**Additional file 1**

**Appendix S1.** PRISMA Checklist

**Appendix S2.** Methods

**Appendix S3.** Search strategy

**Appendix S4.** Characteristics of included studies that assessed the association of omega-3 fatty acids biomarkers with type 2 diabetes

**Appendix S5.** Characteristics of included studies that assessed the association of omega-3 fatty acids biomarkers with cardiovascular disease

**Appendix S6.** Characteristics of included studies that assessed the association of omega-3 fatty acids biomarkers with coronary heart disease

**Appendix S7.** Characteristics of included studies that assessed the association of omega-3 fatty acids biomarkers with stroke

**Appendix S8.** Characteristics of included studies that assessed the association of omega-3 fatty acids biomarkers with cancer

**Appendix S9.** Characteristics of included studies that assessed the association of omega-3 fatty acids biomarkers with mortality

**Appendix S10.** 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

**Appendix S11.** 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

**Appendix S12.** Subgroup analyses of fatty acid biomarkers and type 2 diabetes

**Appendix S13.** Subgroup analyses of fatty acid biomarkers and cardiovascular disease and coronary heart disease

**Appendix S14.** Subgroup analyses of fatty acid biomarkers and stroke

**Appendix S15.** Subgroup analyses of fatty acid biomarkers and prostate cancer

**Appendix S16.** Subgroup analyses of fatty acid biomarkers and all-cause mortality

**Appendix S1. PRISMA Checklist.**

|  |  |  |  |
| --- | --- | --- | --- |
| **Section/topic** | **#** | **Checklist item** | **Reported on page #** |
| **TITLE** | | |  |
| Title | 1 | Identify the report as a systematic review, meta-analysis, or both. | 1 |
| **ABSTRACT** | | |  |
| Structured summary | 2 | Provide a structured summary including, as applicable: background; objectives; data sources; study eligibility criteria, participants, and interventions; study appraisal and synthesis methods; results; limitations; conclusions and implications of key findings; systematic review registration number. | 2-3 |
| **INTRODUCTION** | | |  |
| Rationale | 3 | Describe the rationale for the review in the context of what is already known. | 5-6 |
| Objectives | 4 | Provide an explicit statement of questions being addressed with reference to participants, interventions, comparisons, outcomes, and study design (PICOS). | 5-6 |
| **METHODS** | | |  |
| Protocol and registration | 5 | Indicate if a review protocol exists, if and where it can be accessed (e.g., Web address), and, if available, provide registration information including registration number. | 6 |
| Eligibility criteria | 6 | Specify study characteristics (e.g., PICOS, length of follow-up) and report characteristics (e.g., years considered, language, publication status) used as criteria for eligibility, giving rationale. | 6-7 |
| Information sources | 7 | Describe all information sources (e.g., databases with dates of coverage, contact with study authors to identify additional studies) in the search and date last searched. | 6-7 |
| Search | 8 | Present full electronic search strategy for at least one database, including any limits used, such that it could be repeated. | 6-7, Supplementary  Appendix |
| Study selection | 9 | State the process for selecting studies (i.e., screening, eligibility, included in systematic review, and, if applicable, included in the meta-analysis). | 6-7, Supplementary  Appendix |
| Data collection process | 10 | Describe method of data extraction from reports (e.g., piloted forms, independently, in duplicate) and any processes for obtaining and confirming data from investigators. | 6-7, Supplementary  Appendix |
| Data items | 11 | List and define all variables for which data were sought (e.g., PICOS, funding sources) and any assumptions and simplifications made. | 6-7, Supplementary  Appendix |
| Risk of bias in individual studies | 12 | Describe methods used for assessing risk of bias of individual studies (including specification of whether this was done at the study or outcome level), and how this information is to be used in any data synthesis. | 7,  Supplementary  Appendix |
| Summary measures | 13 | State the principal summary measures (e.g., risk ratio, difference in means). | 7-8,  Supplementary  Appendix |
| Synthesis of results | 14 | Describe the methods of handling data and combining results of studies, if done, including measures of consistency (e.g., I2) for each meta-analysis. | 7-8,  Supplementary  Appendix |
| Risk of bias across studies | 15 | Specify any assessment of risk of bias that may affect the cumulative evidence (e.g., publication bias, selective reporting within studies). | 8 |
| Additional analyses | 16 | Describe methods of additional analyses (e.g., sensitivity or subgroup analyses, meta-regression), if done, indicating which were pre-specified. | 8 |
| **RESULTS** | | |  |
| Study selection | 17 | Give numbers of studies screened, assessed for eligibility, and included in the review, with reasons for exclusions at each stage, ideally with a flow diagram. | 8 |
| Study characteristics | 18 | For each study, present characteristics for which data were extracted (e.g., study size, PICOS, follow-up period) and provide the citations. | 8-9, Supplementary  Appendix |
| Risk of bias within studies | 19 | Present data on risk of bias of each study and, if available, any outcome level assessment (see item 12). | 9, Supplementary  Appendix |
| Results of individual studies | 20 | For all outcomes considered (benefits or harms), present, for each study: (a) simple summary data for each intervention group (b) effect estimates and confidence intervals, ideally with a forest plot. | Supplementary  Appendix |
| Synthesis of results | 21 | Present results of each meta-analysis done, including confidence intervals and measures of consistency. | 9-12, Supplementary  Appendix |
| Risk of bias across studies | 22 | Present results of any assessment of risk of bias across studies (see Item 15). | 9-12, Supplementary  Appendix |
| Additional analysis | 23 | Give results of additional analyses, if done (e.g., sensitivity or subgroup analyses, meta-regression [see Item 16]). | 12, Supplementary  Appendix |
| **DISCUSSION** | | |  |
| Summary of evidence | 24 | Summarize the main findings including the strength of evidence for each main outcome; consider their relevance to key groups (e.g., healthcare providers, users, and policy makers). | 12 |
| Limitations | 25 | Discuss limitations at study and outcome level (e.g., risk of bias), and at review-level (e.g., incomplete retrieval of identified research, reporting bias). | 16 |
| Conclusions | 26 | Provide a general interpretation of the results in the context of other evidence, and implications for future research. | 16-17 |
| **FUNDING** | | |  |
| Funding | 27 | Describe sources of funding for the systematic review and other support (e.g., supply of data); role of funders for the systematic review. | 18 |

**Appendix S2. Methods**

Literature search

Three authors HJ, LNW, and MW) independently searched PubMed (www.ncbi.nlm.nih.gov/pubmed), EMBASE (www.embase.com/), Web of Science (www.isiknowledge.com), and Cochrane Library (<http://www.thecochranelibrary.com>) for relevant published articles from inception to Jan 2020, 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 Additional file 1: Appendix S3 for detail search strategy). 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.

