Omega-3 polyunsaturated fatty acid biomarkers and risk of type 2 diabetes, cardiovascular disease, cancer, and mortality

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Abstract:

Background & aims: Considerable attention has focused on the role of omega-3 polyunsaturated fatty acids (PUFA) in the prevention of cardiometabolic diseases, which has led to dietary recommendations to increase omega-3 fatty acid intake. A meta-analysis was conducted to summarize evidence from prospective studies regarding associations between omega-3 PUFA biomarkers and risk of developing major chronic diseases.

Methods: Four electronic databases were searched for articles from inception to March 1, 2022. Random-effects model was used to estimate the pooled relative risk (RR) and 95% confidence intervals (CIs) for the association of omega-3 PUFAs, including α- linolenic acid (ALA), eicosapentaenoic acid (EPA), docosapentaenoic acid (DPA), and docosahexaenoic acid (DHA), with risk of developing type 2 diabetes (T2D), cardiovascular disease (CVD), including coronary heart disease (CHD) and stroke, cancer, and mortality. The Grades of Recommendation, Assessment, Development and Evaluation assessment tool was used to rates the confidence in estimates.

Results: A total of 67 prospective studies comprised of 310,955 participants were identified. Individual omega-3 PUFAs showed divergent associations with the study outcomes of interest. A significant inverse association with T2D risk was observed across categories of ALA (relative risk [RR]:0.89, 95% confidence interval [CI]: 0.82-0.96), EPA (RR: 0.85, 95%CI: 0.72-0.99) and DPA (RR: 0.84, 95%CI:0.73-0.96) biomarkers. The marine-origin omega-3 fatty acids biomarkers but not ALA was significantly associated with lower risks of total CVD, CHD, and overall mortality, with RRs ranging from 0.70 for DHA-CHD association to 0.85 for EPA-CHD association. A lower risk of colorectal cancer was observed at higher levels of DPA

(RR:0.76, 95%CI:0.59-0.98) and DHA (RR:0.80;95%CI:0.65-0.99), whereas no association was noted for other outcomes. In addition, a dose-response relationship was observed between an increasing level of EPA, DPA, or DHA biomarker and lower risk of CVD.

Conclusions: Higher concentrations of marine-derived omega-3 PUFA biomarkers were associated with a significantly reduced risk of total CVD, CHD, and total mortality. Levels of ALA were inversely associated with a lower risk of T2D but not CVD-related outcomes. These data support the dietary recommendations advocating the role of omega-3 PUFAs in maintaining an overall lower risk of developing cardiovascular disease and premature deaths.

Key words: omega-3 polyunsaturated fatty acid biomarker, type 2 diabetes, cardiovascular disease, cancer, mortality, meta-analysis

Abbreviations:

ALA, alpha-linolenic acid, AMD, age-related macular degeneration; CIs, confidence intervals; CHD, coronary heart disease; CVD, cardiovascular disease; DHA, docosahexaenoic acid; DPA, docosapentaenoic acid; EPA, eicosapentaenoic acid; PUFA, polyunsaturated fatty acids; SD, standard deviation; T2D, type 2 diabetes; RR, relative risk.

1. Introduction

Increasing polyunsaturated fatty acid (PUFA) consumption, especially seafood-derived omega-3 PUFAs, has been considered as a key component of prevention strategy in tackling the current epidemic of chronic disorders in the past half century [1,2]. Dietary guidelines of the American Heart Association recommend a daily consumption of 250 mg eicosapentaenoic acid (EPA) and docosahexaenoic acid (DHA) for decreasing the risk of cardiac deaths among individuals with and without pre-existing cardiovascular disease (CVD) [2]. Accumulating evidence from experimental studies has demonstrated that omega-3 PUFAs have multiple critical health benefits including inhibiting inflammation, regulating lipid metabolism, reducing arrhythmias, and improving endothelial function and insulin resistance [3,4]. Recently, the efficacy of marine-derived omega-3 PUFA supplementation for CVD risk reduction has been demonstrated in the Reduction of Cardiovascular Events with Icosapent Ethyl-Intervention Trial (REDUCE-IT) and the Vitamin D and Omega-3 Trial (VITAL) which consistently reported a beneficial effect of these fatty acids on reducing coronary heart disease (CHD) risk in the overall population or subgroups [5,6]. However, whether these effects can be extrapolated to general populations with lower habitual intake of omega-3 PUFAs or to other related health conditions is unclear.

Existing prospective cohort studies have examined associations of dietary omega-3 PUFAs with the incidence of major chronic diseases and mortality in free-living individuals, and findings of these studies were mixed [7,8]. For instance, some studies showed that intake of plant-derived fatty acid, such as α-linolenic acid (ALA), was associated with decreased risk of type 2 diabetes (T2D), whereas others have reported no such association [9-11]. The conflicting results might be related to variation in

different background diet, measurement errors of dietary assessments, as well as bioavailability of these fatty acids [12]. Meaningful amounts of omega-3 PUFAs could also be obtained from various fortified foods, making an accurate assessment of these fatty acids intake more challenging [13]. Biomarkers of omega-3 PUFAs are valuable when evaluating the associations between the intake of these PUFAs and disease risk because they are free of reporting bias and other measurement errors intrinsic to questionnaire-based assessments [14,15]. Previous researches to date have focused mainly on the association with CVD risk and most suggested inverse associations [16-18]. Compared with the literature on CVD, less evidence exists regarding the associations of omega-3 PUFA fatty acid biomarkers and other chronic conditions, such as T2D, cancer or mortality. In addition, much existing evidence surrounds EPA and DHA, with relatively little evidence generated for the association between other omega-3 PUFAs, such as ALA or docosapentaenoic acid (DPA).

We therefore conducted a comprehensive meta-analysis of prospective studies to evaluate associations of omega-3 PUFA biomarkers with incident of T2D, total CVD, CHD, stroke, cancer and mortality.

2. Methods

This study was reported according to the Meta-analyses of Observational Studies in Epidemiology (MOOSE) guideline and the protocol was registered in an international prospective register of systematic reviews (www.crd.york.ac.uk/PROSPERO/; identifier CRD42021297231) [19].

2.1 Search strategy and selection criteria

PubMed (www.ncbi.nlm.nih.gov/pubmed), EMBASE (www.embase.com/), Web of Science (www.isiknowledge.com), and Cochrane Library (http://www.thecochranelibrary.com) were searched for relevant published articles

from inception to March 1, 2022, using the search terms: “polyunsaturated fatty acids”, “omega-3 fatty acids”, “eicosapentaenoic acid”, “docosahexaenoic acid”, “docosapentaenoic acid”, “alpha linolenic acid”, AND “type 2 diabetes”, “cardiovascular disease”, “heart disease”, “stroke”, “cancer”, “mortality”, AND “serum”, “plasma”, “blood”, “erythrocytes”, “cholesteryl esters”, “phospholipids”, “triacylglycerol”, “adipose tissue”, AND “observational”, “prospective”, “follow-up”, “cohort”, “case-cohort”, “nested case-control” (see Table 1 in Supplementary Appendix 1 for details). No restrictions for language were applied. Reference lists of retrieved articles, review articles, and meta-analyses were also hand searched for additional eligible studies. Authors of included studies and consulted experts were also contacted for any further published or unpublished work.

Studies were eligible for inclusion if they met the following criteria: (1) study design was prospective (including prospective cohort, nested case-control, and case-cohort study) with a follow-up more than 1 year; 2) the exposures of interest were omega-3 PUFA concentration (ALA, and total or individual marine-derived omega-3 fatty acids [EPA, DPA, and DHA]) in any type of tissue (circulating blood [whole blood/serum/plasma/erythrocyte] or adipose tissue); (3) the endpoints of interest included incident T2D, total CVD, CHD, stroke, cancer, all-cause mortality, and cause-specific mortality; (4) the risk estimate with corresponding 95% confidence intervals (95% CIs) or standard error was presented. In the case of overlapping reports, only the one with the most updated data was retained to eliminate potential duplicates. Three investigators (HJ, LNW, and MW) independently searched and further assessed the eligibility of all identified citations, and disagreements were resolved through discussion.

2.2 Data extraction and quality assessment

For each included article, three authors (HJ, NY, and MW) independently extracted data using a piloted data-extraction form that collects information on relevant study details, including study characteristics (the name of the first author, year of publication, geographical location, study design, follow-up year, study name and population size), participant characteristics (age and proportion of men), exposure (omega-3 PUFAs type, exposure source, and assessment method), outcome (type and number of cases or deaths), covariates adjusted in the analysis, and the risk estimate with 95% CIs for all categories of each biomarkers. When studies provided estimates with different degrees of statistical adjustment for confounding, the fully adjusted associations were extracted and considered in the analysis.

Study quality was scrutinized by the same authors following the validated Newcastle-Ottawa scale, which awards 0-9 points and incorporates information on selection (range 0-4 points), comparability (range 0-2 points), and outcome assessment (range 0-3 points). We defined studies as low, moderate, and high quality for those scored 0-3, 4-6, and 7-9, respectively [20]. Any discrepancies in data extraction and quality assessment were resolved by discussion or it would be deferred to a senior independent reviewer (LM), if any uncertainty remained.

The Grades of Recommendation, Assessment, Development and Evaluation (GRADE) was used to rate the overall quality and the strength of each outcome [21]. The GRADE approach basically categorizes the quality of observational studies as low-quality evidence. The following five criteria downgraded the quality of evidence: included study design and execution limitations, inconsistency, indirectness, imprecision, and publication bias.

2.3 Data synthesis and statistical analysis

Methods previously described were used to derive estimates of associations

corresponding to the comparison between the top and bottom thirds of omega-3 PUFA distributions [22]. This strategy was to harmonize different comparison groups used in individual studies, such as quartiles, quintiles, or other categorizations, or per standard deviation [SD] change. In brief, for studies that provided relative risks [RR] per SD change of omega-3 PUFAs, we applied a factor of 2.18 to the log RR to derive the RR comparing extreme thirds, assuming a normal distribution. Similarly, the factor of 2.54 or 2.80 was applied to convert estimates for comparing extreme quartiles or quintiles, respectively. The standard error (SE) of the transformed log RR was calculated after applying the same factors [23]. When studies used multiple measures as biomarker (phospholipids, plasma, cholesterol esters, and adipose tissue), the overall risk estimate was based on different duration of intake reflection according to the following list: adipose tissue, erythrocyte phospholipids, plasma phospholipids, total plasma or serum, and cholesterol esters. For each included study, the most fully adjusted estimates of rate, hazard, or odds ratios from prospective studies were all valid estimates of the RR. Studies that reported results by sex or other subgroups separately were pooled to derive a single effect size for the study by using fixed-effects model. When CHD and stroke outcomes were separately provided in the same study, we did not combine it to obtain total CVD risk estimates, and therefore, the CVD analysis only considered studies that examined total CVD incidence.

Random effects model, which allows consideration of interstudy variation, was used to pooled data across studies because the expected heterogeneity in varying factors (i.e., ages, ethnicities, and methods) making it difficult to assume identical true effect size in every study. Heterogeneity across study effects was assessed using Q test and I^2 statistic. A Cochran's Q $P < 0.10$ and $I^2 > 50\%$ were considered as the threshold of presence of statistically significant heterogeneity. If ≥10 studies were available,

prespecified subgroup and meta-regression analyses were carried out, with the following variables: study design (prospective cohort study, or prospective nested case-cohort study, nested case-control study), sex (men, women, or both), geographical location (America, Australia, Asia, or Europe), duration of follow-up (< 10 or ≥ 10 years), assessment method (gas chromatography [GC] or gas-liquid chromatography [GLC]), biological sample type (total plasma, phospholipids, cholesterol esters, or adipose tissue), number of cases (< 300, 300-500, or ≥ 500), and study quality (moderate or high) [24].

Dose-response analyses were assessed using the method proposed by Greenland and Longnecker to calculate study linear trends and 95% CIs from the natural logs of the RRs and CIs across categories of omega-3 PUFA exposure [25]. The dose-response outcomes were limited to studies that reported circulating omega-3 PUFA due to the small number of included studies with other exposures. The reported midpoint (median/mean level extracted from the original articles) or estimated midpoint (the average of the upper and the lower cut-off point reported in the original articles) of omega-3 PUFA markers level for each category was assigned to corresponding risk estimate [26]. When the highest category did not have an upper bound, the midpoint of the category was set at 1.5 times the lower boundary. If the lowest categories were open ended, the lower boundary was set to zero [27]. For studies without data on number of participants for each category, we then used the average participants number by categories (total participants divided by the number of categories). The method proposed by Bekkering et al., which considering the numbers of cases and the reported risk estimate, was used to impute missing data when the number of cases in each category was not available [28]. Furthermore, only studies that reported RR with 95% CIs for at least three exposure categories were included in

the dose-response estimation. In addition, restricted cubic spline regression model with three knots at 10th, 50th, and 90th percentiles of the biomarkers were used to examine any potential non-linear dose-response relationships [29]. The dose-response outcomes were presented on the basis of per SD increment for the concentration of circulating omega-3 PUFA. When the mean (SD) for per category was not available, a method proposed by McGrath et al. was used to impute category specific mean (SD) based on median, quartile, or extreme values [30]. Sensitivity analyses were carried out by excluding one study at each turn and recalculating the pooled estimates for the remainder of the studies (i.e., the "leave one out" approach) to test the impact by individual studies on the pooled study estimates. A study was considered as being influential when the significance level of the overall association changed (such as from < 0.05 to ≥ 0.05) or the combined risk estimates changed by 10% or more upon its removal. Potential publication bias was examined by Begg's and Egger's tests, as well as the trim and fill method. In addition, we depicted this graphically with a funnel plot if the analysis including 10 or more studies. All analyses were performed using Stata, version 10.2 (Stata Corp, Texas). $P < 0.05$ was considered statistically significant for all analyses unless otherwise specified.

3. Results

3.1 Study characteristics

Fig. 1 summarizes the literature search and selection process. We identified 18,341 citations in the primary search, of which 186 were retrieved for full text evaluation after the initial screening of abstracts and titles. In addition, 13 studies were identified through manual examination of reference lists. Overall, a total of 67 studies reported in 65 articles were included in our main analysis (**Table S2-S7** in Supplementary Appendix 1, **Fig. 1**) [16-18, 31-92].

The included studies comprised of 27 prospective cohort studies [16-18,31,36-38, 40,41,42,44-52,55,63,65-67,70,76,92], nine case-cohort studies [32,39,57,69,72,78, 80, 90,91], one nested case-cohort study [35], and 28 nested case-control studies [33, 34,43, 53,54,56,58,59-62,64,68,71,73-75,77,79,81-89]. Twenty-seven studies were conducted in Europe [16,17,33-35,37-39,42,44,46,49,57,58,63,64,67,69,70,74-76,78,79,81,84,87], 26 in the United States [18,31,36,40,43,47,48,50,51,53-56,59,65,66,68,71,77,82,83, 85,86,88-90], seven in Asia [41,45,52,60-62,73], and five in Australia [32,72,80,91,92]. Mean age of participants in the individual studies ranged from 41.1 to 80.5 years. For the measurements of omega-3 PUFA levels, 51 studies used GC analytic approach [16-18,32,34-41,43-54,57-59,61-64,66-70,73-79,81,83,85,87-90,92], 13 used GLC [31,33,43,55,56,65,71,72,80,82,84,86,91], one in nuclear magnetic resonance-based profiling [42], and one used GC-tandem mass spectrometry [60]. The **Fig. 1** in Supplementary Appendix 2 shows the mean (SD) proportion of each objective omega-3 PUFA relative to the total fatty acid contents in blood compartments. The study quality scores were shown in **Table S8** and **Table S9** in Supplementary Appendix 1. Fifty-seven studies were deemed to be of high quality [16-18,31-40,42,44,46-48,50-52,54-59,61,62,64-82,84-89,91,92], and the others judged as having a moderate quality [41,43,45,49,53,60,63,83,90].

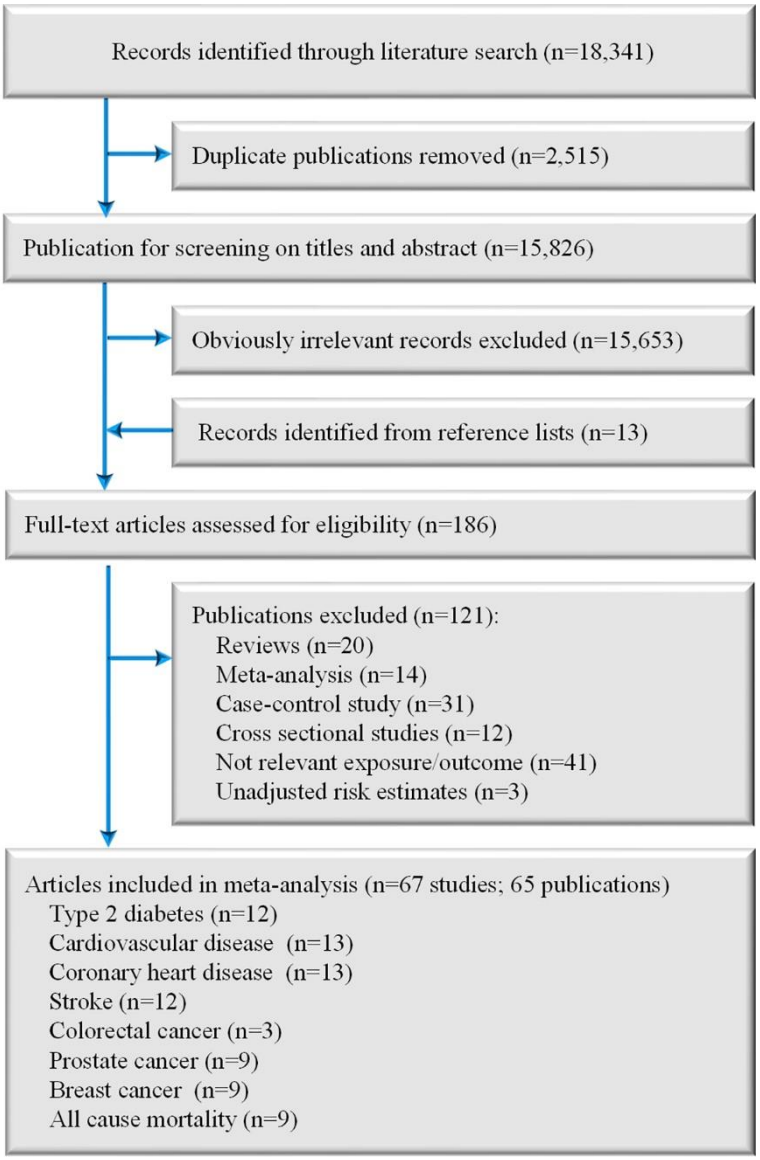

Figure 1. Flow chart of study selection

3.2 Omega-3 PUFA biomarkers and risk of type 2 diabetes

12 studies comprised of 18,510 T2D incidence and 148,865 participants investigated the relationship between the concentrations of omega-3 PUFA biomarkers and T2D risk [31-42]. Compared with participants in the lowest tertile of ALA level, those in the highest tertile had a lower risk of T2D (RR: 0.89, 95%CI: 0.82-0.96, P=0.005; $P_{heterogeneity}$=0.39; **Fig. 2** and **Fig. S2** in Supplementary Appendix 2). Similar results

were observed for EPA (RR: 0.85, 95%CI: 0.72-0.99, P =0.04; $P_{heterogeneity}$ <0.001; **Fig. 2** and **Fig. S3** in Supplementary Appendix 2) and DPA (RR: 0.84, 95%CI:0.73-0.96, P =0.11; $P_{heterogeneity}$ =0.005; **Fig. 2** and **Fig. S4** in Supplementary Appendix 2). A non-significant inverse trend was found for the sum of EPA+DPA+DHA (RR: 0.81, 95%CI: 0.60-1.09, P =0.16; $P_{heterogeneity}$ =0.15; **Fig. 2**, **Fig. S5-S6** in Supplementary Appendix 2) with T2D risk. The result of analyses stratified by length of follow-up, sex, geographic location, number of cases, exposure assessment, biomarkers type, and study design showed that these variables did not substantially alter the association between level of omega-3 PUFA biomarkers and T2D risk (**Table S10** in Supplementary Appendix 1).

3.3 Omega-3 PUFA biomarkers and risk of CVD

Association of omega-3 PUFA levels with total CVD was assessed in 13 studies, which included a total of 4,706 cases among 36,921 participants [16-18, 43-54]. When comparing the extreme tertiles, the risk of total CVD was significantly lower by 21% for EPA (RR: 0.79, 95%CI: 0.70-0.89, P<0.001; $P_{heterogeneity}$=0.20; **Fig. 2** and **Fig. S7** in Supplementary Appendix 2), 22% for DPA (RR: 0.78, 95%CI:0.70-0.86, P<0.001; $P_{heterogeneity}$=0.94; **Fig. 2** and **Fig. S8** in Supplementary Appendix 2), and 24% for DHA (RR: 0.76, 95%CI:0.66-0.88, P<0.001; $P_{heterogeneity}$=0.02; **Fig. 2** and **Fig. S9** in Supplementary Appendix 2, **Table S11** in Supplementary Appendix 1). The sum of EPA+DPA+DHA was also associated with a significantly lower total CVD risk by 55% (RR: 0.45, 95%CI:0.27-0.74, P=0.002; $P_{heterogeneity}$=0.15; **Fig. 2** and **Fig. S10** in Supplementary Appendix 2). No association was observed between ALA and risk of

CVD when comparing the highest with lowest categories (RR: 1.09, 95%CI: 0.98-1.20, P=0.10; $P_{heterogeneity}$=0.74; **Fig. 2** and **Fig. S11** in Supplementary Appendix 2). Results from the dose-response analyses showed a significant linear decrease in the risk of CVD of individuals with increasing values of circulating EPA and DPA concentration, and per 1-SD increment was associated with 22% (RR: 0.78, 95%CI: 0.71-0.86, P<0.001) and 8% (RR: 0.91, 95%CI: 0.87-0.95, P<0.001) lower risk of CVD, respectively (**Fig. 3**). A potential non-linear dose-response curve was detected for DHA-CVD association in that the CVD risk did not decrease until the DHA levels exceeded about 2% ($P_{non\text{-}linearity}$=0.01; **Fig. 3**).

3.4 Omega-3 PUFA biomarkers and risk of CHD

The association between omega-3 PUFA biomarker levels and CHD was evaluated in 13 studies, which consisted of 7,626 cases and 27,624 participants [18,47,48,53-62]. The overall effect estimates of CHD comparing the top tertile with bottom tertile was 0.98 for ALA (95% CI: 0.95-1.02, P=0.30; $P_{heterogeneity}$=0.89; **Fig. 2** and **Fig. S12** in Supplementary Appendix 2), 0.85 for EPA (95%CI: 0.77-0.95, P=0.003; $P_{heterogeneity}$=0.41; **Fig. 2** and **Fig. S13** in Supplementary Appendix 2, **Table S12** in Supplementary Appendix 1), 0.83 for DPA (95%CI: 0.76-0.92, P<0.001; $P_{heterogeneity}$=0.84; **Fig. 2** and **Fig. S14** in Supplementary Appendix 2), 0.70 for DHA (95%CI: 0.58-0.84, P<0.001; $P_{heterogeneity}$=0.02; **Fig. 2** and **Fig. S15** in Supplementary Appendix 2, **Table S12** in Supplementary Appendix 1), and 0.67 for the sum of EPA+DPA+DHA (95%CI: 0.47-0.96, P=0.03; $P_{heterogeneity}$=0.34; **Fig. 2** and **Fig. S16** in Supplementary Appendix 2). For the dose-response analyses, a linear association was observed for marine-derived omega-3 PUFA biomarkers and risk of CHD. For every 1-SD increase in levels of EPA, DPA, and DHA in circulating, the RR of CHD

decreased by 10% (RR: 0.90, 95%CI: 0.83-0.98, *P*=0.02), 4% (RR: 0.96, 95%CI: 0.89-1.02, *P*=0.20), and 7% (RR:0.93, 95%CI: 0.88-0.98, *P*=0.008), respectively (**Fig. 3**).

3.5 Omega-3 PUFA biomarkers and risk of stroke

Twelve studies provided information on omega-3 PUFA levels and the subsequent risk of stroke, including a total of 7,036 events in 77,163 participants [48,51,63-70]. The pooled estimate indicated that high DHA status was associated with a lower risk of stroke (RR: 0.84, 95%CI: 0.72-0.99, *P*=0.03; $P_{heterogeneity}$=0.03; **Fig. 2** and **Fig. S17** in Supplementary Appendix 2), while there was no significant association for biomarkers of ALA, EPA, DPA, or the sum of EPA+DPA+DHA (**Fig. 2** and **Fig. S18-S21** in Supplementary Appendix 2). A linear relation was noted between DHA biomarker and stroke in the dose-response analysis (**Fig. 2** and **Fig. S22** in Supplementary Appendix 2), and the RR was 0.97 (95%CI: 0.93-1.01, *P*=0.15) for each 1-SD increment of DHA concentration in circulating.

3.6 Omega-3 PUFA biomarkers and risk of cancer

Twenty-one studies were included in the analysis of omega-3 PUFA biomarker status and colorectal, breast, or prostate cancers [71-91]. For colorectal cancer (n=3) [71-73], in comparison with the lowest category, the highest level category of DPA and DHA were associated with 24% (RR: 0.76, 95%CI: 0.59-0.98, *P*=0.04; $P_{heterogeneity}$=0.87; **Fig. 2** and **Fig. S23** in Supplementary Appendix 2) and 20% (RR: 0.80, 95%CI: 0.65-0.99, *P*=0.04; $P_{heterogeneity}$=0.56; **Fig. 2** and **Fig. S24** in Supplementary Appendix 2) reduced risk of colorectal cancer, respectively. ALA and EPA biomarker had a non-significant association with incident colorectal cancer (**Fig. 2**, **Fig. S25** and **Fig. S26** in Supplementary Appendix 1). No association was observed between ALA, EPA, DPA, and DHA concentrations and incidence of breast cancer

(study n=9; **Fig. 2** and **Fig. S27-S30** in Supplementary Appendix 2) [74-82]. No significant association was detected for prostate cancer (n=9) [83-91] (**Fig. 2** and **Fig. S31-S34** in Supplementary Appendix 2).

3.7 Omega-3 PUFA biomarkers and total mortality

Nine studies investigated the relation of omega-3 PUFA biomarker levels with mortality with a total of 7,995 deaths from 27,616 participants [16,18,43,46-50,69,92]. Pooled RR for the comparison of extreme tertiles was 0.78 for EPA (95%CI: 0.69-0.88, P<0.001; $P_{\text{heterogeneity}}$=0.01; **Fig. 2** and **Fig. S35** in Supplementary Appendix 2), 0.82 for DPA (95%CI: 0.74-0.90, P<0.001; $P_{\text{heterogeneity}}$=0.73; **Fig. 2** and **Fig. S36** in Supplementary Appendix 2,), and 0.83 for DHA (95%CI: 0.75-0.92, P<0.001; $P_{\text{heterogeneity}}$=0.08; **Fig. 2** and **Fig. S37** in Supplementary Appendix 2). Nonsignificant association was observed for ALA biomarker (RR: 0.98, 95%CI: 0.89-1.07, P=0.63; $P_{\text{heterogeneity}}$=0.18; **Fig. 2** and **Fig. S38** in Supplementary Appendix 2). No significant evidence of heterogeneity was detected in these analyses.

