraded for imprecision.
[136] Median NOS= 9 (range: 7 to 9). Two studies (1 publication, 2 arms) that measured trans fat showed non-significant benefit of PUFA (mvRR=0.67; 95% CI: 0.38 to 1.17); that which did not showed no effect (1.01; 95% CI: 0.65 to 1.59).
[137] I-squared = 45%. Two studies (1 publication, 2 arms) that measured trans fat showed non-significant benefit of PUFA (mvRR=0.67; 95% CI: 0.38 to 1.17); that which did not showed no effect (1.01; 95% CI: 0.65 to 1.59).
[138] 95% CI consistent with 48% reduced risk through 18% increased risk; <500 events.
[139] Due to small number of studies (n<10), publication bias not formally assessed.
[140] Prospective cohort studies begin with GRADE of LOW. Downgraded for inconsistency, imprecision.

	Stroke fatal	129,568 (5 studies)	No serious risk of bias[141]	No serious inconsistency [142]	No serious indirectness	Serious imprecision [143]	Unable to assess reliably[144]	⊕OOO **VERY LOW[145]**	2,414/129,568 (1.9%)	5 fewer (from 11 fewer to 2 more)	0.90 (0.79 to 1.04)	IMPORTANT
	Stroke ischemic	40,743 (4 studies)	No serious risk of bias[146]	No serious inconsistency [147]	No serious indirectness	Serious imprecision [148]	Unable to reliably assess[149]	⊕OOO **VERY LOW[150]**	899/40,743 (2.2%)	0 (from 51 fewer to 68 more)	1.00 (0.81 to 1.25)	IMPORTANT
	Stroke hemorrhagic	20,069 (2 studies)	No serious risk of bias[151]	No serious inconsistency [152]	No serious indirectness	Serious imprecision [153]	Unable to reliably assess[154]	⊕OOO **VERY LOW[155]**	47/20,069 (0.23%)	31 fewer (from 49 fewer to 0)	0.39 (0.15 to 1.00)	IMPORTANT
	Stroke thrombotic	0 (0 studies)			-	-	-	-	-	-	0.39 (0.15 to 1.00)	IMPORTANT
	Atrial fibrillation	79,169 (5 studies)	No serious risk of bias[156]	Serious inconsistency [157]	No serious indirectness	Serious imprecision [158]	Unable to reliably assess[159]	⊕OOO **VERY LOW[160]**	2,978/79,169 (3.8%)	1 more (from 7 fewer to 10 more)	1.02 (0.82 to 1.28)	IMPORTANT

[141] Median NOS = 7 (range: 7 to 9)
[142] I-squared =0; 4/5 point estimates < 1.0
[143] 95% CI consistent with a 21% decreased risk through 4% increased risk; n>2400 events.
[144] Due to small number of studies (n<10), publication bias not formally assessed.
[145] Prospective cohort studies begin with GRADE of LOW. Downgraded for imprecision.
[146] All studies had NOS =9; Wallstrom did not adjust for trans-fat; de Goede did; pooled results in opposite directions (Wallstrom: 1.08; 95% CI: 0.85 to 1.38 and de Goede: 0.75; 95% CI: 0.46 to 1.22).
[147] I-squared = 0%
[148] 95% CI consistent with 19% decreased risk through 25% increased risk.
[149] Due to small number of studies (n<10), publication bias not formally assessed.
[150] Prospective cohort studies begin with GRADE of LOW. Downgraded for imprecision.
[151] NOS = 9 for both studies
[152] I-squared = 0% 95% CI consistent with 85% reduced risk through 0%; n=47 events.
[153] 95% CI consistent with 85% reduced risk through 0%; n=47 events.
[154] Due to small number of studies (n<10), publication bias not formally assessed.
[155] Prospective cohort studies begin with GRADE of LOW. Downgraded for imprecision.
[156] Median NOS = 8 (range: 7 to 9); 1 study that measured trans fats (Brouwer) showed results compatible with other 3 studies.
[157] I-squared = 71%; 2 studies (Virtanen, Gronroos) show possible benefit (pooled mvRR: 0.78, 95% CI: 0.53 to 1.16); 3 show possible harm (pooled mvRR: 1.24; 95% CI: 1.05 to 1.47)

	Heart failure	5,300 (1 study)	No serious risk of bias[161]	Not assessed	No serious indirectness	Serious imprecision [162]	Not assessed	⊕OOO **VERY LOW[163]**	669/5,300 (12.6%)	222 fewer (from 640 fewer to 285 more)	0.89 (0.69 to 1.14)	IMPORTANT
	Type 2 diabetes	479,647 (12 studies)	No serious risk of bias[164]	Serious inconsistency [165]	No serious indirectness	Serious imprecision [166]	No evidence of publication bias[167]	⊕OOO **VERY LOW[168]**	22,513/479,647 (4.7%)	50 more (from 11 fewer to 118 more)	1.09 (0.98 to 1.21)	IMPORTANT
	Dementia	0 (0 studies)	-	-	-	-	-	-	-	-	-	-
	Cognitive decline	6,284 (2 studies)	Serious risk of bias[169]	No serious inconstancy[170]	No serious indirectness	Serious imprecision [171]	Unable to reliably assess[172]	⊕OOO **VERY LOW[173]**	824/6,284 (13.1%)	501 fewer (from 961 fewer to 489 more)	0.62 (0.28 to 1.37)	IMPORTANT

[158] 95% CI of pooled estimate consistent with 18% lower through 28% higher risk.
[159] Due to small number of studies (n<10), publication bias not formally assessed.
[160] Prospective cohort studies begin with GRADE of LOW. Downgraded for inconsistency, imprecision.
[161] NOS= 9; study adjusted for trans-fats
[162] 1 study; 95% CI consistent with a 31% decreased through 14% increased risk; >500 events.
[163] Prospective cohort studies begin with GRADE of LOW. Downgraded for imprecision.
[164] Median NOS = 8 (range: 6 to 9); the two low quality studies (NOS=6) reported significantly increased risk of LC-n3 PUFA (1.20; 1.04 to 1.39) but weight in overall analysis <15%; mvRR in remaining studies: 1.07 (0.95 to 1.20)
[165] I-squared = 77%; 7 of 10 studies have point estimates >1.0 (3 statistically significant- Kaushik (NHS I and II), Djousse); 1 of 3 (Villegas 2011) showing protection was statistically significant.
[166] 95% CI consistent with 2% decreased risk through 21% increased risk.
[167] Begg's test P=0.244; Egger's test P=0.365
[168] Prospective cohort studies begin with GRADE of LOW. Downgraded for inconsistency, imprecision. Assuming linearity, a 0.5-g increase in long chain n-3 PUFA was associated with a 19% increased risk of type 2 diabetes (mvRR: 1.19, 95% CI: 1.12 to 1.27). Assuming linearity, a 0.5% increase in energy from long chain n-3 PUFA was associated with a 46% increased risk of type 2 diabetes (mvRR: 1.46, 95% CI: 1.31 to 1.64).
[169] 2 studies; NOS of Vercambre =9; of Gao=6 (failure to adjust sufficiently for covariates, very high LTFU).
[170] I-squared = 71%; 2 studies only and both point estimates <0.9
[171] 95% CI consistent with 72% decreased risk through 37% increased risk. Fixed-effect model consistent with 35% decreased risk through 0% increased risk; >500 events.
[172] Due to small number of studies (n<10), publication bias not formally assessed.

[173] Prospective cohort studies begin with GRADE of LOW. Downgraded for risk of bias, imprecision.

	Depression	57,949 (3 studies)	No serious risk of bias[174]	No serious inconsistency [175]	No serious indirectness	Serious imprecision [176]	Unable to reliably assess[177]	⊕OOO **VERY LOW**[178]	3,567/57,949 (6.2%)	245 fewer (from 630 fewer to 175 more)	0.93 (0.82 to 1.05)	IMPORTANT
	Suicide	101,507 (2 studies)	No serious risk of bias[179]	No serious inconsistency [180]	No serious indirectness	Serious imprecision [181]	Unable to reliably assess[182]	⊕OOO **VERY LOW**[183]	298/101,507 (0.29%)	0 (from 0 to 1 more)	1.02 (0.69 to 1.50)	IMPORTANT
	Crohn's Disease	171,168 (2 studies)	No serious risk of bias[184]	No serious inconsistency [185]	No serious indirectness	Serious imprecision [186]	Unable to reliably assess[187]	⊕OOO **VERY LOW**[188]	342/171,168 (0.20%)	0 (from 3 fewer to 1 more)	0.85 (0.59 to 1.23)	IMPORTANT
	Ulcerative colitis	170,805 (1 study)	No serious risk of bias[189]	Not assessed	No serious indirectness	Serious imprecision [190]	Not assessed	⊕OOO **VERY LOW**[191]	338/170,805 (0.20%)	1 fewer (from 4 fewer to 0)	0.72 (0.52 to 1.00)	IMPORTANT
	All breast cancer	266,408 (6 studies)	No serious	Serious inconsistency	No serious indirectness	Serious imprecision	Unable to reliably	⊕OOO	5,158/266,408	7 fewer (from 13	0.82 (0.66 to	CRITICAL

[174] Median NOS = 7 (range: 7 to 9)
[175] I-squared = 0%; all point estimates protective.
[176] 95% CI crosses 1; pooled estimate consistent with 18% decreased though 5% increased risk; >500 events
[177] Due to small number of studies (n<10), publication bias not formally assessed.
[178] Prospective cohort studies begin with GRADE of LOW. Downgraded for risk of bias, imprecision.
[179] NOS = 8 for both studies; no measure of trans fats
[180] I-squared = 0%; both studies show now association
[181] 95% CI crosses 1; pooled estimate consistent with 31% decreased though 50% increased risk; <500 events
[182] Due to small number of studies (n<10), publication bias not formally assessed.
[183] Prospective cohort studies begin with GRADE of LOW. Downgraded for risk of imprecision.
[184] Median NOS: 8.5 (range: 8 to 9); 1 study measured trans fat, the other did not.
[185] I-squared=0%; both 95% CI overlap
[186] 95% CI of pooled estimate consistent with 41% decreased through 23% increased risk; <500 events.
[187] Due to small number of studies (n<10), publication bias not formally assessed.
[188] Prospective cohort studies begin with GRADE of LOW. Downgraded for risk of imprecision.
[189] NOS =9; trans fat measured
[190] 95% CI of estimate includes 1; consistent with 48% reduced risk through 0 change); <500 events.
[191] Prospective cohort studies begin with GRADE of LOW. Downgraded for risk of imprecision.

			risk of bias[192]	[193]		[194]	assess[195]	**VERY LOW**[196]	(1.9%)	fewer to 1 more)	1.02)	
	Premenopausal breast cancer	179,450 (2 studies)	No serious risk of bias[197]	No serious inconsistency [198]	No serious indirectness	Serious imprecision [199]	Unable to reliably assess[200]	⊕◯◯◯ **VERY LOW**[201]	1,498/179,450 (0.83%)	3 more (from 2 fewer to 9 more)	1.09 (0.96 to 1.23)	CRITICAL
	Postmenopausal breast cancer	106,333 (2 studies)	No serious risk of bias[202]	Serious inconsistency [203]	No serious indirectness	Serious imprecision [204]	Unable to reliably assess[205]	⊕◯◯◯ **VERY LOW**[206]	1,989/106,333 (1.9%)	31 fewer (from 102 fewer to 112 more	0.82 (0.40 to 1.66)	CRITICAL

[192] Median NOS = 9 (range: 7 to 9); 2 studies adjusted for trans fats (Holmes, Bell)
[193] I-squared = 77%. 2 studies adjusted for trans fats (Holmes, Bell) and mvRR in these studies: 1.08 (1.03 to 1.13); mvRR in remaining 4: 0.76 (0.61 to 0.94). Further, RE estimate: 0.82 (95% CI: 0.66 to 1.02) with 29% of weight carried by Holmes (1999), but FE estimate: 1.05 (95% CI: 1.00 to 1.09) with 89% of weight carried by Holmes (1999).
[194] 95% CI (of RE model) consistent with 34% reduced through 2% increased risk; 95% CI (of FE model) consistent with 0% through 9% increased risk; >5000 cases.
[195] Due to small number of studies (n<10), publication bias not formally assessed.
[196] Prospective cohort studies begin with GRADE of LOW. Downgraded for risk of imprecision, inconsistency.
[197] Both studies had NOS = 9 and measured trans fat.
[198] I-squared = 0%; both studies point estimates >1.0
[199] 95% CI of summary estimate consistent with 4% decreased through 23% increased risk; >1400 events.
[200] Due to small number of studies (n<10), publication bias not formally assessed.
[201] Prospective cohort studies begin with GRADE of LOW. Downgraded for risk of imprecision, inconsistency.
[202] Both studies had NOS = 9; Holmes reported trans fat but Wakai did not.
[203] I-squared = 77%; Holmes finds 9% increased risk (P<0.05) but Wakai finds 48% decreased risk (from 74% decreased through 4% increased); >1900 events.
[204] RE estimate: 0.82 (95 % CI: 0.40 to 1.66) [weight: 61.5% Holmes; 38.5% Wakai]; FE estimate: 1.08 (95% CI: 1.01 to 1.16) [Weight: 99% Holmes; 1% Wakai]
[205] Due to small number of studies (n<10), publication bias not formally assessed.
[206] Prospective cohort studies begin with GRADE of LOW. Downgraded for risk of imprecision, inconsistency.