Data extraction

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.

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.1 This strategy was to harmonize different comparison groups used in individual studies, such as quartiles, quintiles, or other categorizations, or per SD change. In brief, for studies that provided relative risks per SD change of omega-3 PUFAs, we applied a factor of 2.18 to the log relative risk to derive the relative risk 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 relative risk was calculated after applying the same factors.2

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.3 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.4 For studies without data on number of cases or participants for each category, we then used the average case number by categories (that is, total participants or cases divided by the number of categories). Furthermore, only studies that reported relative risk (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 was used to examine any potential non-linear dose-response relationships. 6

# Appendix S3. Search strategy.

|  |
| --- |
| 1. “fatty acids” |
| 2. “omega 3” |
| 3. “omega 3 fatty acids” |
| 4. “omega-3 fatty acids” |
| 5. “n-3” |
| 6. “n-3 fatty acids” |
| 7. “α-linolenic acid” |
| 8. “polyunsaturated fatty acid” |
| 9. “eicosapentaenoic acid” |
| 10. “docosapentaenoic acid” |
| 11. “docosahexaenoic acid” |
| 12. “long-chain n-3” |
| 13. “long-chain omega-3” |
| 14. (1 OR 2 OR 3 OR 4 OR 5 OR 6 OR 7 OR 8 OR 9 OR 10 OR 11 OR 12 OR 13) |
| 15. “coronary heart disease” |
| 16. “heart disease” |
| 17. “ischemic heart disease” |
| 18. “coronary artery disease” |
| 19. “myocardial infarction” |
| 20. stroke |
| 21. “ischemic stroke” |
| 23. “haemorrhagic stroke” |
| 24. "intracranial hemorrhages" |
| 25. "cerebral infarction" |
| 26. “metabolic diseases” |
| 27. “diabetes mellitus” |
| 26. “type 2 diabetes” |
| 28. “impaired glucose” |
| 29. “impaired fasting insulin” |
| 30. cancer |
| 31. neoplasms |
| 32. mortality |
| 33. “all-cause mortality” |
| 34. “total mortality |
| 35. survival |
| 36. (14 OR 15 OR 16 OR 17 OR 18 OR 19 OR 20 OR 21 OR 22 OR 23 OR 24 OR 25 OR 26 OR 27 OR 28 OR 29 OR 30 OR 31 OR 32 OR 33 OR 34 OR 35) |
| 37. blood |
| 38. marker |
| 39. biomarker |
| 40. serum |
| 41. plasma |
| 42. “whole blood" |
| 43. “adipose tissue” |
| 44. circulating |
| 45. erythrocytes |
| 46. "red blood cell" |
| 47. “Cholesteryl esters” |
| 48. (37 OR38 OR 39 OR 40 OR 41 OR 42 OR 43 OR 44 OR 45 OR 46 OR 47) |
| 49. “nested case control studies” |
| 50. cohorts |
| 51. "Prospective Studies |
| 52. "Follow-up Studies" |
| 53. longitudinal |
| 54. “follow-up” |
| 55. “relative risk” |
| 56. “population-based” |
| 57. “odds ratio” |
| 58. “hazard ratio” |
| 59. “incidence rate ratio” |
| 60. (49 OR 50 OR 51 OR 52 OR 53 OR 54 OR 55 OR 56 OR 57 OR 58 OR 59) |
| 61. 14 AND 36 AND 48 AND 60 |

# Appendix S4. Characteristics of included studies that assessed the association of omega-3 fatty acids biomarkers with type 2 diabetes.

|  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |
| --- | --- | --- | --- | --- | --- | --- | --- | --- | --- | --- | --- | --- | --- | --- | --- |
| **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, USA6 | 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, Australia7 | 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, Sweden8 | 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 |
| Kroger et al, 2011, Europe9 | 1991 | NCCD | 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 |
| Djousse et al, 2011, USA10 | 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, Finland11 | 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, Finland12 | 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, blood glucose | High |
| Forouhi et al, 2016, Europe13 | 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, USA14 | 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 |
| Akter et al, 2017, Japan15 | 2008-09 | NCCS | 5.0 | HHS | 1,014 | 34-69 | 90.9 | GC | Serum | Phospholipid | ALA, EPA, DPA, DHA | Records | 336 | Age, sex, smoking, alcohol, examination year, BMI, physical activity, shift work, sleep duration, family history of diabetes and hypertension | High |
| Zheng et al, 2018, China16 | 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 |

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; 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.

# Appendix S5. Characteristics of included studies that assessed the association of omega-3 fatty acids biomarkers with cardiovascular disease.

|  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |
| --- | --- | --- | --- | --- | --- | --- | --- | --- | --- | --- | --- | --- | --- | --- | --- |
| **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, USA17 | 1982-84 | NCCS | 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, Finland18 | 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, Sweden19 | 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, Scotland20 | 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, Finland21 | 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)22 | 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, USA23 | 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, USA24 | 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, USA25 | 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, Swedish26 | 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, USA27 | 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, USA28 | 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 |

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; NCCS, nested case-control study; 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.

# Appendix S6. Characteristics of included studies that assessed the association of omega-3 fatty acids biomarkers with coronary heart disease.

|  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |
| --- | --- | --- | --- | --- | --- | --- | --- | --- | --- | --- | --- | --- | --- | --- | --- |
| **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, USA29 | 1973-76 | NCCS | 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, USA30 | 1992-93 | NCCS | 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, USA31 | 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, USA32 | 1989-90 | NCCS | 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, diabetes, hypercholesterolemia, energy intake, blood fatty acids | High |
| Joensen et al, 2011, Denmark33 | 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, UK34 | 1993-97 | NCCS | 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, USA24 | 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, USA23 | 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, USA35 | 1982-84 | NCCS | 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, USA25 | 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, Singapore36 | 1993-98 | NCCS | 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, Japan37 | 1990-93 | NCCS | 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, Japan38 | 1984, 1989, 1997, 1998 | NCCS | 11.0 (mean) | CIRCS | 608 | 40–85 | - | 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; NCCS, nested case-control 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.

# Appendix S7. Characteristics of included studies that assessed the association of omega-3 fatty acids biomarkers with stroke.

|  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |
| --- | --- | --- | --- | --- | --- | --- | --- | --- | --- | --- | --- | --- | --- | --- | --- |
| **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** | **Ascertainment method** | **Cases (n)** |
| Wiberg et al, 2006, Sweden39 | 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 | High |
| De Goede et al, 2013, Holland40 | 1993-97 | NCCS | 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, USA41 | 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, USA42 | 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, USA25 | 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, Finland43 | 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, USA44 | 1992-93 | NCCS | 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, menopausal status, family history of CVD and diabetes, aspirin treatment, dietary factors | High |
| Saber et al, 2017, USA44 | 1989-90 | NCCS | 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, USA44 | 1993-94 | NCCS | 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, Denmark45 | 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, USA28 | 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, Denmark46 | 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; NCCS, nested case-control 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.