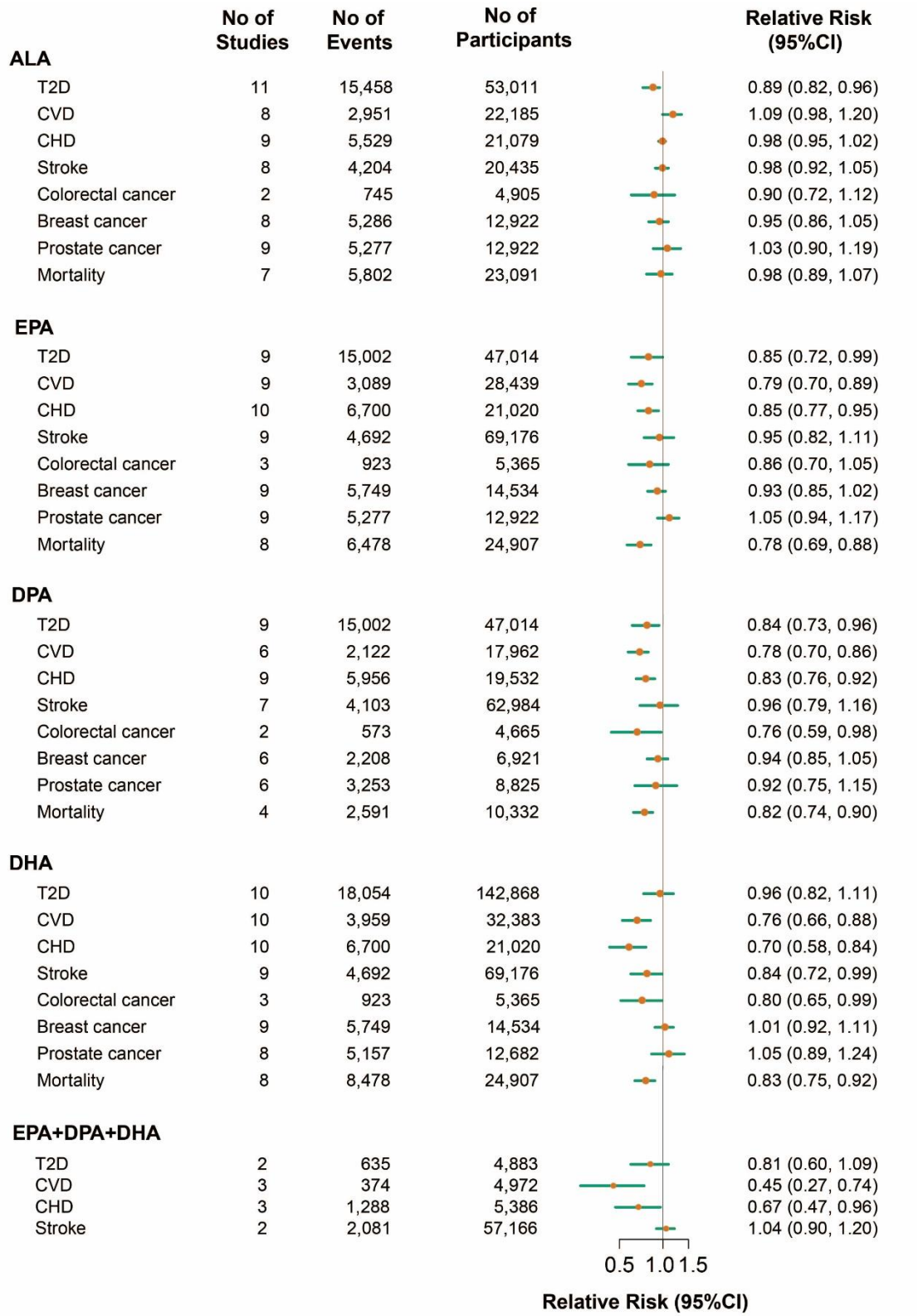

Figure 2. **Pooled relative risks of T2D, CVD, CHD, stroke, colorectal cancer, prostate cancer, and all-cause mortality comparing the highest with the lowest tertile of omega-3 fatty acids biomarkers.**

ALA, α-linolenic acid; CHD, coronary heart disease; CI, confidence interval; CVD, cardiovascular

disease; DHA, docosahexaenoic acid; DPA, docosapentaenoic acid; EPA, eicosapentaenoic acid; RR, relative risk; T2D, type 2 diabetes.

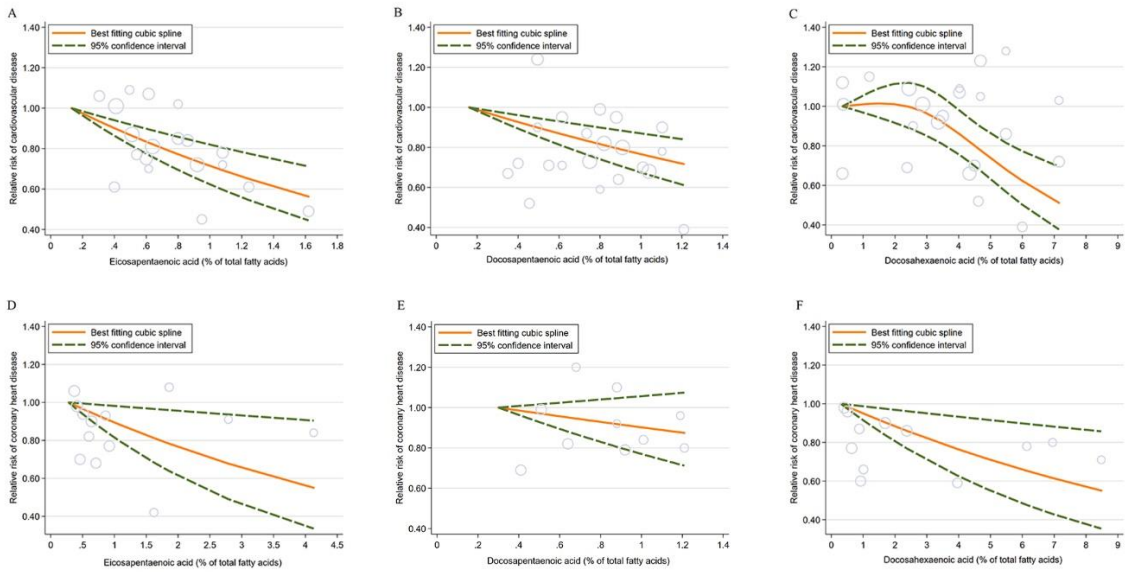

Figure 3. Dose-response analysis for linear or non-linear association of EPA, DPA, and DHA biomarkers with CVD (A, B, and C) and CHD (D, E, and F) risk.

Circles represent point estimates plotted over precision measures. Long dash represent summary estimates and 95% confidence intervals of spline model. CHD, coronary heart disease; CVD, cardiovascular disease; DHA, docosahexaenoic acid; DPA, docosapentaenoic acid; EPA, eicosapentaenoic acid; T2D, type 2 diabetes.

3.8 Sensitivity analysis and publication bias

In sensitivity analyses omitting one study at a time from each analysis, the combined estimate did not substantially change for most omega-3 PUFA biomarkers, except for the studies that evaluated the association between DHA level and prostate cancer: the pooled RR (95% CI) was strengthened to 1.14 (95%CI: 1.00-1.30, P=0.04; I^2=30.6%, $P_{\text{heterogeneity}}$=0.20) when the study by Chavarro et al. was removed [86]. No indication of substantial publication bias was found for most outcomes with either Egger's test or Begg's test (P >0.05 for both tests; **Fig. S39-S42** in Supplementary Appendix 2).

3.9 GRADE profile evidence

Supplementary **Table S13** in Appendix 1 summarize the GRADE quality of evidence for each outcome. All outcomes were downgraded for risk of heterogeneity or indirectness, and outcomes could be upgraded with significant inverse dose-response gradient. Overall, the quality of evidence in four outcomes was rated as "moderate" (EPA-CVD association, EPA-CHD association, DPA-CVD association, and DPA-CHD association), 11 as "low" (ALA-T2D association, ALA-CVD association, ALA-breast cancer association, ALA-prostate cancer association, ALA-mortality association, EPA-breast cancer association, EPA-mortality association, DHA-CVD association, DHA-CHD association, DHA-breast cancer association, and DHA-mortality association), and the remaining outcomes were rated as "very low" based on the GRADE classification.

4. Discussion

This comprehensive meta-analysis demonstrated robust inverse associations between marine-derived omega-3 PUFA in circulation or adipose tissue and lower risk of total CVD, CHD and overall mortality. No significant association was found between these omega-3 PUFA biomarkers and cancer risk, except for modest reduction in the risk of colorectal cancer with DPA and DHA. The associations between ALA and disease outcomes are less clear except an inverse association for T2D. The results largely persisted in dose-response meta-analyses or sub-group analyses. These findings therefore suggest that omega-3 PUFAs have important implications in chronic diseases prevention.

Several biologic mechanisms have been proposed through which marine-derived omega-3 PUFA can reduce biological pathways related to the occurrence of cardiometabolic diseases and cancer, including anti-inflammatory, anti-oxidation,

regulation of lipid metabolism, and amelioration of insulin resistance. Chronic systematic inflammation is recognized as an important contributor to endothelial dysfunction, phospholipid oxidation, insulin resistance, as well as tumor development and growth, all of which are believed to play a role in the subsequent development of metabolic diseases, certain cancers and mortality [93, 94]. Marine-derived omega-3 PUFAs may suppress the expression of inflammation related genes through directly interacting with the nuclear receptor, including peroxisome proliferator-activated receptors, hepatocyte nuclear factor- 4α, and liver X receptor, or through mitigating the activation of the NF-κB transcription factor pathway by blocking IkB phosphorylation [93, 94]. EPA and DHA could limit the inflammatory effect of the arachidonic acid (AA)-derived pro-inflammatory eicosanoids via competing with AA for enzymes (cyclooxygenase and the lipoxygenase) that catalyze the conversion of omega-6 fatty acids to the 2-series prostaglandins and the 4-series leukotrienes [97-99]. Moreover, the metabolites of EPA (3-series prostaglandins and 5-series leukotrienes) could also competitively inhibit the pro-inflammatory triggering of eicosanoids derived from AA as they shared partially same trigger receptors [100]. Another potential mechanism that links beneficial role of marine omega-3 PUFA to chronic condition was the effects of omega-3 PUFA in protecting DNA and lipids from oxidative damage. In H_2O_2-induced DNA damage response in human aortic endothelial cells, Sakai et al. reported that treatment with EPA and DHA significantly diminished the level of intracellular reactive oxygen species and DNA double-strand breaks through upregulation of Nrf2-mediated antioxidant response [101]. Using an alloxan-induced diabetes mellitus rat model, De Assis et al. demonstrated a significant reduction in the content of superoxide dismutase/catalase (SOD/CAT) enzymatic ratio, CAT immunocontent and increase in SOD2 levels after 4 weeks omega-3 PUFA

treatment [102]. In certain circumstances, marine-derived omega-3 PUFA has favorable effect on blood lipid concentration *in vivo* [103]. Furthermore, evidence suggests that DHA and EPA significantly reduce membrane electrical excitability of cardiac myocytes via downregulation of resting membrane potential and the duration of refractory period by directly modulating ion fluxes (e.g., Na^+ and Ca^{2+}), which is generally considered as the mechanism underlying the antiarrhythmic effects of marine-derived omega-3 PUFAs [104].

Results from large-scale randomized controlled trials (RCTs) that investigated the effects of omega-3 PUFA supplementation on composite cardiovascular end points have shown conflicting results. Data from a previous meta-analysis of 10 RCTs (77,917 patients with existing cardiovascular conditions) reported no significant adverse or beneficial effects of omega-3 fatty acid supplements on CHD and major vascular events risk for a mean of 4.4 years treatment [105]. However, two recent randomized trials, which were conducted among individuals who were free of pre-existing CVD, suggested cardiovascular benefits by omega-3 fatty acid supplementation. According to the REDUCE-IT report, supplementation with pure EPA at 4g/day decreased the risk of ischemic events (including cardiovascular death, nonfatal myocardial infarction, nonfatal stroke, coronary revascularization, or hospitalization for unstable angina) by 19-30% during a median of 4.9 y among 8,179 patients with elevated CVD risk or diabetes [5]. Results from the VITAL also showed a significant 39% reduction in myocardial infarction (MI) risk by 1 g/day EPA+DHA supplementation over a median 5.3 years period [6]. In agreement with the results of these two trials, we also found a significant, lower risk of developing CHD and CVD with higher levels of EPA, DPA, and DHA biomarkers among largely healthy individuals, which lends further support for the role of these nutrients in the primary

prevention of CHD and CVD.

With regard to cancer, large intervention trials exploring omega-3 PUFA supplementation in the primary prevention of cancer are scarce. In the VITAL trial, omega-3 PUFA supplementation showed no effect on the incidence of cancer or death from cancer [6]. However, these results should be interpreted with caution as the number of cases was small in the trial which made it difficult to draw small to modest effects. Observational studies also yielded inconsistent results regarding the associations of fish and omega-3 PUFA consumption with breast cancer, prostate cancer, or colorectal cancer, with some studies that reported inverse association, whereas others produced no association or positive association [106-110]. In the present analyses, comparison with cardiometabolic disorders, no apparent association was found for most associations between marine-derived omega-3 PUFA and cancer outcomes. Whether such differences in findings are result from biological differences or other factors is remains unclear, but the fact that, in general, people at high risk of cardiovascular event could obtained more health benefit from omega-3 PUFA cholesterol-lowering effect than the patients with cancer [111-113]. For instance, dyslipidemia induced atherosclerosis is thought to play an important role in the cause of CVD, but it seems not directly involved in the process of cancer incidence and development [112,113]. Considering the complex and multifactorial association of nutrients in relation to disease, additional research is needed to insight into the underlying biological mechanisms of the benefit effects of omega-3 PUFA related to health in order to further understand the association between omega-3 PUFAs and cancer.

Several potential limitations should be considered when interpreting the results. First, fatty acid biomarker levels were measured only once at baseline and changes of

fatty acid levels over time were not accounted for. However, a recent analysis suggested that the omega-3 PUFA concentrations, with the exceptions of DPA, in serum cholesteryl ester, triglyceride and phospholipid fractions remained fairly stable in 8-10 years [114]. Second, although most included studies in our analysis adjusted for multiple major risk factors, such as sociodemographic, lifestyle, clinical, and other dietary risk factors, we cannot exclude the impact of residual and unmeasured confounding on the observed associations [115]. In particular, participants with high omega-3 PUFA status might be more likely to adhere to a healthier dietary pattern or have a higher socioeconomic status, which might distort the true associations. Third, variation in fatty acids metabolism and *de novo* lipogenesis between individuals and between populations may introduce extraneous heterogeneity to the current analysis. In the era of precision medicine, further studies are needed to incorporate factors that account for individual variation in response to omega-3 PUFA intake and subsequent chronic disease risk. Finally, we cannot exclude the possibility of publication bias, although our trim-and-fill analyses suggested such a bias is likely to be small.

5. Conclusions

Our meta-analysis of existing prospective evidence indicated that the marine-derived omega-3 PUFAs were associated with a lower risk of developing major chronic diseases, including CVD, CHD, and overall mortality, although associations for other disease outcomes were unclear. These findings further support the current recommendations of increasing intakes of marine-derived omega-3 PUFAs to facilitate the primary and secondary prevention of chronic conditions, especially CVD.

Author Contributions: LM, DLW and JL generated the idea for the study, formulated an analytical plan. All authors acquired, analyzed, or interpreted the data. HJ and LNW designed the search strategy, and HJ and LNW, and FW performed the literature search and screened studies for eligibility. HJ, YN, and MW extracted data. WRJ and XL assessed the risk of bias. HJ, JXL, and YL performed data analysis. HJ, MW, LM, BBM, FW, FYC, and CL interpreted the data analysis and assessed the certainty of evidence. HJ drafted the manuscript and all other authors revised the manuscript. LM supervised the study. The corresponding author attests that all listed authors meet authorship criteria and that no others meeting the criteria have been omitted.

Conflict of interest: The authors declare no conflicts of interest.

Funding: This work was Supported by the National Natural Science Foundation of China (NSFC-82022062; NSFC-81973025), Nutrition Science Research Foundation of BY-HEALTH (TY0181101); Key Research and Development Program of Shaanxi (2022SF-185), and the Fundamental Research Funds for the Central Universities (qngz2016004, xzy032019008). The funders had no role in the design and conduct of the study; collection, management, analysis, and interpretation of the data; preparation, review, or approval of the manuscript; and decision to submit the manuscript for publication.

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Supplementary Appendix 1

Table of Contents

Table 1. Search strategy

Database and search terms	
PUBMED	**EMBASE**
#1. "fatty acids, omega 3"[Mesh] OR "n-3 fatty acid*"[tiab] OR "omega 3 fatty acid*"[tiab] OR essential fatty acid*"[tiab] OR polyunsaturated fatty acid*"[tiab] OR "ALA"[tiab] OR "alpha-linolenic acid"[tiab] OR "flaxseed oil"[tiab] OR "eicosapentaenoic*" [tiab] OR "Icosapent"[tiab] OR "docosahexaenoic*"[tiab] OR "Docosahexaenoate"[tiab] OR "docosapentaenoic*"[tiab] OR "Timnodonic Acid"[tiab] OR "clupanodonic acid "[tiab]	**#1.** 'fatty acids, omega 3'/exp OR 'n 3 fatty acid*':ab,ti OR 'omega 3 fatty acid*':ab,ti OR 'essential fatty acid*':ab,ti OR 'polyunsaturated fatty acid*':ab,ti OR 'ALA':ab,ti OR 'alpha-linolenic acid':ab,ti OR 'flaxseed oil':ab,ti OR 'eicosapentaenoic*':ab,ti OR 'Icosapent':ab,ti OR 'docosahexaenoic*':ab,ti OR 'Docosahexaenoate':ab,ti OR 'docosapentaenoic*':ab,ti OR 'Timnodonic Acid':ab,ti OR 'clupanodonic acid': ab,ti
#2. "Neoplasms"[Mesh] OR "cancer*"[tiab] OR "carcinoma*"[tiab] OR "tumor*"[tiab] OR "Cerebrovascular Disorders"[Mesh] OR "stroke* "[tiab] OR "cerebrovascular accident*"[tiab] OR "Cardiovascular diseases"[Mesh] OR "cardiovascular"[tiab] OR "heart"[tiab] OR "myocardial infarction*"[tiab] OR "sudden death*"[tiab] OR "Diabetes Mellitus" [Mesh] OR "diabet*"[tiab] OR "Mortality"[Mesh] OR "Death"[Mesh] OR "mortality"[tiab] OR "death"[tiab] OR "fatal"[tiab]	**#2.** 'Neoplasms'/exp OR 'cancer*':ab,ti OR 'carcinoma*':ab,ti OR 'tumor*':ab,ti OR 'Cerebrovascular Disorders'/exp OR 'stroke*':ab,ti OR 'cerebrovascular accident*':ab,ti OR 'Cardiovascular diseases'/exp OR 'cardiovascular':ab,ti OR 'heart':ab,ti OR 'myocardial infarction*':ab,ti OR 'sudden death*':ab,ti OR 'Diabetes Mellitus'/exp OR 'diabet*':ab,ti OR 'Mortality'/exp OR 'Death'/exp OR 'mortality':ab,ti OR 'death':ab,ti OR 'fatal':ab,ti
#3. "Epidemiology" [MESH] OR "Epidemiologic Studies" [MESH] OR "Intervention Studies" [MESH] OR "cohort*"[tiab] OR "incident*"[tiab] OR "incidence*"[tiab] OR "prospective"[tiab] OR "follow-up"[tiab] OR "predict*" [tiab] OR "prognos*"[tiab] OR "case-control"[tiab] OR "cross-sectional"[tiab] OR "intervention*" [tiab] OR "clinical trial*"[tiab] OR "randomized*"[tiab]	**#3.** 'Epidemiology '/exp OR 'Epidemiologic Studies'/exp OR 'Intervention Studies'/exp OR 'cohort*':ab,ti OR 'incident*':ab,ti OR 'incidence*':ab,ti OR 'prospective':ab,ti OR 'follow-up':ab,ti OR 'predict*':ab,ti OR 'prognos*':ab,ti OR 'case-control':ab,ti OR 'cross-sectional':ab,ti OR 'intervention*':ab,ti OR 'clinical trial*':ab,ti OR 'randomized*':ab,ti
#4. "Blood" [MESH] OR "marker*" [tiab] OR "biomarker*" [tiab] OR "serum*" [tiab] OR "plasma*" [tiab] OR "whole blood*" [tiab] OR "adipose tissue*" [tiab] OR "fat*" [tiab] OR "circulating" [tiab] OR "erythrocyte*" [tiab] OR "red blood cell" [tiab] OR "cholesteryl esters" [tiab]	**#4.** 'Blood'/exp OR 'marker*':ab,ti OR 'biomarker*':ab,ti OR 'serum*':ab,ti OR 'plasma*':ab,ti OR 'whole blood*':ab,ti OR 'adipose tissue*':ab,ti OR 'fat*':ab,ti OR 'circulating':ab,ti OR 'erythrocyte*':ab,ti OR 'red blood cell':ab,ti OR 'cholesteryl esters':ab,ti
	#5. #1 AND #2 AND #3 AND #4

#5. #1 AND #2 AND #3 AND #4	
Web of Science **#1.** TS=(fatty acids, omega 3 OR n 3 fatty acid* OR omega 3 fatty acid* OR essential fatty acid* OR polyunsaturated fatty acid* OR ALA OR alpha-linolenic acid OR flaxseed oil OR eicosapentaenoic* OR Icosapent OR docosahexaenoic* OR Docosahexaenoate OR docosapentaenoic* OR Timnodonic Acid OR osbond acid OR clupanodonic acid) **#2.** TS=(Neoplasms OR cancer* OR carcinoma* OR tumor* OR Cerebrovascular Disorders OR stroke* OR cerebrovascular accident* OR Cardiovascular diseases OR cardiovascular OR heart OR myocardial infarction* OR sudden death* OR Diabetes Mellitus OR diabet* OR Mortality OR Death OR mortality OR death OR fatal) **#3.** TS= (Epidemiology OR Epidemiologic Studies OR Intervention Studies OR cohort* OR incident* OR incidence* OR prospective OR follow-up OR predict* OR prognos* OR case-control OR cross-sectional OR intervention* OR clinical trial* OR randomized*) **#4.** TS= (blood OR marker* OR biomarker* OR serum* OR plasma* OR whole blood* OR adipose tissue* OR fat* OR circulating OR erythrocyte* OR red blood cell OR Ccholesteryl esters) **#5.** #1 AND #2 AND #3 AND #4	**Cochrane Library** **#1.** (fatty acids, omega 3):ti,ab,kw OR (n 3 fatty acid*):ti,ab,kw OR (omega 3 fatty acid*):ti,ab,kw OR (essential fatty acid*):ti,ab,kw OR (polyunsaturated fatty acid*):ti,ab,kw OR (ALA):ti,ab,kw OR (alpha-linolenic acid):ti,ab,kw OR (flaxseed oil):ti,ab,kw OR (eicosapentaenoic*):ti,ab,kw OR (Icosapent):ti,ab,kw OR (docosahexaenoic*):ti,ab,kw OR (Docosahexaenoate):ti,ab,kw OR (docosapentaenoic*): ti,ab,kw OR (Timnodonic Acid):ti,ab,kw OR (osbond acid):ti,ab,kw OR (clupanodonic acid):ti,ab,kw **#2.** (Neoplasms):ti,ab,kw OR (cancer*):ti,ab,kw OR (carcinoma*):ti,ab,kw OR (tumor*):ti,ab,kw OR (Cerebrovascular Disorders):ti,ab,kw OR (stroke*): ti,ab,kw OR (cerebrovascular accident*):ti,ab,kw OR (Cardiovascular diseases):ti,ab,kw OR (cardiovascular):ti,ab,kw OR (heart):ti,ab,kw OR (myocardial infarction*):ti,ab,kw OR (sudden death*):ti,ab,kw OR (Diabetes Mellitus): ti,ab,kw OR (diabet*):ti,ab,kw OR (Mortality):ti,ab,kw OR (Death):ti,ab,kw OR (mortality):ti,ab,kw OR (death):ti,ab,kw OR (fatal):ti,ab,kw **#3.** (blood):ti,ab,kw OR (marker*):ti,ab,kw OR (biomarker*):ti,ab,kw OR (serum*):ti,ab,kw OR (plasma*):ti,ab,kw OR (whole blood*):ti,ab,kw OR (adipose tissue*):ti,ab,kw OR (fat*):ti,ab,kw OR (circulating):ti,ab,kw OR (crythrocyte*):ti,ab,kw OR (red blood cell):ti,ab,kw OR (cholesteryl esters):ti,ab,kw **#4.** #1 AND #2 AND #3

Table 2. Summary of prospective studies on biomarkers of omega-3 fatty acids and type 2 diabetes (n=12)

Author, publication year, country	Characteristics of the study				Characteristics of the participant			Characteristics of the exposure				Characteristics of the outcome		Adjustment for confounding factors	Study quality*
	Baseline survey year	Design	Follow up (year)	Study name	No.	Age range (year)	Men (%)	Assay method	Biological sample	Lipid fraction measured	Exposure	Ascertainment method	Cases (n)		
Wang et al, 2003, USA[31]	1987-89	PC	9.0	ARIC	2,909	45-64	46.0	GLC	Plasma	Total fatty acid fraction	ALA	Biomarkers	252	Age, sex, smoking, alcohol, education, BMI, physical activity, WHR, family history of diabetes	High
Hodge et al, 2007, Australia[32]	1990-94	CCD	4.0	MCCS	3,737	36-72	44.1	GC	Plasma	Phospholipid fraction	ALA, EPA, DPA, DHA	Self-report	346	Age, sex, region, alcohol, BMI, physical activity, WHR, family history of diabetes	High
Krachler et al, 2008, Sweden[33]	1985-94	NCCD	8.8	VIP	450	30-60	NR	GLC	Erythrocyte membrane	Phospholipid fraction	ALA, EPA, DPA, DHA	Records	159	Smoking, alcohol, BMI, physical activity, HbA1c	High
Patel et al, 2010, UK[34]	1993-97	NCCS	10.0	EPIC-Norfolk	383	40-79	53.3	GC	Erythrocyte membrane	Phospholipid fraction	ALA, EPA, DPA, DHA	Self-report, medication use	199	Age, sex, smoking, alcohol, BMI, physical activity, family history of diabetes	High
Kröger et al, 2011, Europe[35]	1991	NCCS	7.0 (mean)	EPIC-Potsdam	2,724	23-71	44.5	GC	Erythrocyte membrane	Phospholipid fraction	ALA, EPA, DPA, DHA	Self-report, records, medication use	673	Sex, smoking, alcohol, education, BMI, WHR, occupational activity, physical activity, dietary factors	High
Djoussé et al, 2011, USA[36]	1989-90	PC	10.6 (median)	CHS	3,088	≥ 65	38.9	GC	Plasma	Phospholipid fraction	ALA	Biomarkers	204	Age, sex, race, smoking, alcohol, region, BMI, physical activity, blood lipid, plasma fatty acids	High
Virtanen et al, 2014, Finland[37]	1984-89	PC	19.3 (mean)	KIHD	2,212	42-60	100.0	GC	Serum	Total fatty acid fraction	ALA,EPA+DPA+DHA, EPA, DPA， DHA	Self-report, records, biomarkers	422	Age, examination year, smoking, alcohol, education, BMI, physical activity, family history of diabetes, serum fatty acids	High
Takkunen et al, 2016, Finland[38]	1993-98	PC	11.0 (median)	FDP	407	40–65	32.6	GC	Serum	Total fatty acid fraction	ALA, EPA, DPA, DHA	Biomarkers	155	Age, sex, study group, smoking, alcohol, physical activity, WHR, energy intake, dietary factors, serum lipid, plasma fasting and 2h blood glucose	High
Forouhi et al, 2016, Europe[39]	1991	CCD	9.8 (mean)	EPIC-InterAct	28,051	23-71	41.6	GC	Plasma	Phospholipid fraction	ALA, EPA, DPA, DHA	Self-report, records	12,132	Age, sex, smoking, alcohol, education, BMI, physical activity, energy intake, dietary factors	High
Harris et al, 2016, USA[40]	1995	PC	11.0	WHIMS	6,379	65-80	0.0	GC	Erythrocyte membrane	Phospholipid fraction	ALA, EPA, DHA, DPA	Self-report	703	Age, race, smoking, alcohol, education, physical activity, WHR, family history of diabetes, dietary glycemic load	High

Zheng et al, 2018, China[41]	2008-10	PC	5.6 (median)	GNHS	2,671	40-75	-	GC	Erythrocyte membrane	Phospholipid fraction	ALA, EPA+DHA+DPA, EPA, DPA, DHA	Biomarkers	213	Age, sex, alcohol, smoking, education, household income, physical activity, BMI, WHR, family history of diabetes, dietary factors, fasting serum, glucose and erythrocyte total omega-6 PUFA	Moderate
Zhuang et al, 2022, UK[42]	2006-10	PC	11.6 (mean)	UK Biobank	95,854	37-73	44.5	NMR	Plasma	Total fatty acid fraction	DHA	Records	3,052	Age, sex, race, smoking, alcohol, education, physical activity, Townsend deprivation index, household income, history of hypertension and high cholesterol, family history of diabetes, vitamin supplement use, mineral supplement use, aspirin use, remaining plasma fatty acids	High

ALA, α-linolenic acid; ARIC, Atherosclerosis Risk in Communities Study; BMI, body mass index; CCD, case-cohort design study; CHS, Cardiovascular Health Study; DHA, docosahexaenoic acid; DPA, docosapentaenoic acid; EPIC-InterAct, European Prospective Investigation into Cancer (EPIC)- InterAct study; EPIC- Potsdam, European Prospective Investigation into Cancer and Nutrition (EPIC)-Potsdam Study; EPA, eicosapentaenoic acid; GC, gas chromatography; GNHS, Guangzhou Nutrition and Health Study; GLC, gas-liquid chromatography; HHS, Hitachi Health Study; FDP, Finnish Diabetes Prevention Study; KIHD, Kuopio Ischemic Heart Disease Risk Factor study; NMR, nuclear magnetic resonance-based profiling; NR, not reported; MCCS, Melbourne Collaborative Cohort Study; NCCD, nested case-control design study; NCCS, nested case-cohort study; PC, prospective cohort study; USA, the United States of America; VIP, Västerbotten Intervention Programme; WHIMS, Women's Health Initiative Memory Study; WHR, waist hip rate.

