# Omega-3 Fatty Acid Biomarkers and Incident Atrial Fibrillation

**Brief Title**: Omega-3 Fatty Acids and Atrial Fibrillation

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**Tweet**: New study with 54,799 adults finds higher blood levels of omega-3 fatty acids are not linked with increased risk for atrial fibrillation.

**Abstract**

**Background:** The relationship between omega-3 fatty acids and atrial fibrillation (AF) remains controversial.

**Objective:** We aimed to determine the prospective associations of blood or adipose tissue levels of eicosapentaenoic, docosapentaenoic, and docosahexaenoic acids (EPA, DPA, and DHA, respectively) with incident AF.

**Methods:** We used participant-level data from a global consortium of 17 prospective cohort studies, each with baseline data on blood or adipose tissue omega-3 fatty acid levels and AF outcomes. Each participating study conducted a *de novo* analyses using a prespecified analytical plan with harmonized definitions for exposures, outcome, covariates, and subgroups. Associations were pooled using inverse-variance weighted meta-analysis.

**Results:** Among 54,799 participants from 17 cohorts, 7,720 incident cases of AF were ascertained after a median 13.3 years of follow-up. In multivariable analysis, EPA levels were not associated with incident AF, hazard ratio (HR) and 95% confidence intervals (CI) per interquintile range (i.e., the difference between the $90^{th}$ and $10^{th}$ percentiles) was 1.00 (0.95, 1.05). HR (95%CI) for higher levels of DPA, DHA, and EPA+DHA, were 0.89 (0.83, 0.95), 0.90 (0.85, 0.96), and 0.93 (0.87, 0.99), respectively.

**Conclusions:** *In vivo* levels of omega-3 fatty acids including EPA, DPA, DHA, and EPA+DHA were not associated with increased risk of incident AF. Our data suggest the safety of habitual dietary intakes of omega-3 fatty acids with respect to AF risk. Coupled with the known benefits of these fatty acids in the prevention of adverse coronary events, our study suggests that current dietary guidelines recommending fish/omega-3 fatty acid consumption can be maintained.

**Condensed Abstract:** To address current controversies regarding omega-3 fatty acids and risk for atrial fibrillation (AF), we analyzed data from 17 cohorts including 54,799 adults using *de novo* analyses with harmonized definitions of outcomes and covariates. We found that the risk for developing AF was unrelated to eicosapentaenoic acid (EPA) levels and lower for individuals with higher docosahexaenoic (DHA) and docosapentaenoic acid (DPA) levels. Although high-dose omega-3 products may increase AF risk in populations at high cardiovascular risk, greater dietary intakes as reflected by circulating or adipose tissue fatty acid levels do not appear to be associated with increased risk.

**Key Words**: eicosapentaenoic acid, docosahexaenoic acid, docosapentaenoic acid, biomarkers, observational epidemiology

**Abbreviations**:

AF, atrial fibrillation

CI, confidence interval

CVD, cardiovascular disease

DHA, docosahexaenoic acid

DPA, docosapentaenoic acid

EPA, eicosapentaenoic acid

HR, Hazard Ratio

RCT, randomized controlled trial

## Introduction

Atrial fibrillation (AF) is the most common chronic cardiac arrhythmia, affecting approximately 9% of adults≥65 years. Long-chain omega-3 polyunsaturated fatty acids have been shown in experimental settings to have possible anti-arrhythmic properties.[1] Observational studies and randomized controlled trials (RCTs) have demonstrated that omega-3 fatty acids may reduce the risk of sudden cardiac death, with mechanisms related to prevention of acute, ischemia-induced ventricular arrythmias.[2-4] However, the potential effects of omega-3 fatty acids on other arrhythmias, particularly atrial fibrillation (AF), remain less well understood.

Prior RCTs have tested short-term supplemental doses of omega-3 fatty acids on the development of AF, predominantly among patients undergoing elective cardiac surgery. A meta-analysis showed that omega-3 fatty acid supplementation did not affect overall risk of post-surgical AF, with significant heterogeneity.[5] Recently, several RCTs conducted among individuals with pre-existing cardiovascular disease (CVD) or at high CV risk found that high-dose (up to 4 g/d) omega-3 fatty acids *increased* risk of AF hospitalization, though these were mostly secondary or exploratory outcomes.[3,6,7] On the other hand, the VITamin D and OmegA-3 TriaL (VITAL)-Rhythm,[8] an RCT testing 1 g/d of EPA+DHA among individuals without a history of CVD, did not demonstrate significant benefits or harms with omega-3 fatty acids on AF incidence.

Due to the relatively short duration of these RCTs (median duration ~5 years), frequent use of high-dose omega-3 preparations, and the enrichment for individuals with elevated CV risk, their generalizability to habitual dietary intakes of omega-3 fatty acid-rich foods among the general population is uncertain. Several prospective cohort studies assessing self-reported estimates of dietary fish or omega-3 fatty acid intake have shown beneficial or neutral

associations with AF.[9] However, self-reported estimates of omega-3 fatty acid intake have the limitations of measurement error as well as potential recall and memory biases. Moreover, most of these reports were in Western populations, and studies on the association between omega-3 fatty acids and AF risk outside of Europe and the US remain sparse.

Few studies have examined circulating or tissue omega-3 fatty acids, as objective measures of endogenous omega-3 status as well as a reliable biomarker of habitual dietary intake,[10] in relation to incident AF. To our knowledge, only five prospective cohort studies from the US and Europe have examined this question.[11-15] Interestingly, all five studies tended to show inverse associations between omega-3 fatty acids and AF, with DHA being most consistently associated with lower risk. Moreover, the widespread exposure to omega-3 fatty acids through intake of fish/seafood or fortified foods as well as supplements render the association between these fatty acids and incident AF an important clinical and public health question. Notably, as highlighted by recent RCTs, possible heterogeneity among high-risk vs. general populations may also exist for the effect of omega-3 fatty acid on AF, which has not been examined in prior studies. The limited statistical power in prior analyses to assess potential effect modification by baseline CV risk, as well as publication bias in both studies of fish intake and omega-3 fatty acid biomarkers with respect to AF risk, are indications that further studies are warranted.

In the context of these questions, we evaluated the relationship between circulating and tissue omega-3 fatty acids across 17 prospective international studies with data on incident AF.

## Methods

### *Study selection*

We utilized the same methodologic approach as prior studies from the Fatty Acids and Outcomes Research Consortium (FORCE), details of which has been previously published.[16,17]

In our study, 17 cohorts were included based on availability of ascertained AF, fatty acid exposures [one or more of eicosapentaenoic acid (EPA), docosapentaenoic acid (DPA), and docosahexaenoic acid (DHA)], and prospective design (cohort or nested case-cohort). Participants were included if they were age≥18 years and free of prevalent or a history of AF at the time of blood/adipose tissue collection. Participating studies received approval from their corresponding institutional review boards, and all participants gave informed consent. Cohort-specific inclusion/exclusion criteria at baseline enrollment are provided in the **Supplementary Methods**.

*Exposure assessment*

Each cohort measured fatty acids using gas chromatography in one or more of the following lipid compartments: erythrocyte or plasma/serum phospholipids, total plasma/serum, cholesterol esters, and adipose tissue. Fatty acids of interest were quantified as a percentage of the total measured fatty acids. Detailed information on the measurement protocol, specific fatty acids assessed, and coefficients of variation can be found in **Supplementary Methods**.

*Ascertainment of incident atrial fibrillation*

Incident AF was assessed using one or more of the following criteria: 1) characteristic findings on standard 12-lead ECG or event monitors, 2) hospital discharge or outpatient diagnostic codes (ICD-8: 427.4; ICD-9: 427.3; ICD-10: I48), or 3) medical record review. We did not include incident cases that were diagnosed using self-report only. Cohort-specific methods for AF ascertainment can be found in **Supplementary Table 1**.

*Statistical analyses*

Spearman's correlation coefficients were used to assess the correlations between individual omega-3 fatty acids. In each cohort, multivariable-adjusted Cox proportional hazards

models were used to calculate the hazard ratio (HR) and 95% confidence intervals (CI) per interquintile range (difference between the 90$^{th}$ and 10$^{th}$ percentile of each fatty acid). We also analyzed the associations by cohort-specific quintiles. Multivariable models adjusted for the following prespecified covariates: age (years), sex (male, female), field site (when applicable), race/ethnicity (when applicable), education (<high school, high school graduate, college or higher), physical activity, smoking (never, former, current), alcohol use (drinks/day), BMI (kg/m$^2$), beta blocker use (yes, no), biomarker levels of linoleic acid (LA; 18:2n-6) and arachidonic acid (AA; 20:4n-6) (both assessed as a % of total fatty acids), and prevalent hypertension (yes, no), dyslipidemia (yes, no), diabetes (yes, no), atherosclerotic CVD [coronary heart disease (CHD), stroke, or peripheral artery disease (PAD)] (yes, no), or heart failure (HF) (yes, no). In a secondary model, we additionally adjusted for intakes of fish/seafood, fruits, vegetables, and coffee/tea (servings/day or grams/day).

For the pooled analysis, we used inverse-variance weighted meta-analysis to calculate the HR and 95% CI, overall and within each lipid fraction. Since some cohorts measured omega-3 fatty acids in multiple lipid fractions, for the overall analysis we used the following order to select one lipid fraction that best reflects long-term dietary intake: adipose tissue>erythrocyte phospholipid>plasma/serum phospholipid>total plasma/serum>cholesterol ester. Heterogeneity was calculated using the $I^2$-statistic.

To test for possible nonlinear associations between each of the omega-3 fatty acids and AF, we conducted a pooled analysis of the cohort-specific quintiles. We additionally modeled the associations with meta-regression restricted cubic splines using the median % fatty acid in each cohort-specific quintile and the respective multivariable-adjusted HR and 95% CI. Due to variations in omega-3 fatty acid concentrations in each lipid compartment, this analysis was done

for each compartment separately. We used three knots placed at the $10^{th}$, $50^{th}$, and $90^{th}$ percentiles.

*Subgroup and sensitivity analyses*

To explore for heterogeneity and effect modification, we prespecified three subgroup analyses, each evaluated within each cohort and then pooled. These included by age (< or ≥65 years), by sex (male, female), and among individuals with elevated cardiovascular risk at baseline [defined as one or more of the following: established atherosclerotic CVD, heart failure, diabetes mellitus, baseline triglycerides≥150 mg/dL (≥1.70 mmol/L), or, if triglyceride data was unavailable, non-HDL cholesterol≥160 mg/dL (≥4.14 mmol/L)]. We used meta-regressions to assess the statistical significance of between-subgroup heterogeneity. Due to the exploratory nature of subgroup analyses, we used a Bonferroni-adjusted *P*-value of 0.004 (0.05 divided by 4 fatty acid exposures×3 subgroups) to denote statistically significant heterogeneity. We additionally conducted a post-hoc subgroup analysis by global region, for which we also used meta-regressions to assess for between-subgroup heterogeneity. Finally, we conducted a meta-regression analysis to assess for potential heterogeneity by duration of follow-up (above and below the median duration of follow-up).