Table 6. GRADE Evidence Profile for prospective cohort studies of Eicosapentaenoic acid (EPA) and health outcomes.

Quality Assessment									Summary of Findings			
Exposure	**Outcome**	**Participants (# studies)**	**Risk of bias**	**Inconsistency**	**Indirectness**	**Imprecision**	**Publication bias**	**Overall quality of evidence**	**Study event rate (%)**	**Absolute -adjusted (per 10,000)[207]**	**Most-adjusted MV RR**	**Importance**
Eicosapentaenoic acid	All-cause mortality	134,296 (1 study)	No serious risk of bias[208]	Not assessed	No serious indirectness	No serious imprecision[209]	Not assessed	⊕⊕◯◯ **LOW[210]**	5,836/134,296 (4.4%)	296 fewer (from 395 fewer to 183 fewer)	0.79 [0.72, 0.87]	CRITICAL
	CVD Total	0 (0 studies)	-	-	-	-	-	-	-	-		CRITICAL
	CVD Fatal	134,296 (1 study)	No serious risk of bias[211]	Not assessed	No serious indirectness	No serious imprecision[212]	Not assessed	⊕⊕◯◯ **LOW[213]**	1,789/134,296 (1.3%)	99 fewer (from 148 fewer to 40 fewer)	0.75 [0.63, 0.90]	CRITICAL
	CHD Fatal	135,669 (2 studies)	No serious	No serious inconsistency[215]	No serious indirectness	Serious imprecision[216]	Unable to assess	⊕◯◯◯ **VERY**	824/135,669 (0.61%)	61 fewer (from 115	0.78 [0.59,	CRITICAL

[207] See APPENDIX for sources of absolute event rates
[208] NOS = 8; no adjustment for trans fat.
[209] 95% CI of estimate consistent with 28% through 13% reduced risk; n>5000 deaths.
[210] Prospective cohort studies begin with GRADE of LOW. Not downgraded.
[211] NOS = 8; no adjustment for trans fat.
[212] 95% CI of estimate consistent with 37% through 10% reduced risk; n>1700 events
[213] Prospective cohort studies begin with GRADE of LOW. Not downgraded.

			risk of bias[214]				reliably[217]	**LOW[218]**		fewer to 6 more)	1.02]	
	Sudden cardiac death	0 (0 studies)	-	-	-	-	-	-	-	-	-	IMPORTANT
	Myocardial infarction total	0 (0 studies)	-	-	-	-	-	-	-	-	-	IMPORTANT
	Fatal myocardial infarction	0 (0 studies)	-	-	-	-	-	-	-	-	-	IMPORTANT
	Nonfatal myocardial infarction	0 (0 studies)	-	-	-	-	-	-	-	-	-	IMPORTANT
	Fatal arrhythmia	0 (0 studies)	-	-	-	-	-	-	-	-	-	IMPORTANT
	CHD Total	94,894 (4 studies)	No serious risk of bias[219]	No serious inconsistency[220]	No serious indirectness	Serious imprecision[221]	Unable to assess reliably[222]	⊕◯◯◯ **VERY LOW[223]**	1,733/94,894 (1.8%)	36 fewer (from 132 fewer to 84 more)	0.94 [0.78, 1.14]	CRITICAL
	Stroke total	2,313 (1 study)	Serious risk of bias[224]	Not assessed	No serious indirectness	Serious imprecision[225]	Not assessed	⊕◯◯◯ **VERY LOW[226]**	421/2,313 (18.2%)	58 more (from 42 fewer to 166	1.07 [0.95, 1.20]	IMPORTANT

[215] I-squared = 0%; both studies point estimates <1.0
[216] 95% CI of summary estimate consistent with 41% decreased through 2% increased risk; n>800 events.
[214] NOS of both studies = 8; neither study reported trans fats.
[217] Due to small number of studies (n<10), publication bias not formally assessed.
[218] Prospective cohort studies begin with GRADE of LOW. Downgraded for imprecision.
[219] Median NOS=8.5 (range: 8 to 9); no study adjusted for trans fat.
[220] I-squared = 26%; 3 of 4 point estimates <1.0 but none exclude small benefit or harm.
[221] 95% CI of summary estimate consistent with 22% decreased through 14% increased risk; n>1700 cases.
[222] Due to small number of studies (n<10), publication bias not formally assessed.
[223] Prospective cohort studies begin with GRADE of LOW. Downgraded for imprecision.
[224] NOS=5; did not report validity/reliability measures for fatty acids, did not independently adjudicate cases, and did not adjust for important confounders: socioeconomic status, family history, and energy
[225] 95% CI of study estimate consistent with 5% reduced through 20% increased risk; n<500 events.
[226] Prospective cohort studies begin with GRADE of LOW. Downgraded for risk of bias, imprecision.

										more)		
	Stroke fatal	0 (0 studies)	-	-	-	-	-	-	-	-	-	IMPORTANT
	Stroke ischemic (fatal)	134,296 (1 study)	No serious risk of bias[227]	Not assessed	No serious indirectness	No serious imprecision[228]	Not assessed	⊕⊕◯◯ **LOW**[229]	404/134,296 (0.3%)	22 fewer (from 37 fewer to 6 fewer)	0.56 [0.36, 0.87]	IMPORTANT
	Stroke hemorrhagic (fatal)	134,296 (1 study)	No serious risk of bias[230]	Not assessed	No serious indirectness	Serious imprecision[231]	Not assessed	⊕◯◯◯ **VERY LOW**[232]	460/134,296 (0.34%)	6 fewer (from 13 fewer to 4 more)	0.81 [0.58, 1.13]	IMPORTANT
	Stroke thrombotic	0 (0 studies)	-	-	-	-	-	-	-	-	-	IMPORTANT
	Atrial fibrillation	50,051 (3 studies)	No serious risk of bias[233]	No serious inconsistency[234]	No serious indirectness	Serious imprecision[235]	Unable to assess reliably[236]	⊕◯◯◯ **VERY LOW**[237]	3,285/50,051 (6.6%)	2 fewer (from 7 fewer to 3 more)-	0.94 [0.82, 1.07]	IMPORTANT
	Heart failure	0 (0 studies)	-	-	-	-	-	-	-	-	-	IMPORTANT
	Type 2 diabetes	45,480 (3 studies)	Serious risk of bias[238]	Serious inconsistency[239]	No serious indirectness	Serious imprecision[240]	Unable to assess reliably[241]	⊕◯◯◯ **VERY**	2,880/45,480 (6.3%)	78 more (from 106 fewer to	1.14 [0.81, 1.61]	IMPORTANT

[227] NOS = 8; no adjustment for trans fats.
[228] 95% CI of estimate consistent with 64% through 13% reduced risk; <500 events.
[229] Prospective cohort studies begin with GRADE of LOW. Downgraded for imprecision.
[230] NOS = 8; no adjustment for trans fats.
[231] 95% CI of estimate consistent with 42% decreased through 13% increased risk; <500 events.
[232] Prospective cohort studies begin with GRADE of LOW. Downgraded due to imprecision.
[233] Median NOS = 9 (range: 7 to 9). Only Chiuve adjusted for trans fats.
[234] I-squared = 0%; all CI's overlap.
[235] 95% CI of summary estimate consistent with 18% reduced through 7% increased risk.
[236] Due to small number of studies (n<10), publication bias not formally assessed.
[237] Prospective cohort studies begin with GRADE of LOW. Downgraded due to imprecision.
[238] Median NOS = 6 (range: 6 to 8). Two studies (Patel, Alhazmi) have NOS =6.

								LOW[242]		342 more)		
	Dementia	815 (1 study)	No serious risk of bias[243]	Not assessed	No serious indirectness	Serious imprecision[244]	Not assessed	⊕OOO **VERY LOW**[245]	131/815 (16.1%)	92 fewer (from 585 fewer to 1,086 more)	0.90 [0.38, 2.16]	IMPORTANT
	Cognitive decline	0 (0 studies)	-	-	-	-	-	-	-	-	-	IMPORTANT
	Depression	3,317 (2 studies)	No serious risk of bias[246]	No serious inconsistency[247]	No serious indirectness	Serious imprecision[248]	Unable to assess reliably[249]	⊕OOO **VERY LOW**[250]	744/3,317 (22.4%)	595 fewer (from 1,296 fewer to 315 more)	0.83 [0.63, 1.09]	IMPORTANT
	Suicide	101,507 (2 studies)	No serious risk of bias[251]	No serious inconsistency[252]	No serious indirectness	Serious imprecision[253]	Unable to assess reliably[254]	⊕OOO **VERY LOW**[255]	298/101,507 (0.29%)	0 (from 0 to 2 more)	1.11 [0.74, 1.65]	IMPORTANT

[239] I-squared = 67%; Djousse adjusted for trans fats, and found significant increased risk (mvRR: 1.38; 95% CI: 1.20 to 1.58); Alhazmi and Patel (NOS = 6) find opposite associations (0.66; Patel) and (1.24; Alhazmi)—neither statistically significant.
[240] 95% CI consistent with 19% reduced through 61% increased risk; n>2800 cases. Fixed-effect model yields mvRR = 1.32 (95% CI: 1.16 to 1.49).
[241] Due to small number of studies (n<10), publication bias not formally assessed.
[242] Prospective cohort studies begin with GRADE of LOW. Downgraded due to imprecision, risk of bias, and inconsistency.
[243] NOS = 8 with measurement of trans fats.
[244] 95% CI of estimate consistent with 62% reduced through 116% increased risk; <200 events.
[245] Prospective cohort studies begin with GRADE of LOW. Downgraded due to imprecision.
[246] NOS = 7; no adjustment for trans fats.
[247] 1 study, estimated separately for M and W (both results similar)
[248] 95% CI of summary estimate consistent with 37% decreased through 9% increased risk; n>700 events.
[249] Due to small number of studies (n<10), publication bias not formally assessed.
[250] Prospective cohort studies begin with GRADE of LOW. Downgraded due to imprecision.
[251] NOS = 9; adjustment for trans fats.
[252] 1 study presents estimates separately for M and W; both effects suggest no association.
[253] 95% CI of pooled estimate consistent with 26% reduced through 65% increased risk; n<300 cases.
[254] Due to small number of studies (n<10), publication bias not formally assessed.
[255] Prospective cohort studies begin with GRADE of LOW. Downgraded due to imprecision.