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

# Appendix S8. Characteristics of included studies that assessed the association of omega-3 fatty acids biomarkers with cancer.

|  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |
| --- | --- | --- | --- | --- | --- | --- | --- | --- | --- | --- | --- | --- | --- | --- | --- | --- | --- | --- |
| **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** a |
| **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)** |
| C**olorectal cancer** | | |  |  |  |  |  |  |  |  |  | | |  |  |  | |  |
| Hall et al, 2007, USA47 | 1982-84 | | NCCS | 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, Australia48 | 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, Singapore49 | 1993-98 | | NCCS | 3.3  (median) | SCHS | 700 | 45-74 | 58.86 | GC | Plasma | Total fatty acid fraction | ALA, EPA, DHA | | | Records | 350 | Smoking, alcohol, education, BMI, physical activity, diabetes | High |
| **Breast cancer** | | |  | | |  |  |  |  |  |  | | |  |  |  | |  |
| Chajes et al, 1999, Sweden50 | 1986-97 | | NCCS | 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, France51 | 1985-91 | | NCCS | 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 |
| Chajes et al, 2008, France52 | 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, USA53 | 1985-94 | | NCCS | 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, Denmark54 |  | | CCD | 4.8  (median) | DCH | 1,561 | 50–64 | 0.00 | GC | Adipose tissue | - | EPA+EPA+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, UK55 | 1994-95 | | NCCS | 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, Australia56 | 1990-94 | | CCD | 8.9 (mean) | MCCS | 2491 | 40-69 | 100.00 | GLC | Plasma | Phospholipid fraction | ALA, EPA, DPA, DHA | | | Records | 470 | Age, smoking, alcohol, region, education, physical activity, menopausal status, hormone therapy, oral contraceptive use, energy intake, family history of cancer | High |
| Chajes et al, 2017, Europe57 | 1993-2002 | | NCCS | 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, USA58 | 1996-99 | | NCCS | 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, USA59 | 1982 | | NCCS | NR | PHS | 240 | 40-84 | 100.00 | GC | plasma | Cholesterol fraction | ALA, EPA | | | Records | 120 | Age, smoking | Moderate |
| Harvei et al, 1997, Norway60 | 1973-94 | | NCCS | 11.6  (mean) | - | 423 | NR | 100.00 | GLC | Serum | Phospholipid fraction | ALA, EPA, DPA, DHA | | | Records | 141 | Multiplicative risk | High |
| Männistö et al, 2003, USA61 | 1995-98 | | NCCS | 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,USA62 | 1982 | | NCCS | 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, Europe63 | 1992 | | NCCS | 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, USA64 | 1993-96 | | NCCS | 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, USA65 | 1994-2003 | | NCCS | 7.0 | PCPT | 3,461 | 55-84 | 100.00 | GC | Serum | Phospholipid fraction | ALA, EPA+DHA, EPA, DHA | | | Annual prostate-specific antigen and digital rectal examination | 1,658 | Age, race, family history of prostate cancer, treatment arm | High |
| Brasky et al, 2013, USA66 | 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 | High |
| Bassett et al, 2013, Australia67 | 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, the Melbourne Collaborative Cohort Study; MCS, The Multiethnic Cohort Study; MONICA, Monitoring of Trends and Cardiovascular Disease study; MSP, The Mammary-Screening Project; NCCS, nested case-control 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.

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

# Appendix S9. Characteristics of included studies that assessed the association of omega-3 fatty acids biomarkers with mortality.

|  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |
| --- | --- | --- | --- | --- | --- | --- | --- | --- | --- | --- | --- | --- | --- | --- | --- |
| **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** a |
| **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, Sweden19 | 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)22 | - | 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, USA23 | 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, USA25 | 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, Swedish3 | 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, Australia68 | 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, USA27 | 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, USA28 | 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 |

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; NSCS, the Nambour Skin Cancer Study; 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.