**Results**

*Baseline characteristics*

The 17 cohort studies comprised 54,799 participants from 21 nations from North America, Europe, Asia, and Africa. The baseline characteristics for each study are shown in **Table 1**. Sixteen studies employed a prospective cohort design; and one (MERLIN TIMI-36), a prospective case-cohort design. Mean age of participants was 63 years, and approximately half were women. Mean BMI was in the overweight range (25.0-29.9 $kg/m^2$; except in the Hisayama

Study where mean BMI was 23.0), and each cohort included participants with a wide range of BMI values. Baseline CVD prevalence was generally<20%, except for 5 cohorts with baseline CVD prevalence>20%, and 3 cohorts with HF prevalence>20%. Baseline year of blood or adipose tissue collection ranged from the 1990s to early 2000s, with the exception of ULSAM, where blood collection was performed in the 1970s. Fatty acid distributions for each cohort are shown in **Supplementary Figures 1A-D**. There were moderate correlations between each of the individual omega-3 fatty acids as well as with EPA+DHA, in the 0.5 to 0.9 range (**Supplementary Table 2**).

*Association with incident atrial fibrillation*

During a median follow-up ranging from 0.9 to 29.1 years (weighted median: 13.3 years), 7,720 incident AF cases were ascertained (**Table 1**). In multivariable-adjusted analyses, EPA levels were not associated with incident AF: HR (95% CI) per interquintile range: 1.00 (0.95, 1.05), with moderate heterogeneity, $I^2$=52.2% (**Figure 1A**). In contrast, each of DPA, DHA, and EPA+DHA were associated with lower incidence of AF, with HRs (95% Cis) of 0.89 (0.83, 0.95), 0.90 (0.85, 0.96), and 0.93 (0.87, 0.99), respectively (**Figure 1B-D**). The pooled categorical analyses comparing extreme quintiles yielded similar associations (**Table 2, Supplementary Figures 2-5**). There was low heterogeneity for DPA ($I^2$=0%) and moderate heterogeneity for DHA ($I^2$=47.5%) and EPA+DHA ($I^2$=60.7%).

*Subgroup and sensitivity analyses*

We found little evidence that the associations significantly varied by age, sex, global region, or across the various lipid compartments, ($P_{het}$>0.05 for each fatty acid exposure) (**Table 3**). Moreover, the relationship between omega-3 fatty acids and AF did not significantly differ among individuals at higher CV risk (**Table 3**). In 13 of the 17 cohorts with dietary information,

additional adjustment for dietary intakes did not appreciably alter these associations (**Online Figure 6**). The pooled categorical analyses did not demonstrate apparent non-linear trends (**Supplementary Table 3**). Restricted cubic splines of the compartment-specific associations was suggestive of possible non-linear relationships between plasma phospholipid DPA, DHA, and EPA+DHA with incident AF, though the confidence limits did not exclude 1 (**Supplementary Figures 7 A-N**). No such plateauing was seen for erythrocyte phospholipid DPA, DHA, and EPA+DHA. We also did not find any evidence of heterogeneity by the duration of follow-up in meta-regression analyses ($P_{het}>0.05$ for each fatty acid exposure).

**Discussion**

In this global consortium of 17 cohort studies including 54,799 participants from 21 nations with 7,720 incident cases of AF, we observed that circulating and adipose tissue EPA was not associated with incident AF and higher levels of DPA, DHA, and EPA+DHA were each associated with a statistically significant lower risk of AF (**Central Illustration**). Associations were generally consistent by lipid compartments, demographic characteristics, and global region.

Our findings are consistent with, and greatly expand upon, the limited number of prior observational studies demonstrating an inverse association between omega-3 fatty acid biomarkers and incident AF.[12-15] However, our results are in contrast with those of a recent meta-analysis of intervention trials which found that treatment with omega-3 fatty acid products, particularly at high doses (1.8 to 4 g/day), was associated with increased risk of AF.[18] There are several potential reasons why the results of the present study may differ from recent omega-3 RCTs. First, the majority of the participants included in our study were community-dwelling individuals who were free of CVD or at relatively low CV risk. In contrast, except for VITAL-Rhythm,[8] the studies included in the aforementioned meta-analysis typically enrolled individuals

with baseline CVD or were at elevated CV risk. It is conceivable that the effects of omega-3 fatty acids on atrial arrhythmias may differ in those with existing cardiovascular disease versus without. To afford a more direct comparison to the populations in the typical trials represented in the meta-analysis, we prespecified a subgroup analysis to examine a "REDUCE-IT"-like cohort, with established CVD and/or elevated CV risk. In this subgroup, we observed a lack of association for EPA and inverse associations for DPA, DHA, and EPA+DHA with incident AF.

Secondly, the prevalence of omega-3 fatty acid supplement use in our cohorts was very low,[16,19] meaning that biomarker levels of these fatty acids largely reflect habitual dietary intake. Based on a global survey of seafood omega-3 intake by country,[20] the mean (SD) intake in the countries represented in our study was 0.43 (0.35) g/day, far less than the >1.8 g/d added to the background diets in the RCTs. RCTs of generally short-term, high-dose encapsulated omega-3 agents are unlikely to mimic the long-term impact of habitual dietary omega-3 fatty acid intake on AF risk. Moreover, the duration of follow-up in the majority of our cohorts was longer than that for most RCTs. In particular, as omega-3 fatty acids appear to exert influences on multiple upstream risk factors related to AF such as blood pressure,[3,21-23] type 2 diabetes,[16,24] systemic inflammation,[25] and chronic sympathetic activation,[26] the timeline to seeing a possible benefit with respect to AF may be longer than what can be feasibly achieved in an RCT, particularly coupled with the typical decline in adherence to study therapy over time.[27] More research is needed to confirm our findings and to explore potential mechanisms for which long-term dietary omega-3 fatty acid intake may relate to lower AF risk.

The modest protective association of omega-3 fatty acid levels with incident AF appeared to be more prominent in phospholipids, as compared with cholesterol esters or total plasma. The reason for this difference is unclear, though it might relate to the larger number of studies in our

consortium that assessed fatty acid in phospholipids, which would increase statistical power and precision. Future studies are warranted to assess how fatty acid levels in different lipid compartments may influence AF.

To our knowledge, our study is the largest investigation to date to examine the association between in vivo omega-3 fatty acid status and incident AF. Whether these associations represent a true independent effect or merely a reflection of the correlation with seafood intake and related lifestyle factors warrants further research. The difference in associations for EPA vs. DHA, both of which are correlated with seafood consumption; and the significantly lower risk for DPA, which is endogenously regulated and very weakly correlated with diet suggest that confounding by diet and lifestyle is unlikely to fully explain our observations. Our novel results highlight the need for additional studies to examine how varying intakes of dietary omega-3 fatty acids and omega-3 formulations (e.g., DHA-rich products) may affect intermediary risk factors, such as blood pressure, type 2 diabetes, and inflammation, as well as clinical AF.

The strengths our study include the harmonized exposure, outcome, and analysis plan which can reduce between-study heterogeneity. Nevertheless, moderate unexplained heterogeneity remained for EPA and DHA. By identifying and contacting the majority of cohorts with the necessary exposure and outcome information, and conducting *de novo* analyses in each of the cohorts, our study reduces the likelihood of publication bias and is thus more likely to present accurate and unbiased associations for omega-3 fatty acids and incident AF. We adjusted for a wide range of major AF risk factors in a harmonized fashion, reducing the potential for residual confounding. Heterogeneity was generally low to moderate in the overall pooled analyses. The large number of AF events allowed for greater statistical precision in the

estimation of the overall associations as well as in several subgroups, including among participants at elevated CV risk.

Potential limitations warrant mention. Due to the observational nature of our study, we cannot rule out residual or unmeasured confounding as possible explanations for the associations we observed despite extensive adjustments for known AF risk factors. Nevertheless, the robustness of our findings from pooling multiple cohorts from different populations with varying dietary backgrounds and baseline AF risk, as well as in sensitivity analyses, suggest that our findings are likely not merely due to uncontrolled confounding or chance. We did not prespecify within-cohort subgroup analyses by race/ethnicity or BMI, and thus potential effect modification by these factors was not explored. There was moderate heterogeneity for several of the fatty acid exposures, as evidenced by the $I^2$-statistic (ranging from 47.5% to 60.7%), for which we were not readily able to identify the source of through subgroup and meta-regression analyses. To partially account for this heterogeneity, we also conducted a random-effects meta-analysis for each fatty acid exposure and found similar risk estimates (data not shown). Individuals with paroxysmal AF may have only been partially captured. Fatty acids were only measured at one timepoint, and changes in dietary intakes, other lifestyle exposures, and health conditions over time may alter the endogenous levels of omega-3 fatty acids. Nevertheless, previous studies have shown reasonable reproducibility for omega-3 fatty acid levels over time, ranging from 0.59 to 0.80 for EPA, DPA, and DHA over 6 to 13 years of follow-up.[28] At the same time, since all of the included cohorts were conducted in a prospective fashion, changes in omega-3 fatty acid levels over time would likely be non-differential with respect to the outcome of interest, and would generally tend to bias associations toward the null.

In conclusion, in a global, harmonized, pooled analysis, higher circulating and tissue omega-3 fatty acid biomarkers were not associated with an increased incidence of AF. Our data indicates that high-dose omega-3 supplementation in populations with established or are at high-risk for CVD may not necessarily be generalizable to lower-dose habitual dietary omega-3 intakes. Coupled with the more consistent benefits of these fatty acids in the prevention of adverse coronary events, our study suggests that current dietary guidelines recommending fish/omega-3 fatty acid consumption should be maintained.

**Clinical Perspectives**

Competency in Patient Care and Procedural Skills: Dietary intake of long-chain omega-3 polyunsaturated fatty acids (PUFAs), reflected by circulating fatty acid biomarkers, does not increase the risk of atrial fibrillation (AF) in the general population.

Translational Outlook: Future clinical trials of omega-3 PUFA supplementation should include AF as an adjudicated outcome.