	Crohn's Disease	362 (1 study)	No serious risk of bias[256]	Not assessed	No serious indirectness	Serious imprecision[257]	Unable to assess reliably[258]	⊕OOO **VERY LOW**[259]	73/362 (20.2%)[260]	24 more (from 0 to 436 more)	8.56 [0.88, 83.07]	IMPORTANT
	Ulcerative colitis	203,193 (1 study)	No serious risk of bias[261]	Not assessed	No serious indirectness	Serious imprecision[262]	Unable to assess reliably[263]	⊕OOO **VERY LOW**[264]	126/203,193 (0.06%)	12 more (from 3 fewer to 90 more)	2.58 [0.66, 10.07]	IMPORTANT
	All breast cancer	107,771 (2 studies)	No serious risk of bias[265]	Serious inconsistency[266]	No serious indirectness	Serious imprecision[267]	Unable to assess reliably[268]	⊕OOO **VERY LOW**[269]	2,869/107,771 (2.7%)	25 fewer (from 86 fewer to 67 more)	0.88 [0.59, 1.32]	IMPORTANT
	Premenopausal breast cancer	0 (0 studies)	-	-	-	-	-	-	-	-		IMPORTANT
	Postmenopausal breast cancer	1,598 (1 study)	No serious risk of bias[270]	Not assessed	No serious indirectness	Serious imprecision[271]	Unable to assess reliably[272]	⊕OOO **VERY LOW**[273]	941/1,598 (58.9%)[274]	3 fewer (from 48 fewer to 58 more)	0.98 [0.72, 1.34]	IMPORTANT

[256] NOS = 8; no adjustment for trans fats.
[257] 95% CI of study estimate consistent with 12% decreased through 8,207% increased risk; n<100 cases.
[258] Due to small number of studies (n<10), publication bias not formally assessed.
[259] Prospective cohort studies begin with GRADE of LOW. Downgraded due to imprecision.
[260] Prospective case-control study
[261] NOS = 8; no adjustment for trans fats.
[262] 95% CI of study estimate consistent with 34% decreased through 907% increased risk; n<200 cases.
[263] Due to small number of studies (n<10), publication bias not formally assessed.
[264] Prospective cohort studies begin with GRADE of LOW. Downgraded due to imprecision.
[265] 2 studies, NOS =9 for both; Holmes adjusted for trans fats, Sczaniecka did not.
[266] Holmes (1999) finds significantly increased risk (mvRR: 1.06; 95% CI: 1.02 to 1.10); Sczaniecka (mvRR: 0.70; 95% CI: 0.54 to 0.90). FE model mvRR = 1.05 (1.01 to 1.09; 98% of weight taken by Holmes)
[267] 95% CI of summary estimate consistent with 41% decreased through 32% increased risk; n>2800 cases.
[268] Due to small number of studies (n<10), publication bias not formally assessed.
[269] Prospective cohort studies begin with GRADE of LOW. Downgraded due to imprecision, inconsistency.
[270] NOS =8 with adjustment for trans fats.
[271] 95% CI of estimate consistent with 28% decreased risk through 34% increased risk; n>900 cases.
[272] Due to small number of studies (n<10), publication bias not formally assessed.
[273] Prospective cohort studies begin with GRADE of LOW. Downgraded due to imprecision.
[274] Prospective case-control study

Table 7. GRADE Evidence Profile for prospective cohort studies of Docosahexaenoic acid (DHA) and health outcomes.

Quality Assessment									Summary of Findings			
Exposure	**Outcome**	**Participants (# studies)**	**Risk of bias**	**Inconsistency**	**Indirectness**	**Imprecision**	**Publication bias**	**Overall quality of evidence**	**Study event rate (%)**	**Absolute -adjusted (per 10,000)[275]**	**Most-adjusted MV RR**	**Importance**
Docosahexaenoic acid	All-cause mortality	134,296 (1 study)	No serious risk of bias[276]	Not assessed	No serious indirectness	No serious imprecision[277]	Not assessed	⊕⊕◯◯ **LOW[278]**	5,836/134,296 (4.35%)	310 fewer (409 fewer to 197 fewer)	0.78 [0.71, 0.86]	CRITICAL
	CVD Total	0 (0 studies)	-	-	-	-	-	-	-	-		CRITICAL
	CVD Fatal	134,296 (1 study)	No serious risk of bias[279]	Not assessed	No serious indirectness	No serious imprecision[280]	Not assessed	⊕⊕◯◯ **LOW[281]**	1,789/134,296 (1.33%)	95 fewer (from 144 fewer to 36 fewer)	0.76 [0.64, 0.91]	CRITICAL
	CHD Fatal	134,296 (1 study)	No serious risk of bias[282]	Not assessed	No serious indirectness	Serious imprecision[283]	Not assessed	⊕◯◯◯ **VERY LOW[284]**	476/134,296 (0.35%)	59 fewer (from 121 fewer to 25 more)	0.79 [0.57, 1.09]	CRITICAL

[275] See APPENDIX for sources of absolute event rates
[276] NOS = 8; no adjustment for trans fats.
[277] 95% CI of estimate consistent with 29% through 14% decreased risk; >5,000 events
[278] Prospective cohort studies begin with GRADE of LOW. Not downgraded.
[279] NOS = 8; no adjustment for trans fats.
[280] 95% CI of estimate consistent with 36% through 9% decreased risk; >1,700 events
[281] Prospective cohort studies begin with GRADE of LOW. Not downgraded.
[282] NOS = 8; no adjustment for trans fats.
[283] 95% CI of estimate consistent with 43% decreased through 9% increased risk; <500 events
[284] Prospective cohort studies begin with GRADE of LOW. Downgraded for imprecision.

	Sudden cardiac death	0 (0 studies)	-	-	-	-	-	-	-	-	-	IMPORTANT
	Myocardial infarction total	0 (0 studies)	-	-	-	-	-	-	-	-	-	IMPORTANT
	Fatal myocardial infarction	0 (0 studies)	-	-	-	-	-	-	-	-	-	IMPORTANT
	Nonfatal myocardial infarction	0 (0 studies)	-	-	-	-	-	-	-	-	-	IMPORTANT
	Fatal arrhythmia	0 (0 studies)	-	-	-	-	-	-	-	-	-	IMPORTANT
	CHD Total	94,894 (4 studies)	No serious risk of bias[285]	No serious inconsistency[286]	No serious indirectness	Serious imprecision[287]	Unreliable to assess[288]	⊕◯◯◯ **VERY LOW**[289]	1,733/94,894 (1.83%)	42 fewer (from 126 fewer to 60 more)	0.93 [0.79, 1.10]	CRITICAL
	Stroke total	2,313 (1 study)	Serious risk of bias[290]	Not assessed	No serious indirectness	Serious imprecision[291]	Not assessed	⊕◯◯◯ **VERY LOW**[292]	421/2,313 (18.2%)	0 (from 4 fewer to 6 more)	1.01 [0.91, 1.12]	IMPORTANT
	Stroke fatal	0 (0 studies)	-	-	-	-	-	-	-	-	-	IMPORTANT
	Stroke ischemic (fatal)	134,296 (1 study)	No serious risk of bias[293]	Not assessed	No serious indirectness	No serious imprecision[294]	Not assessed	⊕⊕◯◯ **LOW**[295]	404/134,296 (0.3%)	122 fewer (from 174 to 43	0.55 [0.36, 0.84]	IMPORTANT

[285] NOS = 8 and 9; neither study assessed trans fats.
[286] I-squared = 0%; ¾ point estimates <1.0; all 95% CI overlap
[287] 95% CI consistent with 21% decreased through 10% increased risk; n>1700 events.
[288] Due to small number of studies (n<10) risk of publication bias not formally assessed.
[289] Prospective cohort studies begin with GRADE of LOW. Downgraded for imprecision.
[290] NOS=5; did not report validity/reliability measures for fatty acids, did not independently adjudicate cases, and did not adjust for important confounders: socioeconomic status, family history, and energy
[291] 95% CI of study estimate consistent with 9% reduced through 12% increased risk; n<500 events.
[292] Prospective cohort studies begin with GRADE of LOW. Downgraded for risk of bias, imprecision.
[293] NOS = 8; no adjustment for trans fats.

										fewer)		
	Stroke hemorrhagic (fatal)	134,296 (1 study)	No serious risk of bias[296]	Not assessed	No serious indirectness	Serious imprecision[297]	Not assessed	⊕◯◯◯ **VERY LOW**[298]	460/134,296 (0.34%)	2 fewer (from 16 fewer to 25 more)	0.95 [0.50, 1.81]	IMPORTANT
	Stroke thrombotic	0 (0 studies)	-	-	-	-	-	-	-	-	-	IMPORTANT
	Atrial fibrillation	50,051 (3 studies)	No serious risk of bias[299]	Serious inconsistency[300]	No serious indirectness	Serious imprecision[301]	Unreliable to assess[302]	⊕◯◯◯ **VERY LOW**[303]	3,285/50,051 (6.56%)	4 fewer (from 8 fewer to 1 more)	0.89 [0.79, 1.02]	IMPORTANT
	Heart failure	0 (0 studies)	-	-	-	-	-	-	-	-	-	IMPORTANT
	Type 2 diabetes	45,480 (3 studies)	Serious risk of bias[304]	Serious inconsistency[305]	No serious indirectness	Serious imprecision[306]	Unable to assess reliably[307]	⊕◯◯◯ **VERY LOW**[308]	2,880/45,480 (6.33%)	73 more (from 146 fewer to 415 more)	1.13 [0.74, 1.74]	IMPORTANT
	Dementia	1,980 (3	No serious	No serious	No serious	No serious	Unable to assess	⊕⊕◯◯	272/1,980	549 fewer	0.40 [0.24,	IMPORTANT

[294] 95% CI of estimate consistent with 64% through 16% decreased risk; <500 events
[295] Prospective cohort studies begin with GRADE of LOW. Not downgraded.
[296] NOS = 8; no adjustment for trans fats.
[297] 95% CI of estimate consistent with 50% decreased through 81% increased risk; <500 events
[298] Prospective cohort studies begin with GRADE of LOW. Downgraded for imprecision.
[299] Median NOS = 9 (range: 7 to 9); 1 study (Chiuve) adjusted for trans fats
[300] I-squared = 62%; 1 study (Virtanen) found significant protection (mvRR: 0.58, 95% CI: 0.39 to 0.87); other two (Gronroos, Chiuve) find no association (mvRR: 0.94, 95% CI: 0.82 to 1.08)
[301] 95% CI of summary estimate consistent with 37% decreased through 13% increased risk (R-E); fixed-effect estimate: 0.89 (95% CI: 0.79 to 1.02).
[302] Due to small number of studies (n<10) risk of publication bias not formally assessed.
[303] Prospective cohort studies begin with GRADE of LOW. Downgraded for imprecision, inconsistency.
[304] Median NOS = 6 (range: 6 to 8). Two studies (Patel, Alhazmi) have NOS =6.
[305] I-squared = 78%; Djousse adjusted for trans fats, and found significant increased risk (mvRR: 1.52; 95% CI: 1.33 to 1.74); Alhazmi and Patel (NOS = 6) find opposite associations (0.63; Patel) and (1.19; Alhazmi)—neither statistically significant.
[306] 95% CI consistent with 26% reduced through 74% increased risk; n>2800 cases. Fixed-effect model yields mvRR = 1.42 (95% CI: 1.25 to 1.61; 85% of weight to Djousse).
[307] Due to small number of studies (n<10), publication bias not formally assessed.
[308] Prospective cohort studies begin with GRADE of LOW. Downgraded due to imprecision, risk of bias, and inconsistency.