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

# Appendix S10. 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 |
| **T2D** |  |  |  |  |  |  |  |  |  |
| Wang, 20036 | 1 | 1 | 1 | 1 | 2 | 1 | 1 | 1 | 9 |
| Hodge, 20077 | 1 | 1 | 1 | 1 | 2 | 1 | 0 | 0 | 7 |
| Krachler, 20088 | 1 | 1 | 1 | 1 | 0 | 1 | 1 | 1 | 7 |
| Kroger, 20119 | 1 | 1 | 1 | 1 | 2 | 1 | 1 | 1 | 9 |
| Djousse, 201110 | 0 | 1 | 1 | 1 | 2 | 1 | 1 | 1 | 8 |
| Virtanen, 201411 | 1 | 1 | 1 | 1 | 2 | 1 | 1 | 1 | 9 |
| Takkunen, 201512 | 0 | 1 | 1 | 1 | 2 | 1 | 1 | 0 | 7 |
| Forouhi, 201613 | 0 | 1 | 1 | 1 | 2 | 1 | 1 | 1 | 8 |
| Harris, 201614 | 0 | 1 | 1 | 1 | 2 | 1 | 1 | 0 | 7 |
| Zheng, 201816 | 0 | 1 | 1 | 0 | 2 | 1 | 1 | 0 | 6 |
| **CVD** |  |  |  |  |  |  |  |  |  |
| Laaksonen, 200518 | 1 | 1 | 1 | 1 | 2 | 1 | 1 | 1 | 9 |
| Warensjö, 200819 | 1 | 1 | 1 | 1 | 2 | 1 | 1 | 1 | 9 |
| Woodward, 201120 | 0 | 1 | 1 | 1 | 2 | 1 | 1 | 0 | 7 |
| Virtanen, 201221 | 1 | 1 | 1 | 1 | 2 | 1 | 1 | 1 | 9 |
| Chien, 201322 | 0 | 1 | 1 | 0 | 2 | 1 | 1 | 0 | 6 |
| Mozaffarian, 201323 | 0 | 1 | 1 | 1 | 2 | 1 | 1 | 1 | 8 |
| de Oliveira Otto, 201324 | 1 | 1 | 1 | 1 | 2 | 1 | 1 | 0 | 8 |
| Fretts, 201425 | 0 | 1 | 1 | 1 | 2 | 1 | 1 | 1 | 8 |
| Marklund, 201526 | 1 | 1 | 1 | 1 | 0 | 1 | 1 | 0 | 6 |
| Harris, 201727 | 0 | 1 | 1 | 1 | 2 | 1 | 1 | 0 | 7 |
| Harris, 201828 | 1 | 1 | 1 | 1 | 2 | 1 | 1 | 1 | 9 |
| **CHD** |  |  |  |  |  |  |  |  |  |
| Wang, 200331 | 1 | 1 | 1 | 1 | 2 | 1 | 1 | 1 | 9 |
| Joensen, 201133 | 0 | 1 | 1 | 1 | 2 | 1 | 1 | 1 | 8 |
| de Oliveira Otto, 201324 | 1 | 1 | 1 | 1 | 2 | 1 | 1 | 0 | 8 |
| Mozaffarian, 201323 | 0 | 1 | 1 | 1 | 2 | 1 | 1 | 1 | 8 |
| Fretts, 201425 | 0 | 1 | 1 | 1 | 2 | 1 | 1 | 1 | 8 |
| **Stroke** |  |  |  |  |  |  |  |  |  |
| Wiberg, 200639 | 1 | 1 | 1 | 1 | 2 | 1 | 1 | 1 | 9 |
| Yamagishi, 201341 | 1 | 1 | 1 | 1 | 2 | 1 | 1 | 1 | 9 |
| Yaemsiri, 201342 | 0 | 1 | 1 | 1 | 2 | 1 | 1 | 0 | 7 |
| Fretts, 201425 | 0 | 1 | 1 | 1 | 2 | 1 | 1 | 1 | 8 |
| Daneshmand, 201643 | 1 | 1 | 1 | 1 | 2 | 1 | 1 | 1 | 9 |
| Bork, 201845 | 0 | 1 | 1 | 1 | 2 | 1 | 1 | 1 | 8 |
| Harris, 201828 | 1 | 1 | 1 | 1 | 2 | 1 | 1 | 1 | 9 |
| Venø, 201946 | 1 | 1 | 1 | 1 | 2 | 1 | 1 | 0 | 8 |
| **Colorectal cancer** |  |  |  |  |  |  |  |  |  |
| Hodge, 201448 | 0 | 1 | 1 | 1 | 2 | 1 | 1 | 1 | 8 |
| **Prostate cancer** |  |  |  |  |  |  |  |  |  |
| Brasky, 201366 | 0 | 1 | 1 | 1 | 0 | 1 | 1 | 1 | 6 |
| Bassett, 201367 | 0 | 1 | 1 | 1 | 2 | 1 | 1 | 1 | 8 |
| **Breast cancer** |  |  |  |  |  |  |  |  | 0 |
| Chajes, 199950 | 1 | 1 | 1 | 1 | 0 | 1 | 1 | 1 | 7 |
| Chajes, 200852 | 0 | 1 | 1 | 1 | 2 | 1 | 1 | 1 | 8 |
| Witt, 200954 | 0 | 1 | 1 | 1 | 1 | 1 | 1 | 1 | 7 |
| Bassett, 201656 | 0 | 1 | 1 | 1 | 2 | 1 | 1 | 1 | 8 |
| **All-cause mortality** |  |  |  |  |  |  |  |  |  |
| Laaksonen, 200518 | 1 | 1 | 1 | 1 | 2 | 1 | 1 | 1 | 9 |
| Warensjö, 200819 | 1 | 1 | 1 | 1 | 2 | 1 | 1 | 1 | 9 |
| Chien, 201322 | 0 | 1 | 1 | 0 | 2 | 1 | 1 | 0 | 6 |
| Mozaffarian, 201323 | 0 | 1 | 1 | 1 | 2 | 1 | 1 | 1 | 8 |
| Fretts, 201425 | 0 | 1 | 1 | 1 | 2 | 1 | 1 | 1 | 8 |
| Marklund, 201526 | 1 | 1 | 1 | 1 | 0 | 1 | 1 | 0 | 6 |
| Miura, 201668 | 1 | 1 | 1 | 1 | 2 | 1 | 1 | 1 | 9 |
| Harris, 201727 | 0 | 1 | 1 | 1 | 2 | 1 | 1 | 0 | 7 |
| Harris, 201828 | 1 | 1 | 1 | 1 | 2 | 1 | 1 | 1 | 9 |

T2D, type 2 diabetes; CHD, coronary heart disease; CVD, cardiovascular disease.

# Appendix S11. 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 |
| **T2D** |  |  |  |  |  |  |  |  |  |
| Akter, 201715 | 1 | 1 | 1 | 1 | 2 | 1 | 1 | 1 | 9 |
| **CVD** |  |  |  |  |  |  |  |  |  |
| Albert, 200217 | 0 | 1 | 1 | 1 | 0 | 1 | 1 | 1 | 6 |
| **CHD** |  |  |  |  |  |  |  |  |  |
| Simon, 199529 | 0 | 1 | 1 | 0 | 0 | 1 | 1 | 1 | 5 |
| Lemaitre, 200330 | 0 | 1 | 1 | 1 | 2 | 1 | 1 | 1 | 8 |
| Sun, 200832 | 0 | 1 | 1 | 1 | 2 | 1 | 1 | 1 | 8 |
| Khaw, 201234 | 1 | 1 | 1 | 1 | 2 | 1 | 1 | 1 | 9 |
| Matsumoto, 201335 | 0 | 1 | 1 | 1 | 2 | 1 | 1 | 1 | 8 |
| Sun, 201636 | 0 | 1 | 1 | 1 | 0 | 1 | 1 | 1 | 6 |
| Hamazaki, 201737 | 0 | 1 | 1 | 1 | 2 | 1 | 1 | 1 | 8 |
| Chei, 201838 | 0 | 1 | 1 | 1 | 2 | 1 | 1 | 1 | 8 |
| **Stroke** |  |  |  |  |  |  |  |  |  |
| De Goede, 201340 | 0 | 1 | 1 | 1 | 2 | 1 | 1 | 1 | 8 |
| Saber, 2017(CHS)44 | 0 | 1 | 1 | 1 | 2 | 1 | 1 | 1 | 8 |
| Saber, 2017(HPFS) 44 | 0 | 1 | 1 | 1 | 2 | 1 | 1 | 1 | 8 |
| Saber, 2017(FHS) 44 | 0 | 1 | 1 | 1 | 2 | 1 | 1 | 1 | 8 |
| **Colorectal cancer** |  |  |  |  |  |  |  |  |  |
| Hall, 200747 | 0 | 1 | 1 | 1 | 2 | 1 | 1 | 1 | 8 |
| Butler, 201749 | 0 | 1 | 1 | 1 | 2 | 1 | 1 | 1 | 8 |
| **Prostate cancer** |  |  |  |  |  |  |  |  |  |
| Gann, 199459 | 0 | 1 | 1 | 1 | 0 | 1 | 1 | 1 | 6 |
| Harvei, 199760 | 0 | 1 | 1 | 1 | 1 | 1 | 1 | 1 | 7 |
| Mannisto, 200361 | 0 | 1 | 1 | 1 | 2 | 1 | 1 | 1 | 8 |
| Chavarro, 200762 | 0 | 1 | 1 | 1 | 2 | 1 | 1 | 1 | 8 |
| Park, 200864 | 0 | 1 | 1 | 1 | 2 | 1 | 1 | 1 | 8 |
| Crowe, 200863 | 0 | 1 | 1 | 1 | 2 | 1 | 1 | 1 | 8 |
| Brasky, 201165 | 0 | 1 | 1 | 1 | 2 | 1 | 1 | 1 | 8 |
| **Breast cancer** |  |  |  |  |  |  |  |  |  |
| Saadatian-Elahi, 200251 | 0 | 1 | 1 | 1 | 2 | 1 | 1 | 1 | 8 |
| Takata, 200953 | 0 | 1 | 1 | 1 | 2 | 1 | 1 | 1 | 8 |
| Pouchieu, 201455 | 0 | 1 | 1 | 1 | 2 | 1 | 1 | 1 | 8 |
| Chajes, 201757 | 0 | 1 | 1 | 1 | 2 | 1 | 1 | 1 | 8 |
| Hirko, 201858 | 0 | 1 | 1 | 1 | 2 | 1 | 1 | 1 | 8 |