**References**

1. London B, Albert C, Anderson ME et al. Omega-3 fatty acids and cardiac arrhythmias: prior studies and recommendations for future research: a report from the National Heart, Lung, and Blood Institute and Office Of Dietary Supplements Omega-3 Fatty Acids and their Role in Cardiac Arrhythmogenesis Workshop. Circulation 2007;116:e320-35.
2. Dietary supplementation with n-3 polyunsaturated fatty acids and vitamin E after myocardial infarction: results of the GISSI-Prevenzione trial. Gruppo Italiano per lo Studio della Sopravvivenza nell'Infarto miocardico. Lancet 1999;354:447-55.
3. Bhatt DL, Steg PG, Miller M et al. Cardiovascular Risk Reduction with Icosapent Ethyl for Hypertriglyceridemia. N Engl J Med 2019;380:11-22.
4. Siscovick DS, Raghunathan TE, King I et al. Dietary intake and cell membrane levels of long-chain n-3 polyunsaturated fatty acids and the risk of primary cardiac arrest. JAMA 1995;274:1363-7.
5. Mariani J, Doval HC, Nul D et al. N-3 polyunsaturated fatty acids to prevent atrial fibrillation: updated systematic review and meta-analysis of randomized controlled trials. J Am Heart Assoc 2013;2:e005033.
6. Nicholls SJ, Lincoff AM, Garcia M et al. Effect of High-Dose Omega-3 Fatty Acids vs Corn Oil on Major Adverse Cardiovascular Events in Patients at High Cardiovascular Risk: The STRENGTH Randomized Clinical Trial. JAMA 2020;324:2268-2280.
7. Kalstad AA, Myhre PL, Laake K et al. Effects of n-3 Fatty Acid Supplements in Elderly Patients after Myocardial Infarction: A Randomized Controlled Trial. Circulation 2020.

8. Albert CM, Cook NR, Pester J et al. Effect of Marine Omega-3 Fatty Acid and Vitamin D Supplementation on Incident Atrial Fibrillation: A Randomized Clinical Trial. JAMA 2021;325:1061-1073.

9. Djousse L, Akinkuolie AO, Wu JH, Ding EL, Gaziano JM. Fish consumption, omega-3 fatty acids and risk of heart failure: a meta-analysis. Clin Nutr 2012;31:846-53.

10. Serra-Majem L, Nissensohn M, Overby NC, Fekete K. Dietary methods and biomarkers of omega 3 fatty acids: a systematic review. Br J Nutr 2012;107 Suppl 2:S64-76.

11. Wu JH, Lemaitre RN, King IB et al. Association of plasma phospholipid long-chain omega-3 fatty acids with incident atrial fibrillation in older adults: the cardiovascular health study. Circulation 2012;125:1084-93.

12. Virtanen JK, Mursu J, Voutilainen S, Tuomainen TP. Serum long-chain n-3 polyunsaturated fatty acids and risk of hospital diagnosis of atrial fibrillation in men. Circulation 2009;120:2315-21.

13. Gronroos NN, Chamberlain AM, Folsom AR et al. Fish, fish-derived n-3 fatty acids, and risk of incident atrial fibrillation in the Atherosclerosis Risk in Communities (ARIC) study. PLoS One 2012;7:e36686.

14. Rix TA, Joensen AM, Riahi S, Lundbye-Christensen S, Overvad K, Schmidt EB. Marine n-3 fatty acids in adipose tissue and development of atrial fibrillation: a Danish cohort study. Heart 2013;99:1519-24.

15. Rix TA, Dinesen P, Lundbye-Christensen S et al. Omega-3 fatty acids in adipose tissue and risk of atrial fibrillation. Eur J Clin Invest 2022;52:e13649.

16. Qian F, Ardisson Korat AV, Imamura F et al. n-3 Fatty Acid Biomarkers and Incident Type 2 Diabetes: An Individual Participant-Level Pooling Project of 20 Prospective Cohort Studies. Diabetes Care 2021;44:1133-1142.

17. Harris WS, Tintle NL, Imamura F et al. Blood n-3 fatty acid levels and total and cause-specific mortality from 17 prospective studies. Nat Commun 2021;12:2329.

18. Gencer B, Djousse L, Al-Ramady OT, Cook NR, Manson JE, Albert CM. Effect of Long-Term Marine Omega-3 Fatty Acids Supplementation on the Risk of Atrial Fibrillation in Randomized Controlled Trials of Cardiovascular Outcomes: A Systematic Review and Meta-Analysis. Circulation 2021.

19. Del Gobbo LC, Imamura F, Aslibekyan S et al. omega-3 Polyunsaturated Fatty Acid Biomarkers and Coronary Heart Disease: Pooling Project of 19 Cohort Studies. JAMA Intern Med 2016;176:1155-66.

20. Micha R, Khatibzadeh S, Shi P et al. Global, regional, and national consumption levels of dietary fats and oils in 1990 and 2010: a systematic analysis including 266 country-specific nutrition surveys. BMJ 2014;348:g2272.

21. Miller PE, Van Elswyk M, Alexander DD. Long-chain omega-3 fatty acids eicosapentaenoic acid and docosahexaenoic acid and blood pressure: a meta-analysis of randomized controlled trials. Am J Hypertens 2014;27:885-96.

22. Cabo J, Alonso R, Mata P. Omega-3 fatty acids and blood pressure. Br J Nutr 2012;107 Suppl 2:S195-200.

23. Emdin CA, Anderson SG, Salimi-Khorshidi G et al. Usual blood pressure, atrial fibrillation and vascular risk: evidence from 4.3 million adults. Int J Epidemiol 2017;46:162-172.

24. Aune D, Feng T, Schlesinger S, Janszky I, Norat T, Riboli E. Diabetes mellitus, blood glucose and the risk of atrial fibrillation: A systematic review and meta-analysis of cohort studies. J Diabetes Complications 2018;32:501-511.

25. Li K, Huang T, Zheng J, Wu K, Li D. Effect of marine-derived n-3 polyunsaturated fatty acids on C-reactive protein, interleukin 6 and tumor necrosis factor alpha: a meta-analysis. PLoS One 2014;9:e88103.

26. Gray B, Steyn F, Davies PS, Vitetta L. Omega-3 fatty acids: a review of the effects on adiponectin and leptin and potential implications for obesity management. Eur J Clin Nutr 2013;67:1234-42.

27. Bhatt DL, Steg PG, Miller M et al. Effects of Icosapent Ethyl on Total Ischemic Events: From REDUCE-IT. J Am Coll Cardiol 2019;73:2791-2802.

28. Lai HT, de Oliveira Otto MC, Lemaitre RN et al. Serial circulating omega 3 polyunsaturated fatty acids and healthy ageing among older adults in the Cardiovascular Health Study: prospective cohort study. BMJ 2018;363:k4067.

**Figure Legends**

**Figure 1. Pooled hazard ratio of incident atrial fibrillation (AF) for A) EPA, B) DPA, C) DHA, and D) EPA+DHA.** The association between marine omega-3 fatty acid levels and incident AF were assessed in multivariable models for each cohort, and the results were pooled using inverse-variance weighted fixed-effect meta-analysis. In each cohort, a multivariable model was used to assess the association with adjustment for age, sex, field site (if applicable), race/ethnicity, education, smoking, alcohol use, BMI, beta blocker use, linoleic acid (18:2n-6) level, arachidonic acid (20:4n-6) level, and prevalent hypertension, dyslipidemia, diabetes mellitus, atherosclerotic cardiovascular disease (defined as the presence of one or more of the following: coronary heart disease, stroke, or peripheral artery disease), and heart failure. If multiple biomarkers were available for a study, only one was used for the overall analysis based on the best ability to reflect long-term dietary intake (in the following order of preference): adipose tissue, erythrocyte phospholipids, plasma/serum phospholipids, total plasma/serum, and cholesterol ester. Please note that phospholipids include both erythrocyte and plasma/serum phospholipids. Abbreviations for individual cohorts are spelled out in the footnote for Table 1.

**Central Illustration: Omega-3 levels and Risk for Atrial Fibrillation.** Compared with individuals in the lowest quintile, those in the highest quintile of blood/tissue omega-3 levels were between 6% and 13% lower risk for developing atrial fibrillation. These multivariable-adjusted estimates were derived from a pooled study of 17 cohorts including 54,799 individuals followed for a median of 13.3 years and analyzed using pre-specified and harmonized outcome definitions, covariates, and statistical methods. EPA, eicosapentaenoic acid; DPA, docosapentaenoic acid; DHA, docosahexaenoic acid.

**Table 1.** Baseline characteristics of studies assessing omega-3 fatty acid biomarker levels and incident atrial fibrillation

| Study | Country | Study design* | Baseline year | Total N/ number of AF cases | Median follow-up, y | Age, mean (SD), y | Women, % | Mean BMI, kg/m$^2$ | Baseline ASCVD, %† | Baseline HF, % |
|---|---|---|---|---|---|---|---|---|---|---|
| 60YO | Sweden | Prospective cohort | 1997-99 | 3987/581 | 16.5 | 60.0 | 51.7 | 26.8 | 8.2 | 1.2 |
| ARIC | USA | Prospective cohort | 1987 | 3821/896 | 29.1 | 54.0 | 52.0 | 27.1 | 5.3 | 3.5 |
| CHS | USA | Prospective cohort | 1992-93 | 3526/1459 | 9.5 | 74.9 | 60.0 | 26.8 | 24.3 | 4.9 |
| DCH | Denmark | Prospective cohort | 1993-97 | 3187/183 | 13.5 | 56.7 | 46.1 | 26.2 | 3.0 | 0.2 |
| EPIC-Norfolk | UK | Prospective cohort | 1993-98 | 7383/1070 | 14.3 | 63.3 | 19.2 | 26.6 | 5.0 | - |
| FHS | USA | Prospective cohort | 2005-08 | 2488/329 | 11.3 | 65.9 | 55.5 | 28.2 | 13.1 | 1.1 |
| Hisayama | Japan | Prospective cohort | 2002-03 | 3126/153 | 9.0 | 62.0 | 57.5 | 23.0 | 5.6 | - |
| HPFS | USA | Prospective cohort | 1994 | 1529/64 | 17.4 | 64.6 | 0.0 | 25.8 | 0.0 | 0.0 |
| KIHD | Finland | Prospective cohort | 1998-01 | 1774/435 | 18.1 | 62.8 | 53.0 | 27.8 | 30.1 | 6.7 |
| MERLIN TIMI-36 | 17 countries‡ | Prospective case-cohort | 2004 | 1769/161 | 0.9 | 63.2 | 37.4 | 29.1 | 100.0 | 21.0 |
| MESA | USA | Prospective cohort | 2000 | 6203/816 | 12.9 | 62.0 | 52.7 | 28.2 | 0.0 | 0.0 |
| PIVUS | Sweden | Prospective cohort | 2001-04 | 950/205 | 15.0 | 70.2 | 51.1 | 27.0 | 10.0 | 4.7 |
| PRE-DETERMINE | USA and Canada | Prospective cohort | 2007-13 | 4732/505 | 7.9 | 63.0 | 23.9 | 30.2 | 100.0 | 23.3 |
| RS | Netherlands | Prospective cohort | 2002-05 | 2361/299 | 9.9 | 74.9 | 58.8 | 27.4 | 14.7 | 4.9 |
| RUTI-HF | Spain | Prospective cohort | 2006-20 | 700/84 | 2.9 | 64.8 | 30.4 | 27.4 | 32.4 | 100.0 |
| ULSAM | Sweden | Prospective cohort | 1971-74 | 2006/406 | 33.3 | 49.7 | 0.0 | 25.0 | 1.2 | 2.7 |
| WHIMS | USA | Prospective cohort | 1995 | 5257/74 | 6.0 | 70.0 | 100.0 | 28.3 | 18.1 | 0.8 |
| **Total** | | | | **54799/7720** | **13.3** | **63.4** | **46.7** | **27.3** | **19.6** | **6.6** |