		studies)	risk of bias[309]	inconsistency[310]	indirectness	imprecision[311]	reliably[312]	**LOW[313]**	(13.74%)	(from 774 fewer to 278 fewer)	0.67]	
	Cognitive decline	0 (0 studies)	-	-	-	-	-	-	-	-	-	IMPORTANT
	Depression	3,317 (2 studies)	No serious risk of bias[314]	No serious inconsistency[315]	No serious indirectness	Serious imprecision[316]	Unable to assess reliably[317]	⊕OOO **VERY LOW[318]**	744/3,317 (22.43%)	735 fewer (from 1,401 fewer to 140 more)	0.79 [0.60, 1.04]	IMPORTANT
	Suicide	101,507 (2 studies)	No serious risk of bias[319]	No serious inconsistency[320]	No serious indirectness	Serious imprecision[321]	Unable to assess reliably[322]	⊕OOO **VERY LOW[323]**	298/101,507 (0.29%)	0 (from 0 to 2 more)	1.09 [0.74, 1.60]	IMPORTANT
	Crohn's Disease	362 (1 study)	No serious risk of bias[324]	Not assessed	No serious indirectness	Serious imprecision[325]	Unable to assess reliably[326]	⊕OOO **VERY LOW[327]**	73/362 (20.17%)	3 fewer (from 13 fewer to 1 fewer)	0.06 [0.01, 0.51]	IMPORTANT

[309] Median NOS = 8 (range: 7 to 8); Morris adjusted for trans fats.
[310] I-squared = 0%; all studies point estimates contained by each other's 95% CI. Study that adjusted for trans fats (Morris) observed similar association as other 2 studies.
[311] 95% CI of summary estimate consistent with 33% through 76% reduced risk; n =272 events.
[312] Due to small number of studies (n<10), publication bias not formally assessed.
[313] Prospective cohort studies begin with GRADE of LOW. Not downgraded.
[314] NOS = 7; no adjustment for trans fats.
[315] 1 study, estimated separately for M and W (both results similar)
[316] 95% CI of summary estimate consistent with 40% decreased through 4% increased risk; n>700 events.
[317] Due to small number of studies (n<10), publication bias not formally assessed.
[318] Prospective cohort studies begin with GRADE of LOW. Downgraded due to imprecision.
[319] NOS = 9; adjustment for trans fats.
[320] 1 study presents estimates separately for M and W; both effects suggest no association.
[321] 95% CI of pooled estimate consistent with 26% reduced through 60% increased risk; n<300 cases.
[322] Due to small number of studies (n<10), publication bias not formally assessed.
[323] Prospective cohort studies begin with GRADE of LOW. Downgraded due to imprecision.
[324] NOS = 8; no adjustment for trans fats.
[325] 95% CI of study estimate consistent with 49% decreased through 99% increased risk; n<100 cases.

	Ulcerative colitis	203,193 (1 study)	No serious risk of bias[328]	Not assessed	No serious indirectness	Serious imprecision[329]	Unable to assess reliably[330]	⊕OOO **VERY LOW**[331]	126/203,193 (0.06%)	6 fewer (from 15 fewer to 0)	0.23 [0.06, 0.92]	IMPORTANT
	All breast cancer	107,791 (2 studies)	No serious risk of bias[332]	Serious inconsistency[333]	No serious indirectness	Serious imprecision[334]	Unable to assess reliably[335]	⊕OOO **VERY LOW**[336]	2,869/107,791 (2.66%)	31 fewer (from 94 fewer to 65 more)	0.85 [0.55, 1.31]	IMPORTANT
	Premenopausal breast cancer	0 (0 studies)	-	-	-	-	-	-	-	-		IMPORTANT
	Postmenopausal breast cancer	1,598 (1 study)	No serious risk of bias[337]	Not assessed	No serious indirectness	Serious imprecision[338]	Unable to assess reliably[339]	⊕OOO **VERY LOW**[340]	941/1,598 (58.89%)	0 (from 48 fewer to 65 more)	1.00 [0.72, 1.38]	IMPORTANT

[326] Due to small number of studies (n<10), publication bias not formally assessed.
[327] Prospective cohort studies begin with GRADE of LOW. Downgraded due to imprecision.
[328] NOS = 8; no adjustment for trans fats.
[329] 95% CI of study estimate consistent with 8% through 94% decreased risk; n<200 cases.
[330] Due to small number of studies (n<10), publication bias not formally assessed.
[331] Prospective cohort studies begin with GRADE of LOW. Downgraded due to imprecision.
[332] 2 studies, NOS =9 for both; Holmes adjusted for trans fats, Sczaniecka did not.
[333] Holmes (1999) finds significantly increased risk (mvRR: 1.04; 95% CI: 1.02 to 1.07); Sczaniecka (mvRR: 0.67; 95% CI: 0.52 to 0.87). FE model mvRR = 1.04 (1.01 to 1.06; 99% of weight taken by Holmes)
[334] 95% CI of summary estimate consistent with 45% decreased through 31% increased risk; n>2800 cases.
[335] Due to small number of studies (n<10), publication bias not formally assessed.
[336] Prospective cohort studies begin with GRADE of LOW. Downgraded due to imprecision, inconsistency.
[337] NOS =8 with adjustment for trans fats.
[338] 95% CI of estimate consistent with 28% decreased risk through 38% increased risk; n>900 cases.
[339] Due to small number of studies (n<10), publication bias not formally assessed.
[340] Prospective cohort studies begin with GRADE of LOW. Downgraded due to imprecision.

Table 8. GRADE Evidence Profile for prospective cohort studies of docosapentaenoic acid (DPA) and health outcomes.

Quality Assessment									Summary of Findings			
Exposure	**Outcome**	**Participants (# studies)**	**Risk of bias**	**Inconsistency**	**Indirectness**	**Imprecision**	**Publication bias**	**Overall quality of evidence**	**Study event rate (%)**	**Absolute -adjusted (per 10,000)[341]**	**Most-adjusted MV RR**	**Importance**
docosapentaenoic acid	All-cause mortality	0 (0 studies)	-	-	-	-	-	-	-	-		CRITICAL
	CVD Total	0 (0 studies)	-	-	-	-	-	-	-	-		CRITICAL
	CVD Fatal	0 (0 studies)	-	-	-	-	-	-	-	-		CRITICAL
	CHD Fatal	0 (0 studies)	-	-	-	-	-	-	-	-	-	CRITICAL
	Sudden cardiac death	0 (0 studies)	-	-	-	-	-	-	-	-		IMPORTANT
	Myocardial infarction total	0 (0 studies)	-	-	-	-	-	-	-	-	-	IMPORTANT
	Fatal myocardial infarction	0 (0 studies)	-	-	-	-	-	-	-	-	-	IMPORTANT
	Nonfatal myocardial infarction	0 (0 studies)	-	-	-	-	-	-	-	-	-	IMPORTANT
	Fatal arrhythmia	0 (0 studies)	-	-	-	-	-	-	-	-	-	IMPORTANT
	CHD Total	53,803 (2 studies)	No serious	No serious inconsistency[343]	No serious indirectness	Serious imprecision[344]	Not assessed	⊕◯◯◯ **VERY**	1,124/53,803	54 fewer (from 168	0.91 (0.72 to	CRITICAL

[341] See APPENDIX for sources of absolute event rates

			risk of bias[342]					**LOW**[345]	(2.1%)	fewer to 90 more)	1.15)	
	Stroke total	0 (0 studies)	-	-	-	-	-	-	-	-	-	IMPORTANT
	Stroke fatal	0 (0 studies)	-	-	-	-	-	-	-	-	-	IMPORTANT
	Stroke ischemic (fatal)	0 (0 studies)	-	-	-	-	-	-	-	-	-	IMPORTANT
	Stroke hemorrhagic (fatal)	0 (0 studies)	-	-	-	-	-	-	-	-	-	IMPORTANT
	Stroke thrombotic	0 (0 studies)	-	-	-	-	-	-	-	-	-	IMPORTANT
	Atrial fibrillation	2,174 (1 study)	No serious risk of bias[346]	Not assessed	No serious indirectness	Serious imprecision[347]	Not assessed	⊕◯◯◯ **VERY LOW**[348]	1,124/53,803 (2.1%)	2 fewer (from 13 fewer to 15 more)	0.95 (0.65 to 1.39)	IMPORTANT
	Heart failure	0 (0 studies)	-	-	-	-	-	-	-	-	-	IMPORTANT
	Type 2 diabetes	0 (0 studies)	-	-	-	-	-	-	-	-	-	IMPORTANT
	Dementia	0 (0 studies)	-	-	-	-	-	-	-	-	-	IMPORTANT
	Cognitive decline	0 (0 studies)	-	-	-	-	-	-	-	-	-	IMPORTANT

[343] Study presented associations for M and W separately; I-squared =0%
[344] 95% CI of study estimate consistent with 28% reduced through 15% increased risk; n>1000 events.
[342] NOS = 8; no adjustment for trans fats.
[345] Prospective cohort studies begin with GRADE of LOW. Downgraded for imprecision.
[346] NOS =7; no adjustment for trans fats.
[347] 95% CI of study estimate consistent with 35% reduced through 39% increased risk; n<300 events.
[348] Prospective cohort studies begin with GRADE of LOW. Downgraded for imprecision.

	Depression	0 (0 studies)	-	-	-	-	-	-	-	-	-	IMPORTANT
	Suicide	0 (0 studies)	-	-	-	-	-	-	-	-	-	IMPORTANT
	Crohn's Disease	0 (0 studies)	-	-	-	-	-	-	-	-	-	IMPORTANT
	Ulcerative colitis	0 (0 studies)	-	-	-	-	-	-	-	-	-	IMPORTANT
	All breast cancer	0 (0 studies)	-	-	-	-	-	-	-	-	-	IMPORTANT
	Premenopausal breast cancer	0 (0 studies)	-	-	-	-	-	-	-	-	-	IMPORTANT
	Postmenopausal breast cancer	0 (0 studies)	-	-	-	-	-	-	-	-	-	IMPORTANT

APPENDIX: Methodology and sources for estimates of absolute risks.

Absolute risk was estimated using the method of Newcombe et al. (*Evid Based Med 2014;19;6-8*).

Lifetime estimates of baseline risk of premature all-cause mortality, CHD mortality, CVD mortality, total CHD, and type 2 diabetes and the associated 95% confidence levels, were obtained from various studies. The Emerging Risk Factors Consortium (*Lancet 2010 Jun 26;375(9733):2215-22*) which included 691,872 people from 102 prospective studies. Overall, the mean age of participants at entry was 52 (SD 13) years, and 297,081 (43%) were women. (96%) were in Europe, North America, and Australasia, with the remainder in Japan or the Caribbean. These risks were 11.4% (11.2% to 11.6%) for total mortality; 2.0% (1.9% to 2.2%) for CHD mortality, 4.2% (4.1% to 4.4%) for total CHD; and 5.6% (5.5% to 5.8%) for type 2 diabetes.

Lifetime risk of total CVD was obtained from Lloyd-Jones (Framingam Heart Study; Circulation. 2006;113:791-798) to be: 51.7% (49.3 to 54.2%) in men; and 39.2 (37.0 to 41.4) in women; both groups: 45.5 (43.2 to 47.8). The risk for sudden cardiac death was 0.75% (0.58 to 0.92%) from Fishman et al. (Circulation 2010;122;2335-2348) who used estimates from U.S.A. and Europe; for total stroke it was 0.48% (95% CI: 0.475 to 0.481), and fatal stroke 0.24% (95% CI: 0.236 to 0.243.