CHS, Cardiovascular Health Study; CHD, coronary heart disease; CVD, cardiovascular disease; FHS, Framingham Heart Study; HPFS, Health Professionals Follow-Up Study; T2D, type 2 diabetes.

# Appendix S12. Subgroup analyses of fatty acid biomarkers and type 2 diabetes.

|  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |
| --- | --- | --- | --- | --- | --- | --- | --- | --- | --- | --- | --- | --- | --- | --- | --- |
|  | **EPA** | | | | | **DPA** | | | | | **DHA** | | | | |
|  | n | RR (95% CI) | *I2* | *Ph* a | *Phb* | n | RR (95% CI) | *I2* | *Pha* | *Phb* | n | RR (95% CI) | *I2* | *Pha* | *Phb* |
| All studies | 9 | 0.87 (0.75, 1.00) | 77.7 | <0.001 |  | 9 | 0.87 (0.76, 1.00) | 69.9 | 0.001 |  | 9 | 0.92 (0.78, 1.10) | 69.0 | 0.005 |  |
| Study type |  |  |  |  | 0.22 |  |  |  |  | 0.02 |  |  |  |  | 0.97 |
| PC | 4 | 0.70 (0.55, 0.89) | 72.0 | 0.01 |  | 4 | 0.72 (0.63, 0.83) | 9.9 | 0.34 |  | 4 | 0.85 (0.60, 1.20) | 82.9 | 0.001 |  |
| CCD | 2 | 1.09 (0.91, 1.31) | 61.5 | 0.11 |  | 2 | 0.97 (0.95, 1.00) | 0.0 | 0.35 |  | 2 | 1.05 (0.69, 1.60) | 81.4 | 0.03 |  |
| NCCD | 2 | 0.92 (0.77, 1.09) | 0.0 | 0.44 |  | 2 | 0.89 (0.70, 1.15) | 0.0 | 0.71 |  | 2 | 1.07 (0.83, 1.36) | 0.0 | 0.94 |  |
| NCCS | 1 | 1.00 (0.82, 1.22) |  |  |  | 1 | 1.30 (0.99, 1.71) |  |  |  | 1 | 0.79 (0.60, 1.05) |  |  |  |
| Gender |  |  |  |  | 0.77 |  |  |  |  | 0.34 |  |  |  |  | 0.40 |
| Males | 1 | 0.77 (0.63, 0.95) |  |  |  | 1 | 0.75 (0.60, 0.94) |  |  |  | 1 | 0.70 (0.56, 0.88) |  |  |  |
| Women | 1 | 0.89 (0.74, 1.07) |  |  |  | 1 | 0.79 (0.64, 0.97) |  |  |  | 1 | 0.98 (0.80, 1.20) |  |  |  |
| Men and women | 7 | 0.87 (0.73, 1.04) | 80.1 | <0.001 |  | 7 | 0.91 (0.76, 1.08) | 67.1 | 0.006 |  | 7 | 0.96 (0.77, 1.20) | 70.2 | 0.003 |  |
| Geographic location |  |  |  |  | 0.28 |  |  |  |  | 0.24 |  |  |  |  | 0.11 |
| Europe | 5 | 0.80 (0.64, 1.00) | 83.7 | 0.05 |  | 5 | 0.81 (0.66, 1.00) | 70.5 | 0.09 | 0.11 | 5 | 0.81 (0.65, 1.01) | 64.2 | 0.03 |  |
| USA | 1 | 0.89 (0.74, 1.07) |  |  |  | 1 | 0.79 (0.64, 0.97) |  |  |  | 1 | 0.79 (0.60, 1.05) |  |  |  |
| Asia | 2 | 0.81 (0.51, 1.29) | 80.9 | 0.02 |  | 2 | 0.94 (0.49, 1.80) | 87.9 | 0.004 |  | 2 | 1.05 (0.59, 1.88) | 84.2 | 0.01 |  |
| Australia | 1 | 1.24 (0.99, 1.56) |  |  |  | 1 | 1.12 (0.83, 1.52) |  |  |  | 1 | 1.34 (0.97, 1.86) |  |  |  |
| Duration of follow-up |  |  |  |  | 0.08 |  |  |  |  | 0.25 |  |  |  |  | 0.40 |
| < 10 years | 5 | 1.10 (0.87, 1.38) | 54.0 | 0.07 |  | 5 | 0.97 (0.76, 1.23) | 59.0 | 0.45 |  | 5 | 1.03 (0.81, 1.32) | 49.9 | 0.11 |  |
| ≥ 10 years | 4 | 0.79 (0.64, 0.97) | 69.7 | 0.02 |  | 4 | 0.78 (0.63, 0.98) | 82.0 | 0.001 |  | 4 | 0.86 (0.68, 1.09) | 77.2 | 0.002 |  |
| Number of cases |  |  |  |  | 0.06 |  |  |  |  | 0.25 |  |  |  |  | 0.83 |
| < 300 | 3 | 0.58 (0.47, 0.73) | 4.3 | 0.35 |  | 3 | 0.63 (0.49, 0.81) | 0.0 | 0.52 |  | 3 | 0.91 (0.45, 1.83) | 83.8 | 0.002 |  |
| 300-500 | 3 | 0.98 (0.75, 1.28) | 78.8 | 0.009 |  | 3 | 1.02 (0.72, 1.44) | 79.9 | 0.007 |  | 3 | 0.89 (0.62, 1.28) | 80.9 | 0.005 |  |
| ≥ 500 | 3 | 0.99 (0.92, 1.06) | 11.7 | 0.32 |  | 3 | 0.91 (0.80, 1.04) | 49.7 | 0.14 |  | 3 | 0.94 (0.84, 1.05) | 0.0 | 0.37 |  |
| Assessment method |  |  |  |  | 0.75 |  |  |  |  | 0.84 |  |  |  |  | 0.81 |
| GC | 8 | 0.87 (0.75, 1.01) | 80.0 | <0.001 |  | 8 | 0.87 (0.76, 1.01) | 73.3 | 0.001 |  | 8 | 0.92 (0.77, 1.01) | 73.2 | <0.001 |  |
| GLC | 1 | 0.76 (0.46, 1.25) |  |  |  | 1 | 0.79 (0.40, 1.57) |  |  |  | 1 | 1.04 (0.52, 2.08) |  |  |  |
| Biomarkers type |  |  |  |  | 0.10 |  |  |  |  | 0.41 |  |  |  |  | 0.08 |
| Total plasma | 2 | 1.09 (0.91, 1.31) | 61.5 | 0.11 |  | 2 | 0.97 (0.95, 1.00) | 0.0 | 0.35 |  | 2 | 1.05 (0.69, 1.60) | 81.4 | 0.02 |  |
| Serum | 3 | 0.74 (0.52, 1.05) | 85.0 | 0.001 |  | 3 | 0.81 (0.50, 1.31) | 87.0 | 0.008 |  | 3 | 0.69 (0.56, 0.85) | 28.3 | 0.25 |  |
| Phospholipids | 4 | 0.85 (0.72, 0.99) | 32.5 | 0.22 |  | 4 | 0.80 (0.69, 0.93) | 0.0 | 0.60 |  | 4 | 1.07 (0.92, 1,24) | 4.2 | 0.37 |  |