* Study quality was assessed with the Newcastle-Ottawa scale.

Table 3. Summary of prospective studies on biomarkers of omega-3 fatty acids and cardiovascular disease (n=13)

Author, publication year, country	Characteristics of the study				Characteristics of the participant				Characteristics of the exposure			Characteristics of the outcome		Adjustment for confounding factors	Study quality*
	Baseline survey year	Design	Follow up (year)	Study name	No	Age range (year)	Men (%)	Assay method	Biological sample	Lipid fraction measured	Exposure	Ascertainment method	Cases (n)		
Albert et al, 2002, USA[43]	1982-84	NCCD	8.7 (mean)	PHS	278	40-84	100.0	GLC	Blood	Total fatty acid fraction	EPA+DPA+DHA	Records, interview	94 sudden deaths	Alcohol, BMI, physical activity, aspirin use, beta carotene or placebo treatment, diabetes, hypertension, hypercholesterolemia, family history of MI, blood fatty acids	Moderate
Laaksonen et al, 2005, Finland[44]	1984-89	PC	14.6 (median)	KIHD	1,551	42-60	100.0	GC	Serum	Total fatty acid fraction	ALA	Records	78 CVD deaths	Age, smoking, alcohol, socioeconomic status, examination year, BMI, physical activity, SBP, BP medication, family history of IHD, energy intake, dietary factors, blood lipid, plasma fatty acids, insulin concentration	High
Warensjö et al, 2008, Sweden[16]	1920-24	PC	30.7 (median)	ULSAM	3,894	≥ 50	100.0	GC	Serum	Cholesteryl fraction	ALA, EPA, DHA	Records	461 CVD deaths	Smoking, BMI, physical activity, hypertension, blood lipid	High
Woodward et al, 2011, Scotland[17]	1984-87	PC	19.5 (median)	SHHECS	3,944	40-59	53.1	GC	Adipose tissue	-	DPA, DHA	Records	870 CVD	Age, sex, smoking, socioeconomic status, SBP, BP treatment, diabetes, and family history, blood lipid	High
Virtanen et al, 2012, Finland[46]	1984-89	PC	20.1 (mean)	KIHD	1,857	42-60	100.0	GC	Serum	Total fatty acid fraction	EPA+DPA+DHA, EPA, DPA, DHA	Records	91 SCD	Age, smoking, alcohol, examination year, BMI, hair mercury content	High
Chien et al, 2013, China (Taiwan) [45]	1990	PC	9.6 (median)	Cohort in Taiwan	1,833	50-72	NR	GC	Plasma	Total fatty acid fraction	EPA, DHA	Records	275 CVD cases	Age, sex, marital status, occupation, smoking, alcohol, education, BMI, physical activity, hypertension, diabetes, blood lipid	Moderate
Mozaffarian et al, 2013, USA[18]	1992-93	PC	16.0 (max)	CHS	2,692	≥ 65	36.3	GC	Plasma	Phospholipid fraction	EPA, DPA, DHA	Records, interview	570 CVD deaths	Age, sex, race, smoking, alcohol, education, region, BMI, WHR, physical activity, fatty acid measurement batch, diabetes, atrial fibrillation, drug-treated hypertension, dietary factors	High
de Oliveira Otto et al, 2013, USA[47]	2000-02	PC	9.0	MESA	2,837	45-84	46.8	GC	Plasma	Phospholipid fraction	ALA, EPA+DPA+DHA, EPA, DPA, DHA	Records	189 CVD	Age, sex, race, smoking, alcohol, education, region, BMI, physical activity, diabetes, dietary supplement treatment, BP treatment, energy intake, dietary factors	High
Fretts et al, 2014, USA[48]	1992-93	PC	16.0 (max)	CHS	2,709	≥ 65	36.1	GC	Plasma	Phospholipid fraction	ALA	Records, interview	517 CVD deaths	Age, sex, race, smoking, alcohol, education, region, BMI, diabetes, hypertension, energy intake	High
Marklund et al, 2015, Swedish[49]	1997-98	PC	14.5 (median)	Cohort in Swedish	2,193	≥ 60	48.2	GC	Serum	Cholesterol fraction	ALA, EPA, DHA	Records	484 CVD deaths	Smoking, alcohol, education, BMI, physical activity, diabetes, drug-treated hypertension and hypercholesterolemia	Moderate

Harris et al, 2017, USA[50]	1996	PC	14.9 (median)	WHIMS	6,501	65-80	0.0	GC	Erythrocyte membrane	Phospholipid fraction	ALA, EPA, EPA, DHA	Records	617 CVD deaths	Age, race, smoking, alcohol, education, region, BMI, WHR, physical activity, HT assignment, hypertension, diabetes, CVD and/or cancer, family history of cancer and CVD, aspirin treatment, cholesterol medication	High
Harris et al, 2018, USA[51]	1971	PC	7.3 (median)	FHS	2,500	56-75	43.1	GC	Erythrocyte membrane	Phospholipid fraction	ALA, EPA, DPA, DHA	-	245 CVD, 58 CVD deaths	Age, sex, marital status, occupation, smoking, alcohol, education, BMI, physical activity, health insurance status, aspirin treatment, hypertension, cholesterol medication, diabetes, SBP, blood lipid	High
Zhang, et al, 2021, China[52]	2003-04	PC	6.9 (mean)	NHANES	4,132	≥ 18	49.3	GC	Serum	Triglycerides fraction, phospholipid, fraction, cholesterol fraction	EPA, DPA, DHA	National Death Index	157 CVD deaths	Age, sex, BMI, race, smoking, drinking, education, family annual income, physical activity, diabetes, CVD, cancer, ever controlled blood pressure, blood cholesterol or blood glucose, serum triglycerides, serum total cholesterol, SFAs, USFAs, fiber, total energy, carbohydrate, protein intake, AHEI-2010	High

AHEI: alternative healthy eating index; ALA, α-linolenic acid; BP, blood pressure; BMI, body mass index; CHS , Cardiovascular Health Study; CVD, cardiovascular disease; DHA, docosahexaenoic acid; DPA, docosapentaenoic acid; EPA, eicosapentaenoic acid; FHS, The Framingham Heart Study; GC, gas chromatography; GLC, gas-liquid chromatography; HT, heart rate; KIHD, Kuopio Ischemic Heart Disease Risk Factor study; IHD, ischemic heart disease; MI, myocardial infarction; MESA, the Multi-Ethnic Study of Atherosclerosis; NCCD, nested case-control design study; NHANES, National Health and Nutrition Examination Survey; NR, not reported; PC, prospective cohort study; PHS, Physicians' Health Study; SCD, sudden cardiac death; SHHECS, the Scottish Heart Health Extended Cohort Study; SBP, systolic blood pressure; ULSAM, Uppsala Longitudinal Study of Adult Men; USA, the United States of America; WHIMS, Women's Health Initiative Memory Study; WHR, waist hip rate.

* Study quality was assessed with the Newcastle-Ottawa scale.

Table 4. Summary of prospective studies on circulating omega-3 fatty acids and coronary heart disease included in this review (n=13)

Author, publication year, country	Characteristics of the study				Characteristics of the participant			Characteristics of the exposure				Characteristics of the outcome		Adjustment for confounding factors	Study quality*
	Baseline survey year	Design	Follow up (year)	Study name	No	Age range (year)	Men (%)	Assay method	Biological sample	Lipid fraction measured	Exposure	Ascertainment method	Cases (n)		
Simon et al, 1995, USA[53]	1973-76	NCCD	6.9 (mean)	MRFIT	188	35-57	100.0	GC	Serum	Phospholipid fraction, cholesterol fraction	ALA, EPA, DPA, DHA	Records, interview	94 CHD	Age, alcohol, region, recruitment date, blood lipid, serum fatty acids	Moderate
Lemaitre et al, 2003, USA[54]	1992-93	NCCD	1.76	CHS	304	≥ 65	73.0	GC	Plasma	Phospholipid fraction, cholesterol esters fraction	ALA, EPA+DHA	Records	125 fatal MI	Age, sex, education, region, recruitment date, BMI, SBP, fasting plasma glucose	High
Wang et al, 2003, USA[55]	1987-89	PC	10.7 (mean)	ARIC	3,591	45-64	46.0	GLC	Plasma	Total fatty acid fraction	ALA	Records	282 CHD	Age, sex, smoking, alcohol, physical activity, dietary factors	High
Sun et al, 2008, USA[56]	1989-90	NCCD	6.0	NHS	434	30-55	0.0	GLC	Plasma	Total fatty acid fraction	EPA+DPA+DHA, EPA, DPA, DHA	Records	146 nonfatal MI	Blood collection age, smoking, alcohol, fasting status, BMI, physical activity, postmenopausal status, postmenopausal hormone treatment, MI, hypertension, hypercholesterolemia, diabetes, energy intake, blood fatty acids	High
Joensen et al, 2011, Denmark[57]	1993-97	CCD	7.6 (mean)	DCH	2,792	50-64	61.2	GC	Adipose tissue	-	EPA, DPA, DHA	Records	1,012 ACS	Smoking, alcohol, education, BMI, physical activity, history of diabetes, blood pressure, blood lipid, hormone replacement therapy (women)	High
Khaw et al, 2012, UK[58]	1993-97	NCCD	13.0 (mean)	EPIC-Norfolk	7,354	40-79	52.2	GC	Plasma	Phospholipid fraction	ALA, EPA, DPA, DHA	Records	2,424 CHD	Smoking, alcohol, education, socioeconomic status, BMI, physical activity, diabetes, SBP, blood lipid, plasma vitamin C	High
de Oliveira Otto, 2013, USA[47]	2000-02	PC	10.0 (max)	MESA	2,837	45-84	46.8	GC	Plasma	Phospholipid fraction	ALA, EPA+DPA+DHA, EPA, DPA, DHA	Records	189 CHD	Age, sex, race, smoking, alcohol, education, region, BMI, physical activity, diabetes, energy intake, dietary supplement treatment, BP treatment, dietary factors	High
Mozaffarian et al, 2013, USA[18]	1992-93	PC	16.0 (max)	CHS	2,692	≥ 65	36.3	GC	Plasma	Phospholipid fraction	EPA, DPA, DHA	Records	730 CHD deaths	Age, sex, race, smoking, alcohol, education, region, BMI, WHR, physical activity, fatty acid measurement batch, diabetes, atrial fibrillation, drug-treated hypertension, dietary factors	High
Matsumoto et al, 2013, USA[59]	1982-84	NCCD	5.0 (average)	PHS I	2,000	50-92	100.0	GC	Erythrocyte membrane	Phospholipid fraction	ALA, EPA, DPA, DHA	Records	1,000 CHD	Age, smoking, alcohol, recruitment date, blood collection age, BMI, physical activity, hypertension, diabetes, hypercholesterolemia	High

Fretts et al, 2014, USA[48]	1992-93	PC	16.0 (max)	CHS	2,709	≥ 65	36.1	GC	Plasma	Phospholipid fraction	ALA	Records	519 CHD deaths	Age, sex, energy intake, race, smoking, alcohol, education, region, BMI, diabetes, drug-treated hypertension	High
Sun et al, 2016, Singapore[60]	1993-98	NCCD	10.0 (max)	SCHS	1,488	47-83	64.7	GC-MS/MS	Plasma	Total fatty acid fraction	ALA, EPA, DHA,	Records	744 AMI	Age, sex, smoking, alcohol, education, age at blood collection, recruitment date, BMI, physical activity, hours of fasting before blood collection, hypertension, diabetes, energy intake, dietary factors, plasma fatty acids	Moderate
Hamazaki et al, 2017, Japan[61]	1990-93	NCCD	13.5 (mean)	JPHC	627	40-59	63.6	GC	Plasma	Total fatty acid fraction	EPA+DPA+DHA, EPA, DPA, DHA	Records	209 CHD	Age at blood collection, sex, smoking, alcohol, region, recruitment date, BMI, time elapsed since last meal, hypertension, hypercholesterolemia treatment, serum glucose category	High
Chei et al, 2018, Japan[62]	1984, 1989, 1997, 1998	NCCD	11.0 (mean)	CIRCS	608	40-385	-	GC	Serum	Total fatty acid fraction	ALA, EPA, DPA, DHA	Records	152 CAD	Smoking, alcohol, BMI, matching for sex, age, community, year of serum stored, fasting status	High

ALA, α-linolenic acid; ACS, acute coronary syndromes; ARIC, Atherosclerosis Risk in Communities Study; AMI, acute myocardial infarction; BMI, body mass index; CAD, coronary artery disease; CCD, nested case-cohort design study; CHD, coronary heart disease; CHS, Cardiovascular Health Study; DBP, diastolic blood pressure; CIRCS, Circulatory Risk in Communities Study; DCH, the Diet, Cancer and Health study; DHA, docosahexaenoic acid; DPA, docosapentaenoic acid; EPIC-Norfolk, European Prospective Investigation into Cancer (EPIC)-Norfolk study; EPA, eicosapentaenoic acid; GC, gas chromatography; GLC, gas-liquid chromatography; GC-MS/MS, Gas chromatography-triple quadrupole mass spectrometry; JPHC, Japan Public Health Center-based study; MI, myocardial infarction; MESA, the Multi-Ethnic Study of Atherosclerosis; MRFIT, Multiple Risk Factor Intervention Trial; NCCD, nested case-control design study; NHS, Nurses' Health Study; PHS, Physicians' Health Study; PUFA, polyunsaturated fatty acid; SCHS, Singapore Chinese Health Study; UK, the United Kingdom; USA, the United States of America; PC, prospective cohort study; SBP, systolic blood pressure; VIP, Västerbotten Intervention Program; WHR, waist hip rate.

* Study quality was assessed with the Newcastle-Ottawa scale.

Table 5. Summary of prospective studies on circulating omega-3 fatty acids and stroke included in this review (n=12)

Author, publication year, country	Characteristics of the study				Characteristics of the participant			Characteristics of the exposure				Characteristics of the outcome		Adjustment for confounding factors	Study quality*
	Baseline survey year	Design	Follow up (year)	Study name	No	Age range	Men (%)	Assay method	Biological sample	Lipid fraction measured	Exposure (cases/controls)	Ascertainment method	Cases (n)		
Wiberg et al, 2006, Sweden[63]	1920-24	PC	29.3 (median)	ULSAM	2,322	50	100.0	GC	Serum	Cholesterol fraction	ALA, EPA, DHA	Records	421 stroke or TIA	Smoking, physical activity, antihypertensive, antidiabetic, lipid-lowering drugs, hypertension, diabetes, atrial fibrillation, CVD, metabolic syndrome, blood lipid	Moderate
De Goede et al, 2013, Holland[64]	1993-97	NCCD	10.5 (median)	MORGEN	358	20-65	53.0	GC	Plasma	Cholesteryl fraction	ALA	Records	179 stroke	Age, sex, smoking, alcohol, education, enrollment date, BMI, diabetes, hypertension, hypercholesterolemia	High
Yamagishi et al, 2013, USA[65]	1987-89	PC	19.9 (median)	ARIC	3,870	45-64	61.3	GLC	Plasma	Phospholipid fraction, cholesterol fraction	ALA, EPA, DHA	Records	168 ischemic stroke	Age, sex, smoking, cigarette-years, alcohol	High
Yaemsiri et al, 2013, USA[66]	1993-98	PC	10.0 (max)	WHI-OS	1,928	50-79	0.0	GC	Serum	Total fatty acid fraction	ALA, EPA, DPA, DHA	Self-report	964 ischemic stroke	Age, race, smoking, examination year, BMI, SBP, diabetes, aspirin treatment, BP treatment, blood lipid, normalized-triglycerides	High
Fretts et al, 2014, USA[48]	1992-93	PC	16.0 (max)	CHS	2,709	≥ 65	36.1	GC	Plasma	Phospholipid fraction	ALA	Records	430 stroke	Age, sex, race, region, smoking, alcohol, education, BMI, diabetes, BP treatment, energy intake	High
Daneshmand et al, 2016, Finland[67]	1992-93	PC	21.2 (mean)	KIHD	1,828	42-60	100.0	GC	Serum	Total fatty acid fraction	ALA, EPA+EPA+DHA, EPA, DPA, DHA	Records	202 stroke	Age, smoking, alcohol, examination year, BMI, SBP, physical activity, diabetes, blood lipid	High
Saber et al, 2017, USA[68]	1992-93	NCCD	11.2	CHS	516	≥ 65	40.0	GC	Plasma	Phospholipid fraction	EPA, DPA, DHA	Records	516 ischemic stroke	Age, sex, race, smoking, alcohol, BMI, physical activity, hypertension, family history of CVD and diabetes, menopausal status, aspirin treatment, dietary factors	High
Saber et al, 2017, USA[68]	1989-90	NCCD	8.3	NHS	714	30-55	0.0	GC	Plasma	Phospholipid fraction	EPA, DPA, DHA	Records	357 ischemic stroke	Age, sex, race, smoking, alcohol, BMI, physical activity, hypertension, family history of CVD and diabetes, menopausal status, aspirin treatment, dietary factors	High
Saber et al, 2017, USA[68]	1993-94	NCCD	8.3	HPFS	160	40-75	100.0	GC	Plasma	Phospholipid fraction	EPA, DPA, DHA	Records	80 ischemic stroke	Age, sex, race, smoking, alcohol, BMI, physical activity, hypertension, family history of CVD and diabetes, menopausal status, aspirin treatment, dietary factors	High

Bork et al, 2018, Denmark[69]	1993-97	CCD	13.4 (mean)	DCH	4,920	50-64	61.2	GC	Adipose tissue	-	ALA	Records	1,735 ischemic stroke	Age, smoking, alcohol, education, waist circumference, BMI, physical activity, hypercholesterolemia and/or lipid-lowering medication, hypertension and/or antihypertensive medication use, diabetes, atrial fibrillation	High
Harris et al, 2018, USA[48]	1971	PC	7.3	FHS	2,500	56-75	43.1	GC	Erythrocyte membrane	Phospholipid fraction	ALA, EPA, DPA, DHA	-	105 ischemic stroke	Demographic, clinical status, therapeutic, CVD risk factors	High
Venø et al, 2019, Denmark[70]	1993-97	PC	13.5 (median)	DCH	55,338	50-65	48.0	GC	Adipose tissue	-	EPA+EPA+DHA, EPA, DPA, DHA	Records	1,879 ischemic stroke	Age, sex, smoking, alcohol, education, BMI, waist circumference, physical activity, alcohol abstain	High

ALA, α-linolenic acid; ARIC, Atherosclerosis Risk in Communities Study; BP, blood pressure; BMI, body mass index; CCD, nested case-cohort design study; CHS, Cardiovascular Health Study; CVD, cardiovascular disease; DHA, docosahexaenoic acid; DCH, the Diet, Cancer and Health study; DPA, docosapentaenoic acid; EPA, eicosapentaenoic acid; FHS, Framingham Heart Study; GC, gas chromatography; GLC, gas-liquid chromatography; HPFS, Health Professionals Follow-Up Study; KIHD, Kuopio Ischemic Heart Disease Risk Factor study; MORGEN, Monitoring Project on Risk Factors for Chronic Diseases; NCCD, nested case-control design study; NHS, Nurses' Health Study; USA, the United States of America; PC, prospective cohort study; SBP, systolic blood pressure; TIA, Transient ischemic attack; ULSAM, Uppsala Longitudinal Study of Adult Men; WHI-OS, Women's Health Initiative Observational Study.

* Study quality was assessed with the Newcastle-Ottawa scale.

Table 6. Summary of prospective studies on circulating omega-3 fatty acids and cancer included in this review (n=21)

Author, publication year, country	Characteristics of the study				Characteristics of the participant			Characteristics of the exposure				Characteristics of the outcome		Adjustment for confounding factors	Study quality*
	Baseline survey year	Design	Follow up (year)	Study name	No	Age range	Men (%)	Assay method	Biological sample	Lipid fraction measured	Exposure (cases/controls)	Ascertainment method	Cases (n)		
Colorectal cancer															
Hall et al, 2007, USA[71]	1982-84	NCCD	10.0 (max)	PHS	460	40-84	100.00	GLC	Blood	Total fatty acid fraction	EPA +DPA+DHA, EPA, DPA, DHA	Records	178	Alcohol, BMI, physical activity, diabetes, aspirin treatment, multivitamin treatment, dietary factors, blood fatty acids	High
Hodge et al, 2014, Australia[72]	1990–94	CCD	9.0 (mean)	MCCS	4,205	40–69	45.0	GLC	Plasma	Phospholipid fraction	ALA, EPA, DPA, DHA	Records	395	Alcohol, smoking, education, physical activity, energy intake	High
Butler et al, 2017, Singapore[73]	1993-98	NCCD	3.3 (median)	SCHS	700	45-74	58.86	GC	Plasma	Total fatty acid fraction	ALA, EPA, DHA	Records	350 (211 colon, 139 rectal)	Smoking, alcohol, education, BMI, physical activity, diabetes	High
Breast cancer															
Chajès et al, 1999, Sweden[74]	1986-97	NCCD	9.0 (median)	VIP, MONICA, MSP	584	30-60	0.00	GC	Serum	Phospholipid fraction	ALA, EPA, DHA	Records	196	Menarche age, first full-term pregnancy age, number of children, hormone replacement therapy use, height and weight	High
Saadatian-Elahi et al, 2002, France[75]	1985-91	NCCD	4.3 (median)	NYUWHS	394	34-65	0.00	GC	Erythrocyte membrane	Phospholipid fraction	ALA, EPA, DPA, DHA	Records	197	First full-term birth age, family history of breast cancer and benign breast cancer, cholesterol	High
Chajès et al, 2008, France[76]	1989-91	PC	7.0 (mean)	E3N Study	1,065	40–65	0.00	GC	Serum	Phospholipid fraction	ALA, EPA+DPA+DHA, EPA, DHA	Records	363	Alcohol, education, BMI, height, menopausal hormone treatment, parity, family history of breast cancer	High
Takata et al, 2009, USA[77]	1985-94	NCCD	7.5 (median)	CARET	387	50-69	0.00	GC	Serum	Phospholipid fraction	ALA, EPA, DPA, DHA	Self-report, records	130	Age, smoking, alcohol, region, examination year, BMI, intervention arm	High
Witt et al, 2009, Denmark[78]	1997	CCD	4.8 (median)	DCH	1,561	50–64	0.00	GC	Adipose tissue	-	EPA+ DPA+DHA, EPA, DPA, DHA	Records	463	Smoking, alcohol, education, physical activity, BMI, HRT use, menarche, age at first child, number of children	High
Pouchieu et al, 2014, UK[79]	1994-95	NCCD	3.7 (median)	SU.VI.MAX	500	35-60	0.00	GC	Plasma	Total fatty acid	ALA, EPA, DPA, DHA	Self-report	154	Age, sex, smoking, alcohol, education, study group, height, BMI, physical activity, family history of cancer	High

Bassett et al, 2016, Australia[80]	1990-94	CCD	8.9 (mean)	MCCS	2491	40-69	100.00	GLC	Plasma	Phospholipid fraction	ALA, EPA, DPA, DHA	Records	470	Age, region, smoking, alcohol, education, physical activity, menopausal status, hormone therapy, oral contraceptive use, family history of cancer, energy intake,	High
Chajès et al, 2017, Europe[81]	1993-2002	NCCD	11.5 (median)	EPIC	5,964	40-84	0.00	GC	Plasma	Phospholipid fraction	ALA, EPA, DHA	Records	2,982	Alcohol, education, BMI, height, menopausal hormone treatment, first birth age and parity combined, energy intake, family history of breast cancer	High
Hirko et al, 2018, USA[82]	1996-99	NCCD	8.0 (median)	NHS II	1,588	25-42	0.00	GLC	Erythrocyte membrane	Phospholipid fraction	ALA, EPA, DPA, DHA	Self-report	794	Menarche age, first birth/parity age, alcohol, region, BMI, physical activity, family history of breast cancer and benign breast disease, weight change between age 18 and blood collection	High
Prostate cancer															
Gann et al, 1994, USA[83]	1982	NCCD	NR	PHS	240	40-84	100.00	GC	plasma	Cholesterol fraction	ALA, EPA	Records	120	Age, smoking	Moderate
Harvei et al, 1997, Norway[84]	1973-94	NCCD	11.6 (mean)	-	423	NR	100.00	GLC	Serum	Phospholipid fraction	ALA ,EPA, DPA, DHA	Records	141	Multiplicative risk	High
Mannisto et al, 2003, USA[85]	1995-98	NCCD	6.1 (median)	ATBC	396	50-69	100.00	GC	Serum	Total fatty acid fraction	ALA, EPA, DHA	Records	246	Smoking, region, alcohol, education, BMI	High
Chavarro et al, 2007, USA[86]	1982	NCCD	13.0	PHS	952	40-84	100.00	GLC	Whole blood	Total fatty acid fraction	ALA, EPA+DPA+DHA, EPA, DPA, DHA	Records	476	Age, smoking, examination year	High
Crowe et al, 2008, Europe[87]	1992	NCCD	4.2 (median)	EPIC	2,022	40-84	100.00	GC	Plasma	Phospholipid fraction	ALA, EPA, DPA, DHA	Records	962	Age, smoking, alcohol, marital status, education, region, BMI, physical activity	High
Park et al, 2008, USA[88]	1993-96	NCCD	1.9 (mean)	MCS	1,105	45-75	100.00	GC	Erythrocyte membrane	Phospholipid fraction	ALA, EPA, DPA, DHA	Records	376	Blood collection age, education, BMI, fasting hours prior to blood collection, family history of prostate cancer	High
Brasky et al, 2011, USA[89]	1994-2003	NCCD	7.0	PCPT	3,461	55-84	100.00	GC	Serum	Phospholipid fraction	ALA, EPA+DHA, EPA, DHA	Annual prostate-specific antigen and	1,658	Age, race, family history of prostate cancer, treatment arm	High

											digital rectal examination testing				
Brasky et al, 2013, USA[90]	2001-04	CCD	9.0 (max)	SELECT	2,198	≥ 50	100.00	GC	Plasma	Phospholipid fraction	ALA, EPA+DPA+DHA, EPA, DPA, DHA	Records	834	Age, race, education, diabetes, family history of prostate cancer, intervention arm	Moderate
Bassett et al, 2013, Australia[91]	1990-94	CCD	8.9 (mean)	MCCS	2,125	40-69	100.00	GLC	Plasma	Phospholipid fraction	ALA, EPA, DPA, DHA	Records	464	Country of birth, alcohol, education, physical activity, family history of cancer, energy intake	High

ALA, α-linolenic acid; ATBC, the Alpha-Tocopherol, Beta-Carotene Cancer Prevention Study; BMI, body mass index; CCD, case-cohort design study; CARET, the β-Carotene and Retinol Efficacy Trial; DCH, the Diet, Cancer and Health study; DHA, docosahexaenoic acid; DPA, docosapentaenoic acid; EPA, eicosapentaenoic acid; EPIC, European Prospective Investigation into Cancer and Nutrition; E3N Study, Etude Epide´miologique aupre`s des femmes de la Mutuelle Ge´ne´rale de l'Education Nationale; GC, gas chromatography; GLC, gas-liquid chromatography; HRT, hormone replacement treatment; MCCS,; MCS, The Multiethnic Cohort Study; MONICA, Monitoring of Trends and Cardiovascular Disease study; MSP, The Mammary-Screening Project; NCCD, nested case-control design study; NHS, Nurses' Health Study; NYUWHS, New York University Women's Health Study; PC, prospective cohort study; PCPT, Prostate Cancer Prevention Trial; PHS, Physicians' Health Study; SCHS, Singapore Chinese Health Study; SELECT, the Selenium and Vitamin E Cancer Prevention Trial; SU.VI.MAX, the Supplementation en Vitamines et Mine´raux Antioxydants study; USA, the United States of America; VIP, Västerbotten Intervention Program.