Lifetime risk of ischemic stroke taken from Framingham Heart Study (U.S.A.; *Seshadri et al., Stroke. 2006 Feb;37(2):345-50. Epub 2006 Jan 5*.). Risk of atrial fibrillation taken from Chugh et al (Circulation, 2013:113) to be 373/100,000 in women (348 to 402) and 596 (558 to 637) in men.

Lifetime risk of dementia taken from Framingham Heart Study (U.S.A.; Seshadri *et al., Neurology, 1997 Dec 49(6):498-504*): men= 6.3% (3.9 to 8.7%); women=12% (9.2 to 14.8%). Risk of cognitive decline (surrogate- mild cognitive impairment) taken from Li, C-Y (China; Industrial Health, 2002, 40(7-13): 13.2% (11.7 to 14.7%).

Lifetime risk of depression set at 35% (95% CI: 34.1 to 35.8%), using estimates from Kruijshaar et al. (*Eur J Epidemiol. 2005;20(1):103-11*), using data from The Netherlands Mental Health Survey and Incidence Study (NEMESIS) and the Australian Adult Mental Health and Wellbeing Survey. Estimates for IBD (Crohn's Disease and UC) taken from European Prospective cohort study (EPIC; *Eur J Clin Nutr. 2017 Apr;71(4):512-518*): CD= 0.03% and (0.01% to 0.14%); UC=0.075% (0.001% to 0.19%).

Lifetime risk of breast cancer taken from the Pooling Project of Cancer (Smith-Warner et al., *Int J Cancer 2001(92):767-774*): Total = 20.8% (19.4% to 22.3%). Assumed 18.5% of cases are pre-menopausal: 3.9% (2.6 to 5.1%), and 82.5% are post-menopausal: 17.0 (15.6 to 18.4%) (Ghiasvand et al., *BMC Cancer. 2014; 14: 343*).

Lifetime risk of total, fatal, and non-fatal MI from George et al. (*Circulation. 2015 Oct 6;132(14):1320-8*), using data from the Cardiovascular Research Using LInked Bespoke Studies and Electronic Records [CALIBER] research platform MI: 0.84% (0.73 to 0.95%); fatal MI: 0.03 (0.0001 to 0.015); non-fatal MI: 0.81% (0.69 to 0.92%).

Lifetime risk of hemorrhagic stroke estimated from Feigin et al. (*Lancet Neurol. 2009;8(4):355-69*), taken to be 15% of all strokes: 0.3% (0.2 to 0.4%), risk of fatal stroke to be 0.5% (95% CI: 0.30 to 0.71%) from Lund Haheim et al. (*J Epi. Comm. Health, 1996:50:621-24*).

Lifetime risk of heart failure estimated from Framingham Heart study (Lloyd-Jones et al., *Circulation. 2002 Dec 10;106(24):3068-72*) and set at 20.2% (16.1 to 24.2%).

Lifetime risk of suicide estimated from GBD 2014 Mortality and Causes of Death Collaborators (*Lancet*. 2015 Jan 10;385(9963):117-71. doi: 10.1016/S0140-6736(14)61682-2. Epub 2014 Dec 18.) as 0.01% (0.009% to 0.11%).

APPENDIX 2: DOSE-RESPONSE META-ANALYSIS

Objective: To evaluate the dose-response relationship between PUFA and health outcomes using summarized data from prospective cohorts.

Methods: The dose-response relation was estimated by using generalized least squares trend estimation, according to the methods developed by Greenland and Longnecker, implemented in STATA (v. 13, College Station, TX) using the "glst"package written by Orsini[48 49]. The goodness-of-fit test was used to assess whether the linearity assumption was tenable. If the P for this test was <0.05, the interpretation is that a straight line is not a good fit to the data; if P>0.05 then a straight line is an adequate fit. If the straight line was not a good fit (i.e. goodness-of-fit test P<0.05), we used a piecewise-polynomial approach, which assumes linear associations across certain ranges of doses ("segments"), the boundaries of which are called knots. We applied restricted cubic splines to create three equally-spaced knots across the distribution to model the association between knots. The procedure described by Orsini and Greenland was finally used to estimate the pooled RRs for increments of specific exposure values, using the "*lincom*" command in STATA.

Results: Figures 1-42 display the generalized least squares trend estimates for exposure-outcome associations that showed significant associations in the extreme-quantile analyses.

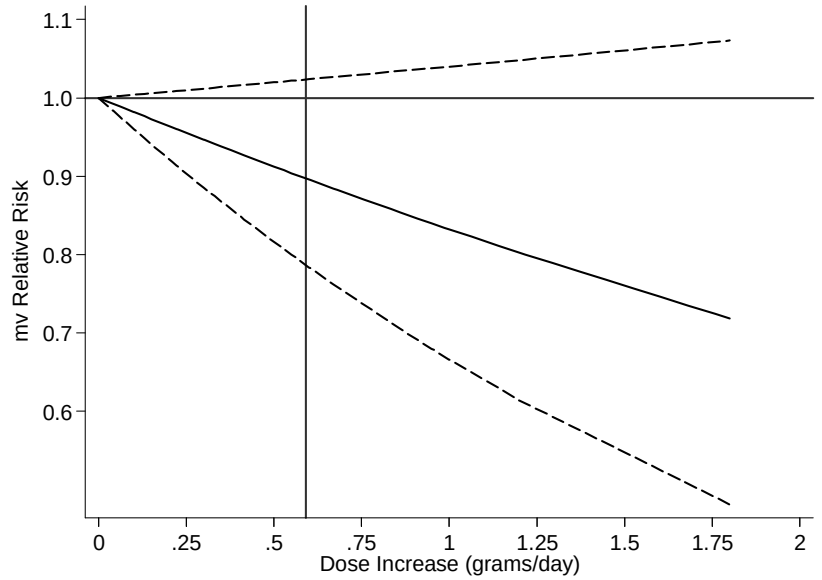

Figure 11. Dose-response association between total n-3 PUFA (g/d) and most-adjusted RR of CHD mortality in 6 studies, assuming linearity (P=0.06 for goodness-of-fit). Assuming linearity, a 2-g increase in n-3 PUFA was associated with a 31% reduced risk of CHD mortality (mvRR: 0.69, 95% CI: 0.44 to 1.08). *Horizontal line represents a RR = 1.0; vertical line represents the median n-3 PUFA intake in the studied populations (590 mg)*

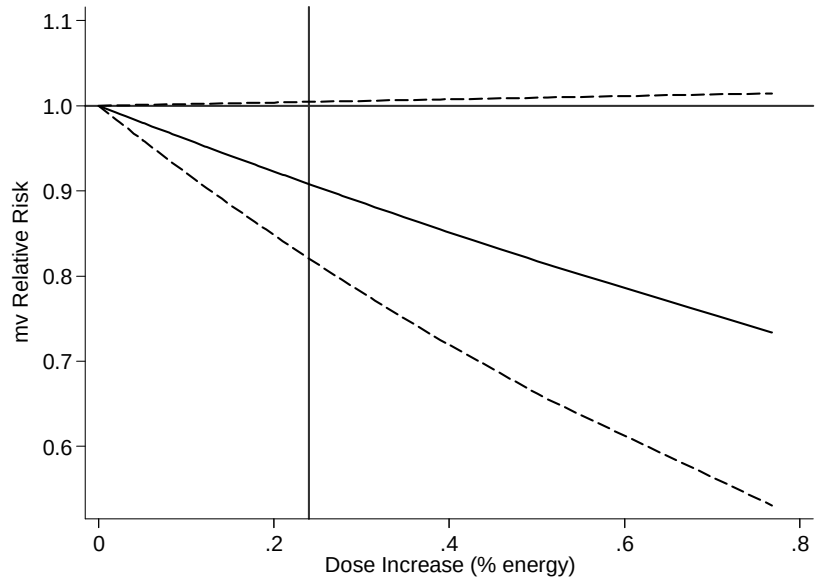

Figure 12. Dose-response association between total n-3 PUFA (% energy) and most-adjusted RR of CHD mortality, assuming linearity (P=0.11 for goodness-of-fit). Assuming linearity, a 0.5% increase in n-3 PUFA was associated with an 18% reduced risk of CHD mortality (mvRR: 0.82, 95% CI: 0.66 to 1.01). *Horizontal line represents a RR = 1.0; vertical line represents the median n-3 PUFA intake in the studied populations (0.2%)*

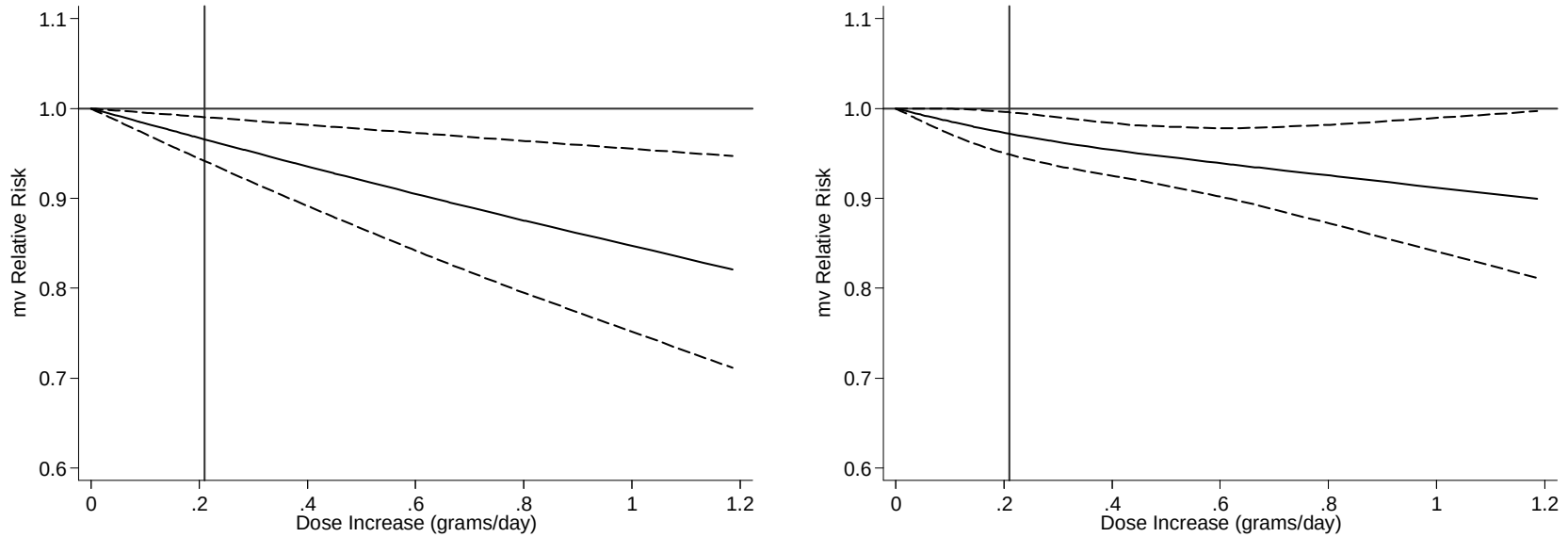

Figure 13. Dose-response association between long-chain n-3 PUFA (g/d) and most-adjusted RR of total mortality in 10 studies, assuming linearity (P<0.002 for goodness-of-fit) (L), and using non-linear, cubic spline approach (R). Assuming linearity, a 0.5-g increase in long chain n-3 PUFA was associated with an 8% reduced risk of all-cause mortality (mvRR: 0.92, 95% CI: 0.87 to 0.98). *Horizontal line represents a RR = 1.0; vertical line represents the median long-chain n-3 PUFA intake in the studied populations (0.1% energy)*