CCD, case-cohort design study; CI, confidence interval; DPA, docosapentaenoic acid; DHA, docosahexaenoic acid; EPA, eicosapentaenoic acid; GC, gas chromatography; GLC, gas-liquid chromatography; NCCS, nested case-control study; NCCD, nested case-cohort design study; PC, prospective cohort study; RR, relative risk; USA, the United States of America.

a P for heterogeneity within each subgroup.

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

# eTable 12. Subgroup analyses of fatty acid biomarkers and cardiovascular disease and coronary heart disease.

|  |  |  |  |  |  |  |  |  |  |  |
| --- | --- | --- | --- | --- | --- | --- | --- | --- | --- | --- |
| **DHA** | **CVD** | | | | | **CHD** | | | | |
| n | RR (95% CI) | *I2* | *Pha* | *Phb* | n | RR (95% CI) | *I2* | *Pha* | *Phb* |
| All studies | 9 | 0.75 (0.65, 0.87) | 59.4 | 0.01 |  | 10 | 0.70 (0.58, 0.84) | 54.1 | 0.02 |  |
| Study type |  |  |  |  | NC |  |  |  |  | 0.50 |
| PC | 9 | 0.75 (0.65, 0.87) | 59.4 | 0.01 |  | 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) |  |  |  |
| Gender |  |  |  |  | 0.83 |  |  |  |  | 0.40 |
| Men | 2 | 0.71 (0.46, 1.09) | 58.6 | 0.12 |  | 2 | 0.63 (0.22, 1.79) | 79.4 | 0.03 |  |
| Women | 1 | 1.00 (0.74, 1.35) |  |  |  | 1 | 0.69 (0.32, 1.48) |  |  |  |
| Men and women | 6 | 0.72 (0.60, 0.87) | 62.9 | 0.02 |  | 7 | 0.68 (0.57, 0.82) | 38.9 | 0.13 |  |
| Geographic location |  |  |  |  | 0.29 |  |  |  |  | 0.73 |
| Europe | 4 | 0.73 (0.62, 0.86) | 45.0 | 0.14 |  | 2 | 0.76 (0.53, 1.09) | 67.9 | 0.08 |  |
| USA | 4 | 0.70 (0.53, 0.93) | 65.6 | 0.03 |  | 5 | 0.61 (0.41, 0.91) | 74.3 | 0.004 |  |
| Asia | 1 | 1.10 (0.80, 1.51) |  |  |  | 3 | 0.69 (0.53, 0.90) | 0.0 | 0.84 |  |
| Duration of follow-up |  |  |  |  | 0.83 |  |  |  |  | 0.79 |
| < 10 years | 6 | 0.76 (0.66, 0.87) | 45.2 | 0.10 |  | 4 | 0.70 (0.48, 1.03) | 67.3 | 0.03 |  |
| ≥ 10 years | 3 | 0.70 (0.42, 1.17) | 81.1 | 0.005 |  | 6 | 0.69 (0.55, 0.86) | 46.1 | 0.10 |  |
| Number of cases |  |  |  |  | 0.11 |  |  |  |  | 0.18 |
| < 300 | 3 | 0.55 (0.43, 0.72) | 0.0 | 0.48 |  | 5 | 0.57 (0.40, 0.81) | 35.4 | 0.19 |  |
| 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 |  | 5 | 0.77 (0.63, 0.94) | 57.3 | 0.05 |  |
| Assessment method |  |  |  |  |  |  |  |  |  |  |
| GC | 9 | 0.75 (0.65, 0.87) | 59.4 | 0.01 | NC | 5 | 0.67 (0.55, 0.81) | 0.0 | 0.97 |  |
| GLC |  |  |  |  |  |  |  |  |  |  |
| Biomarkers type |  |  |  |  | 0.44 |  |  |  |  | 0.19 |
| Plasma | 3 | 0.73 (0.48, 1.12) | 80.3 | <0.001 |  | 6 | 0.68 (0.54, 0.86) | 45.9 | 0.10 |  |
| Serum | 3 | 0.69 (0.55, 0.87) | 53.1 | 0.12 |  | 2 | 0.55 (0.25, 1.20) | 55.6 | 0.13 |  |
| Phospholipids | 2 | 0.82 (0.54, 1.25) | 67.4 | 0.08 |  | 1 | 0.98 (0.79, 1.22) |  |  |  |
| Adipose tissue | 1 | 0.81 (0.66, 0.99) |  |  |  | 1 | 0.63 (0.46, 0.86) |  |  |  |
| Study quality |  |  |  |  | 0.62 |  |  |  |  | 0.33 |
| Moderate | 2 | 0.83 (0.49, 1.41) | 87.4 | 0.005 |  | 2 | 0.53 (0.30, 0.95) | 39.0 | 0.20 |  |
| High | 7 | 0.74 (0.63, 0.87) | 48.8 | 0.07 |  | 8 | 0.73 (0.60, 0.89) | 55.1 | 0.03 |  |

CI, confidence interval; DHA, docosahexaenoic acid; GC, gas chromatography; GLC, gas-liquid chromatography; NCCS, nested case-control study;

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

a P for heterogeneity within each subgroup.