* Study quality was assessed with the Newcastle-Ottawa scale.

Table 7. Summary of prospective studies on circulating omega-3 fatty acids and mortality included in this review (n=9)

Author, publication year, country	Characteristics of the study				Characteristics of the participant				Characteristics of the exposure			Characteristics of the outcome		Adjustment for confounding factors	Study quality*
	Baseline survey year	Design	Follow up (year)	Study name	No.	Age range (year)	Men (%)	Assay method	Biological sample	Lipid fraction measured	Exposure	Ascertainment method	Cases (n)		
Warensjö et al, 2008, Sweden[16]	1920-24	PC	30.7 (median)	ULSAM	2,009	≥ 50	100.0	GC	Serum	Cholesteryl fraction	ALA, EPA, DHA	Records	1,012	Smoking, BMI, physical activity, hypertension, blood lipid	High
Chien et al, 2013, China (Taiwan) [45]	-	PC	9.6 (median)	A cohort study in Taiwan	1,833	50-72	NR	GC	Plasma	Total fatty acid fraction	EPA, DHA	House-to-house visits	568	Age, sex, marital status, occupation, smoking, alcohol, education, BMI, physical activity, hypertension, diabetes, blood lipid	Moderate
Mozaffarian et al, 2013, USA[18]	1992-93	PC	16.0 (max)	CHS	2,692	≥ 65	36.3	GC	Plasma	Phospholipid fraction	EPA, DPA, DHA	Interviews	1,625	Age, sex, race, region, smoking, alcohol, physical activity, education, BMI, WHR, fatty acid measurement batch, diabetes, hypertension, atrial fibrillation, drug-treated, dietary factors	High
Fretts et al, 2014, USA[48]	1992-93	PC	12.0 (max)	CHS	2,709	≥ 65	36.1	GC	Plasma	Phospholipid fraction	ALA	Records	1,517	Age, sex, race, smoking, alcohol, education, region, BMI, diabetes, drug-treated hypertension	High
Marklund et al, 2015, Swedish[49]	1997-98	PC	14.5 (median)	A cohort study in Swedish	4,232	≥ 60	48.2	GC	Serum	Cholesterol fraction	ALA, EPA, DHA	Records	456	Sex, smoking, alcohol, education, BMI, physical activity, diabetes, drug-treated hypertension, drug-treated hypercholesterolemia	Moderate
Miura et al, 2016, Australia[92]	1992-96	PC	17.0	NSCS	1,008	20-69	44.0	GC	Plasma	Phospholipid fraction	ALA, EPA+DPA+DHA, EPA, DPA, DHA	Records	179	Age, sex, smoking, blood cholesterol, jaundice measure, serious medical condition	High
Harris et al, 2017, USA[50]	1996	PC	14.9 (median)	WHIMS	6,501	65-80	0.0	GC	Erythrocyte membrane	Phospholipid fraction	ALA, EPA+DHA, EPA, DHA	Records	1,851	Age, race, region, smoking, alcohol, education, BMI, WHR, heart rate, physical activity, diabetes, hypertension, CVD and/or cancer, family history of cancer and CVD, aspirin treatment, cholesterol medication, supplement intake	High
Harris et al, 2018, USA[51]	1971	PC	7.3 (median)	FHS	2,500	56-75	43.1	GC	Erythrocyte membrane	Phospholipid fraction	ALA, EPA, DPA, DHA	-	350	Age, sex, marital status, occupation, smoking, alcohol, education, BMI, SBP, physical activity, health insurance status, diabetes, hypertension, aspirin treatment, cholesterol medication, blood lipid	High

Zhang, et al, 2021, China[52]	2003-04	PC	6.9 (mean)	NHANES	4,132	≥ 18	49.3	GC	Serum	Triglycerides fraction, phospholipid, fraction, cholesterol fraction	ALA, EPA, DPA, DHA	National Death Index	437	Age, sex, race, smoking, alcohol, education, family annual income, BMI, physical activity, diabetes, CVD, cancer, ever controlled blood pressure, blood cholesterol or blood glucose, serum triglycerides, total cholesterol, SFAs, and USFAs, energy, fiber, carbohydrate, and protein intake, AHEI-2010	High

AHEI: alternative healthy eating index; ALA, α-linolenic acid; BMI, body mass index; CHS, Cardiovascular Health Study; CVD, cardiovascular disease; DHA, docosahexaenoic acid; DPA, docosapentaenoic acid; EPA, eicosapentaenoic acid; FHS, Framingham Heart Study; GC, gas chromatography; NHANES, National Health and Nutrition Examination Survey; NCCD, nested case-control design study; NR, not reported; PC, prospective cohort study; SBP, systolic blood pressure; ULSAM, Uppsala Longitudinal Study of Adult Men; USA, the United States of America; WHIMS, the Women's Health Initiative Memory Study; WHR, waist hip rate.

* Study quality was assessed with the Newcastle-Ottawa scale.

Table 8. Newcastle Ottawa scale assessments for prospective cohort studies and nested case-cohort studies on fatty acids biomarkers and type 2 diabetes, cardiovascular disease, coronary heart disease, stroke, colorectal cancer, prostate cancer, breast cancer, and mortality included in this review

First author, year	Selection				Comparability	Assessment of exposure			Total score
	Representativeness	Selection of the non-exposed cohort	Exposure ascertainment	Demonstration of outcome not present at start	Comparability of cohorts on the basis of the design or analysis	Assessment of outcome	Long enough follow-up	Adequacy of follow up	
Type 2 diabetes									
Wang, 2003[31]	1	1	1	1	2	1	1	1	9
Hodge, 2007[32]	1	1	1	1	2	1	0	0	7
Krachler, 2008[33]	1	1	1	1	0	1	1	1	7
Zhuang, 2022[42]	1	1	1	1	2	1	1	1	9
Kröger, 2011[35]	1	1	1	1	2	1	1	1	9
Djoussé, 2011[36]	0	1	1	1	2	1	1	1	8
Virtanen, 2014[37]	1	1	1	1	2	1	1	1	9
Takkunen, 2015[38]	0	1	1	1	2	1	1	0	7
Forouhi, 2016[39]	0	1	1	1	2	1	1	1	8
Harris, 2016[40]	0	1	1	1	2	1	1	0	7
Zheng, 2018[41]	0	1	1	0	2	1	1	0	6
Cardiovascular disease									
Laaksonen, 2005[44]	1	1	1	1	2	1	1	1	9
Warensjö, 2008[16]	1	1	1	1	2	1	1	1	9
Woodward, 2011[17]	0	1	1	1	2	1	1	0	7
Virtanen, 2012[46]	1	1	1	1	2	1	1	1	9
Chien, 2013[45]	0	1	1	0	2	1	1	0	6
Mozaffarian, 2013[18]	0	1	1	1	2	1	1	1	8
de Oliveira Otto, 2013[47]	1	1	1	1	2	1	1	0	8
Fretts, 2014[48]	0	1	1	1	2	1	1	1	8
Marklund, 2015[49]	1	1	1	1	0	1	1	0	6
Harris, 2017[50]	0	1	1	1	2	1	1	0	7
Harris, 2018[51]	1	1	1	1	2	1	1	1	9
Zhang, 2021[52]	1	1	1	1	2	1	1	0	8
Coronary heart disease									

Wang, 2003[55]	1	1	1	1	2	1	1	1	9
Joensen, 2011[57]	0	1	1	1	2	1	1	1	8
de Oliveira Otto, 2013[47]	1	1	1	1	2	1	1	0	8
Mozaffarian, 2013[18]	0	1	1	1	2	1	1	1	8
Fretts, 2014[48]	0	1	1	1	2	1	1	1	8
Stroke									
Wiberg, 2006[63]	1	1	1	1	0	1	1	0	6
Yamagishi, 2013[65]	1	1	1	1	2	1	1	1	9
Yaemsiri, 2013[66]	0	1	1	1	2	1	1	0	7
Fretts, 2014[48]	0	1	1	1	2	1	1	1	8
Daneshmand, 2016[67]	1	1	1	1	2	1	1	1	9
Bork, 2018[69]	0	1	1	1	2	1	1	1	8
Harris, 2018[51]	1	1	1	1	2	1	1	1	9
Venø, 2019[70]	1	1	1	1	2	1	1	0	8
Colorectal cancer									
Hodge, 2014[72]	0	1	1	1	2	1	1	1	8
Prostate cancer									
Brasky, 2013[90]	0	1	1	1	0	1	1	1	6
Bassett, 2013[91]	0	1	1	1	2	1	1	1	8
Breast cancer									0
Chajès, 1999[74]	1	1	1	1	0	1	1	1	7
Chajès, 2008[76]	0	1	1	1	2	1	1	1	8
Witt, 2009[78]	0	1	1	1	1	1	1	1	7
Bassett, 2016[80]	0	1	1	1	2	1	1	1	8
All-cause mortality									
Warensjö, 2008[16]	1	1	1	1	2	1	1	1	9
Chien, 2013[45]	0	1	1	0	2	1	1	0	6
Mozaffarian, 2013[18]	0	1	1	1	2	1	1	1	8
Fretts, 2014[48]	0	1	1	1	2	1	1	1	8
Marklund, 2015[49]	1	1	1	1	0	1	1	0	6
Miura, 2016[92]	1	1	1	1	2	1	1	1	9
Harris, 2017[50]	0	1	1	1	2	1	1	0	7
Harris, 2018[51]	1	1	1	1	2	1	1	1	9
Zhang, 2021[62]	1	1	1	1	2	1	1	0	8

Table 9. Newcastle Ottawa scale assessments for prospective nested case-control studies on fatty acids biomarkers and type 2 diabetes, cardiovascular disease, coronary heart disease, stroke, colorectal cancer, prostate cancer, breast cancer, and mortality included in this review

First author, year	Selection				Comparability	Assessment of exposure			Total score
	Representativeness	Selection of the non-exposed cohort	Exposure ascertainment	Demonstration of outcome not present at start	Comparability of cohorts on the basis of the design or analysis	Assessment of outcome	Long enough follow-up	Adequacy of follow up	
Type 2 diabetes									
Patel, 2010[34]	1	1	1	1	2	1	1	1	9
Cardiovascular disease									
Albert, 2002[43]	0	1	1	1	0	1	1	1	6
Coronary heart disease									
Simon, 1995[53]	0	1	1	0	0	1	1	1	5
Lemaitre, 2003[54]	0	1	1	1	2	1	1	1	8
Sun, 2008[56]	0	1	1	1	2	1	1	1	8
Khaw, 2012[58]	1	1	1	1	2	1	1	1	9
Matsumoto, 2013[59]	0	1	1	1	2	1	1	1	8
Sun, 2016[60]	0	1	1	1	0	1	1	1	6
Hamazaki, 2017[61]	0	1	1	1	2	1	1	1	8
Chei, 2018[62]	0	1	1	1	2	1	1	1	8
Stroke									
De Goede, 2013[64]	0	1	1	1	2	1	1	1	8
Saber, 2017(CHS) [68]	0	1	1	1	2	1	1	1	8
Saber, 2017(HPFS) [68]	0	1	1	1	2	1	1	1	8
Saber, 2017(FHS) [68]	0	1	1	1	2	1	1	1	8
Colorectal cancer									
Hall, 2007[71]	0	1	1	1	2	1	1	1	8
Butler, 2017[73]	0	1	1	1	2	1	1	1	8
Prostate cancer									
Gann, 199482[83]	0	1	1	1	0	1	1	1	6
Harvei, 1997[84]	0	1	1	1	1	1	1	1	7
Mannisto, 2003[85]	0	1	1	1	2	1	1	1	8
Chavarro, 2007[86]	0	1	1	1	2	1	1	1	8

Crowe, 2008[87]	0	1	1	1	2	1	1	1	8
Park, 2008[88]	0	1	1	1	2	1	1	1	8
Brasky, 2011[89]	0	1	1	1	2	1	1	1	8
Breast cancer									
Saadatian-Elahi, 2002[75]	0	1	1	1	2	1	1	1	8
Takata, 2009[77]	0	1	1	1	2	1	1	1	8
Pouchieu, 2014[79]	0	1	1	1	2	1	1	1	8
Chajès, 2017[81]	0	1	1	1	2	1	1	1	8
Hirko, 2018[82]	0	1	1	1	2	1	1	1	8

CHS, Cardiovascular Health Study; FHS, Framingham Heart Study; HPFS, Health Professionals Follow-Up Study.

Table 10. Subgroup analyses of alpha-linolenic acid and type 2 diabetes

	ALA				
	n	RR (95% CI)	I^2	Ph^*	$Ph^\dagger$
All studies	11	0.89 (0.82, 0.96)	5.1	0.39	
Study type					0.87
PC	6	0.88 (0.74, 1.04)	39.4	0.14	
CCD	4	0.88 (0.80, 0.98)	0.0	0.60	
NCCD	1	0.70 (0.34, 1.46)	NA	NA	
NCCS	-				
Gender					0.93
Males	1	0.84 (0.66, 1.07)	NA	NA	
Women	1	1.00 (0.81, 1.23)	NA	NA	
Men and women	9	0.88 (0.79, 0.97)	10.6	0.34	
Geographic location					0.73
Europe	6	0.87 (0.79, 0.95)	0.0	0.85	
USA	3	0.78 (0.56, 1.08)	66.9	0.05	
Asia	1	1.06 (0.75, 1.49)	NA	NA	
Australia	1	1.05 (0.77, 1.44)	NA	NA	
Duration of follow-up					0.50
< 10 years	5	0.93 (0.80, 1.09)	0.6	0.40	
≥ 10 years	6	0.87 (0.78, 0.97)	16.3	0.31	
Number of cases					0.74
< 300	6	0.84 (0.68, 1.04)	28.2	0.22	
300-500	2	0.92 (0.74, 1.14)	17.1	0.27	
≥ 500	3	0.89 (0.81, 0.98)	0.0	0.38	
Assessment method					0.27
GC	9	0.90 (0.83, 0.98)	4.8	0.39	
GLC	2	0.68 (0.48, 0.98)	0.0	0.95	
Biomarkers type					0.20
Total plasma	4	0..82 (0.68, 0.98)	44.6	0.14	
Serum	3	0.94 (0.78, 1.12)	0.0	0.44	
Phospholipids	4	0.96 (0.82, 1.12)	0.0	0.8	

CCD, case-cohort design study; CI, confidence interval; GC, gas chromatography; GLC, gas-liquid chromatography; NA, not applicable (because only 1 study); NCCS, nested case-control study; NCCD, nested case-cohort design study; PC, prospective cohort study; RR, relative risk; USA, the United States of America.

* *P* for heterogeneity within each subgroup.

† *P* for heterogeneity between subgroups with a meta-regression analysis.

Table 11. Subgroup analyses of docosahexaenoic acid and cardiovascular disease

	n	RR (95% CI)	I^2	Ph^*	$Ph^\dagger$
All studies	10	0.76 (0.66, 0.88)	55.5	0.02	
Study type					NC
PC	10	0.76 (0.66, 0.88)	55.5	0.02	
PNCC	-				
NCCS	-				
Gender					0.17
Men	2	0.71 (0.46, 1.09)	58.6	0.12	
Women	1	1.00 (0.74, 1.35)	NA	NA	
Men and women	7	0.74 (0.62, 0.88)	57.9	0.03	
Geographic location					0.76
Europe	4	0.73 (0.62, 0.86)	45.0	0.14	
USA	4	0.70 (0.53, 0.93)	65.6	0.03	
Asia	2	1.05 (0.80, 1.38)	0.0	0.59	
Duration of follow-up					0.45
< 10 years	6	0.76 (0.66, 0.87)	45.2	0.10	
≥ 10 years	4	0.75 (0.50, 1.11)	72.8	0.01	
Number of cases					0.20
< 300	4	0.61 (0.46, 0.82)	33.0	0.21	
300-500	3	0.82 (0.62, 1.09)	76.4	0.01	
≥ 500	3	0.82 (0.69, 0.97)	31.2	0.23	
Assessment method					NC
GC	10	0.76 (0.66, 0.88)	55.5	0.02	
GLC	-				
Biomarkers type					0.95
Plasma	3	0.73 (0.48, 1.12)	80.3	<0.001	
Serum	4	0.72 (0.59, 0.89)	42.6	0.16	
Phospholipids	2	0.82 (0.54, 1.25)	67.4	0.08	
Adipose tissue	1	0.81 (0.66, 0.99)	NA	NA	
Study quality					0.41
Moderate	2	0.83 (0.49, 1.41)	87.4	0.005	
High	8	0.76 (0.65, 0.87)	42.6	0.09	

CI, confidence interval; GC, gas chromatography; GLC, gas-liquid chromatography; NA, not applicable (because only 1 study); NC, cannot be calculated; NCCS, nested case-control study; PC, prospective cohort study; RR, relative risk; USA, the United States of America.

* *P* for heterogeneity within each subgroup.

† *P* for heterogeneity between subgroups with a meta-regression analysis.

Table 12. Subgroup analyses of fatty acid biomarkers and coronary heart disease

	EPA					DHA				
	n	RR (95% CI)	I^2	Ph^*	$Ph^\dagger$	n	RR (95% CI)	I^2	Ph^*	$Ph^\dagger$
All studies	10	0.85 (0.77, 0.95)	3.0	0.41		10	0.70 (0.58, 0.84)	54.1	0.02	
Study type										0.50
PC	10	0.85 (0.77, 0.95)	3.0	0.41	NC	2	0.51 (0.27, 0.96)	73.1	0.05	
PNCC	-					7	0.80 (0.66, 0.97)	31.2	0.19	
NCCS	-					1	0.63 (0.46, 0.86)	NA	NA	
Gender					0.42					0.40
Men	2	0.85 (0.70, 1.04)	0.0	0.65		2	0.63 (0.22, 1.79)	79.4	0.03	
Women	8	0.84 (0.73, 0.98)	22.8	0.25		1	0.69 (0.32, 1.48)	NA	NA	
Men and women					0.95	7	0.68 (0.57, 0.82)	38.9	0.13	
Geographic location										0.73
Europe	2	0.94 (0.80, 1.12)	0.0	0.50		2	0.76 (0.53, 1.09)	67.9	0.08	
USA	4	0.77 (0.62, 0.95)	40.0	0.17		5	0.61 (0.41, 0.91)	74.3	0.004	
Asia	4	0.83 (0.65, 1.06)	0.0	0.64		3	0.69 (0.53, 0.90)	0.0	0.84	
Duration of follow-up					0.30					0.79
< 10 years	5	0.80 (0.66, 0.97)	29.1	0.23		4	0.70 (0.48, 1.03)	67.3	0.03	
≥ 10 years	5	0.89 (0.77, 1.02)	0.0	0.52		6	0.69 (0.55, 0.86)	46.1	0.10	
Number of cases					0.85					0.18
< 300	5	0.77 (0.57, 1.04)	29.0	0.23		5	0.57 (0.40, 0.81)	35.4	0.19	
300-500	-					-				
≥ 500	5	0.87 (0.78, 0.98)	0.0	0.60		5	0.77 (0.63, 0.94)	57.3	0.05	
Assessment method					NC					
GC	10	0.85 (0.77, 0.95)	3.0	0.41		5	0.67 (0.55, 0.81)	0.0	0.97	
GLC	-					5	0.68 (0.49, 0.95)	75.7	0.002	
Biomarkers type					0.50					0.19
Plasma	3	0.77 (0.58, 1.01)	0.0	0.79		6	0.68 (0.54, 0.86)	45.9	0.10	
Serum	1	1.04 (0.65, 1.66)	NA	NA		2	0.55 (0.25, 1.20)	55.6	0.13	
Phospholipids	5	0.82 (0.68, 0.99)	46.2	011		1	0.98 (0.79, 1.22)	NA	NA	
Adipose tissue	1	0.89 (0.70, 1.13)	NA	NA		1	0.63 (0.46, 0.86)	NA	NA	
Study quality					0.73					0.33
Moderate	2	0.74 (0.56, 0.97)	0.0	0.83		2	0.53 (0.30, 0.95)	39.0	0.20	
High	8	0.87 (0.77, 0.98)	11.6	0.34		8	0.73 (0.60, 0.89)	55.1	0.03	

CI, confidence interval; DHA, docosahexaenoic acid; EPA, eicosapentaenoic acid; GC, gas chromatography; GLC, gas-liquid chromatography; NA, not applicable (because only 1 study); NC, cannot be calculated; NCCS, nested case-control study;

PC, prospective cohort study; RR, relative risk; USA, the United States of America.

* *P* for heterogeneity within each subgroup.

† *P* for heterogeneity between subgroups with a meta-regression analy

Table 13. GRADE assessment of the systematic review and meta-analysis of prospective cohort studies assessing the association between omega-3 polyunsaturated fatty acid biomarkers and the endpoints of interest

tcome	№ of studies	Study design	Risk of bias	Inconsistency	Indirectness	Imprecision	Other considerations	Relative risk (95% CI
etes	11	Observational studies	Not serious	Not serious	Not serious	Not serious	Dose response gradient	0.89 (0.82 to 0.96)
ular disease	8	Observational studies	Not serious	Not serious	Not serious	Not serious	None	1.09 (0.98 to 1.20)
eart disease	9	Observational studies	Not serious	Not serious	Serious [a]	Not serious	None	0.98 (0.95 to 1.02)
	8	Observational studies	Not serious	Not serious	Serious [a]	Not serious	None	0.98 (0.92 to 1.05)
cancer	2	Observational studies	Not serious	Not serious	Serious [c]	Not serious	None	0.90 (0.72 to 1.12)
er	8	Observational studies	Not serious	Not serious	Not serious	Not serious	None	0.95 (0.86 to 1.05)
ncer	9	Observational studies	Not serious	Not serious	Not serious	Not serious	None	1.03 (0.90 to 1.19)
	7	Observational studies	Not serious	Not serious	Not serious	Not serious	None	0.98 (0.89 to 1.07)
etes	9	Observational studies	Not serious	Serious [d]	Not serious	Not serious	None	0.85 (0.72 to 0.99)
ular disease	9	Observational studies	Not serious	Not serious	Not serious	Not serious	Dose response gradient	0.79 (0.70 to 0.89)
eart disease	10	Observational studies	Not serious	Not serious	Not serious	Not serious	Dose response gradient	0.85 (0.77 to 0.95)
	9	Observational studies	Not serious	Serious [e]	Not serious	Not serious	None	0.95 (0.82 to 1.11)
cancer	3	Observational studies	Not serious	Not serious	Serious [f]	Not serious	None	0.86 (0.70 to 1.05)
er	9	Observational studies	Not serious	Not serious	Not serious	Not serious	None	0.93 (0.85 to 1.02)
ncer	9	Observational studies	Not serious	Not serious	Not serious	Not serious	None	1.05 (0.94 to 1.17)
	8	Observational studies	Not serious	Serious [g]	Not serious	Not serious	None	0.78 (0.69 to 0.88)
etes	9	Observational studies	Not serious	Serious [h]	Not serious	Not serious	None	0.84 (0.73 to 0.96)
ular disease	6	Observational studies	Not serious	Not serious	Not serious	Not serious	Dose response gradient	0.78 (0.70 to 0.86)
eart disease	9	Observational studies	Not serious	Not serious	Not serious	Not serious	Dose response gradient	0.83 (0.76 to 0.92)
	7	Observational studies	Not serious	Serious [i]	Not serious	Not serious	None	0.96 (0.79 to 1.16)
cancer	2	Observational studies	Not serious	Not serious	Serious [j]	Not serious	None	0.76 (0.59 to 0.98)
er	6	Observational studies	Not serious	Not serious	Serious [k]	Not serious	None	0.94 (0.85 to 1.05)
ncer	6	Observational studies	Not serious	Serious [l]	Not serious	Not serious	None	0.92 (0.75 to 1.15)
	4	Observational studies	Not serious	Not serious	Serious [m]	Not serious	None	0.82 (0.74 to 0.90)

etes	10	Observational studies	Not serious	Serious [n]	Not serious	Not serious	None	0.96 (0.82 to 1.11)
ular disease	10	Observational studies	Not serious	Serious [o]	Not serious	Not serious	Dose response gradient	0.76 (0.66 to 0.88)
eart disease	10	Observational studies	Not serious	Serious [p]	Not serious	Not serious	Dose response gradient	0.70 (0.58 to 0.84)
	9	Observational studies	Not serious	Serious [q]	Not serious	Not serious	None	0.84 (0.72 to 0.99)
cancer	3	Observational studies	Not serious	Not serious	Serious [r]	Not serious	None	0.80 (0.65 to 0.99)
er	9	Observational studies	Not serious	Not serious	Not serious	Not serious	None	1.01 (0.92 to 1.11)
ncer	8	Observational studies	Not serious	Serious [s]	Not serious	Not serious	None	1.05 (0.89 to 1.24)
	8	Observational studies	Not serious	not serious	Not serious	Not serious	None	0.83 (0.75 to 0.92)
HA								
etes	2	Observational studies	Not serious	Serious [t]	Serious [u]	Not serious	None	0.81 (0.60 to 1.09)
ular disease	3	Observational studies	Not serious	Not serious	Serious [v]	Not serious	None	0.45 (0.27 to 0.74)
eart disease	3	Observational studies	Not serious	Not serious	Serious [w]	Not serious	None	0.67 (0.47 to 0.96)
	2	Observational studies	Not serious	Not serious	Serious [x]	Not serious	None	1.04 (0.90 to 1.20)

ALA, alpha-linolenic acid; EPA, eicosapentaenoic acid; DPA, docosapentaenoic acid; DHA, docosahexaenoic acid.