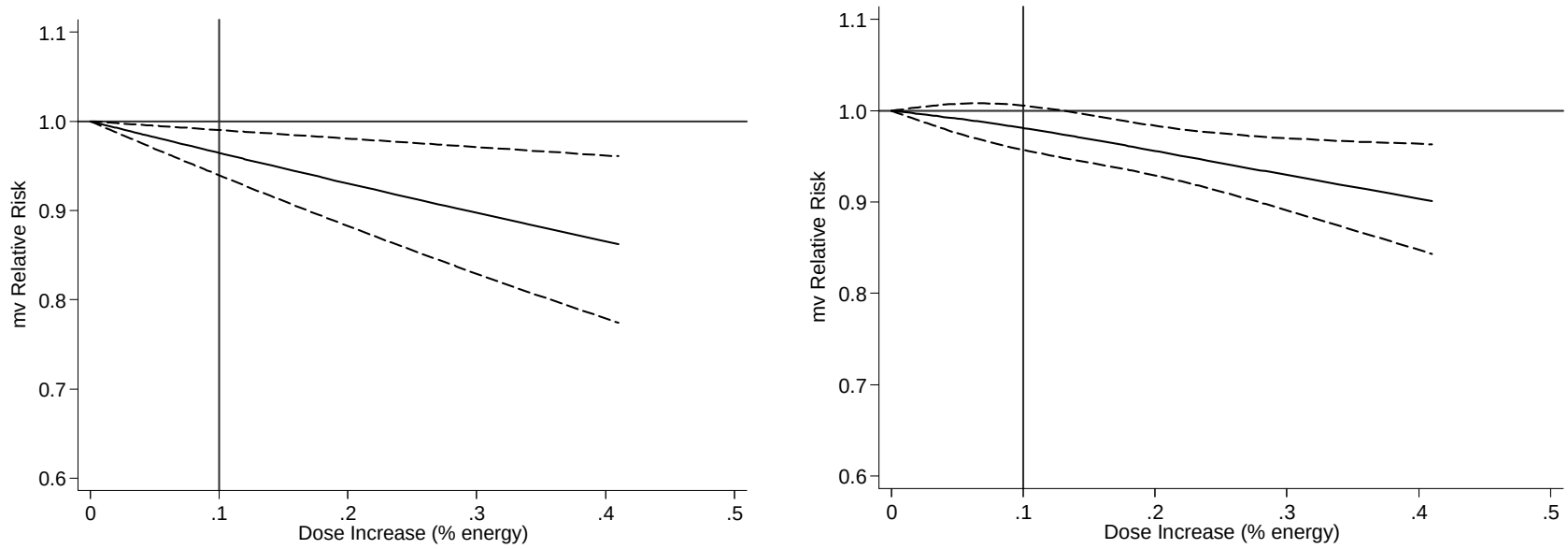

Figure 14. Dose-response association between long-chain n-3 PUFA (% E) and most-adjusted RR of total mortality in 10 studies, assuming linearity (P<0.002 for goodness-of-fit) (L), and using non-linear, cubic spline approach (R). Assuming linearity, a 0.5% increase in long chain n-3 PUFA was associated with an 8% reduced risk of all-cause mortality (mvRR: 0.92, 95% CI: 0.87 to 0.98). *Horizontal line represents a RR = 1.0; vertical line represents the median long-chain n-3 PUFA intake in the studied populations (0.09%)*

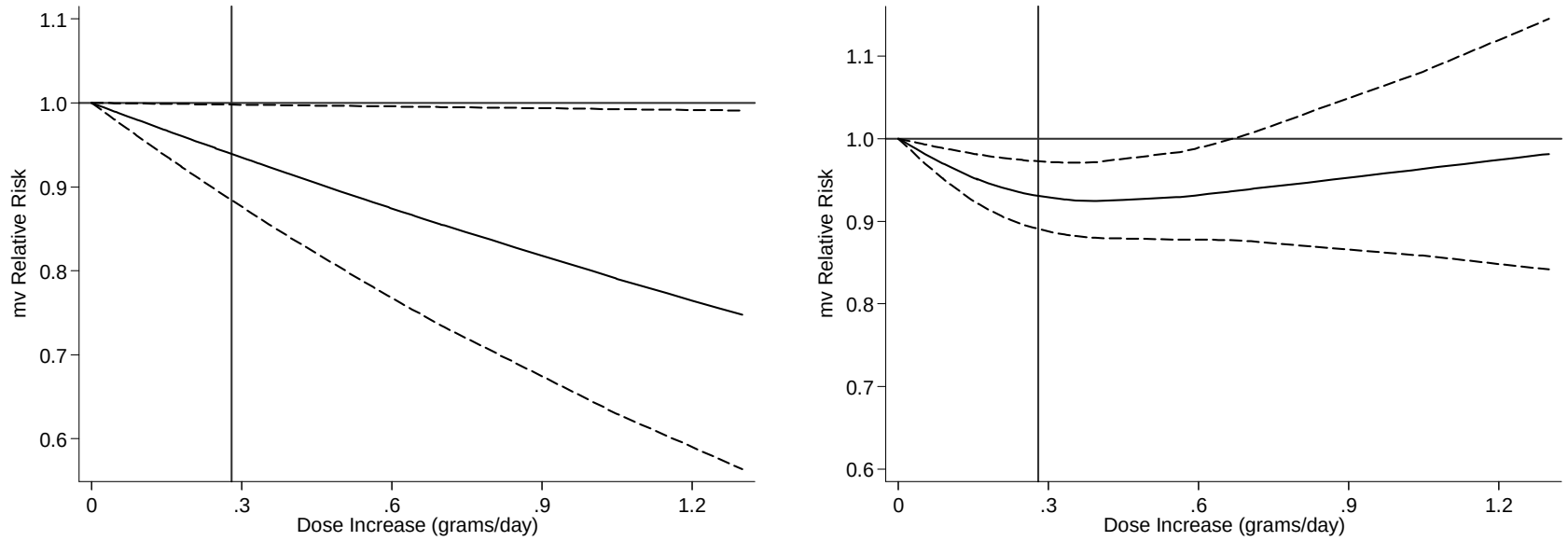

Figure 15. Dose-response association between long-chain n-3 PUFA (g/d) and most-adjusted RR of CVD mortality in 12 studies, assuming linearity (P<0.0001 for goodness-of-fit) (L), and using non-linear, cubic spline approach (R). Assuming linearity, a 0.5-g increase in long chain n-3 PUFA was associated with an 11% reduced risk of CVD mortality (mvRR: 0.894, 95% CI: 0.802 to 0.996). *Horizontal line represents a RR = 1.0; vertical line represents the median long-chain n-3 PUFA intake in the studied populations (280 mg)*

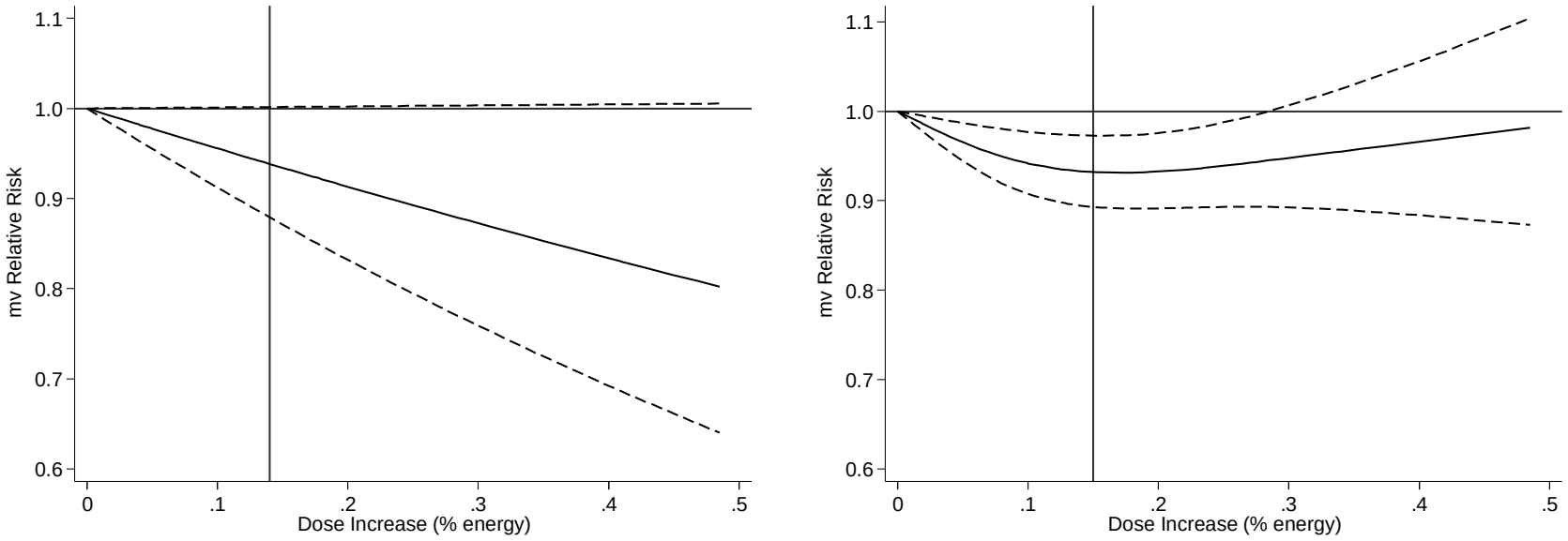

Figure 16. Dose-response association between long-chain n-3 PUFA (% energy) and most-adjusted RR of fatal CVD in 12 studies, assuming linearity (P<0.0001 for goodness-of-fit) (L), and using non-linear, cubic spline approach (R). Assuming linearity, a 0.5% increase in long chain n-3 PUFA was associated with a 20% reduced risk of CVD mortality (mvRR: 0.80, 95% CI: 0.63 to 1.01). *Horizontal line represents a RR = 1.0; vertical line represents the median long-chain n-3 PUFA intake in the studied populations (0.15%)*

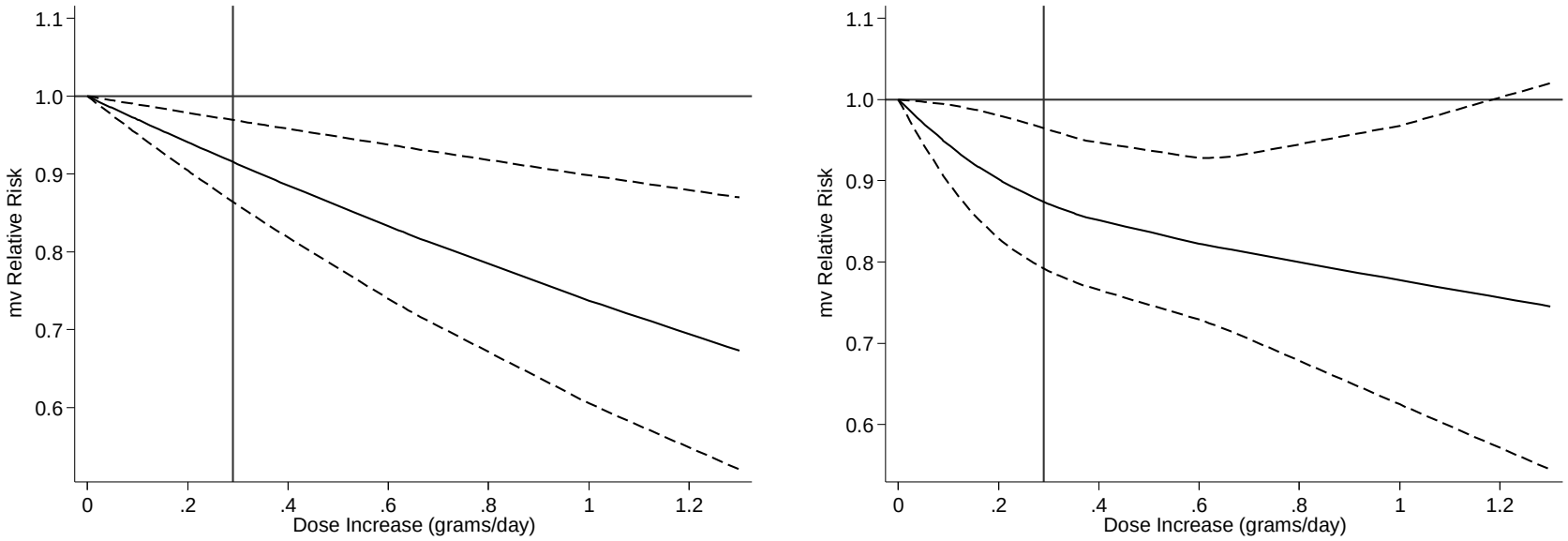

Figure 17. Dose-response association between long-chain n-3 PUFA (g/d) and most-adjusted RR of fatal CHD in 9 studies, assuming linearity (P<0.02 for goodness-of-fit) (L), and using non-linear, cubic spline approach (R). Assuming linearity, a 0.5-g increase in long chain n-3 PUFA was associated with a 14% reduced risk of CHD mortality (mvRR: 0.86, 95% CI: 0.78 to 0.95). *Horizontal line represents a RR = 1.0; vertical line represents the median long-chain n-3 PUFA intake in the studied populations (290 mg/d)*