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

# Appendix S14. Subgroup analyses of fatty acid biomarkers and stroke.

|  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |
| --- | --- | --- | --- | --- | --- | --- | --- | --- | --- | --- | --- | --- | --- | --- | --- |
|  | **EPA** | | |  |  | **DPA** | | | | | **DHA** | | | | |
|  | n | RR (95% CI) | *I2* | *Pha* | *Phb* | n | RR (95% CI) | *I2* | *Pha* | *Phb* | n | RR (95% CI) | *I2* | *Pha* | *Phb* |
| All studies | 9 | 0.95 (0.82, 1.11) | 54.6 | 0.02 |  | 7 | 0.96 (0.79, 1.16) | 73.0 | 0.001 |  | 9 | 0.84 (0.72, 0.99) | 53.2 | 0.03 |  |
| Study type |  |  |  |  | 0.93 |  |  |  |  | 0.17 |  |  |  |  | 0.91 |
| PC | 6 | 0.95 (0.80, 1.12) | 50.3 | 0.70 |  | 4 | 1.06 (0.82, 1.36) | 83.0 | 0.001 |  | 6 | 0.85 (0.70, 1.03) | 59.1 | 0.03 |  |
| NCCS | 3 | 0.99 (0.59, 1.67) | 72.2 | 0.03 |  | 3 | 0.77 (0.63, 0.94) | 0.0 | 0.85 |  | 3 | 0.83 (0.55, 1.25) | 53.4 | 0.12 |  |
| Gender |  |  |  |  | 0.39 |  |  |  |  | 0.70 |  |  |  |  | 0.61 |
| Men | 3 | 1.16 (0.89, 1.50) | 24.5 | 0.27 |  | 2 | 0.97 (0.70, 1.35) | 0.0 | 0.68 |  | 3 | 0.94 (0.81, 1.08) | 0.0 | 0.46 |  |
| Women | 2 | 0.80 (0.61, 1.04) | 31.7 | 0.22 |  | 2 | 0.89 (0.82, 0.96) | 0.0 | 0.97 |  | 2 | 0.59 (0.43, 0.80) | 0.0 | 0.92 |  |
| Men and women | 4 | 0.94 (0.76, 1.16) | 58.5 | 0.07 |  | 3 | 1.02 (0.66, 1.58) | 87.3 | <0.001 |  | 4 | 0.85 (0.59, 1.21) | 62.4 | 0.05 |  |
| Geographic location |  |  |  |  | 0.96 |  |  |  |  | 0.67 |  |  |  |  | 0.03 |
| Europe | 3 | 0.96 (0.73, 1.26) | 75.0 | 0.02 |  | 2 | 1.19 (0.92, 1.53) | 48.8 | 0.16 |  | 3 | 1.01 (0,89, 1.14) | 0.0 | 0.99 |  |
| USA | 6 | 0.96 (0.78, 1.17) | 44.1 | 0.11 |  | 5 | 0.88 (0.81, 0.94) | 0.0 | 0.60 |  | 6 | 0.71 (0.57, 0.89) | 40.1 | 0.14 |  |
| Duration of follow-up |  |  |  |  | 0.60 |  |  |  |  | 0.94 |  |  |  |  | 0.26 |
| < 10 years | 3 | 1.01 (0.51, 1.98) | 65.6 | 0.06 |  | 3 | 0.95 (0.65, 1.37) | 0.0 | 0.78 |  | 3 | 0.70 (0.51, 0.97) | 44.6 | 0.17 |  |
| ≥ 10 years | 6 | 0.96 (0.83, 1.12) | 55.6 | 0.05 |  | 4 | 0.96 (0.76, 1.22) | 86.2 | <0.001 |  | 6 | 0.91 (0.75, 1.09) | 52.7 | 0.06 |  |
| Number of cases |  |  |  |  | 0.15 |  |  |  |  | 0.90 |  |  |  |  | 0.71 |
| < 300 | 4 | 1.16 (0.92, 1.46) | 0.0 | 0.42 |  | 3 | 1.00 (0.75, 1.35) | 0.0 | 0.84 |  | 4 | 0.82 (0.67, 1.00) | 28.3 | 0.24 |  |
| 300-500 | 2 | 0.87 (0.52, 1.43) | 77.8 | 0.03 |  | 1 | 0.88 (0.53, 1.45) |  |  |  | 2 | 0.82 (0.49, 1.36) | 74.1 | 0.05 |  |
| ≥ 500 | 3 | 0.87 (0.73, 1.04) | 57.6 | 0.09 |  | 3 | 0.96 (0.73, 1.27) | 90.8 | <0.001 |  | 3 | 0.90 (0.55, 1.48) | 74.9 | 0.02 |  |
| Assessment method |  |  |  |  | 0.45 |  |  |  |  | NC |  |  |  |  | 0.11 |
| GC | 8 | 0.93 (0.80, 1.09) | 55.9 | 0.03 |  | 9 | 0.84 (0.72, 0.99) | 53.2 | 0.03 |  | 8 | 0.88 (0.76, 1.03) | 44.9 | 0.08 |  |
| GLC | 1 | 1.15 (0.81, 1.64) |  |  |  | - |  |  |  |  | 1 | 0.58 (0.39, 0.87) |  |  |  |
| Biomarkers type |  |  |  |  | 0.17 |  |  |  |  | 0.14 |  |  |  |  | 0.87 |
| Phospholipids | 1 | 0.96 (0.52, 1.78) |  |  |  | 1 | 1.13 (0.60, 2.13) |  |  |  | 1 | 0.50 (0.26, 0.96) |  |  |  |
| Plasma | 4 | 1.01 (0.72, 1.40) | 62.4 | 0.05 |  | 3 | 0.77 (0.63, 0.94) | 0.0 | 0.85 |  | 4 | 0.79 (0.62, 1.01) | 35.4 | 0.20 |  |
| Serum | 3 | 1.00 (0.84, 1.18) | 20.8 | 0.28 |  | 2 | 0.89 (0.83, 0.96) | 0.0 | 0.56 |  | 3 | 0.87 (0.63, 1.20) | 67.5 | 0.05 |  |
| Adipose tissue | 1 | 0.77 (0.66, 0.90) |  |  |  | 1 | 1.30 (1.11, 1.53) |  |  |  | 1 | 1.00 (0.85, 1.17) |  |  |  |

CI, confidence interval; DPA, docosapentaenoic acid; DHA, docosahexaenoic acid; EPA, eicosapentaenoic acid; GC, gas chromatography; GLC, gas-liquid chromatography; NCCS, nested case-control study; PC, prospective cohort study; RR, relative risk; USA, the United States of America.

a P for heterogeneity within each subgroup.