[a] Serious indirectness for coronary heart disease, as >50% of the weight (75.89%) was contributed by a study conducted among males.

[b] Serious indirectness for stroke, as >50% of the weight (52.96%) was contributed by a study conducted among females.

[c] Serious indirectness for colorectal cancer, as there were only 2 available studies s and >50% of the weight (67.92%) was contributed by a study.

[d] Serious inconsistency for type 2 diabetes due to high degree of unexplained heterogeneity (I^2=77.7%, P<0.001).

[e] Serious inconsistency for stroke due to high degree of unexplained heterogeneity (I^2=54.6%, P=0.02).

[f] Serious indirectness for colorectal cancer, as >50% of the weight (65.26%) was contributed by a study.

[g] Serious inconsistency for mortality due to high degree of unexplained heterogeneity (I^2=62.7%, P=0.009).

[h] Serious inconsistency for type 2 diabetes due to high degree of unexplained heterogeneity (I^2=69.9%, P=0.001).

[i] Serious inconsistency for stroke due to high degree of unexplained heterogeneity (I^2=73.0%, P =0.001).

[j] Serious indirectness for colorectal cancer, as there were only 2 available studies and >50% of the weight (80.69%) was contributed to a study.

[k] Serious indirectness for breast cancer, as >50% of the weight (62.59%) was contributed to a study.

[l] Serious inconsistency for prostate cancer due to high degree of unexplained heterogeneity (I^2=58.4%, P=0.034).

[m] Serious indirectness for colorectal cancer, as there were only 3 available studies and >50% of the weight (76.89%) was contributed to a study.

[n] Serious inconsistency for type 2 diabetes due to high degree of unexplained heterogeneity (I^2=69.5%, P=0.001).

[o] Serious inconsistency for cardiovascular disease due to high degree of unexplained heterogeneity (I^2=55.5%, P =0.017).

[p] Serious inconsistency for coronary heart disease due to high degree of unexplained heterogeneity (I^2=54.1%, P =0.020).

[q] Serious inconsistency for stroke due to high degree of unexplained heterogeneity (I^2=54.2%, P =0.026).

[r] Serious indirectness for colorectal cancer, as there were only 3 available studies and >50% of the weight (63.66%) was contributed to one study.

[s] Serious inconsistency for stroke due to high degree of unexplained heterogeneity (I^2=61.5%, P =0.011).

[t] Serious inconsistency for type 2 diabetes due to high degree of unexplained heterogeneity (I^2=52.2%, P=0.148).

[u] Serious indirectness for type 2 diabetes, as there were only 2 available studies and >50% of the weight (59.57%) was contributed by a study.

[v] Serious indirectness for cardiovascular disease, as there were only 3 available studies.

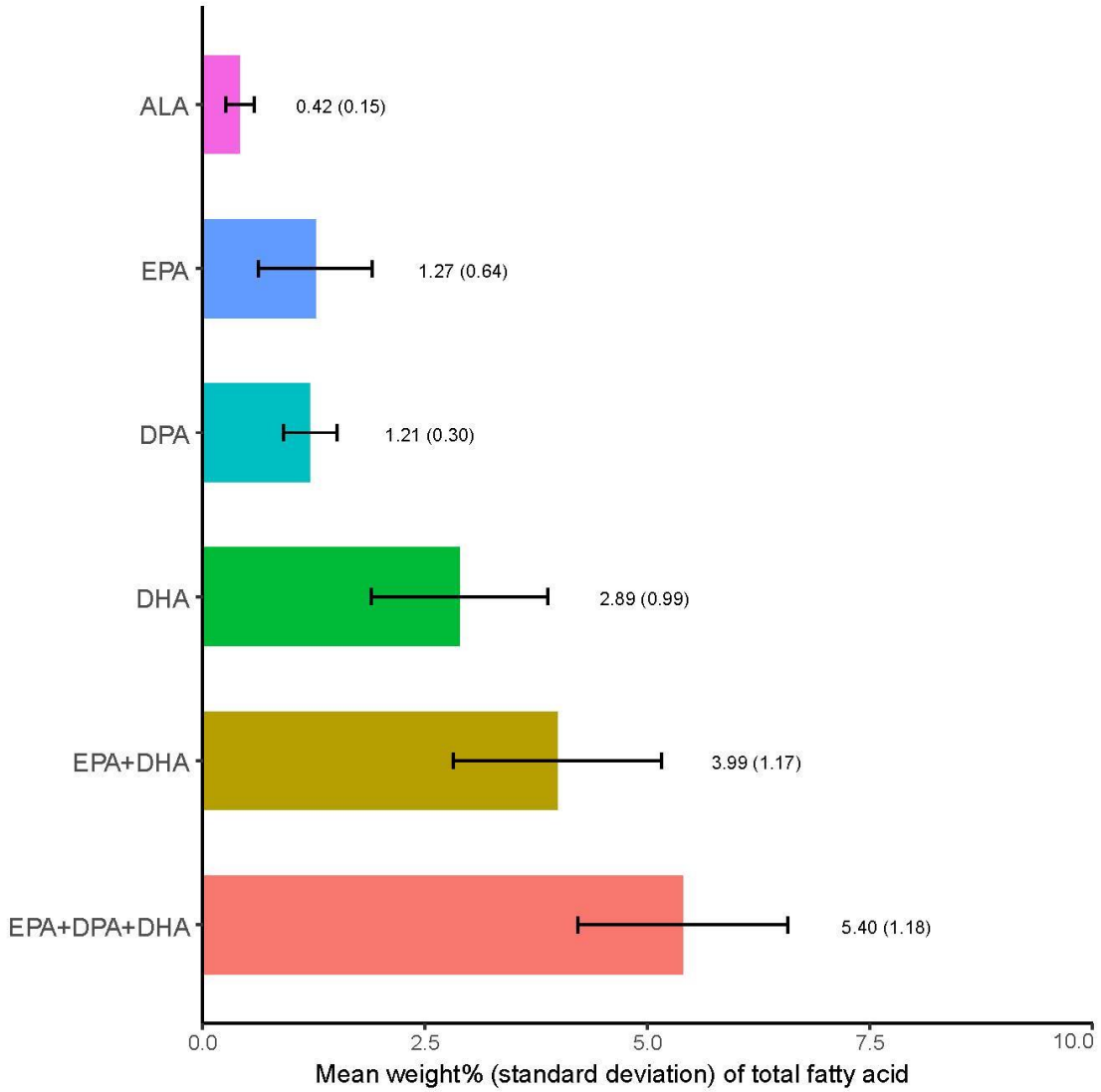
[w] Serious indirectness for coronary heart disease, as there were only 3 available studies.

[x] Serious indirectness for stroke, as there were only 2 available studies and >50% of the weight (82.34%) was contributed by a study.

Supplementary Appendix 2

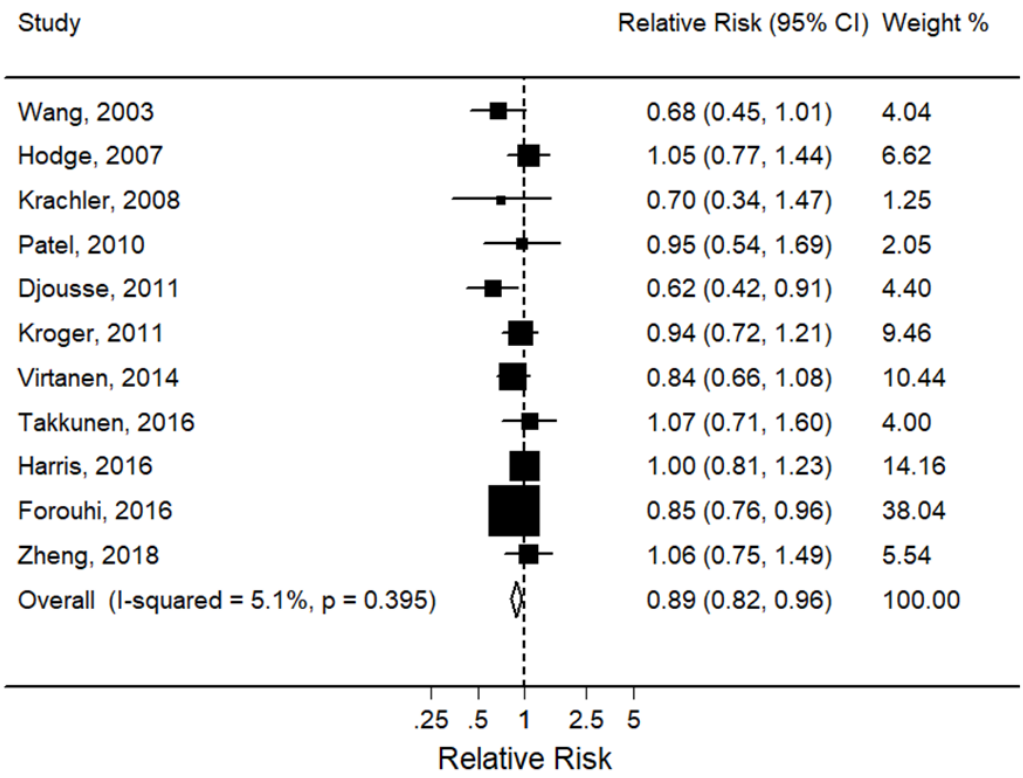
Figure of Contents

Figure 1. Mean (standard deviation) of circulating blood omega-3 polyunsaturated fatty acid composition at baseline in the available studies



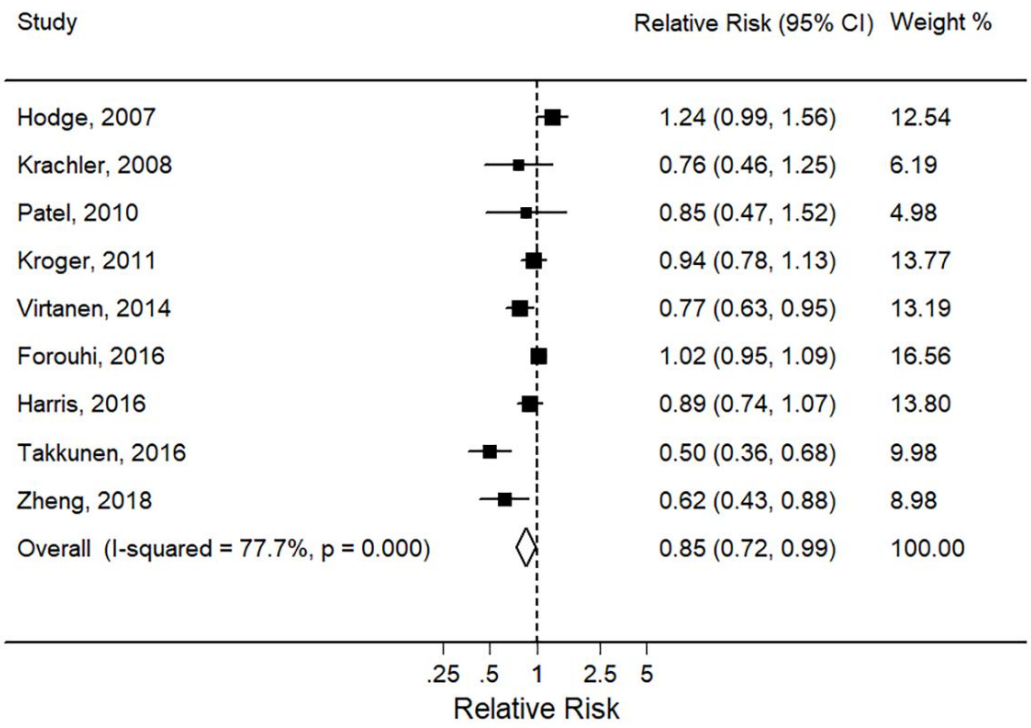
ALA, α-linoleic acid; EPA, eicosapentaenoic acid; DPA, docosapentaenoic acid; DHA, docosahexaenoic acid.

Figure 2. Pooled relative risk of type 2 diabetes for the highest versus lowest categories of α-linolenic acid biomarker level



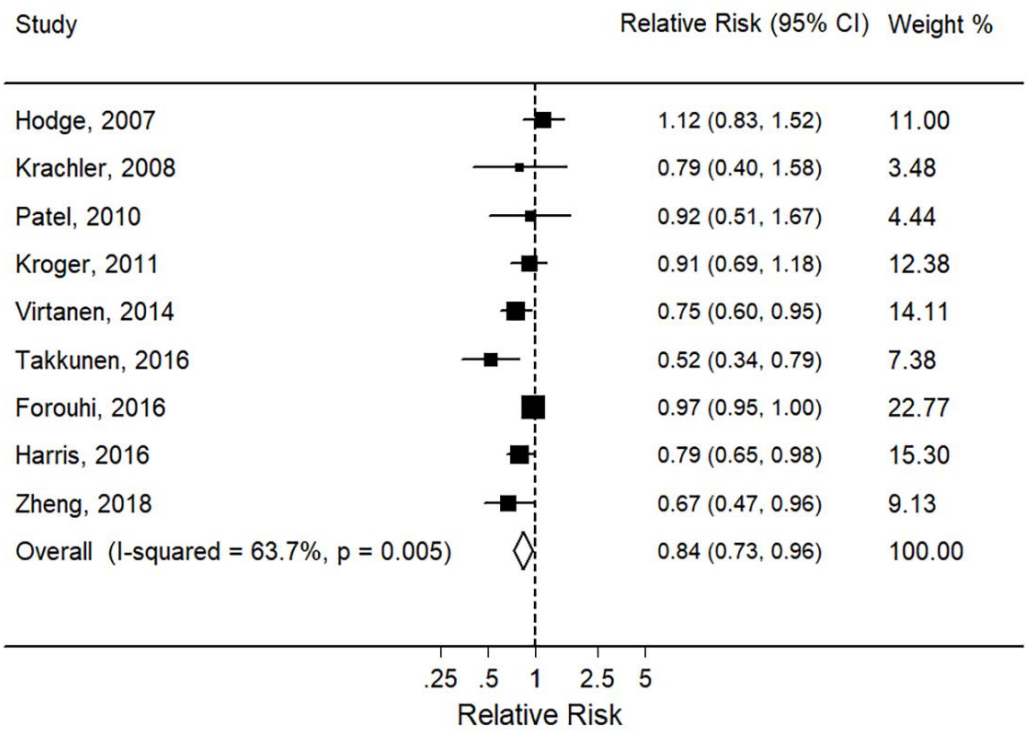
95% CI, 95% confidence interval.

Figure 3. Pooled relative risk of type 2 diabetes for the highest versus lowest categories of eicosapentaenoic acid biomarker level



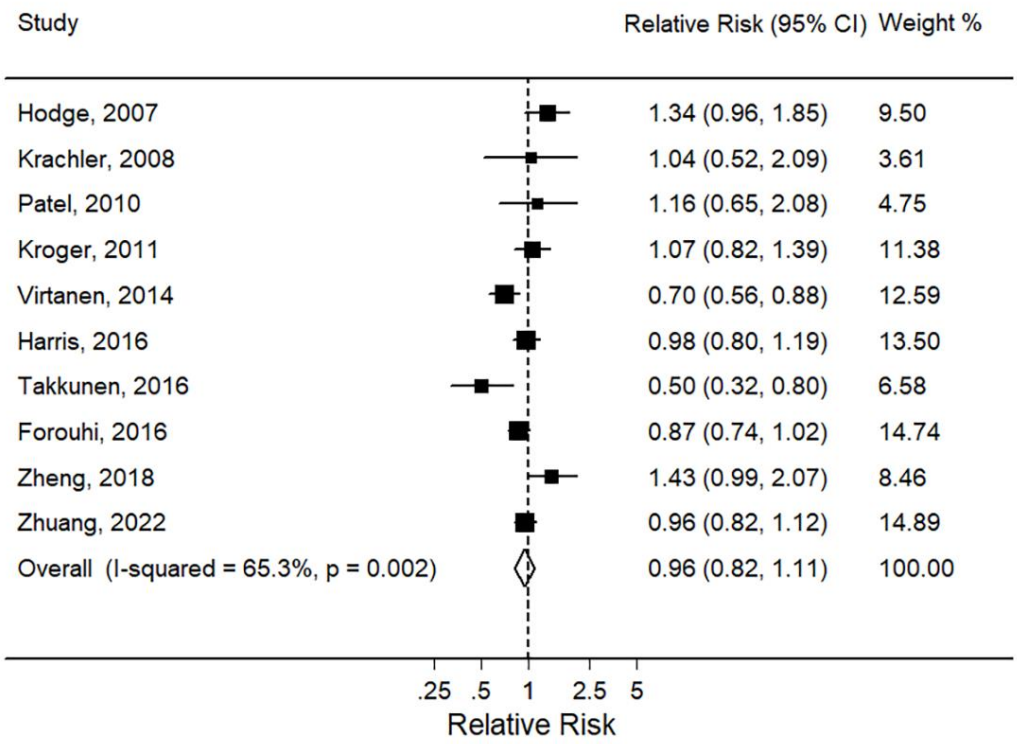
95% CI, 95% confidence interval.

Figure 4. Pooled relative risk of type 2 diabetes for the highest versus lowest categories of docosapentaenoic acid biomarker level



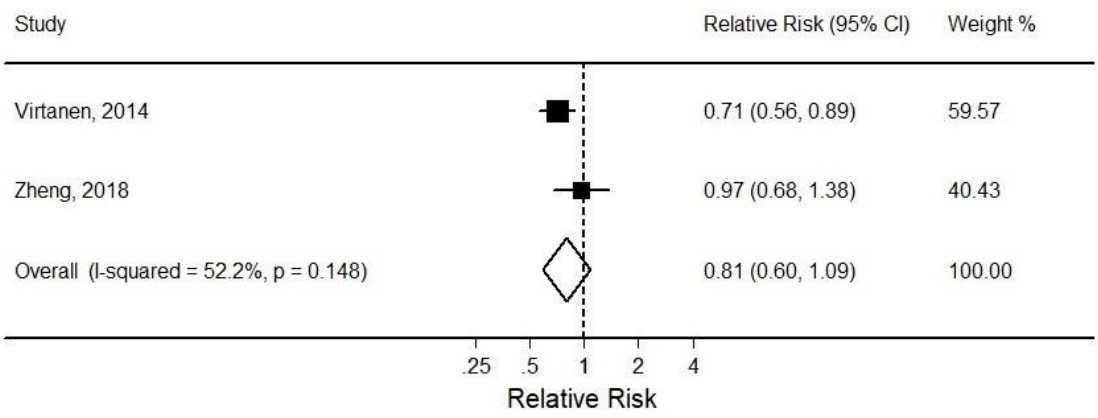
95% CI, 95% confidence interval.

Figure 5. Pooled relative risk of type 2 diabetes for the highest versus lowest categories of docosahexaenoic acid biomarker level



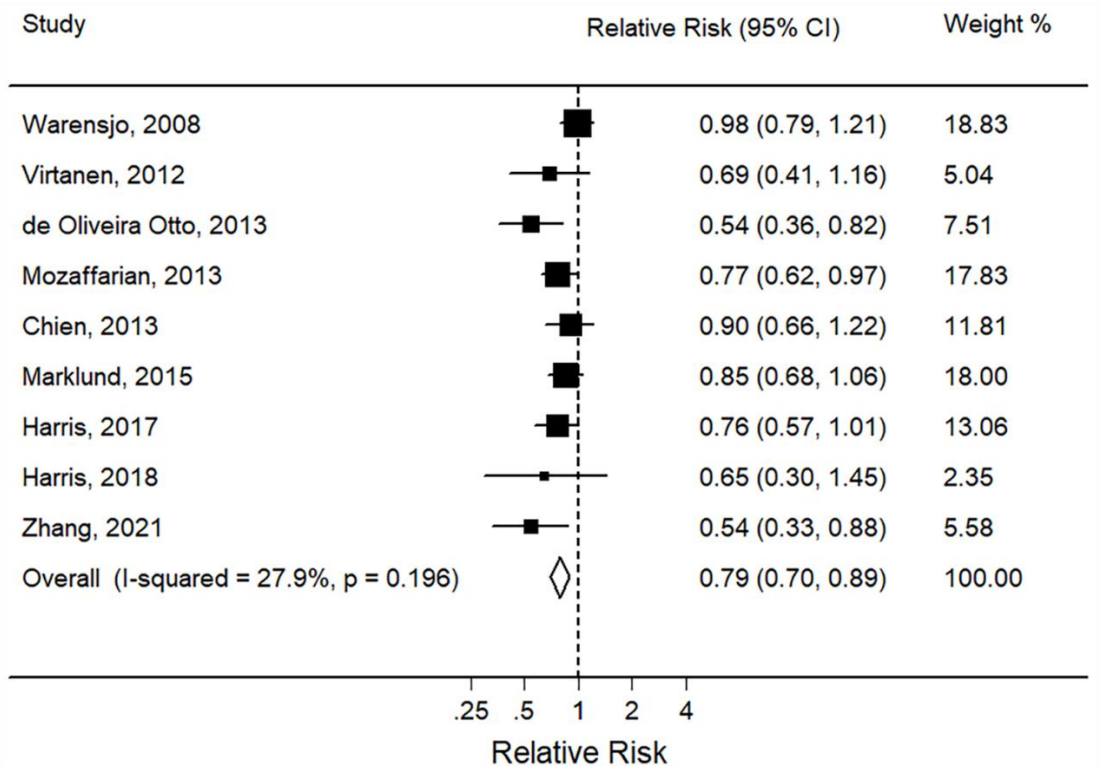
95% CI, 95% confidence interval.

Figure 6. Pooled relative risk of type 2 diabetes for the highest versus lowest categories of long-chain omega-3 polyunsaturated fatty biomarker level



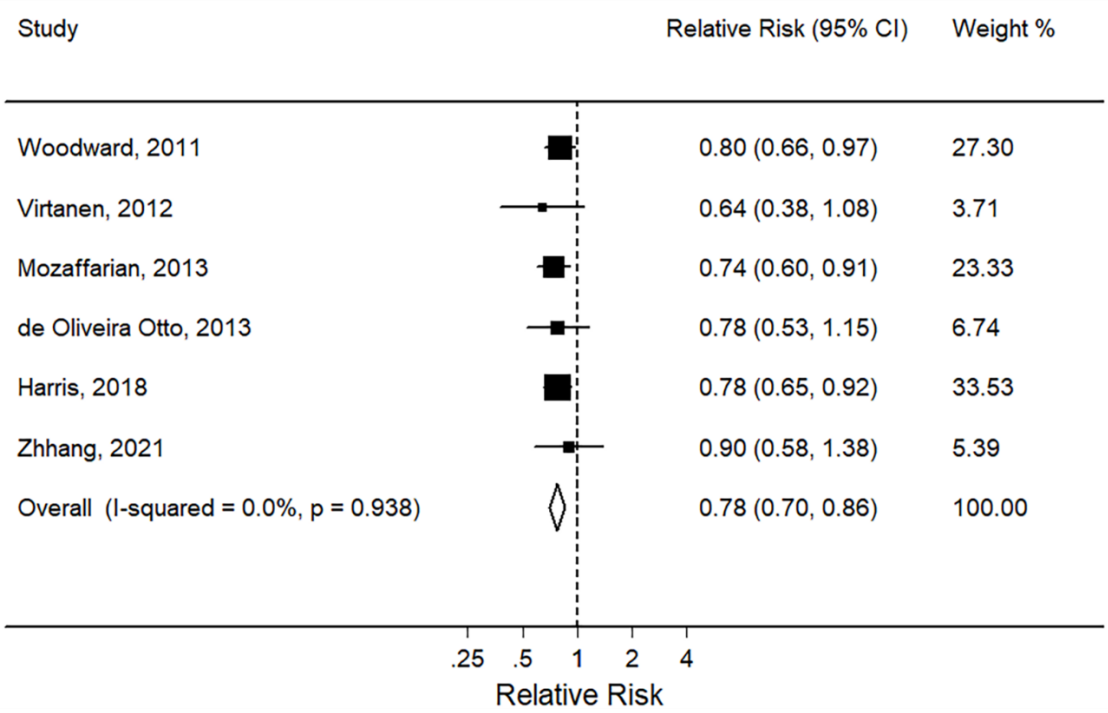
95% CI, 95% confidence interval.

Figure 7. Pooled relative risk of cardiovascular disease for the highest versus lowest categories of eicosapentaenoic acid biomarker level



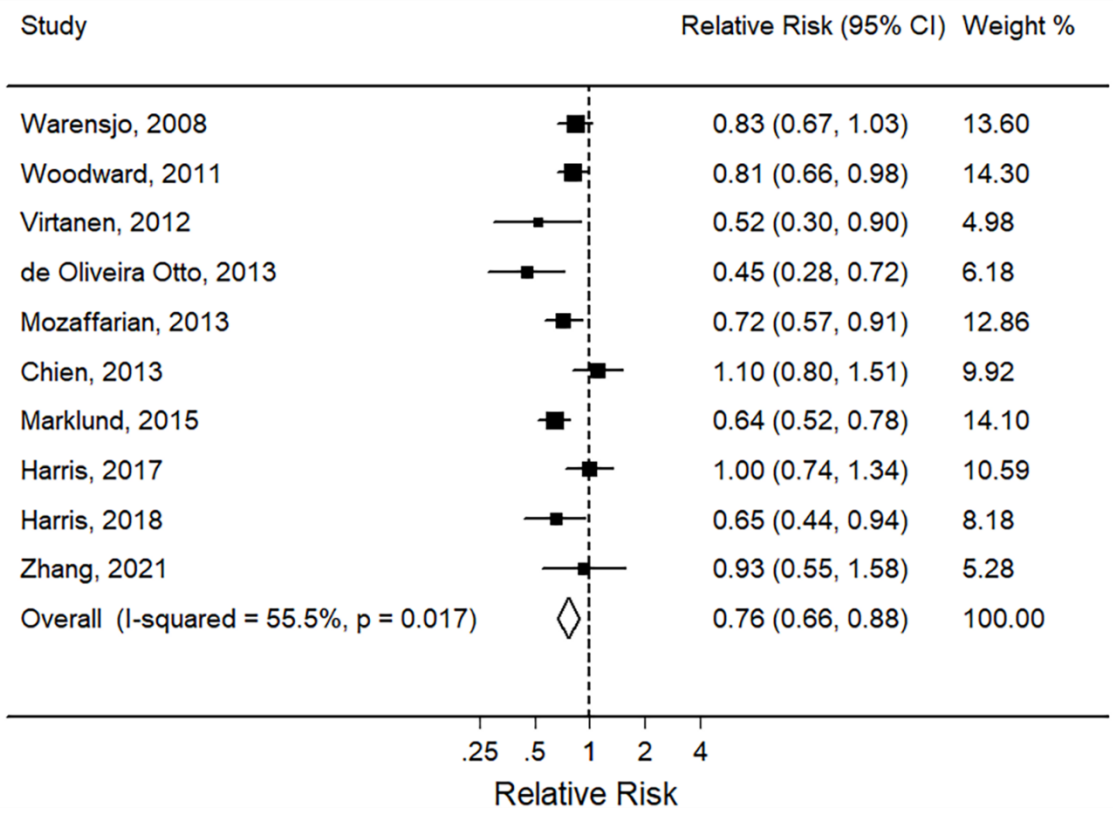
95% CI, 95% confidence interval.