**Notes:**

60YO: The Stockholm Cohort of 60-year-olds; ASCVD: atherosclerotic cardiovascular disease; CHS: Cardiovascular Health Study; DCH: Danish Diet, Cancer and Health Study; EPIC-Norfolk: European Prospective Investigation into Cancer and Nutrition-Norfolk; FHS: Framingham Heart Study; HF: heart failure; Hisayama: Hisayama Study; HPFS: Health Professionals Follow-up Study; KIHD: Kuopio Ischaemic Heart Disease Risk Factor Study; MERLIN TIMI-36: The Metabolic Efficiency With Ranolazine for Less Ischemia in Non-ST-Elevation Acute Coronary Syndromes - Thrombolytics in Myocardial Infarction 36 Trial; MESA: Multi-Ethnic Study of Atherosclerosis; PIVUS: Prospective Investigation of Vasculature in Uppsala Seniors; PRE-DETERMINE: PRE-DETERMINE Biologic Markers and Sudden Cardiac Death Study; RS: Rotterdam Study; RUTI-HF: Can Ruti Heart Failure Cohort; ULSAM: Uppsala Longitudinal Study of Adult Men; WHIMS: Women's Health Initiative Memory Study

*Study details are available in Supplementary Appendix.

†ASCVD is defined as a prior diagnosis of coronary heart disease, stroke, or peripheral artery disease.

[‡]Included participants from Austria, Belgium, Canada, Czech Republic, France, Georgia, Germany, Hungary, Israel, Italy, the Netherlands, Poland, Russia, South Africa, Spain, UK, and US

**Table 2.** Association between omega-3 fatty acid biomarker levels* and incident atrial fibrillation

| Exposure | Studies, n | Cases, n | Continuous analysis per interquintile range† | | Categorical analysis comparing Q5 vs. Q1‡ | |
|---|---|---|---|---|---|---|
| | | | HR (95% CI) | $I^2$ (%) | HR (95% CI) | $I^2$ (%) |
| **EPA** | | | | | | |
| Phospholipids | 10 | 5,502 | 1.00 (0.95, 1.06) | 55.1% | 0.92 (0.83, 1.02) | 60.1% |
| Total plasma/serum | 4 | 810 | 1.09 (0.94, 1.28) | 58.3% | 0.97 (0.75, 1.25) | 62.5% |
| Cholesterol ester | 3 | 1,880 | 0.97 (0.87, 1.08) | 78.0% | 1.06 (0.90, 1.26) | 43.6% |
| Adipose tissue | 1 | 183 | 0.83 (0.58, 1.20) | - | 0.86 (0.54, 1.37) | - |
| **Overall** | **16** | 7,421 | **1.00 (0.95, 1.05)** | **52.2%** | **0.94 (0.86, 1.02)** | **49.6%** |
| **DPA** | | | | | | |
| Phospholipids | 9 | 5,418 | 0.89 (0.82, 0.95) | 8.1% | 0.87 (0.79, 0.97) | 0.0% |
| Total plasma/serum | 4 | 810 | 0.97 (0.79, 1.17) | 0.0% | 1.17 (0.91, 1.51) | 0.0% |
| Adipose tissue | 1 | 183 | 0.71 (0.45, 1.14) | - | 0.72 (0.42, 1.23) | - |
| **Overall** | **13** | 6,350 | **0.89 (0.83, 0.95)** | 0.0% | **0.90 (0.82, 0.98)** | 0.0% |
| **DHA** | | | | | | |
| Phospholipids | 10 | 5,502 | 0.87 (0.80, 0.94) | 57.9% | 0.84 (0.76, 0.92) | 44.7% |
| Total plasma/serum | 5 | 1,109 | 0.97 (0.83, 1.12) | 27.7% | 0.91 (0.74, 1.11) | 0.0% |
| Cholesterol ester | 3 | 1,880 | 0.96 (0.85, 1.08) | 67.8% | 0.94 (0.81, 1.10) | 73.5% |
| Adipose tissue | 1 | 183 | 0.75 (0.50, 1.14) | - | 0.73 (0.45, 1.19) | - |
| **Overall** | **17** | 7,720 | **0.90 (0.85, 0.96)** | **47.5%** | **0.87 (0.80, 0.94)** | **38.4%** |
| **EPA + DHA** | | | | | | |
| Phospholipids | 10 | 5,502 | 0.91 (0.85, 0.98) | 65.6% | 0.84 (0.76, 0.92) | 57.1% |
| Total plasma/serum | 4 | 810 | 1.07 (0.90, 1.27) | 32.0% | 0.95 (0.74, 1.23) | 28.7% |
| Cholesterol ester | 3 | 1,880 | 0.96 (0.86, 1.07) | 80.3% | 1.00 (0.84, 1.18) | 76.9% |
| Adipose tissue | 1 | 183 | 0.77 (0.51, 1.15) | - | 0.73 (0.44, 1.20) | - |
| **Overall** | **16** | 7,421 | **0.93 (0.87, 0.99)** | **60.7%** | **0.88 (0.81, 0.95)** | **57.1%** |

DHA: docosahexaenoic acid; DPA: docosapentaenoic acid; EPA: eicosapentaenoic acid

**Notes:**

*Multiple lipid fractions were available for some studies, but only one lipid fraction was used for the overall analysis.

†An interquintile range refers to the difference between the $10^{th}$ to $90^{th}$ percentile of the respective fatty acid in each cohort.

‡Q1 and Q5 refer to the first and the fifth groups categorized by quintile values in each cohort. Effect estimates were pooled using inverse-variance weighted fixed-effect meta-analysis.

**Table 3.** Stratified analysis of omega-3 fatty acid biomarkers and incident atrial fibrillation

| | EPA | | DPA | | DHA | | EPA + DHA | |
|---|---|---|---|---|---|---|---|---|
| | HR (95% CI)* | $P_{het}$[2] | HR (95% CI)[1] | $P_{het}$[2] | HR (95% CI)[1] | $P_{het}$[2] | HR (95% CI)[1] | $P_{het}$[†] |
| **Overall estimate** | **1.00 (0.95, 1.05)** | | **0.89 (0.83, 0.95)** | | **0.90 (0.85, 0.96)** | | **0.93 (0.87, 0.99)** | |
| Elevated CV risk[‡] | 0.94 (0.88, 1.02) | | 0.88 (0.80, 0.96) | | 0.89 (0.82, 0.97) | | 0.91 (0.85, 0.98) | |
| Global region | | 0.54 | | 0.34 | | 0.09 | | 0.12 |
| North America | 0.98 (0.92, 1.05) | | 0.86 (0.79, 0.94) | | 0.85 (0.78, 0.92) | | 0.86 (0.79, 0.94) | |
| Europe | 1.04 (0.94, 1.14) | | 0.96 (0.85, 1.09) | | 0.97 (0.88, 1.07) | | 1.00 (0.89, 1.12) | |
| East Asia | 1.14 (0.71, 1.83) | | 0.81 (0.51, 1.26) | | 1.04 (0.64, 1.67) | | 1.01 (0.62, 1.62) | |
| Age, years | | 0.29 | | 0.77 | | 0.56 | | 0.92 |
| < 65 | 0.97 (0.89, 1.05) | | 0.88 (0.78, 0.99) | | 0.94 (0.86, 1.04) | | 0.93 (0.85, 1.03) | |
| ≥ 65 | 1.02 (0.96, 1.09) | | 0.90 (0.82, 0.97) | | 0.91 (0.84, 0.98) | | 0.94 (0.86, 1.02) | |
| Sex | | 0.33 | | 0.31 | | 0.39 | | 1.00 |
| Male | 0.98 (0.92, 1.05) | | 0.86 (0.78, 0.95) | | 0.94 (0.87, 1.02) | | 0.94 (0.88, 1.01) | |
| Female | 1.03 (0.96, 1.11) | | 0.93 (0.84, 1.03) | | 0.89 (0.82, 0.98) | | 0.94 (0.87, 1.02) | |

DHA: docosahexaenoic acid; DPA: docosapentaenoic acid; EPA: eicosapentaenoic acid