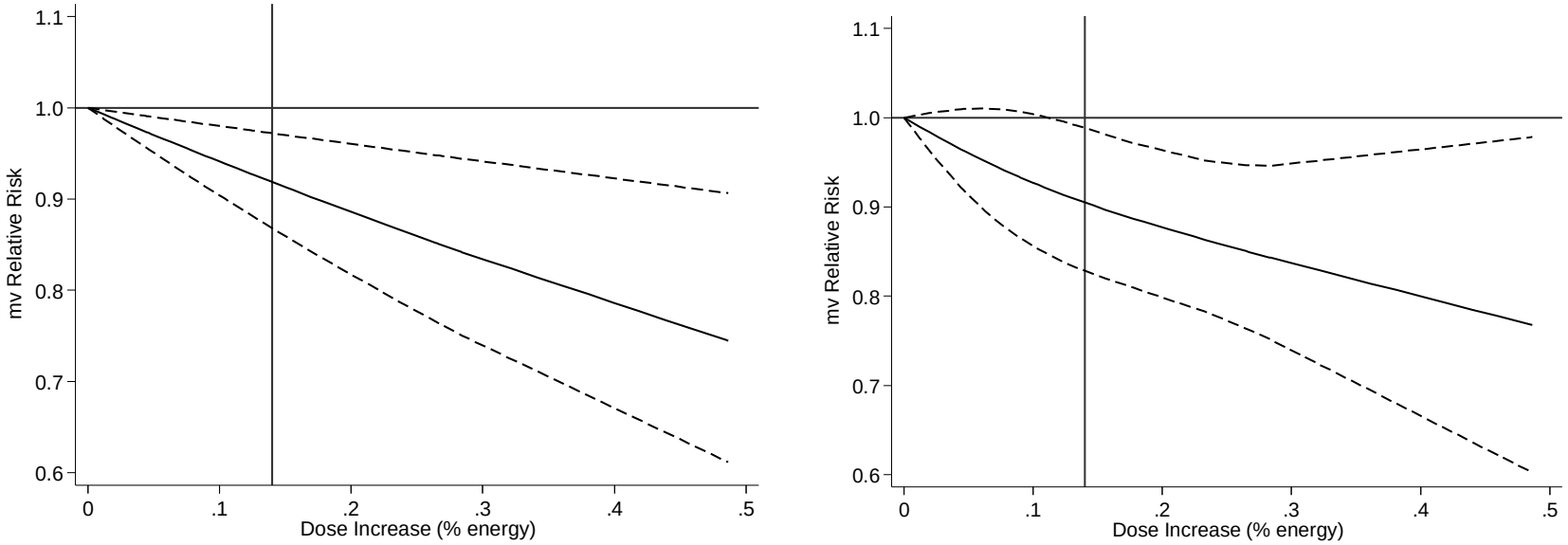

Figure 18. Dose-response association between long-chain n-3 PUFA (% energy) and most-adjusted RR of CHD mortality in 9 studies, assuming linearity (P<0.02 for linearity) (L), and using non-linear, cubic spline approach (R). Assuming linearity, a 0.5% increase in long chain n-3 PUFA was associated with a 26% reduced risk of CHD mortality (mvRR: 0.74, 95% CI: 0.60 to 0.90). *Horizontal line represents a RR = 1.0; vertical line represents the median long-chain n-3 PUFA intake in the studied populations (0.14% E)*

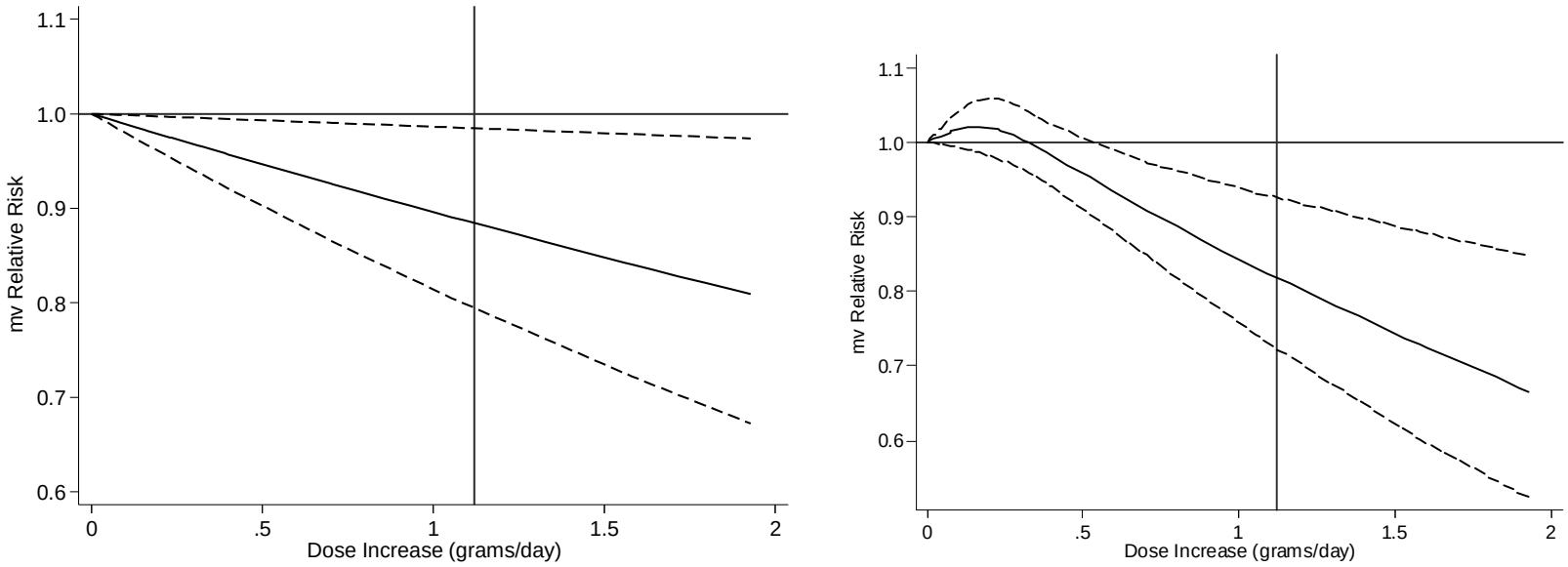

Figure 21. Dose-response association between ALA (g/d) and most-adjusted RR of all-cause mortality in 5 studies, assuming linearity (P<0.01 for goodness-of-fit) (L), and using non-linear, cubic spline approach (R). Assuming linearity, a 0.5 g/d increase in ALA was associated with a 5% decreased risk of all-cause mortality (mvRR: 0.95, 95% CI: 0.90 to 0.99). *Horizontal line represents a RR = 1.0; vertical line represents the median ALA intake in the studied populations (1.14 g/d)*

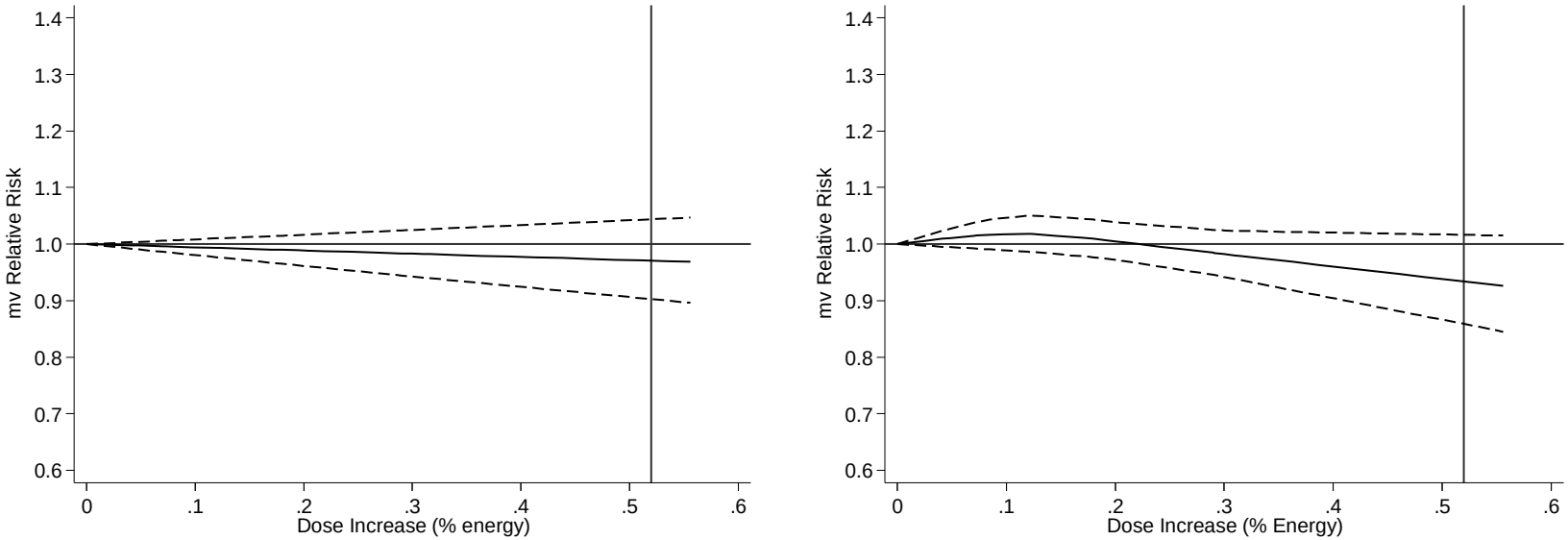

Figure 22. Dose-response association between ALA (%E) and most-adjusted RR of all-cause mortality in 5 studies, assuming linearity (P<0.01 for goodness-of-fit) (L), and using non-linear, cubic spline approach (R). Assuming linearity, a 0.2% increase in energy from ALA was associated with a 2% decreased risk of all-cause mortality (mvRR: 0.98; 95% CI: 0.95 to 1.01). *Horizontal line represents a RR = 1.0; vertical line represents the median ALA intake in the studied populations (0.52%)*