Figure 8. Pooled relative risk of cardiovascular disease for the highest versus lowest categories of docosapentaenoic acid biomarker level



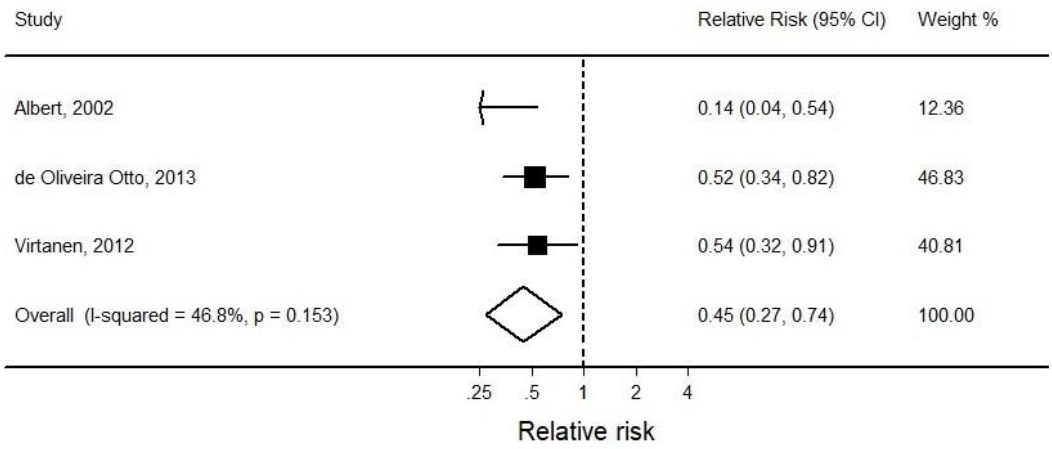
95% CI, 95% confidence interval.

Figure 9. Pooled relative risk of cardiovascular disease for the highest versus lowest categories of docosahexaenoic acid biomarker level



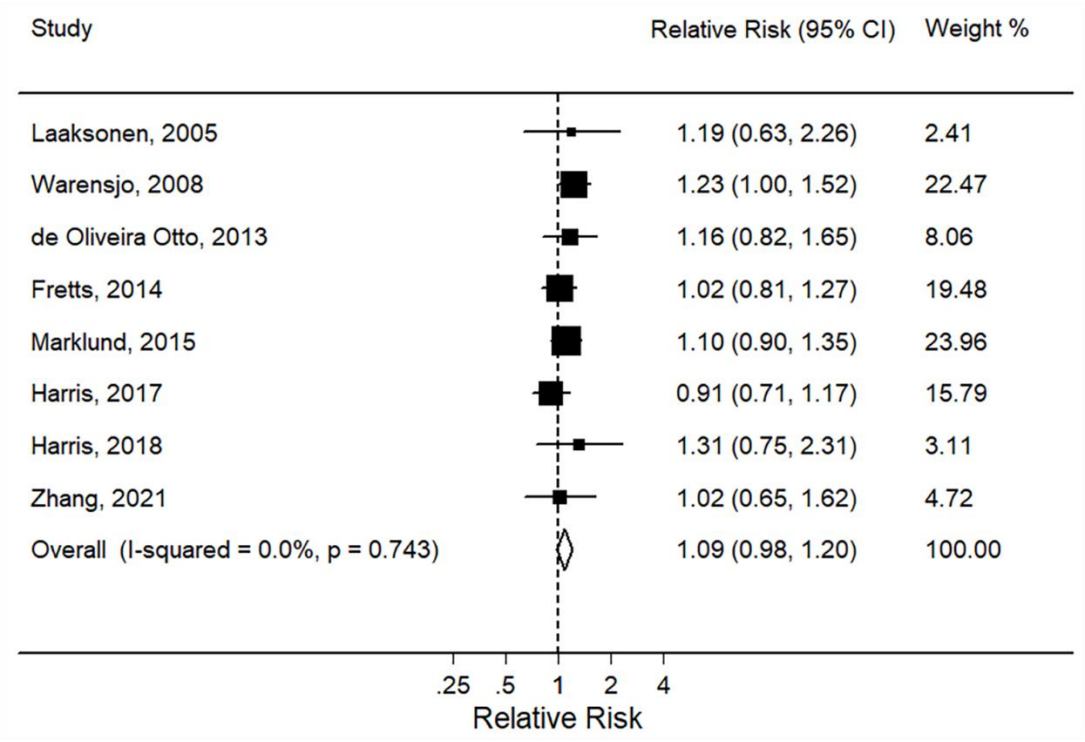
95% CI, 95% confidence interval.

Figure 10. Pooled relative risk of cardiovascular disease for the highest versus lowest categories of long-chain omega-3 polyunsaturated fatty biomarker level



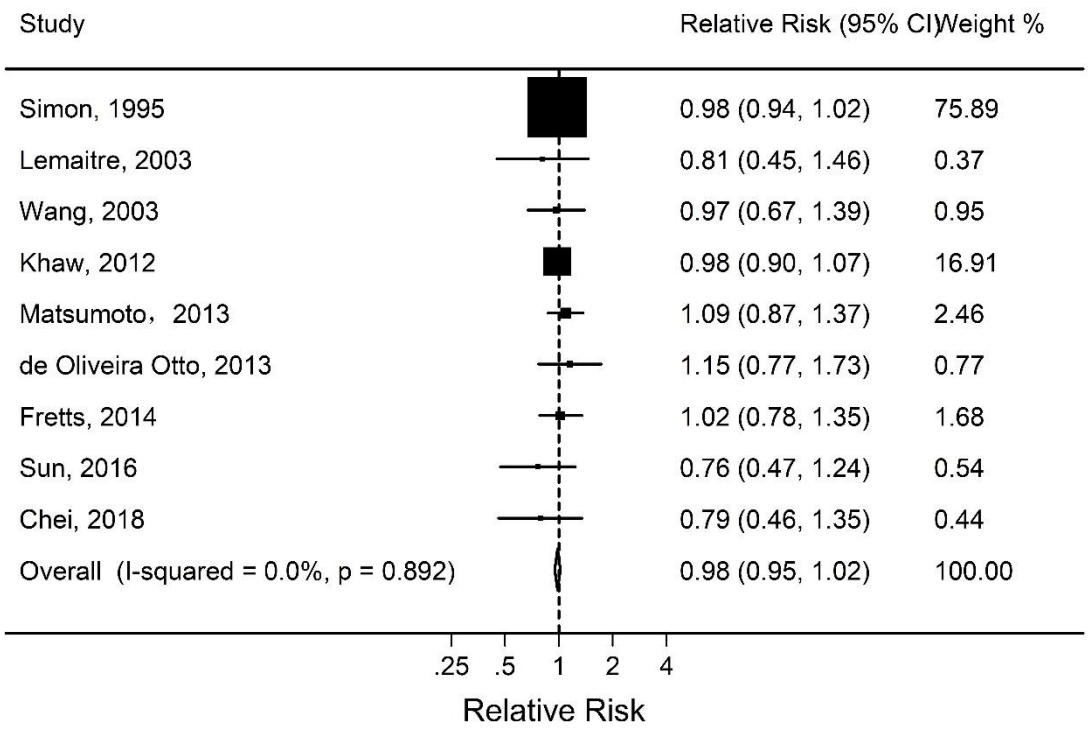
95% CI, 95% confidence interval.

Figure 11. Pooled relative risk of cardiovascular disease for the highest versus lowest categories of α-linolenic acid biomarker level



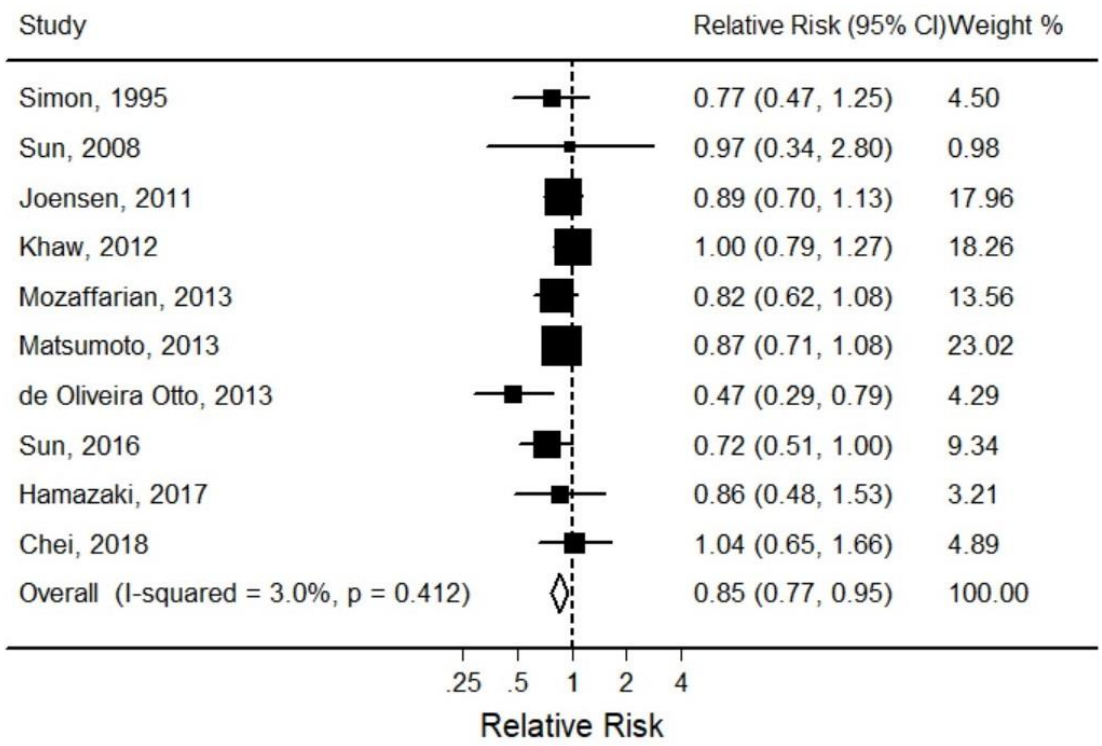
95% CI, 95% confidence interval.

Figure 12. Pooled relative risk of coronary heart disease for the highest versus lowest categories of α-linolenic acid biomarker level



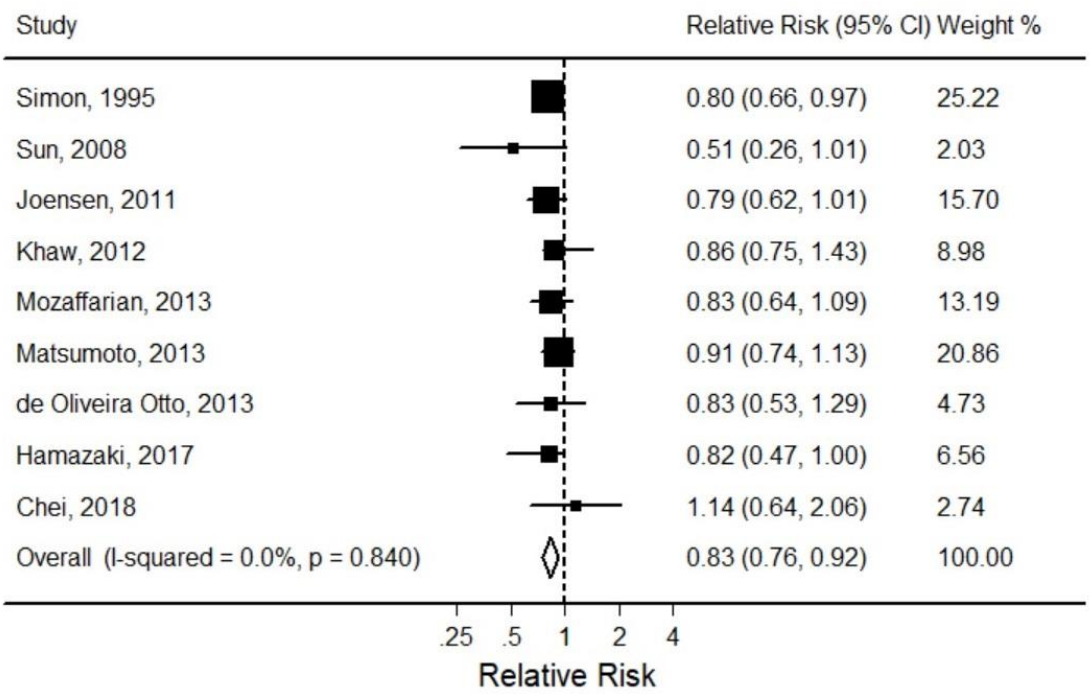
95% CI, 95% confidence interval.

Figure 13. Pooled relative risk of coronary heart disease for the highest versus lowest categories of eicosapentaenoic acid biomarker level



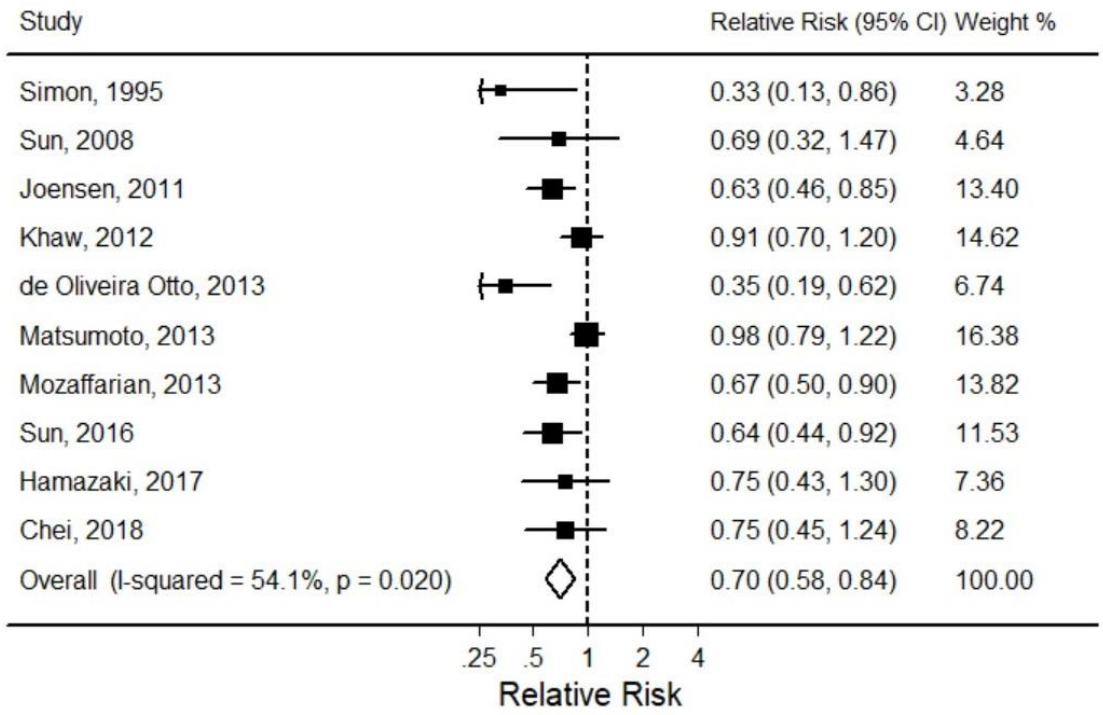
95% CI, 95% confidence interval.

Figure 14. Pooled relative risk of coronary heart disease for the highest versus lowest categories of docosapentaenoic acid biomarker level



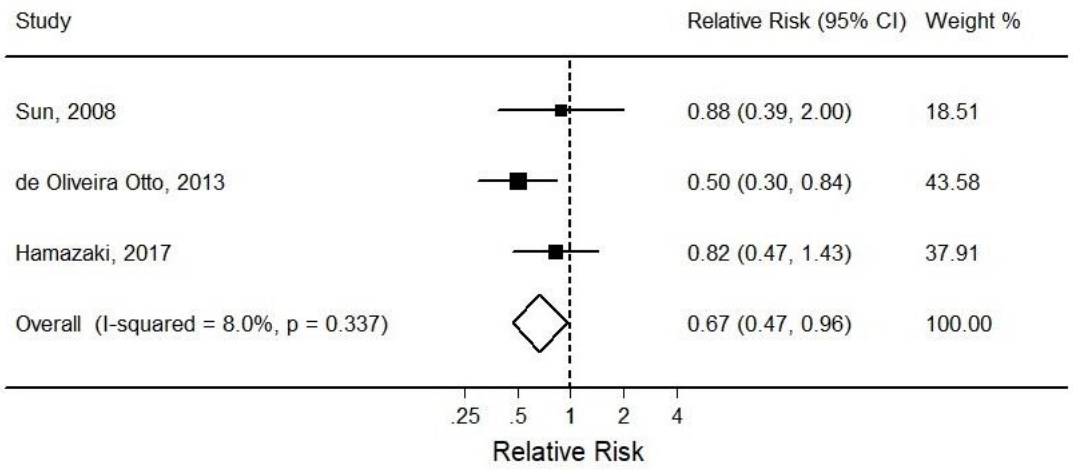
95% CI, 95% confidence interval.

Figure 15. Pooled relative risk of coronary heart disease for the highest versus lowest categories of docosahexaenoic acid biomarker level



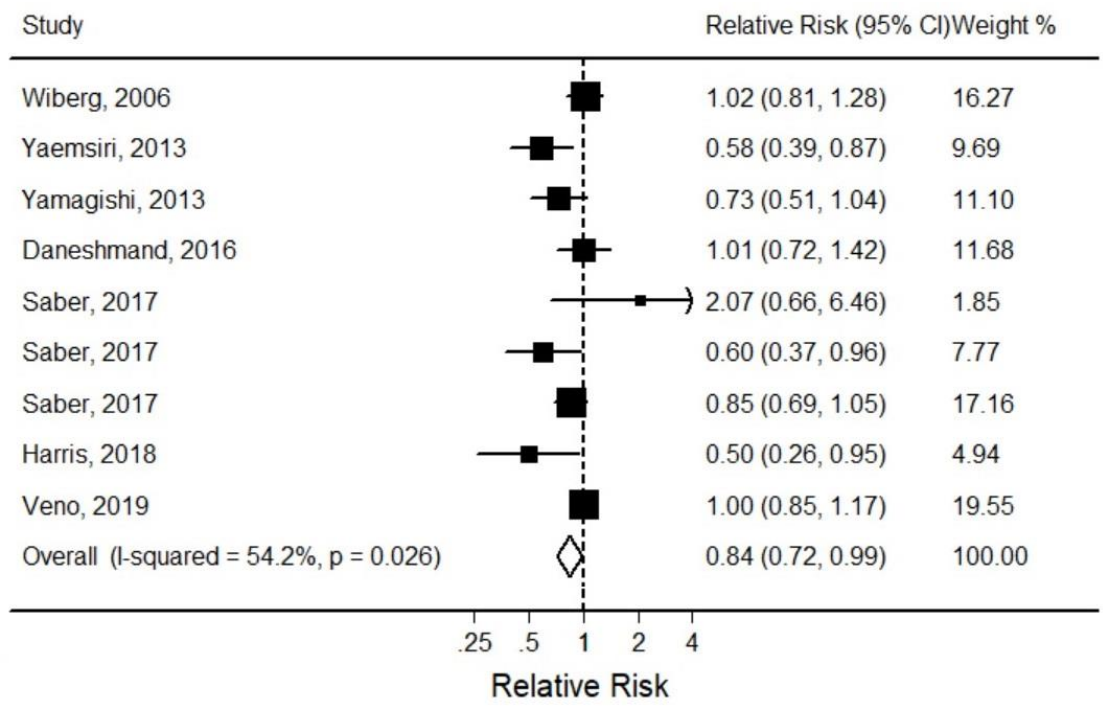
95% CI, 95% confidence interval.

Figure 16. Pooled relative risk of coronary heart disease for the highest versus lowest categories of long-chain omega-3 polyunsaturated fatty acid biomarker level



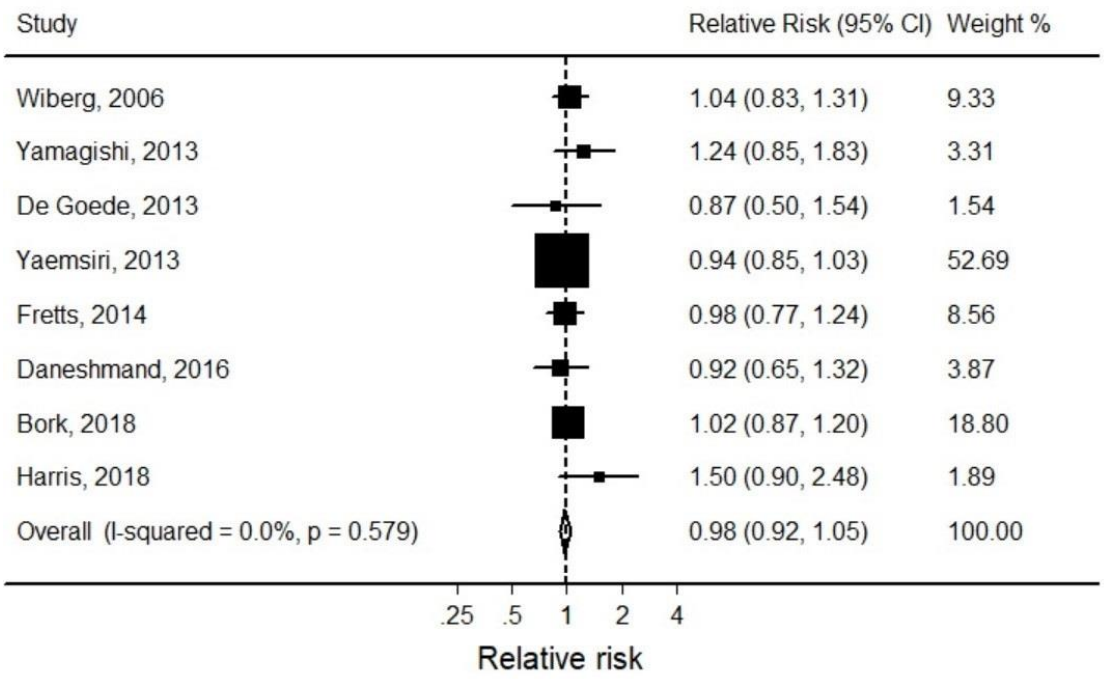
95% CI, 95% confidence interval.

Figure 17. Pooled relative risk of stroke for the highest versus lowest categories of docosahexaenoic acid biomarker level



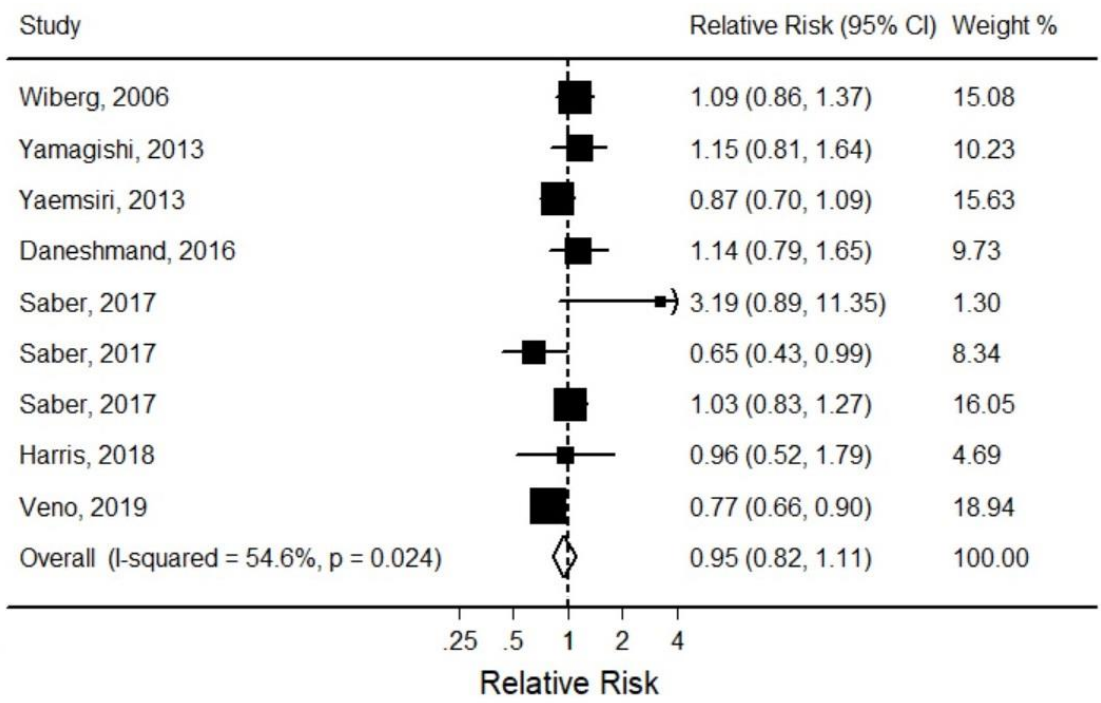
95% CI, 95% confidence interval.

Figure 18. Pooled relative risk of stroke for the highest versus lowest categories of α-linolenic acid biomarker level



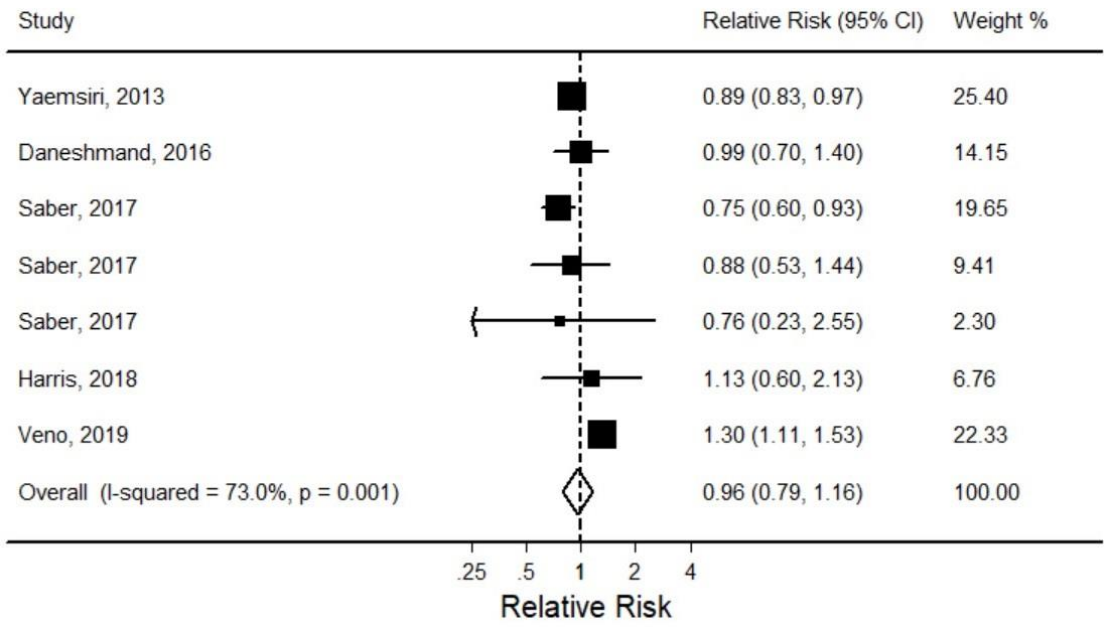
95% CI, 95% confidence interval.