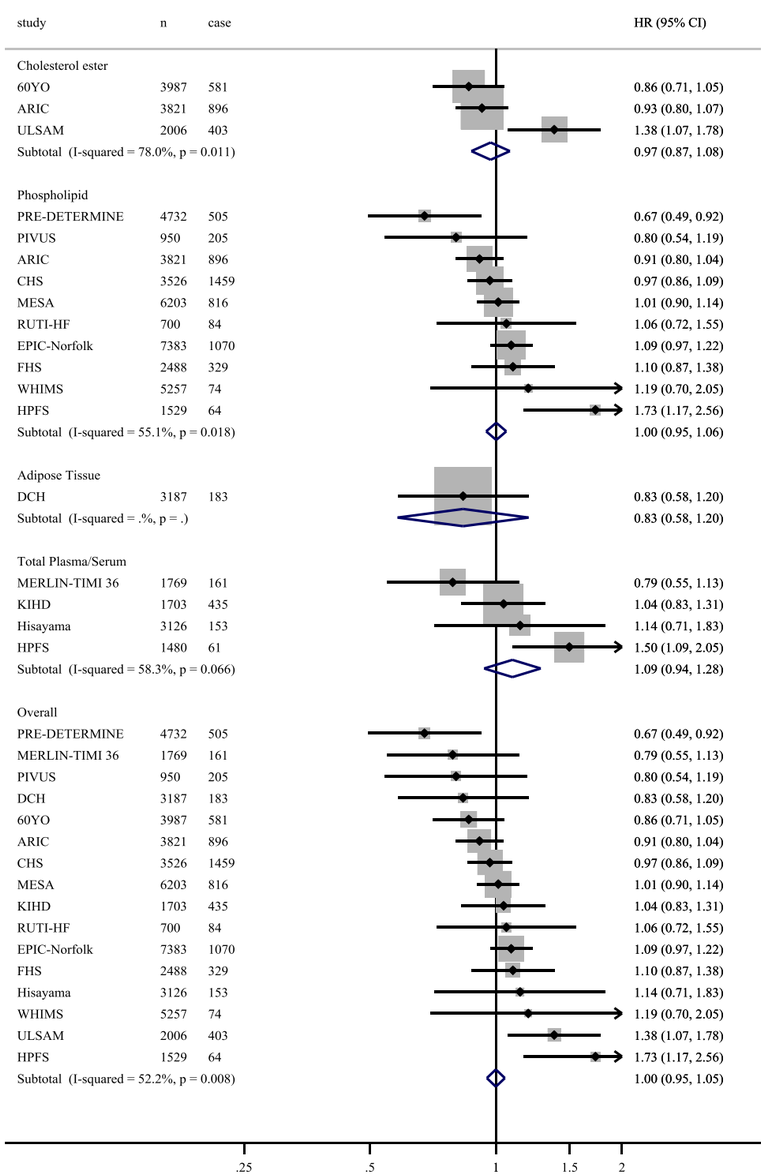
**Notes:**

*Effect estimates were per study-specific interquintile range ($10^{th}$ – $90^{th}$ percentile), multivariable-adjusted as described in the figure legends, and pooled using inverse-variance weighted fixed-effect meta-analysis.

[†]$P_{het}$ between subgroups was calculated using meta-regression

[‡]Elevated CV risk was defined as possessing one or more of the following: 1) fasting triglycerides ≥ 150 mg/dL (or ≥ 1.70 mmol/L), 2) non-HDL cholesterol ≥ 160 mg/dL (or ≥ 4.14 mmol/L), 3) diabetes mellitus, 4) atherosclerotic cardiovascular disease, including coronary heart disease, stroke, or peripheral artery disease, 5) heart failure

A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Cholesterol ester
60YO 3987 581 0.86 (0.71, 1.05)
ARIC 3821 896 0.93 (0.80, 1.07)
ULSAM 2006 403 1.38 (1.07, 1.78)
Subtotal (I-squared = 78.0%, p = 0.011) 0.97 (0.87, 1.08)
Phospholipid
PRE-DETERMINE 4732 505 0.67 (0.49, 0.92)
PIVUS 950 205 0.80 (0.54, 1.19)
ARIC 3821 896 0.91 (0.80, 1.04)
CHS 3526 1459 0.97 (0.86, 1.09)
MESA 6203 816 1.01 (0.90, 1.14)
RUTI-HF 700 84 1.06 (0.72, 1.55)
EPIC-Norfolk 7383 1070 1.09 (0.97, 1.22)
FHS 2488 329 1.10 (0.87, 1.38)
WHIMS 5257 74 1.19 (0.70, 2.05)
HPFS 1529 64 1.73 (1.17, 2.56)
Subtotal (I-squared = 55.1%, p = 0.018) 1.00 (0.95, 1.06)
Adipose Tissue
DCH 3187 183 0.83 (0.58, 1.20)
Subtotal (I-squared = .%, p = .) 0.83 (0.58, 1.20)
Total Plasma/Serum
MERLIN-TIMI 36 1769 161 0.79 (0.55, 1.13)
KIHD 1703 435 1.04 (0.83, 1.31)
Hisayama 3126 153 1.14 (0.71, 1.83)
HPFS 1480 61 1.50 (1.09, 2.05)
Subtotal (I-squared = 58.3%, p = 0.066) 1.09 (0.94, 1.28)
Overall
PRE-DETERMINE 4732 505 0.67 (0.49, 0.92)
MERLIN-TIMI 36 1769 161 0.79 (0.55, 1.13)
PIVUS 950 205 0.80 (0.54, 1.19)
DCH 3187 183 0.83 (0.58, 1.20)
60YO 3987 581 0.86 (0.71, 1.05)
ARIC 3821 896 0.91 (0.80, 1.04)
CHS 3526 1459 0.97 (0.86, 1.09)
MESA 6203 816 1.01 (0.90, 1.14)
KIHD 1703 435 1.04 (0.83, 1.31)
RUTI-HF 700 84 1.06 (0.72, 1.55)
EPIC-Norfolk 7383 1070 1.09 (0.97, 1.22)
FHS 2488 329 1.10 (0.87, 1.38)
Hisayama 3126 153 1.14 (0.71, 1.83)
WHIMS 5257 74 1.19 (0.70, 2.05)
ULSAM 2006 403 1.38 (1.07, 1.78)
HPFS 1529 64 1.73 (1.17, 2.56)
Subtotal (I-squared = 52.2%, p = 0.008) 1.00 (0.95, 1.05)
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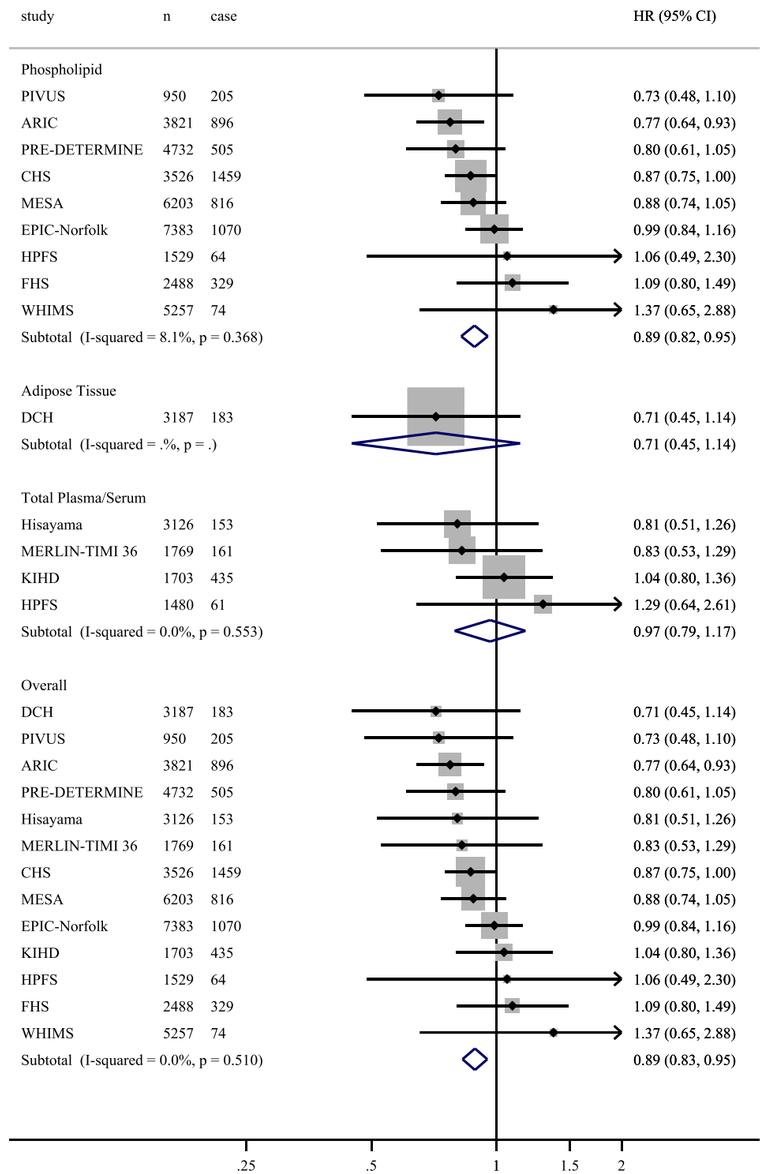
B