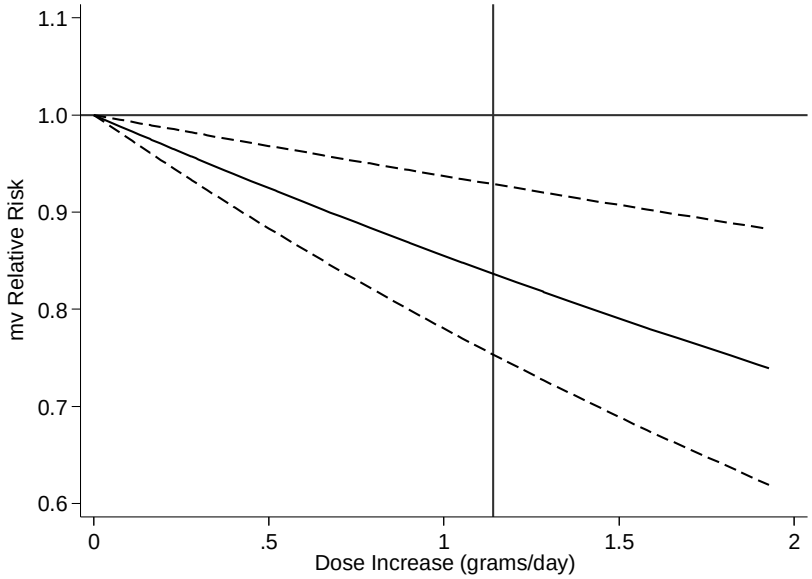

Figure 23. Dose-response association between ALA (g/d) and most-adjusted RR of CVD mortality in 12 studies, assuming linearity (P=0.61 for goodness-of-fit). Assuming linearity, a 0.5 g/d increase in ALA was associated with an 8% decreased risk of CV mortality (mvRR: 0.92, 95% CI: 0.88 to 0.97). *Horizontal line represents a RR = 1.0; vertical line represents the median ALA intake in the studied populations (1.14 g)*

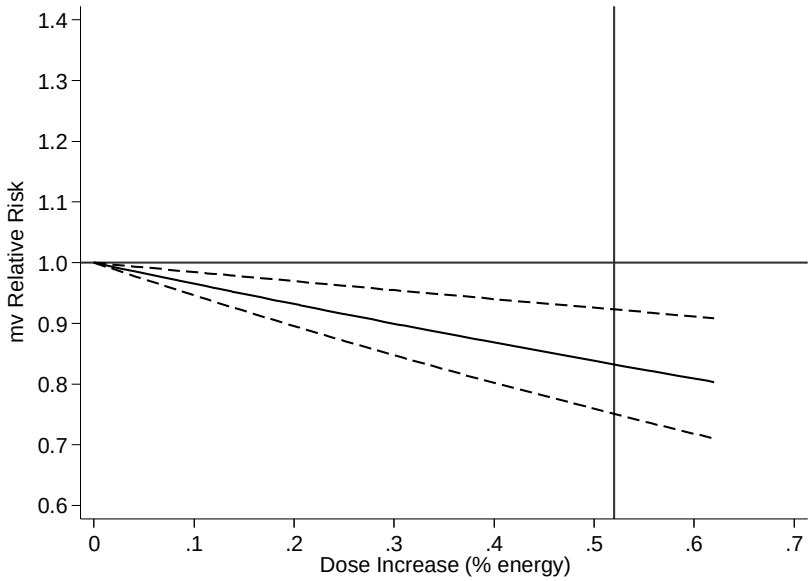

Figure 24. Dose-response association between ALA (%) and most-adjusted RR of CV mortality in 12 studies, assuming linearity (P=0.67 for linearity). Assuming linearity, a 0.5 % increase in energy from ALA was associated with an 7% decreased risk of CV mortality (mvRR: 0.93, 95% CI: 0.90 to 0.97). *Horizontal line represents a RR = 1.0; vertical line represents the median ALA intake in the studied populations (0.52%)*

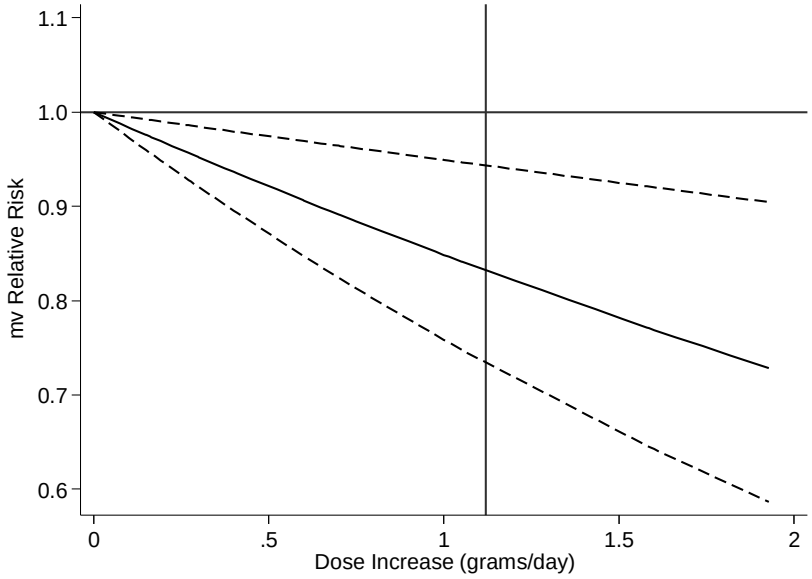

Figure 25. Dose-response association between ALA (g/d) and most-adjusted RR of CHD mortality in 8 studies, assuming linearity (P=0.27 for linearity). Assuming linearity, a 0.5 g/d increase in ALA was associated with an 8% decreased risk of CHD mortality (mvRR: 0.92, 95% CI: 0.87 to 0.97). *Horizontal line represents a RR = 1.0; vertical line represents the median ALA intake in the studied populations (1.12 g/d).*

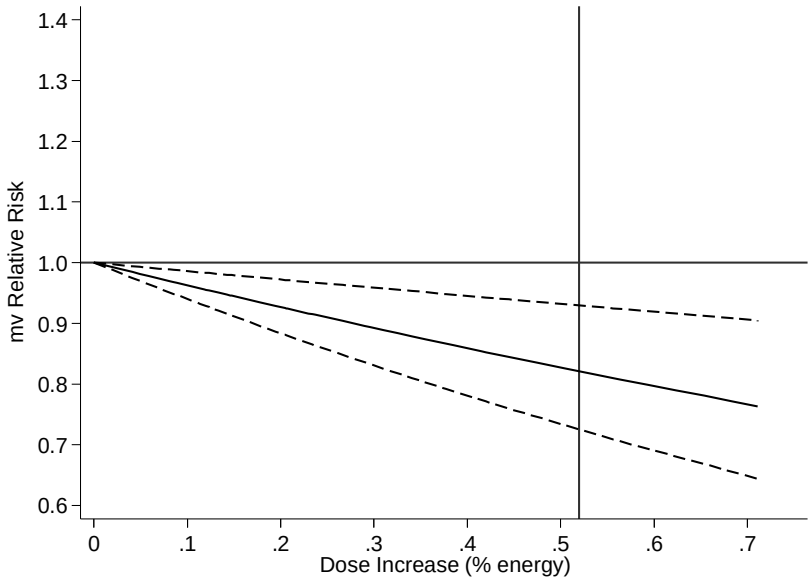

Figure 26. Dose-response association between ALA (%E) and most-adjusted RR of CHD mortality in 8 studies, assuming linearity (P=0.27 for linearity). Assuming linearity, a 0.2 % increase in ALA was associated with an 7% decreased risk of CHD mortality (mvRR: 0.93, 95% CI: 0.88 to 0.97). *Horizontal line represents a RR = 1.0; vertical line represents the median ALA intake in the studied populations (0.52%).*

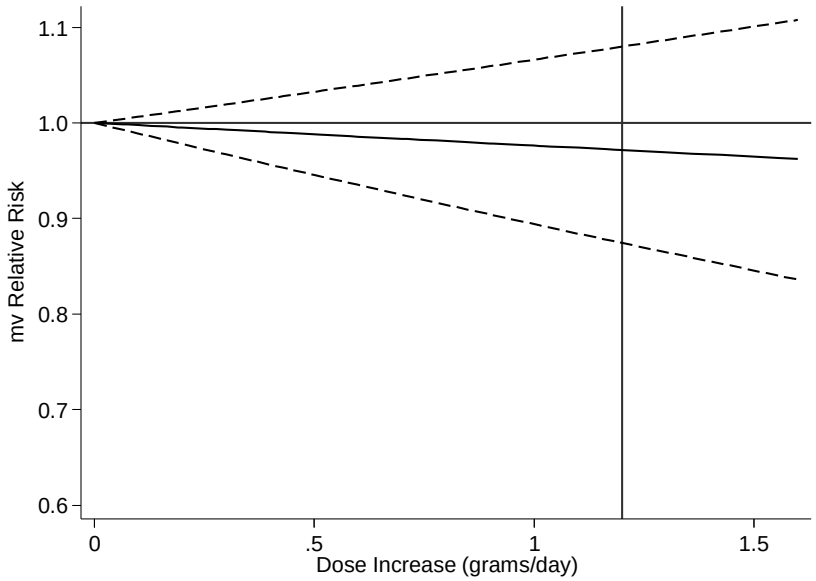

Figure 27. Dose-response association between ALA (g/d) and most-adjusted RR of total CHD in 7 studies, assuming linearity (P=0.69 for goodness-of-fit). There was no evidence of a dose-response association between ALA and risk of CHD (mvRR: 0.99, 95% CI: 0.96 to 1.03 per 0.5-g). *Horizontal line represents a RR = 1.0; vertical line represents the median ALA intake in the studied populations (1.12 g/d).*

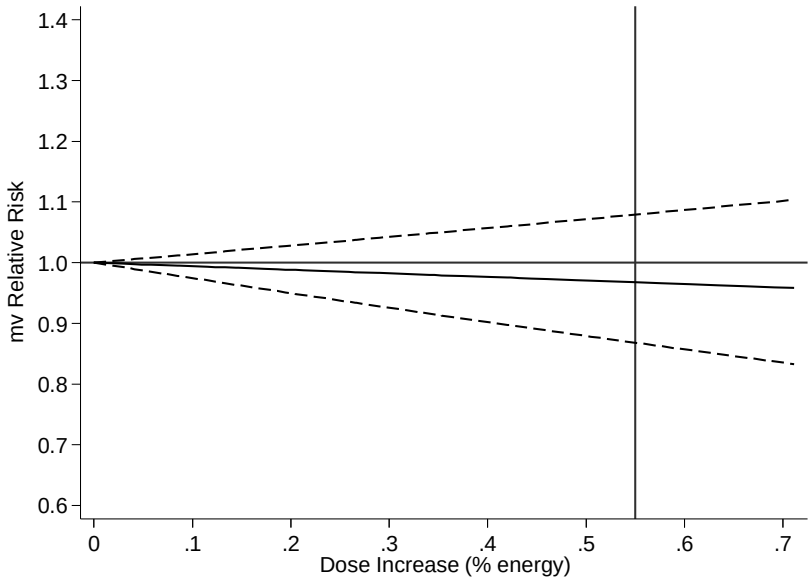

Figure 28. Dose-response association between ALA (%E) and most-adjusted RR of CHD in 7 studies, assuming linearity (P=0.70 goodness-of-fit). There was no evidence of a dose-response association between ALA and risk of CHD (mvRR: 0.99, 95% CI: 0.95 to 1.03 per 0.2%). *Horizontal line represents a RR = 1.0; vertical line represents the median ALA intake in the studied populations (0.55% E).*

Appendix 2 References

1. Orsini N, Bellocco R, Greeland S. Generalized least squares for trend estimation of summarized dose-response data. *Stata J.* 2006;6:40-57
2. Orsini N, Li R, Wolk A, Khudyakov P, Spiegelman D. Meta-analysis for linear and nonlinear dose-response relations: Examples, an evaluation of approximations, and software. *Am J Epidemiol*. 2012;175:66-73