Figure 19. Pooled relative risk of stroke for the highest versus lowest categories of eicosapentaenoic acid biomarker level



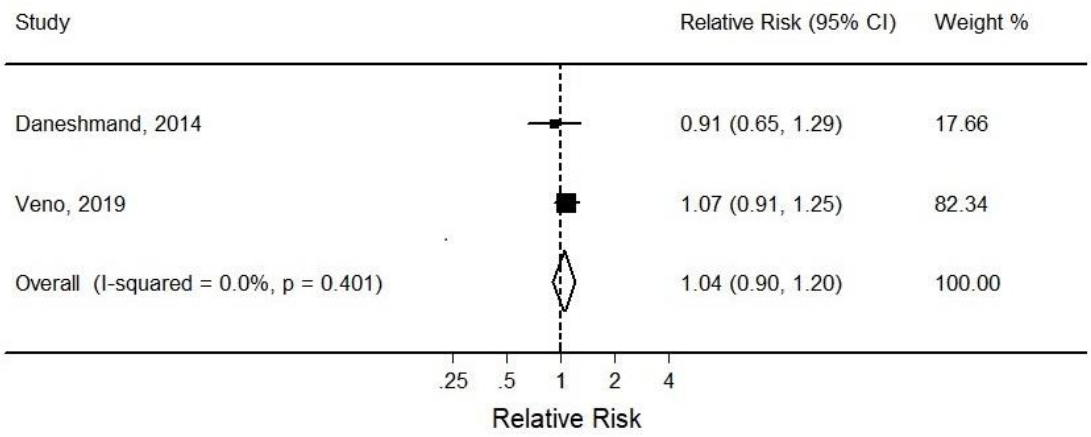
95% CI, 95% confidence interval.

Figure 20. Pooled relative risk of stroke for the highest versus lowest categories of docosapentaenoic acid biomarker level



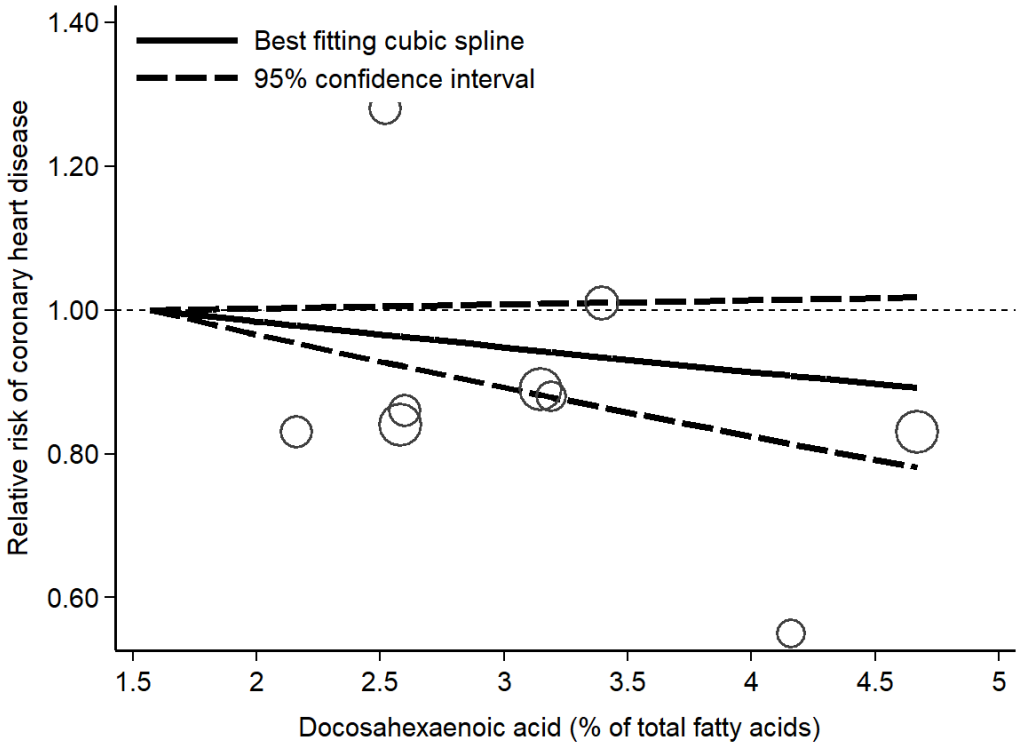
95% CI, 95% confidence interval.

Figure 21. Pooled relative risk of stroke for the highest versus lowest categories of long-chain omega-3 polyunsaturated fatty acid biomarker level



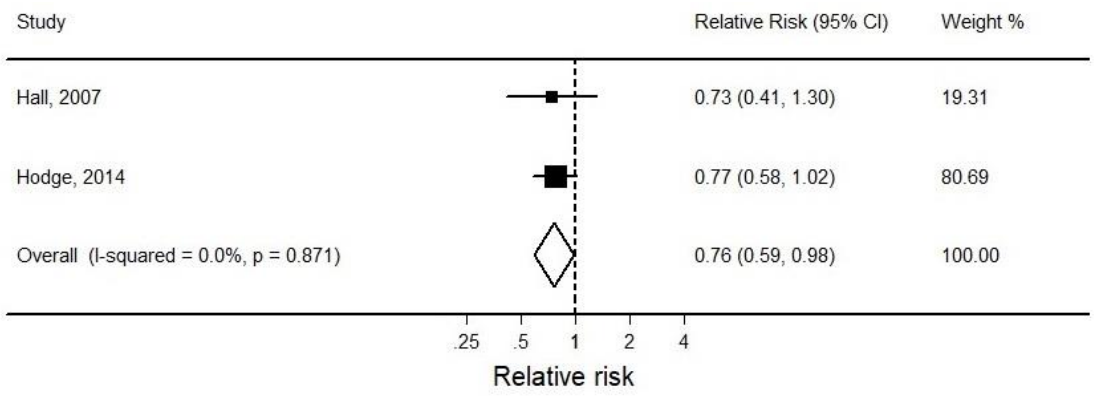
95% CI, 95% confidence interval.

Figure 22. Dose-response analyses of the linear association between docosahexaenoic acid biomarker and the risk of stroke



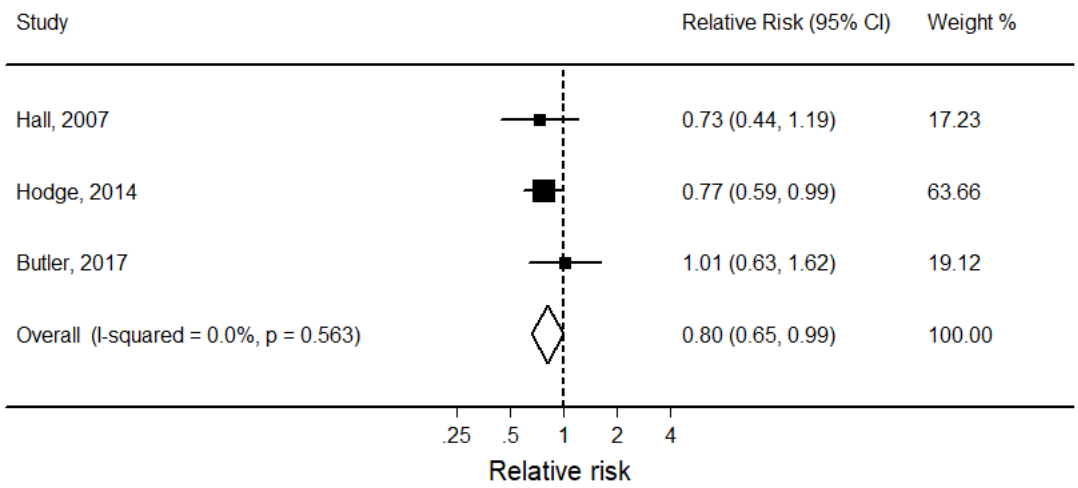
Circles represent point estimates plotted over precision measures. The solid line and the dotted lines represent the estimated relative risks and their 95% confidence interval.

Figure 23. Pooled relative risk of colorectal cancer for the highest versus lowest categories of docosapentaenoic acid biomarker level



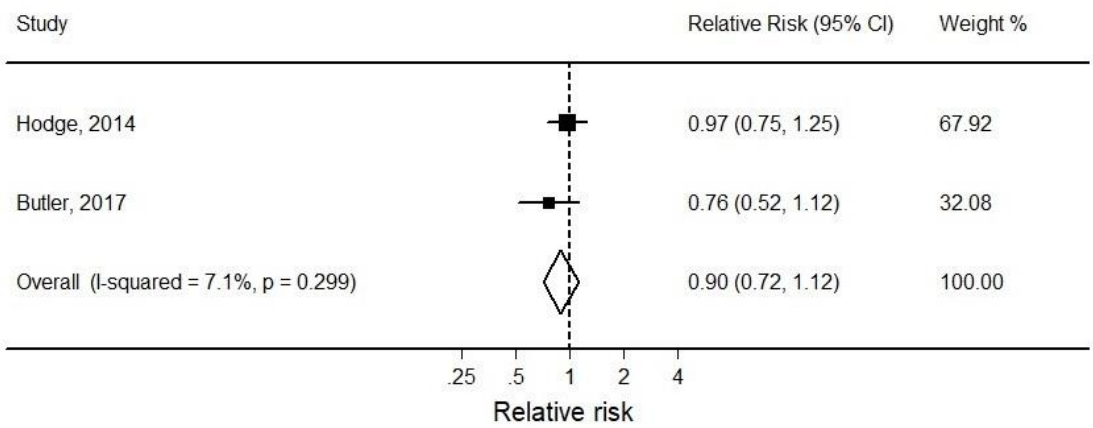
95% CI, 95% confidence interval.

Figure 24. Pooled relative risk of colorectal cancer for the highest versus lowest categories of docosahexaenoic acid biomarker level



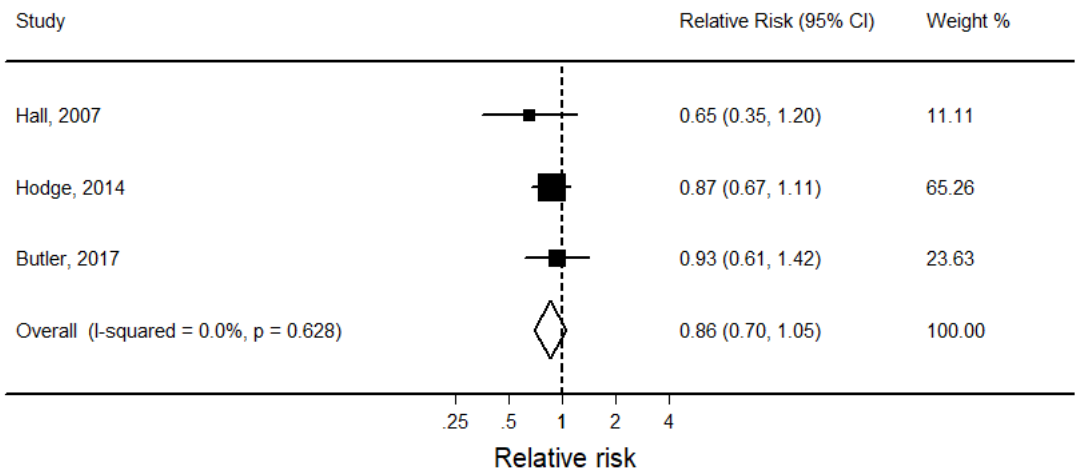
95% CI, 95% confidence interval.

Figure 25. Pooled relative risk of colorectal cancer for the highest versus lowest categories of α-linolenic acid biomarker level



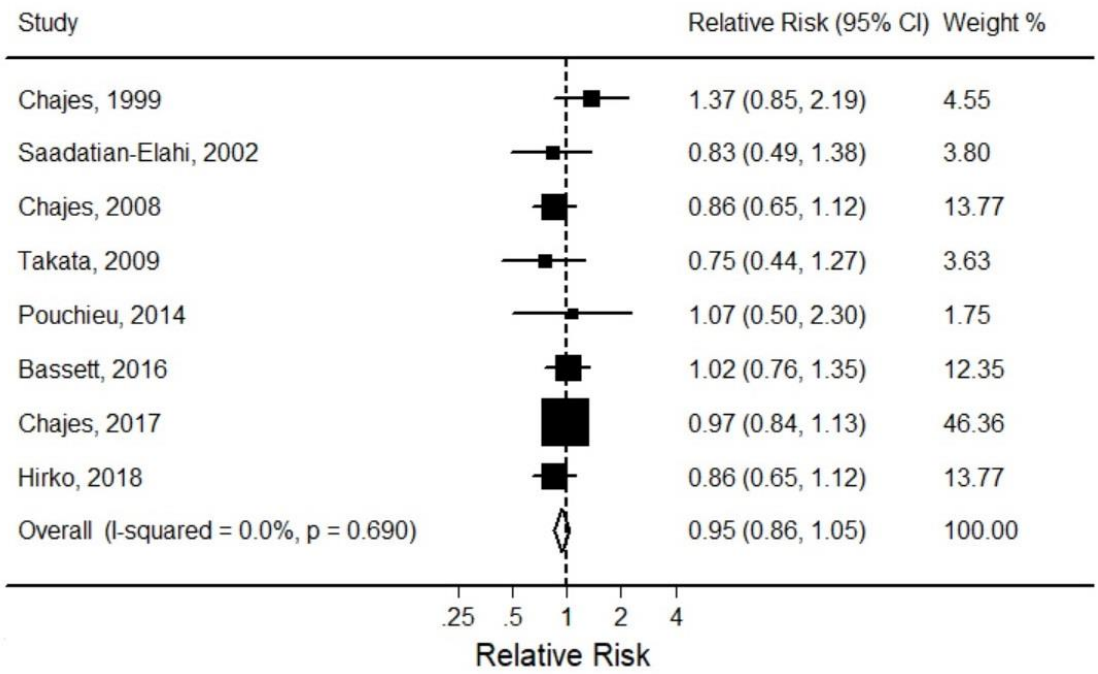
95% CI, 95% confidence interval.

Figure 26. Pooled relative risk of colorectal cancer for the highest versus lowest categories of eicosapentaenoic acid biomarker level



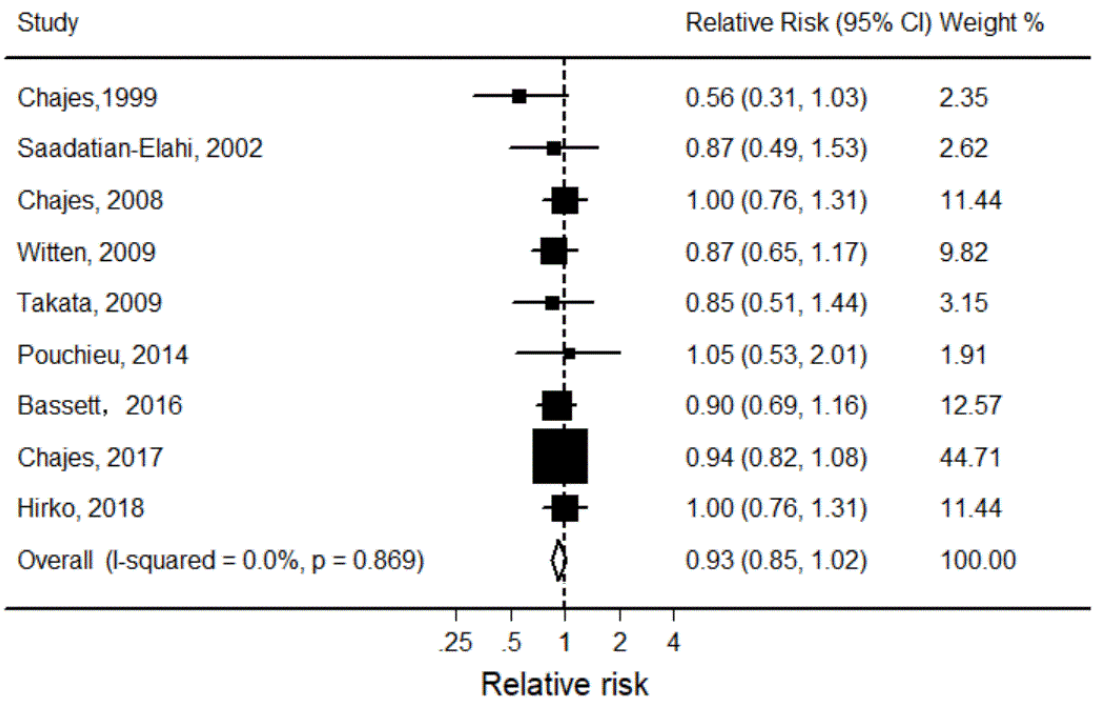
95% CI, 95% confidence interval.

Figure 27. Pooled relative risk of breast cancer for the highest versus lowest categories of α-linolenic acid biomarker level



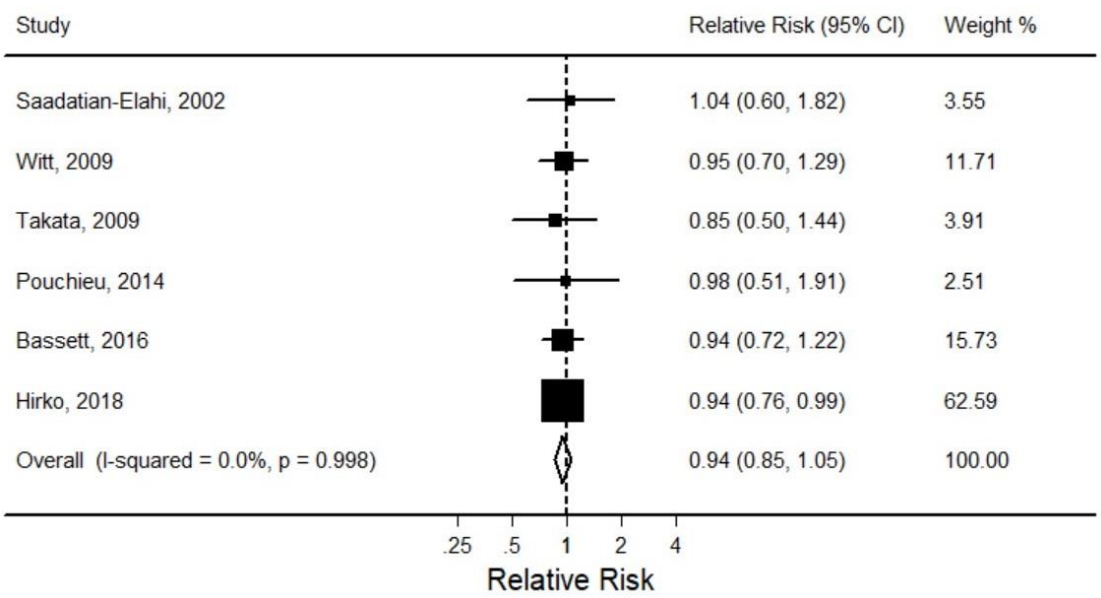
95% CI, 95% confidence interval.

Figure 28. Pooled relative risk of breast cancer for the highest versus lowest categories of eicosapentaenoic acid biomarker level



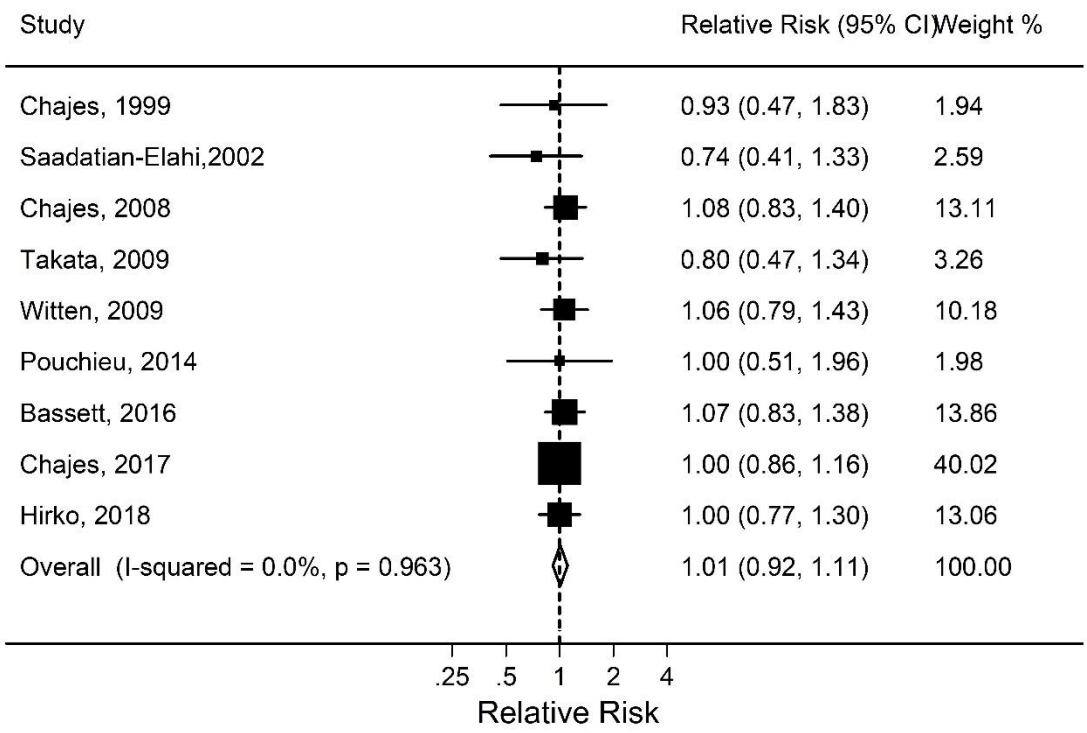
95% CI, 95% confidence interval.

Figure 29. Pooled relative risk of breast cancer for the highest versus lowest categories of docosapentaenoic acid biomarker level



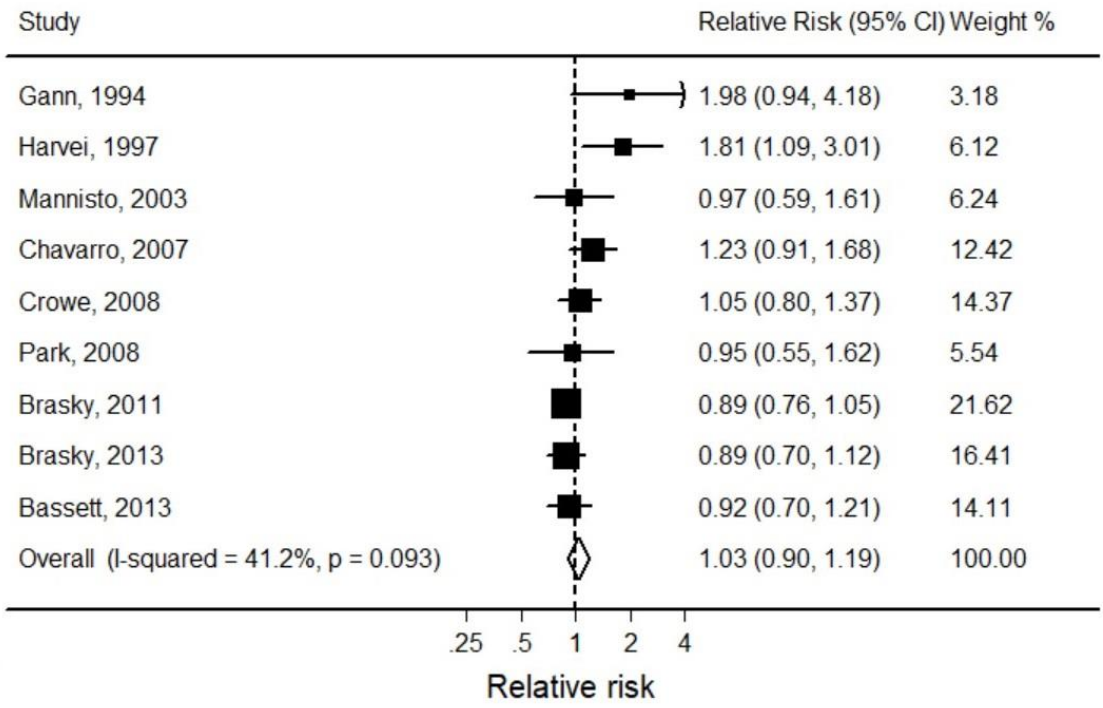
95% CI, 95% confidence interval.

Figure 30. Pooled relative risk of breast cancer for the highest versus lowest categories of docosahexaenoic acid biomarker level



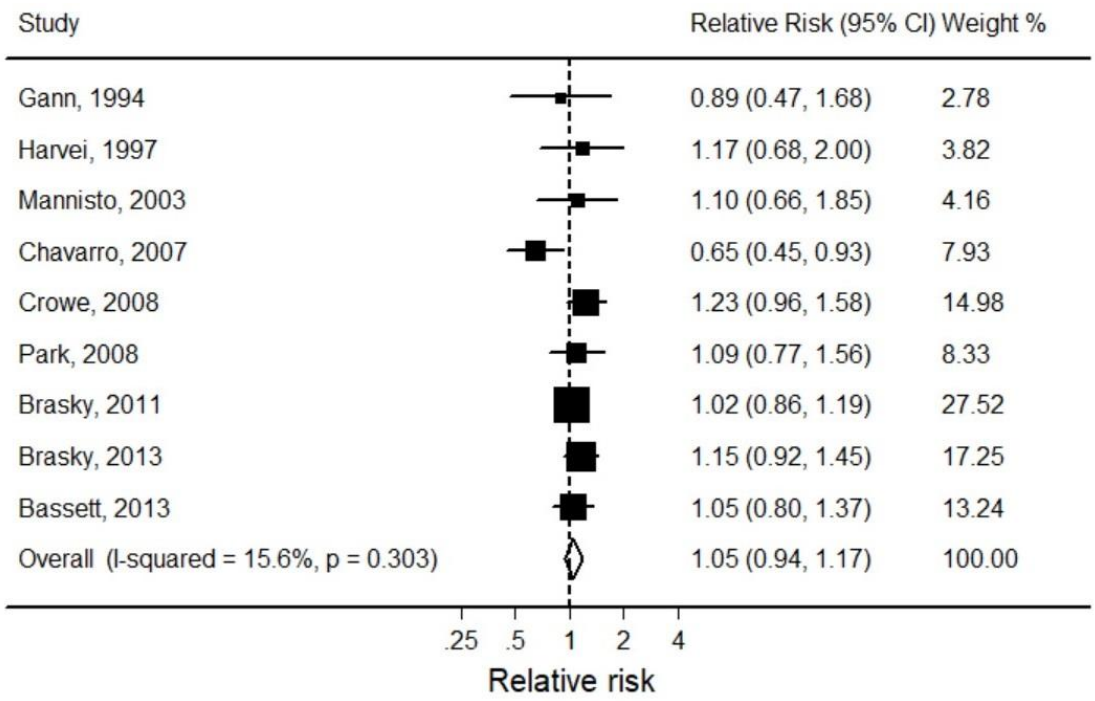
95% CI, 95% confidence interval.