study n case HR (95% CI)
Phospholipid
PIVUS 950 205 0.73 (0.48, 1.10)
ARIC 3821 896 0.77 (0.64, 0.93)
PRE-DETERMINE 4732 505 0.80 (0.61, 1.05)
CHS 3526 1459 0.87 (0.75, 1.00)
MESA 6203 816 0.88 (0.74, 1.05)
EPIC-Norfolk 7383 1070 0.99 (0.84, 1.16)
HPFS 1529 64 1.06 (0.49, 2.30)
FHS 2488 329 1.09 (0.80, 1.49)
WHIMS 5257 74 1.37 (0.65, 2.88)
Subtotal (I-squared = 8.1%, p = 0.368) 0.89 (0.82, 0.95)
Adipose Tissue
DCH 3187 183 0.71 (0.45, 1.14)
Subtotal (I-squared = .%, p = .) 0.71 (0.45, 1.14)
Total Plasma/Serum
Hisayama 3126 153 0.81 (0.51, 1.26)
MERLIN-TIMI 36 1769 161 0.83 (0.53, 1.29)
KIHD 1703 435 1.04 (0.80, 1.36)
HPFS 1480 61 1.29 (0.64, 2.61)
Subtotal (I-squared = 0.0%, p = 0.553) 0.97 (0.79, 1.17)
Overall
DCH 3187 183 0.71 (0.45, 1.14)
PIVUS 950 205 0.73 (0.48, 1.10)
ARIC 3821 896 0.77 (0.64, 0.93)
PRE-DETERMINE 4732 505 0.80 (0.61, 1.05)
Hisayama 3126 153 0.81 (0.51, 1.26)
MERLIN-TIMI 36 1769 161 0.83 (0.53, 1.29)
CHS 3526 1459 0.87 (0.75, 1.00)
MESA 6203 816 0.88 (0.74, 1.05)
EPIC-Norfolk 7383 1070 0.99 (0.84, 1.16)
KIHD 1703 435 1.04 (0.80, 1.36)
HPFS 1529 64 1.06 (0.49, 2.30)
FHS 2488 329 1.09 (0.80, 1.49)
WHIMS 5257 74 1.37 (0.65, 2.88)
Subtotal (I-squared = 0.0%, p = 0.510) 0.89 (0.83, 0.95)
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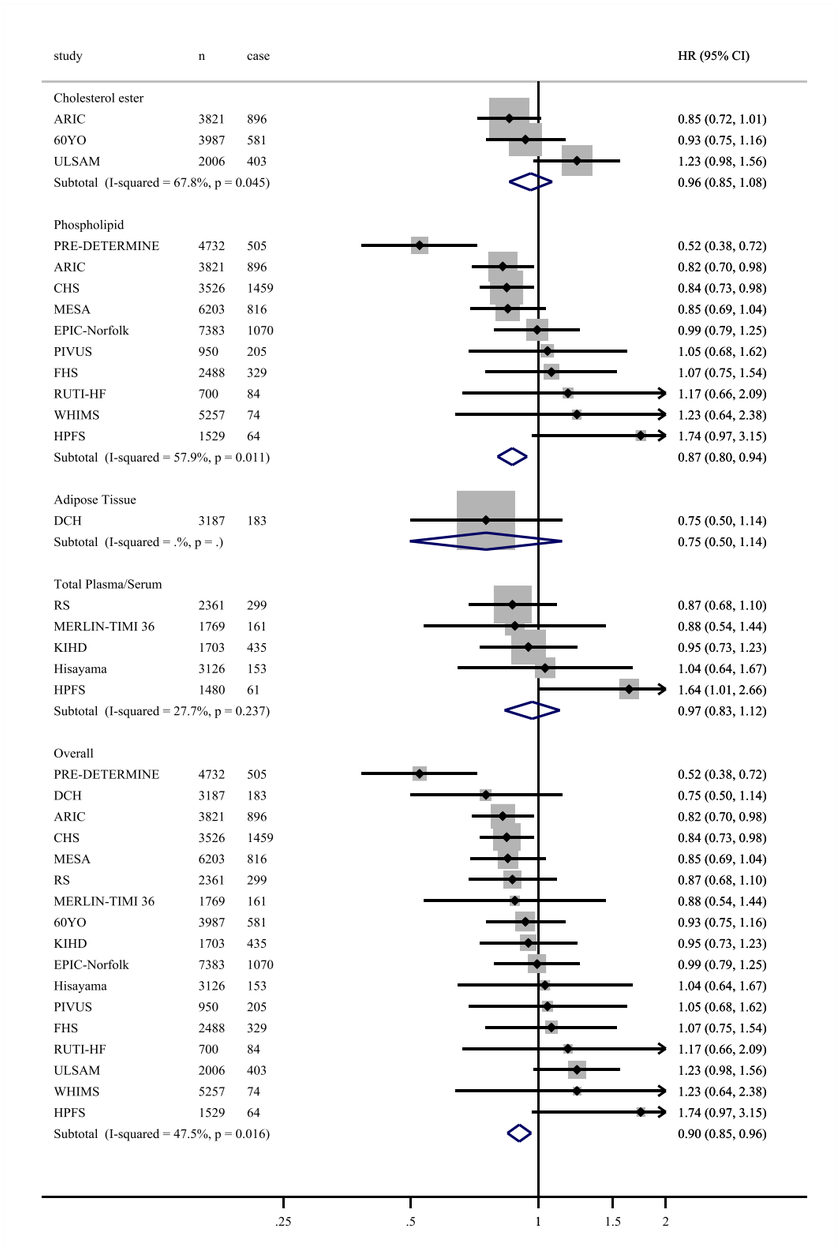
C


study n case HR (95% CI)
Cholesterol ester
ARIC 3821 896 0.85 (0.72, 1.01)
60YO 3987 581 0.93 (0.75, 1.16)
ULSAM 2006 403 1.23 (0.98, 1.56)
Subtotal (I-squared = 67.8%, p = 0.045) 0.96 (0.85, 1.08)
Phospholipid
PRE-DETERMINE 4732 505 0.52 (0.38, 0.72)
ARIC 3821 896 0.82 (0.70, 0.98)
CHS 3526 1459 0.84 (0.73, 0.98)
MESA 6203 816 0.85 (0.69, 1.04)
EPIC-Norfolk 7383 1070 0.99 (0.79, 1.25)
PIVUS 950 205 1.05 (0.68, 1.62)
FHS 2488 329 1.07 (0.75, 1.54)
RUTI-HF 700 84 1.17 (0.66, 2.09)
WHIMS 5257 74 1.23 (0.64, 2.38)
HPFS 1529 64 1.74 (0.97, 3.15)
Subtotal (I-squared = 57.9%, p = 0.011) 0.87 (0.80, 0.94)
Adipose Tissue
DCH 3187 183 0.75 (0.50, 1.14)
Subtotal (I-squared = .%, p = .) 0.75 (0.50, 1.14)
Total Plasma/Serum
RS 2361 299 0.87 (0.68, 1.10)
MERLIN-TIMI 36 1769 161 0.88 (0.54, 1.44)
KIHD 1703 435 0.95 (0.73, 1.23)
Hisayama 3126 153 1.04 (0.64, 1.67)
HPFS 1480 61 1.64 (1.01, 2.66)
Subtotal (I-squared = 27.7%, p = 0.237) 0.97 (0.83, 1.12)
Overall
PRE-DETERMINE 4732 505 0.52 (0.38, 0.72)
DCH 3187 183 0.75 (0.50, 1.14)
ARIC 3821 896 0.82 (0.70, 0.98)
CHS 3526 1459 0.84 (0.73, 0.98)
MESA 6203 816 0.85 (0.69, 1.04)
RS 2361 299 0.87 (0.68, 1.10)
MERLIN-TIMI 36 1769 161 0.88 (0.54, 1.44)
60YO 3987 581 0.93 (0.75, 1.16)
KIHD 1703 435 0.95 (0.73, 1.23)
EPIC-Norfolk 7383 1070 0.99 (0.79, 1.25)
Hisayama 3126 153 1.04 (0.64, 1.67)
PIVUS 950 205 1.05 (0.68, 1.62)
FHS 2488 329 1.07 (0.75, 1.54)
RUTI-HF 700 84 1.17 (0.66, 2.09)
ULSAM 2006 403 1.23 (0.98, 1.56)
WHIMS 5257 74 1.23 (0.64, 2.38)
HPFS 1529 64 1.74 (0.97, 3.15)
Subtotal (I-squared = 47.5%, p = 0.016) 0.90 (0.85, 0.96)
.25
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1.5
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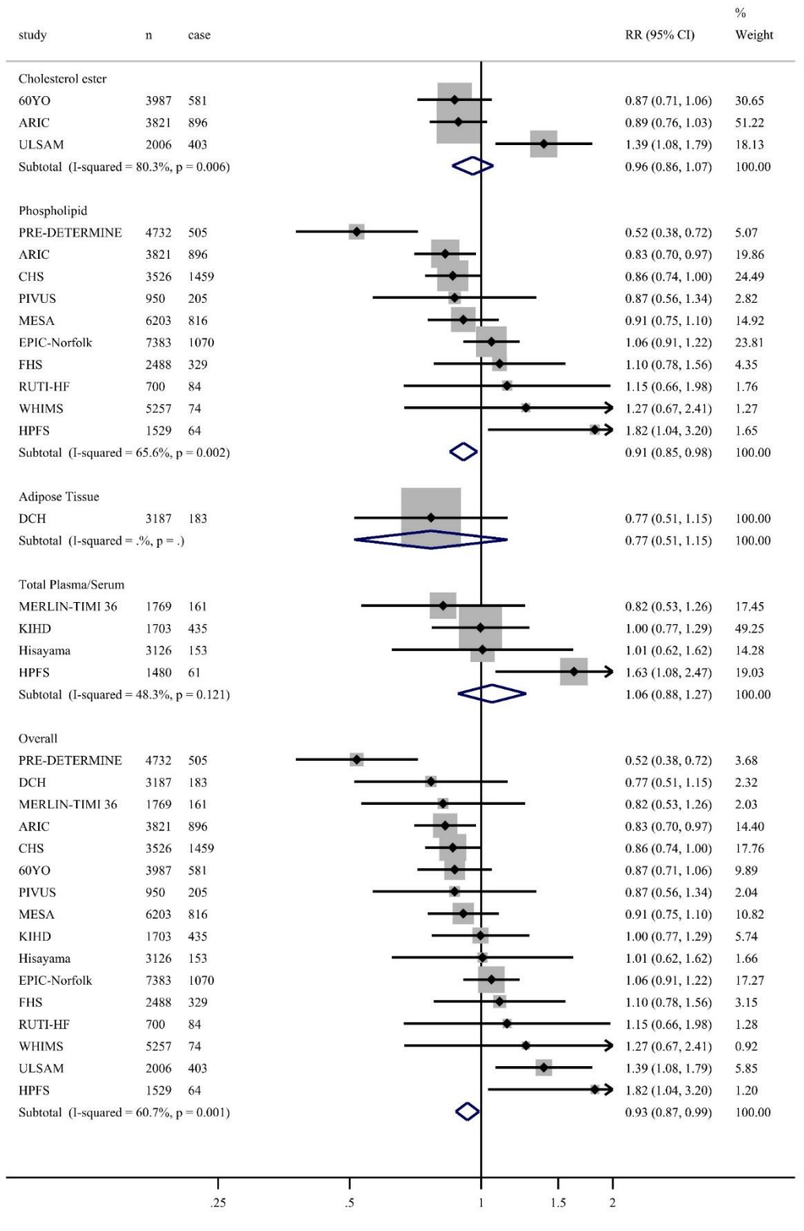
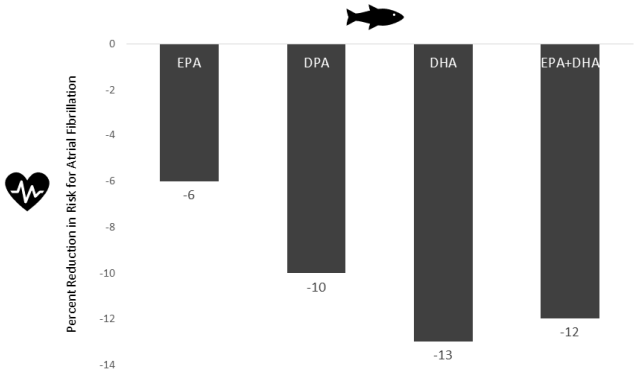
D