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

# Appendix S15. Subgroup analyses of fatty acid biomarkers and prostate cancer.

|  |  |  |  |  |  |  |  |  |  |  |
| --- | --- | --- | --- | --- | --- | --- | --- | --- | --- | --- |
|  | **DPA** | | |  |  | **DHA** | | |  |  |
|  | n | RR (95% CI) | *I2* | *Pha* | *Phb* | n | RR (95% CI) | *I2* | *Pha* | *Phb* |
| All studies | 6 | 0.92 (0.75, 1.15) | 58.4 | 0.03 |  | 8 | 1.05 (0.89, 1.24) | 61.5 | 0.01 |  |
| Study type |  |  |  |  | 0.16 |  |  |  |  | 0.67 |
| CCD | 2 | 1.12 (0.79, 1.57) | 71.2 | 0.06 |  | 2 | 1.11 (0.78, 1.60) | 75.3 | 0.04 |  |
| NCCS | 4 | 0.83 (0.69, 1.00) | 0.0 | 0.44 |  | 6 | 1.01 (0.82, 1.26) | 64.3 | 0.02 |  |
| Geographic location |  |  |  |  | 0.95 |  |  |  |  | 0.46 |
| Europe | 2 | 0.92 (0.72, 1.18) | 0.0 | 0.46 |  | 2 | 1.24 (0.99, 1.55) | 0.0 | 0.41 |  |
| USA | 3 | 0.91 (0.57,1.47) | 81.5 | 0.004 |  | 5 | 1.02 (0.80, 1.30) | 72.9 | 0.005 |  |
| Australia | 1 | 0.93 (0.70, 1.23) |  |  |  | 1 | 0.92 (0.70, 1.21) |  |  |  |
| Duration of follow-up/years |  |  |  |  | 0.13 |  |  |  |  | 0.08 |
| < 10 | 2 | 0.69 (0.50, 0.93) | 0.0 | 0.79 |  | 6 | 1.14 (0.99, 1.32) | 40.0 | 0.14 |  |
| ≥ 10 | 4 | 1.03 (0.84, 1.27) | 47.8 | 0.13 |  | 2 | 0.77 (0.53, 1.12) | 33.2 | 0.22 |  |
| Number of cases |  |  |  |  | 0.12 |  |  |  |  | 0.02 |
| < 300 | 1 | 0.74 (0.39, 1.39) |  |  |  | 2 | 0.85 (0.59, 1.23) | 0.0 | 0.44 |  |
| 300-500 | 3 | 0.82 (0.67, 1.00) | 1.6 | 0.36 |  | 3 | 0.87 (0.67, 1.14) | 49.5 | 0.14 |  |
| ≥ 500 | 2 | 1.13 (0.83, 1.55) | 67.3 | 0.08 |  | 3 | 1.26 (1.12, 1.42) | 0.0 | 0.82 |  |
| Assessment method |  |  |  |  | 0.20 |  |  |  |  | 0.03 |
| GC | 3 | 1.06 (0.80, 1.40) | 57.4 | 0.10 |  | 5 | 1.21 (1.03, 1.37) | 18.7 | 0.30 |  |
| GLC | 3 | 0.81 (0.65, 1.00) | 5.6 | 0.35 |  | 3 | 0.83 (0.66, 1.05) | 21.5 | 0.28 |  |
| Biomarkers type |  |  |  |  | 0.97 |  |  |  |  | 0.32 |
| Whole blood | 1 | 0.67 (0.47, 0.95) |  |  |  | 1 | 0.67 (0.48, 0.94) |  |  |  |
| Plasma | 3 | 1.07 (0.85, 1.34) | 56.4 | 0.10 |  | 3 | 1.18 (0.94, 1.47) | 57.4 | 0.01 |  |
| Serum | 1 | 0.74 (0.39, 0.95) |  |  |  | 3 | 1.04 (0.78, 1.40) | 46.1 | 0.16 |  |
| Phospholipids | 1 | 0.81 (0.49, 1.34) |  |  |  | 1 | 1.09 (0.76, 1.57) |  |  |  |

CCD, case-cohort design study; CI, confidence interval; DPA, docosapentaenoic acid; DHA, docosahexaenoic acid; GC, gas chromatography;

GLC, gas-liquid chromatography; NCCS, nested case-control study; RR, relative risk; USA, the United States of America.

a P for heterogeneity within each subgroup.

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

**Appendix S16. Subgroup analyses of eicosapentaenoic acid and all-cause mortality.**

|  |  |  |  |  |  |
| --- | --- | --- | --- | --- | --- |
|  | n | RR (95% CI) | *I2* | *Pha* | *Phb* |
| All studies | 7 | 0.80 (0.71, 0.90) | 58.9 | 0.02 |  |
| Gender |  |  |  |  | 0.91 |
| Women | 1 | 0.78 (0.66, 0.93) |  |  |  |
| Men and women | 6 | 0.80 (0.66, 0.93) | 64.3 | 0.02 |  |
| Geographic location |  |  |  |  | 0.79 |
| Europe | 3 | 0.74 (0.51, 1.07) | 85.1 | 0.001 |  |
| USA | 3 | 0.83 (0.75, 0.91) | 0.0 | 0.64 |  |
| Asia | 1 | 0.80 (0.64, 1.00) |  |  |  |
| Australia | - |  |  |  |  |
| Duration of follow-up |  |  |  |  | >0.99 |
| < 10 years | 2 | 0.80 (0.67, 0.95) | 0.0 | 0.95 |  |
| ≥ 10 years | 5 | 0.79 (0.68, 0.93) | 72.1 | 0.006 |  |
| Number of cases |  |  |  |  | 0.17 |
| < 300 | 2 | 0.72 (0.55, 0.95) | 38.5 | 0.20 |  |
| 300-500 | 2 | 0.70 (0.56, 0.87) | 31.0 | 0.23 |  |
| ≥ 500 | 3 | 0.88 (0.77, 1.00) | 58.2 | 0.09 |  |
| Biomarkers type |  |  |  |  | 0.20 |
| Phospholipids | 4 | 0.78 (0.69, 0.88) | 39.7 | 0.17 |  |
| Total plasma | 2 | 0.72 (0.55, 0.95) | 38.5 | 0.20 |  |
| Cholesterol esters | 1 | 1.00 (0.86, 1.16) |  |  |  |
| Study quality |  |  |  |  | 0.38 |
| Moderate | 2 | 0.71 (0.57, 0.90) | 49.9 | 0.16 |  |
| High | 5 | 0.84 (0.74, 0.95) | 55.0 | 0.06 |  |

CI, confidence interval; EPA, eicosapentaenoic acid; RR, relative risk; USA, the United States of America.

a P for heterogeneity within each subgroup.

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

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