Figure 31. Pooled relative risk of prostate cancer for the highest versus lowest categories of α-linoleic acid biomarker level



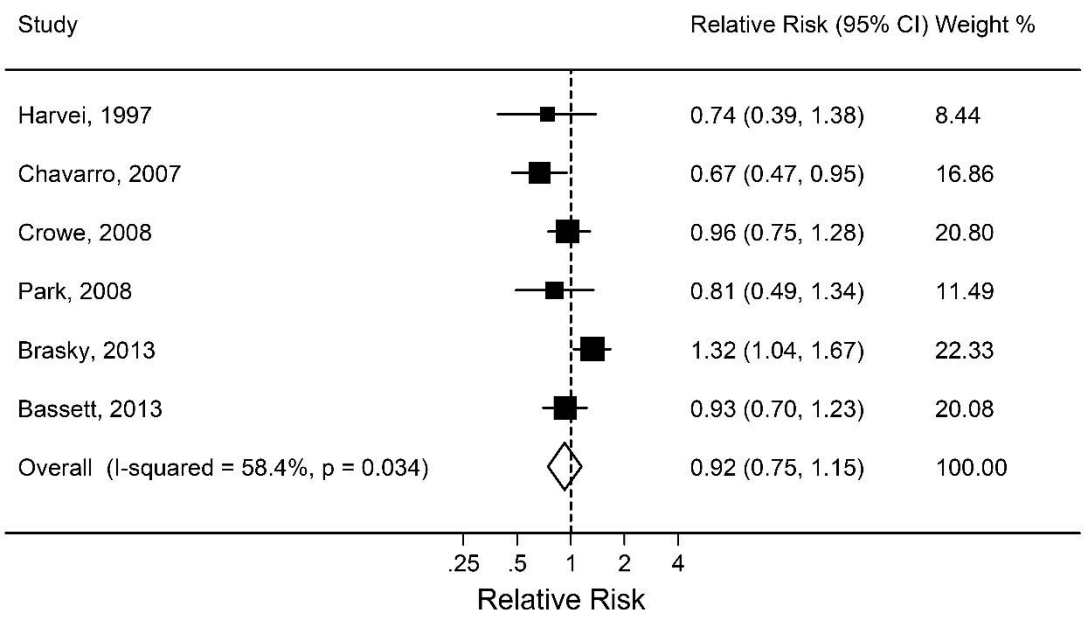
95% CI, 95% confidence interval.

Figure 32. Pooled relative risk of prostate cancer for the highest versus lowest categories of eicosapentaenoic acid biomarker level



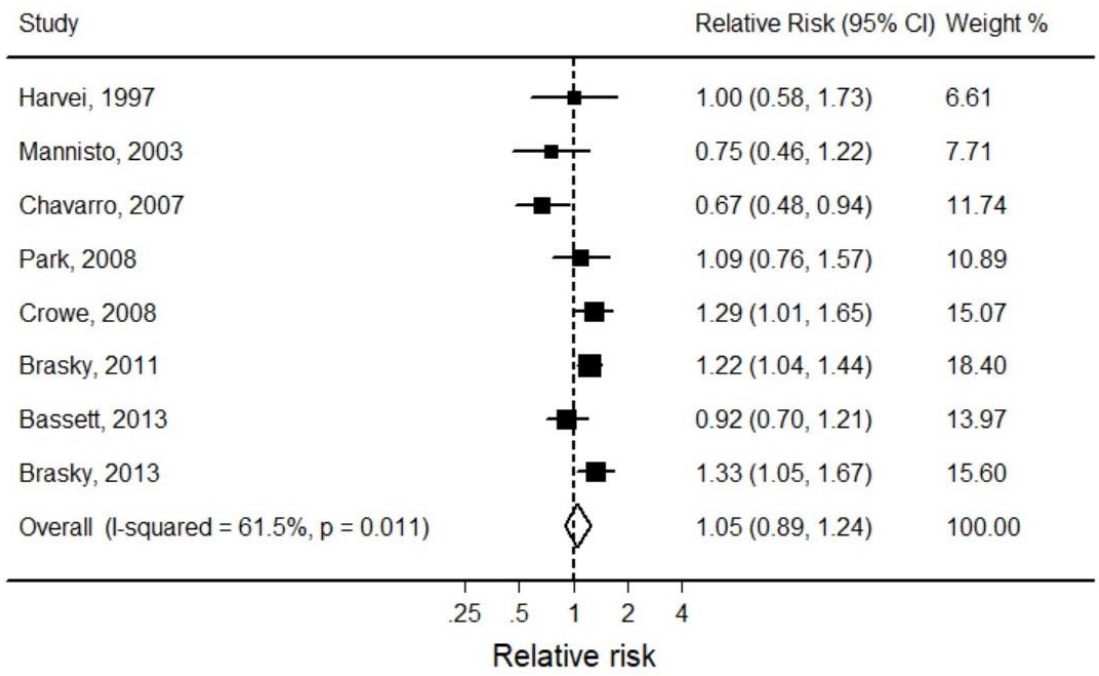
95% CI, 95% confidence interval.

Figure 33. Pooled relative risk of prostate cancer for the highest versus lowest categories of docosapentaenoic acid biomarker level



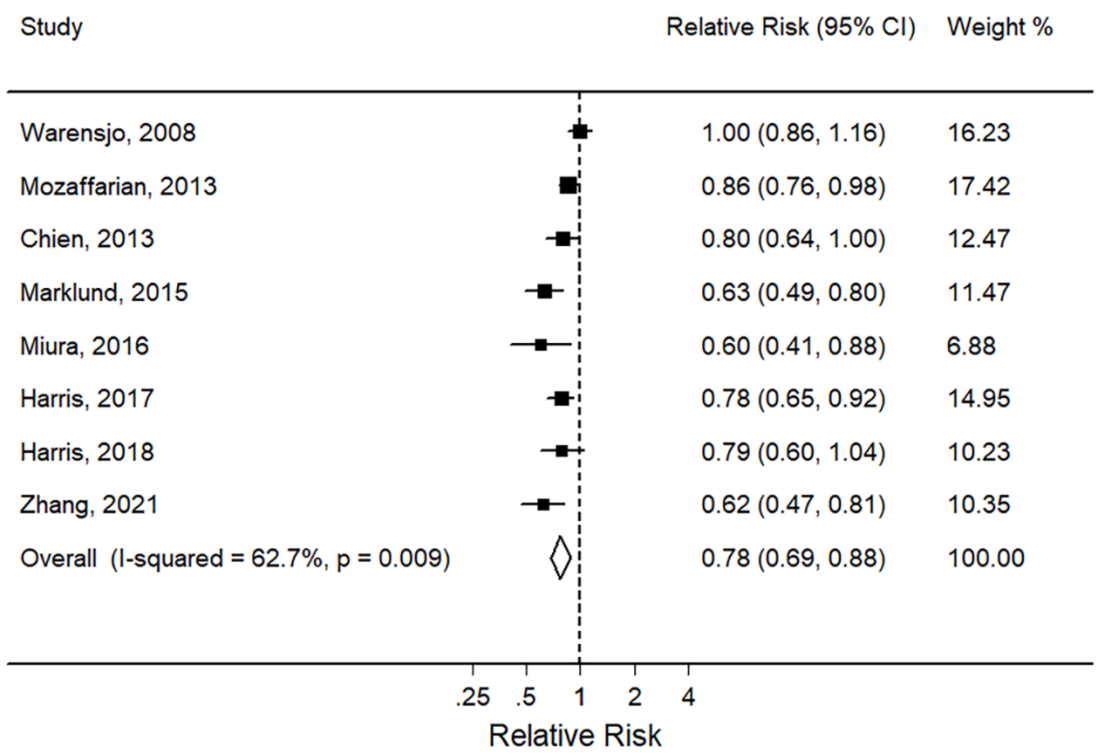
95% CI, 95% confidence interval.

Figure 34. Pooled relative risk of prostate cancer for the highest versus lowest categories of docosahexaenoic acid biomarker level



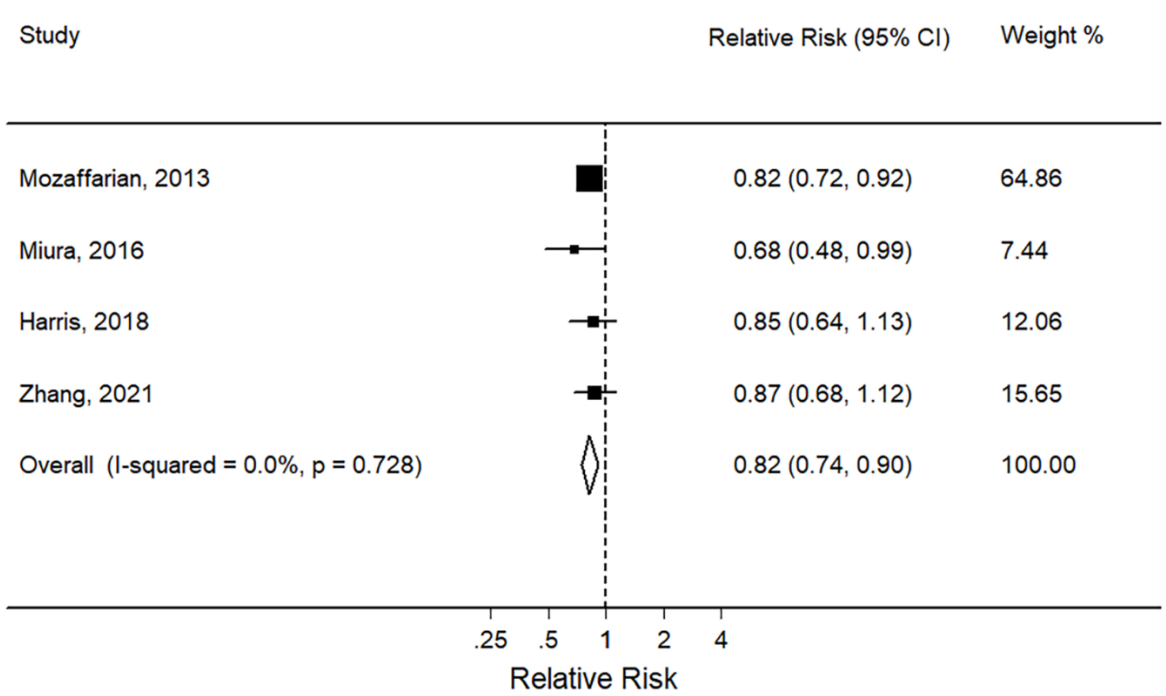
95% CI, 95% confidence interval.

Figure 35. Pooled relative risk of mortality for the highest versus lowest categories of eicosapentaenoic acid biomarker level



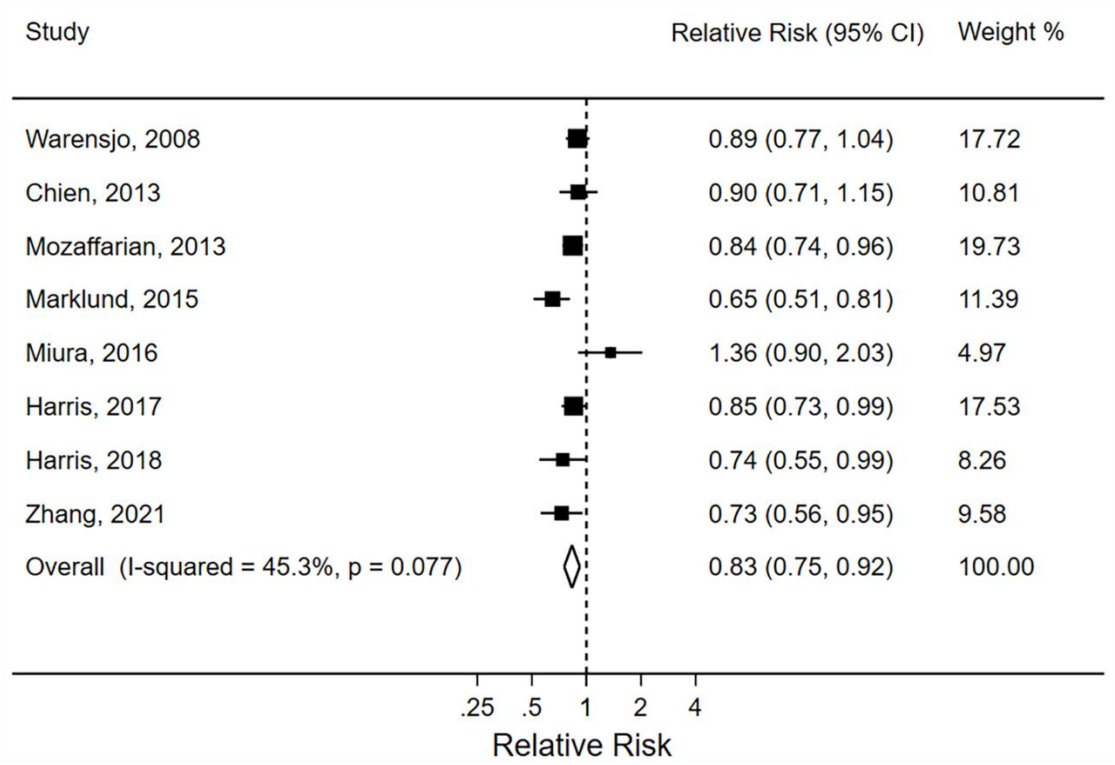
95% CI, 95% confidence interval.

Figure 36. Pooled relative risk of mortality for the highest versus lowest categories of docosapentaenoic acid biomarker level



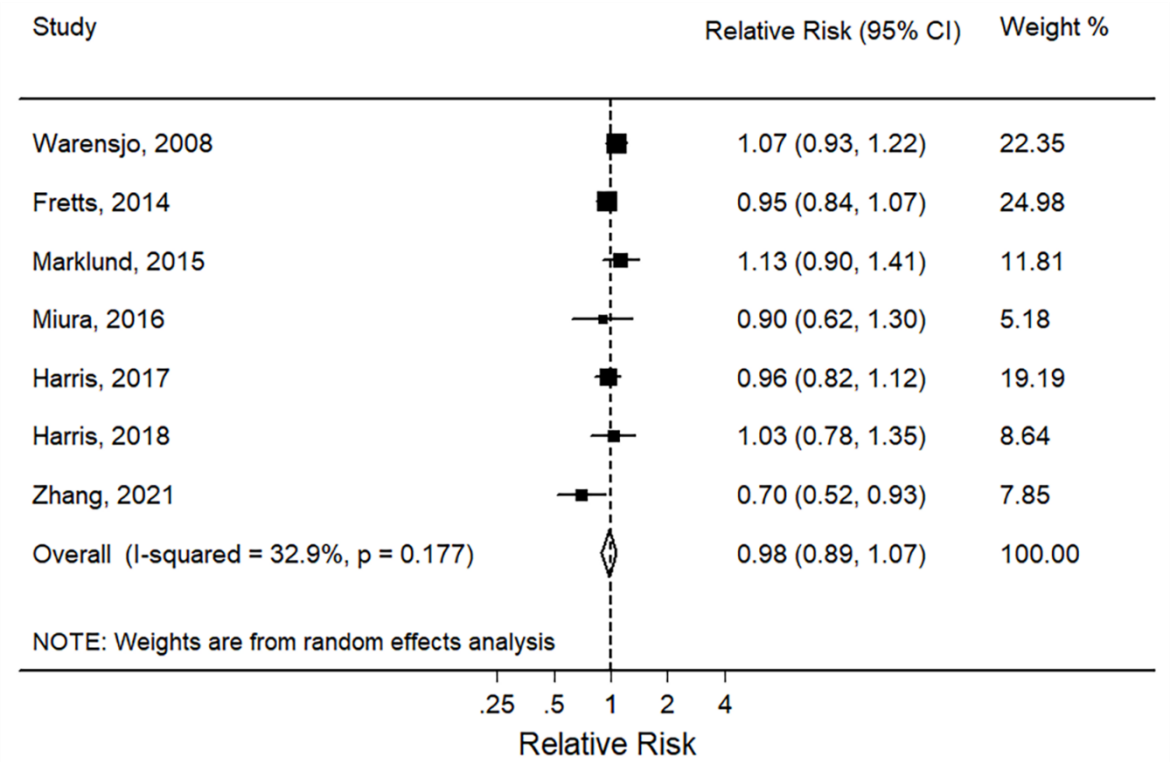
95% CI, 95% confidence interval.

Figure 37. Pooled relative risk of mortality for the highest versus lowest categories of docosahexaenoic acid biomarker level



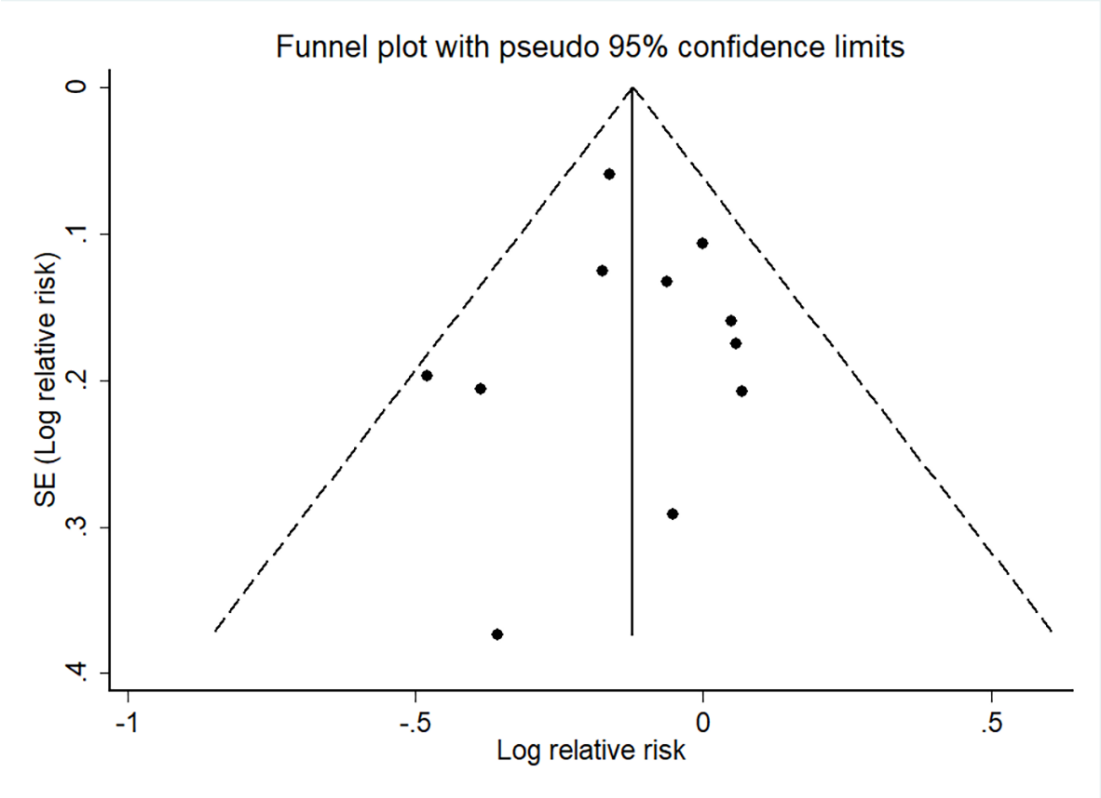
95% CI, 95% confidence interval.

Figure 38. Pooled relative risk of mortality for the highest versus lowest categories of α-linolenic acid biomarker level



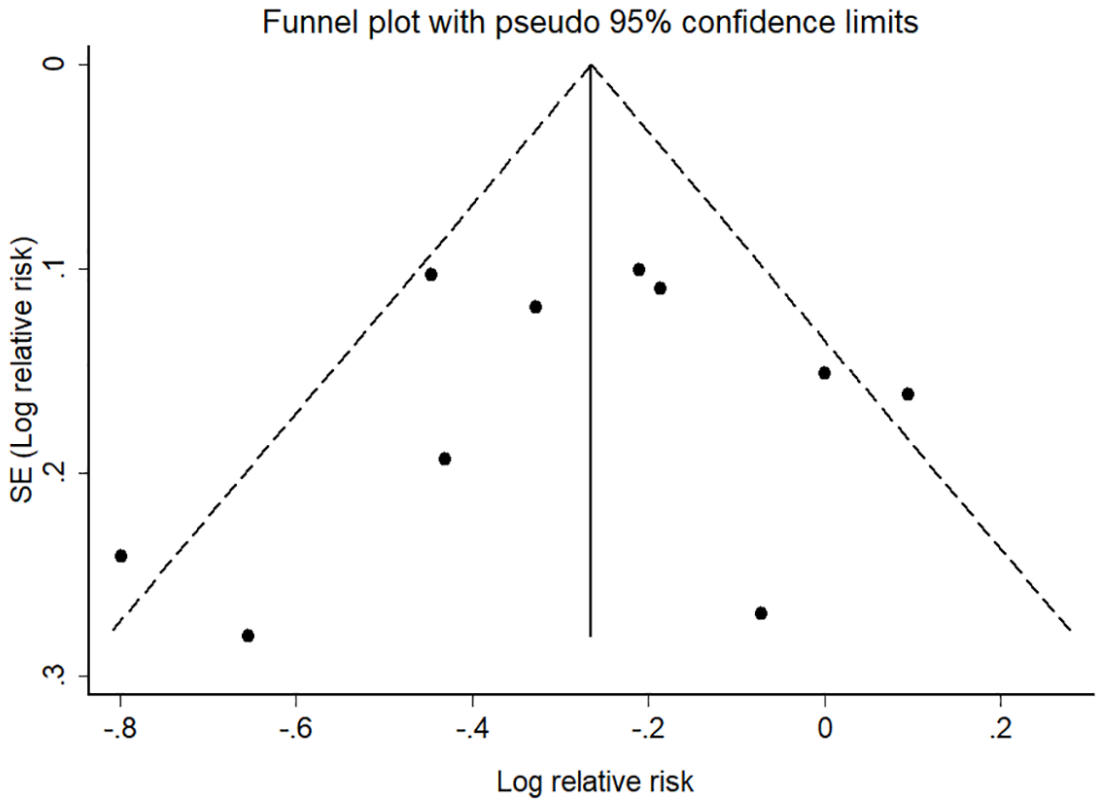
95% CI, 95% confidence interval.

Figure 39. Funnel plot of relative risk (RR) for type 2 diabetes comparing the lowest with the highest categories for α-linolenic acid biomarker level



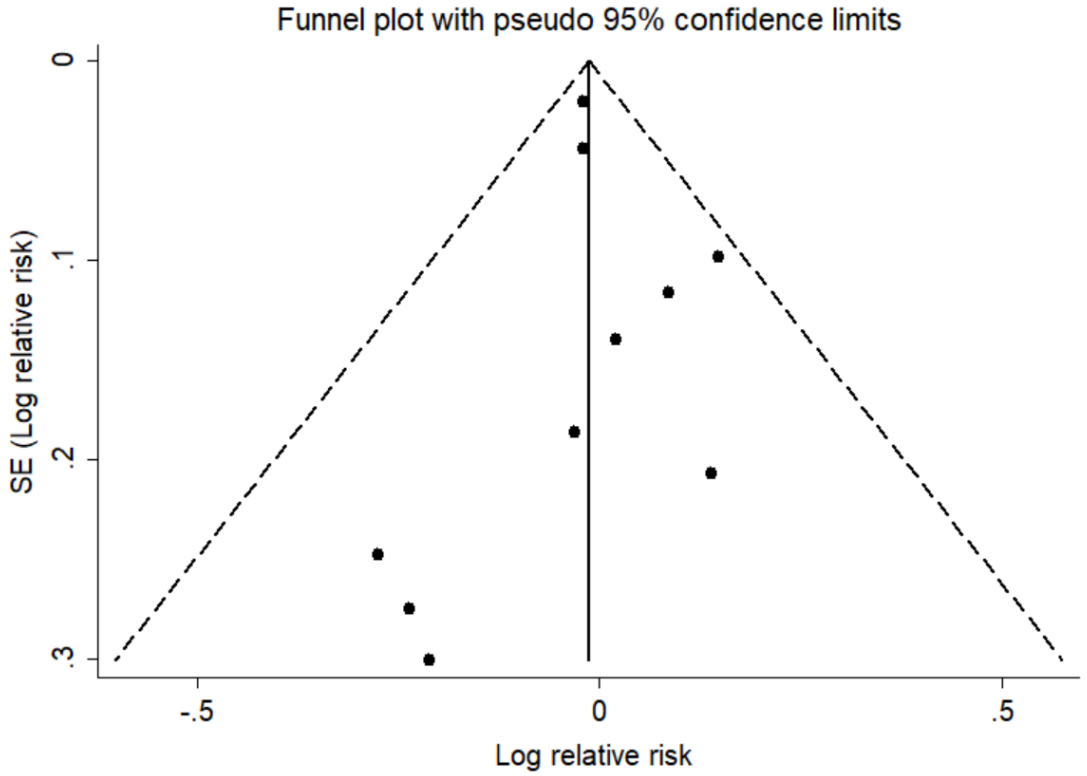
The dashed lines represent the pseudo-95% confidence interval of the RR. The circles represent risk estimates for each cohort, and the horizontal line represents standard errors of the RR**.** RR, relative risk.

Figure 40. Funnel plot of relative risk (RR) for cardiovascular disease comparing the lowest with the highest categories for docosahexaenoic acid biomarker level



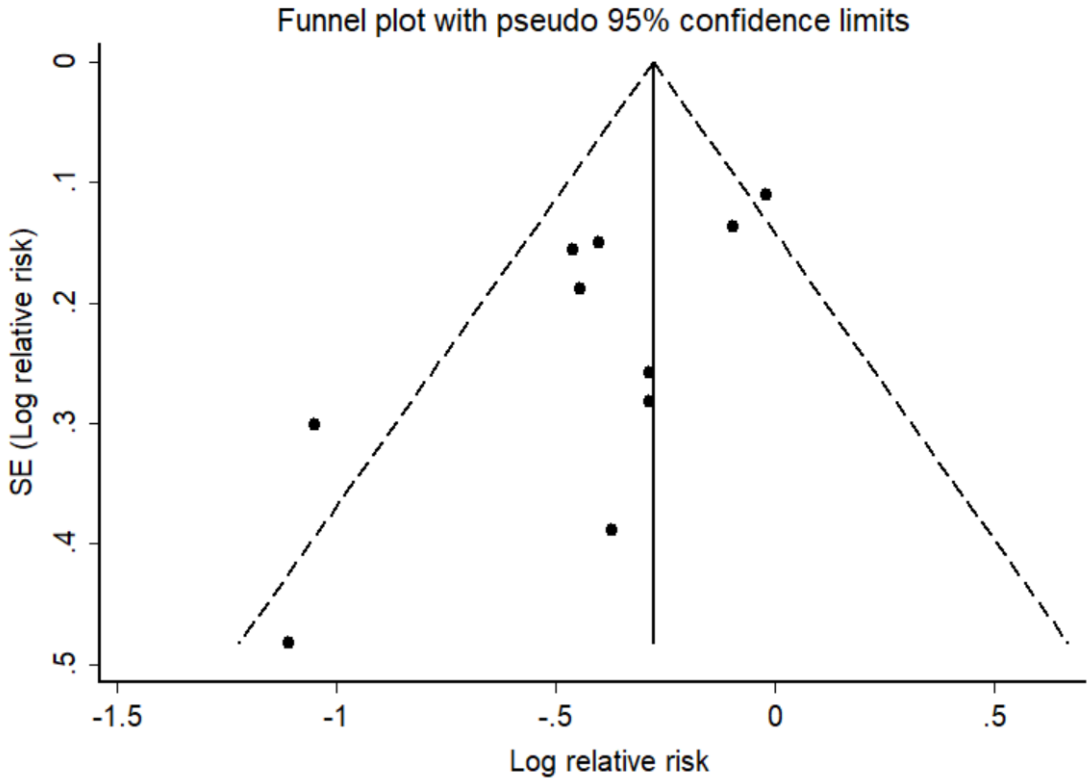
The dashed lines represent the pseudo-95% confidence interval of the RR. The circles represent risk estimates for each cohort, and the horizontal line represents standard errors of the RR. RR, relative risk.

Figure 41. Funnel plot of relative risk (RR) for coronary heart disease comparing the lowest with the highest categories for α-linolenic acid biomarker level



The dashed lines represent the pseudo-95% confidence interval of the RR. The circles represent risk estimates for each cohort, and the horizontal line represents standard errors of the RR**.** RR, relative risk.

Figure 42. Funnel plot of relative risk (RR) for coronary heart disease comparing the lowest with the highest categories for docosahexaenoic acid biomarker level



The dashed lines represent the pseudo-95% confidence interval of the RR. The circles represent risk estimates for each cohort, and the horizontal line represents standard errors of the RR. RR, relative risk.