study n case RR (95% CI) % Weight
Cholesterol ester
60YO 3987 581 0.87 (0.71, 1.06) 30.65
ARIC 3821 896 0.89 (0.76, 1.03) 51.22
ULSAM 2006 403 1.39 (1.08, 1.79) 18.13
Subtotal (I-squared = 80.3%, p = 0.006) 0.96 (0.86, 1.07) 100.00
Phospholipid
PRE-DETERMINE 4732 505 0.52 (0.38, 0.72) 5.07
ARIC 3821 896 0.83 (0.70, 0.97) 19.86
CHS 3526 1459 0.86 (0.74, 1.00) 24.49
PIVUS 950 205 0.87 (0.56, 1.34) 2.82
MESA 6203 816 0.91 (0.75, 1.10) 14.92
EPIC-Norfolk 7383 1070 1.06 (0.91, 1.22) 23.81
FHS 2488 329 1.10 (0.78, 1.56) 4.35
RUTI-HF 700 84 1.15 (0.66, 1.98) 1.76
WHIMS 5257 74 1.27 (0.67, 2.41) 1.27
HPFS 1529 64 1.82 (1.04, 3.20) 1.65
Subtotal (I-squared = 65.6%, p = 0.002) 0.91 (0.85, 0.98) 100.00
Adipose Tissue
DCH 3187 183 0.77 (0.51, 1.15) 100.00
Subtotal (I-squared = .%, p = .) 0.77 (0.51, 1.15) 100.00
Total Plasma/Serum
MERLIN-TIMI 36 1769 161 0.82 (0.53, 1.26) 17.45
KIHD 1703 435 1.00 (0.77, 1.29) 49.25
Hisayama 3126 153 1.01 (0.62, 1.62) 14.28
HPFS 1480 61 1.63 (1.08, 2.47) 19.03
Subtotal (I-squared = 48.3%, p = 0.121) 1.06 (0.88, 1.27) 100.00
Overall
PRE-DETERMINE 4732 505 0.52 (0.38, 0.72) 3.68
DCH 3187 183 0.77 (0.51, 1.15) 2.32
MERLIN-TIMI 36 1769 161 0.82 (0.53, 1.26) 2.03
ARIC 3821 896 0.83 (0.70, 0.97) 14.40
CHS 3526 1459 0.86 (0.74, 1.00) 17.76
60YO 3987 581 0.87 (0.71, 1.06) 9.89
PIVUS 950 205 0.87 (0.56, 1.34) 2.04
MESA 6203 816 0.91 (0.75, 1.10) 10.82
KIHD 1703 435 1.00 (0.77, 1.29) 5.74
Hisayama 3126 153 1.01 (0.62, 1.62) 1.66
EPIC-Norfolk 7383 1070 1.06 (0.91, 1.22) 17.27
FHS 2488 329 1.10 (0.78, 1.56) 3.15
RUTI-HF 700 84 1.15 (0.66, 1.98) 1.28
WHIMS 5257 74 1.27 (0.67, 2.41) 0.92
ULSAM 2006 403 1.39 (1.08, 1.79) 5.85
HPFS 1529 64 1.82 (1.04, 3.20) 1.20
Subtotal (I-squared = 60.7%, p = 0.001) 0.93 (0.87, 0.99) 100.00
.25
.5
1
1.5
2




**Central Illustration:** Omega-3 levels and Risk for Atrial Fibrillation. Compared with individuals in the lowest quintile, those in the highest quintile of blood/tissue omega-3 levels were between 6% and 13% lower risk for developing atrial fibrillation. These multivariable-adjusted estimates were derived from a pooled study of 17 cohorts including 54,799 individuals followed for a median of 13.3 years and analyzed using pre-specified and harmonized outcome definitions, covariates, and statistical methods. EPA, eicosapentaenoic acid; DPA, docosapentaenoic acid; DHA, docosahexaenoic acid.

**Supplementary Online Content**

**Omega-3 Fatty Acid Biomarkers and Incident Atrial Fibrillation**

**Table of Contents**

**Supplementary Methods.** Cohort descriptions and fatty acid measurements

**The Stockholm Cohort of 60-year-olds (60YO), Sweden[1]**

The 60YO is a population-based cohort including Swedish men and women, aged 60 years at the time of enrollment. From August 1997 to March 1999, every third man and woman who was born between 1 July 1937 and 30 June 1938 (60 years old) and living in Stockholm County, Sweden, was invited to participate in a screening for cardiovascular disease (CVD) risk factors. Among the participants invited (n=5,460), 4,232 (78% response rate), 2,039 men and 2,193 women, agreed to participate. At baseline, participants underwent to a physical examination including anthropometric measurements and blood pressure and completed an extensive questionnaire about their disease history, health status, medication therapy, lifestyle, and nutritional habits. Blood samples were also drawn after overnight fasting. Participants were followed-up for CVD and death till December 31st, 2017.

Serum samples, collected at the time of the recruitment (1997-1999), were stored at -80°C until the analyses were performed in 2012. The percentage composition of methylated fatty acids was determined by gas-chromatography (GC) with a flame ionization detector and helium as the carrier gas. To avoid contamination of the GC column, free cholesterol liberated in the reaction was removed using an aluminum oxide column. The gas-liquid chromatography (GLC) system used for the analysis consisted of a 30-m glass capillary column coated with Thermo TR-FAME (Thermo Electron Corporation, Waltham, MA, USA), and an Agilent Technologies system consisting of model GLC 6890N, an autosampler 7683 and Agilent Chem Station (Agilent Technologies Inc., Santa Clara, CA, USA). The temperature was programmed to 150–260°C. Thirteen fatty acids were identified using standards from Nu Check Prep (Elysian, MN, USA). Individual serum cholesterol fatty acids were expressed as a proportion of the sum of all fatty acids measured. Fatty acid composition in one serum sample was repeatedly analyzed in duplicates in all batches for quality control. The intra-assay and inter-assay coefficient variations were ≤0.24 and ≤2.49 %, respectively, for the fatty acids utilized for statistical analyses (EPA, DHA, LA and AA).

**Atherosclerosis Risk in Communities Study (ARIC), USA[2,3]**

ARIC is a multi-center prospective investigation of atherosclerotic disease in a predominantly bi-racial population. Men and women aged 45-64 years at baseline were recruited from 4 communities: Forsyth County, North Carolina; Jackson, Mississippi; suburban areas of Minneapolis, Minnesota; and Washington County, Maryland. A total of 15,792 individuals participated in the baseline examination in 1987-1989, but only baseline fasting blood from the Minnesota field center were analyzed for participants with all data plus plasma fatty acids (n=3494).

Fatty acids were measured in EDTA plasma that had been frozen at -70˚C. Fatty acid assays were performed at the Collaborative Studies Clinical Laboratory at Fairview University Medical Center (Minneapolis, MN) as previously described 8. Lipids were extracted with chloroform/methanol and separated by thin layer chromatography. Fatty acid methyl esters were prepared from the phospholipid fraction and separated by gas chromatography using an HP-5890

gas chromatograph (Hewlett- Packard, Palo Alto, CA) with a 100-m capillary Varian CP7420 column. We identified 29 fatty acids. The concentration of each fatty acid was expressed as to percentage of total fatty acids.

**Cardiovascular Health Study (CHS), USA[4]**

The CHS Study is a prospective population-based cohort study of people ≥ 65 years old at baseline initiated to evaluate risk factors for the development and progression of cardiovascular disease. Participants were recruited at four field centers (Forsyth County, NC; Sacramento County, CA; Washington County, MD; Pittsburgh, PA) from random samples of Medicare eligibility lists. The cohort consists of 5201 non-institutionalized men and women, recruited in 1989-1990, plus an additional 687 black participants recruited in 1992-93.

Plasma phospholipid fatty acids were measured at the Fred Hutchinson Cancer Research Center (Seattle, WA) using stored blood samples from 1992-1993. Total lipids were extracted from plasma using the methods of Folch. A one dimensional thin-layer chromatography was used to separate phospholipids from neutral lipids. Phospholipids fraction was directly trans-esterified using the Lepage and Roy method to prepare fatty acid methyl esters, and individual fatty acid methyl esters were separated using gas chromatography (Agilent 5890 Gas Chromatograph flame ionization detector, Agilent Technologies, Palo Alto, CA; fused silica capillary column SP-2560 [100m x 0.25mm, 0.2μm], Supelco Belefonte, PA; initial 160 degrees Celsius for 16 min, ramp 3 degrees Celsius/min to 240 degrees Celsius, hold 15 minutes). All fatty acids were processed at the Biomarker Laboratory of the Fred Hutchinson Cancer Research Center (Seattle, WA). Coefficient of variation was <5% for each of the fatty acid biomarkers analyzed for the present study.

**Diet, Cancer and Health (DCH), Denmark[5]**

The DCH cohort was initiated to investigate diet and lifestyle factors in relation to development of cancer and other chronic diseases. The study participants were recruited from in and around the two largest cities in Denmark including Copenhagen and Aarhus. All individuals were men and women aged between 50 and 65 years of age, born in Denmark and without a previous diagnosis of cancer at the time of invitation. A total of 57,053 participants were recruited between 1993 and 1997. A sub-cohort of 3,187 participants randomly drawn from the whole cohort was eligible for the current analysis.

The fatty acid composition of adipose tissue was quantified at the lipid research laboratory at Aalborg University Hospital (Aalborg, Denmark) using stored subcutaneous adipose samples from the buttock collected at recruitment. The adipose tissue biopsy was taken using an evacuated Luer-lock system (Terumo, Terumo Corp, Tokyo Japan) consisting of a needle, a venoject multi-sample Luer adaptor and an evacuated blood tube. The fatty acid biopsies were flushed with nitrogen and immediately after collection and stored at -150 $^{o}$C. Before fatty acid analysis, the biopsies were thawed and approximately 1-2 mg tissue was removed to a glass and prewarmed at 50 $^{o}$C for 10 min. Subsequently, the fat was dissolved in heptane at 50 $^{o}$C and the fatty acids were transesterified by 2mol/L potassium hydroxide in methanol at 50 degrees Celsius for two min according to the IUPAC standard methods for analysis of oils, fats and derivates. The fatty acid composition was analyzed using a Varian 3900 gas chromatograph with

a CP-8400 auto sampler (Varian, Middleburg, The Netherlands) equipped with a flame ionization detector. Split injecting mode, a CP-sil 88 60 m x 0.25 mm capillary column and temperature programming (90 to 210 degrees $^{o}$C) and constant flow were used. Helium was used as carrier gas. Peak retention times and area percentages of fatty acid methyl esters were identified using commercially available standards (Nu-check-Prep, Inc., US). Adipose tissue content of fatty acids was expressed as area percentage of total fatty acids. The inter-assay coefficient of variation in adipose tissue was 5.5% for EPA, 3.0% for DPA and 2.9% for DHA.

**The European Prospective Investigation into Cancer and Nutrition – Norfolk (EPIC-Norfolk), UK[6]**

A prospective study of 25,639 men and women aged 40–79 years in Norfolk, UK similar in characteristics to UK general population samples, who participated in a baseline survey in 1993–1997. Participants completed a health and lifestyle questionnaire including data on medical history, smoking, alcohol intake, physical activity, social class, and education and attended a clinic for a health examination. Blood samples were spun, separated into 0.5 ml fractions of serum and citrated plasma, placed in straws, sealed, and stored in liquid nitrogen. Funding was obtained for blood FA analyses in 2003–2008. Selection of participants for fatty acid analyses was based on a series of nested case control studies with incident cases of cancers and cardiovascular disease and up to four disease-free controls for each case. This selection totaled about 10,000 individuals representing two-fifths of the cohort, enriched for incident diseases. Analyses on 8,000 samples were carried out in the WHO International Agency for Cancer Research laboratories, Lyon, France. Because of laboratory constraints in Lyon, an additional 2,000 samples were analyzed in Quotient Laboratories, UK, using the same methods and quality control standards. Atrial fibrillation (AF) was not the incident outcome in the case-control sample but ascertained in the study population as a part of the cohort effort. Because the sampling for each case set and control set was separately operated, we did stratified analyses by case status using the same range or categorization of each fatty acid exposure. Then, estimates per interquartile range or per category were meta-analyzed with a random-effect meta-analysis.

Citrated plasma straws were retrieved from liquid nitrogen storage, thawed at room temperature and 20 µg of di-palmitoyl-D31-phosphatidylcholine (Sigma, St. Louis, MO) internal standard was added to each 200 µl plasma sample. Following extraction of total lipids with chloroform/methanol, phospholipids were further purified by adsorption chromatography (LC-Si SPE, Supelco/Sigma, St. Louis, MO), transmethylated to fatty acid methyl esters and extracted with hexane. Analysis was carried out by gas chromatography with flame ionization detection (220°C) using a 30 m x 0.32 mm x 0.2 um SP2340 fused silica capillary column (Supelco/Sigma, St. Louis, MO). Carrier gas was Helium at a constant flow of 1.3 ml/min. Samples of 0.5 µl were introduced onto the column via on-column injection. The column was held initially for 1 min at 65°C, then programmed at 5°C/min to 135°C, then at 2°C/min to 200°C, and finally at 10°C/min to 220°C. Run time was 60 min.

Identification of individual fatty acid methyl esters was based on comparison with retention times of authentic standards (Sigma, St. Louis, MO). Plasma concentrations were measured by comparison of peak areas of individual fatty acids with the peak area of the palmitoyl-D31-fatty acid methyl ester internal standard using individual calibration curves for each of the 22 fatty acid methyl esters measured. The chromatographic peak for palmitoyl-D31-fatty acid methyl

ester elutes about 1 minute earlier than non-labelled palmitoyl fatty acid methyl ester in a zone free of interference from other peaks. Each chromatogram was integrated automatically and checked for accuracy and specificity by a laboratory technician. The analytical method allowed for the analysis of 22 individual fatty acids with chain lengths between 14 and 22 carbons, including very long-chain omega-3 polyunsaturated fatty acids (20:5n-3, 22:5n-3, 22:6n-3). The quantitative measures were used as mol%. Analytical quality control was carried out by the daily use of standard quality control plasma samples. The CVs for the major fatty acids were between 3% to 13%. Samples were identified only by a code number and cases and controls assorted within each analytic batch.

**Framingham Heart Study - Offspring Cohort (FHS), USA[7]**

FHS is a population based longitudinal study of families living in Framingham, Massachusetts. The offspring study was initiated in 1971 and consisted of a sample of 5,124 individuals, offspring of the original cohort and their spouses. Blood samples for fatty acid measurement and covariate data were collected during wave 8 of the study (2005-2008), and participants were followed until 2019.

The fatty acid composition of erythrocyte samples were analyzed by gas chromatography equipped with a SP 2560 capillary column after direct transesterification for 10 minutes in boron trifluoride/ methanol and hexane at 100 C as previously described.[8] This technique generates fatty acids primarily from erythrocyte glycerophospholipids. Erythrocytes were isolated from blood drawn after a 10–12 h fast and frozen at −80 °C immediately after collection. All fatty acids present at >1% abundance had CVs of ≤7%.

**Hisayama Study (Hisayama), Japan[9,10]**

The Hisayama Study is an ongoing, population-based prospective cohort study of cardiovascular disease and its risk factors in the town of Hisayama, a suburb in the metropolitan in Japan. A total of 3,103 residents who were aged 40 years older, without cardiovascular disease at baseline, and had no missing values for serum fatty acid levels were enrolled in the present study.

Serum fatty acids levels were assayed by gas chromatography (SRL, Tokyo, Japan). Briefly, total lipids in plasma were extracted according to the Folch's procedure, followed by hydrolysis to free fatty acids. Free fatty acids were esterified with potassium methoxide/methanol and boron trifluorideemethanol. The methylated fatty acids were analyzed using GC-17A gas chromatograph (Shimadzu Corporation, Kyoto, Japan) with omegawax-250 capillary column (SUPELCO, SigmaeAldrich Japan, Tokyo, Japan). Reproducibility (i.e. the coefficient of variation) of the determination of serum EPA, DHA, and AA levels by this method was reported to be 4.4%, 2.3%, and 3.8%, respectively.

**Health Professionals Follow-up Study (HPFS), USA[11,12]**

The Health Professionals Follow-up Study (HPFS) started in 1986, with 51,529 male health professionals, who were 40 – 75 years of age at recruitment in 1986. Blood samples were collected from HPFS participants in 1994. For this study we utilized previously measured fatty acid concentrations in stored blood used for nested case-control studies of incident cardiovascular diseases. Subjects were free of cardiovascular diseases and cancer at the time of blood sampling.

Blood samples were sent to the lab with an ice pack via overnight courier and the majority of the samples arrived within 24 hours. Upon arrival, samples were centrifuged and divided into aliquots for plasma, white blood cell, and red blood cells, and stored in liquid nitrogen freezers at ≤-130°C. Fatty acid concentrations were measured in stored total plasma and erythrocyte samples using gas-liquid chromatography. Concentrations of individual circulating fatty acids were expressed as a percentage of total fatty acids either in plasma or erythrocyte membranes. Plasma intra-assay CVs are as follows: EPA: 7%, DPA: 13%, DHA: 10%. The corresponding erythrocyte CVs are as follows: EPA: 12%, DPA: 13%, DHA: 14%.

**Kuopio Ischaemic Heart Disease Risk Factor Study (KIHD), Finland[13]**

The KIHD is an ongoing population-based cohort study designed to investigate risk factors for CVD and other chronic diseases in middle-aged and older men and women in Eastern Finland. The baseline examinations of the KIHD study were conducted between 1984 and 1989 to a random sample of men living in the city of Kuopio and neighboring rural communities. A total of 2682 men who were 42-60 years old at baseline (82.9% of those eligible) were recruited in two cohorts. The first cohort consisted of 1166 men who were 54 years old, enrolled between 1984 and 1986, and the second cohort included 1516 men who were 42, 48, 54 or 60 years old, enrolled between 1986 and 1989. During the years 1998-2001 all men from the second cohort were invited to the 11-year re-examinations of the study, and 854 men (85.6%) participated. These examinations were also the baseline for 920 postmenopausal women (78.4% of the 1173 eligible women) from the same area, aged 53-73 years. A total of 1703 adults with available data on circulating fatty acids were eligible for the current analysis.

Serum total fatty acids were determined from frozen samples with a NB-351 capillary column (HNU-Nordion, Helsinki, Finland) by a Hewlett-Packard 5890 Series II gas chromatograph (Hewlett-Packard Company, Avondale, Pa, USA, since 1999 Agilent Technologies Inc., USA) with a flame ionization detector. Serum was extracted with chloroform-methanol and fatty acids were methylated with methanol and sulphuric acid prior to gas chromatography. Each analyte had an individual reference standard and the analytes were quantified with an internal standard method using eicosane. Results for fatty acids were obtained in μmol/L and in the data analyses proportion of a fatty acid from the total fatty acids was used.

**Metabolic Efficiency With Ranolazine for Less Ischemia in Non−ST-Elevation Acute Coronary Syndromes (MERLIN)-TIMI 36, Multi-national[14]**

The MERLIN-TIMI 36 trial was a randomized, controlled, double-blinded trial that compared ranolazine with placebo in 6560 patients hospitalized with a non–ST segment–elevation ACS within 48 hours of symptoms onset. Patients eligible for enrollment had at least 10 minutes of ischemic symptoms at rest and presented with one of the following additional risk indicators: elevated biomarkers of myonecrosis, ST depression ≥0.1 mV, history of diabetes mellitus, or an intermediate-to-high (≥3) Thrombolysis in Myocardial Infarction (TIMI) Risk Score. Patients were excluded if they had end-stage renal disease requiring dialysis, cardiogenic shock, or a life expectancy of <1 year.

At randomization (median 24 hours from symptom onset), a plasma sample was drawn and stored at −20°C until shipped to the TIMI Clinical Trials Laboratory (Boston, MA), where it was

maintained at −80°C or colder. Samples were recorded as fasting or nonfasting by sites. Plasma samples were collected at randomization, and the composition of fatty acids were assessed through gas chromatography with flame ionization detection in the Nutritional Biomarker Laboratory of the Department of Nutrition at the Harvard T. H. Chan School of Public Health using previously published methodology.

**Multiethnic Study of Atherosclerosis (MESA), USA[15]**

MESA is a National Heart, Lung and Blood Institute-sponsored, population-based investigation of subclinical cardiovascular disease and its progression. A total of 6,814 individuals, aged 45 to 84 years, were recruited from six US communities (Baltimore City and County, MD; Chicago, IL; Forsyth County, NC; Los Angeles County, CA; New York, NY; and St. Paul, MN) between July 2000 and August 2002. Participants were excluded if they had physician-diagnosed cardiovascular disease prior to enrollment, including angina, myocardial infarction, heart failure, atrial fibrillation, stroke or TIA, resuscitated cardiac arrest or a cardiovascular intervention (e.g., CABG, angioplasty, valve replacement, or pacemaker/defibrillator placement). Pre-specified recruitment plans identified four racial/ethnic groups (White European-American, African-American, Hispanic-American, and Chinese-American) for enrollment, with targeted oversampling of minority groups to enhance statistical power. Investigators of MESA can be found at http://www.mesa-nhlbi.org.

Phospholipid fatty acids were extracted and measured at the University of Minnestoa (Minneapolis, MN). Plasma was diluted in saline and lipids were extracted from with a mixture of chloroform:methanol, and cholesterol, triglycerides and phospholipid subclasses were separated on a silica thin-layer chromatography plate in a solvent mixture of petroleum ether, diethyl ether, and glacial acetic acid. The band of phospholipids was harvested for the formation of methyl esters. FAME prepared with 14% boron trifluoride in methanol, incubated at 80ºC for 90 minutes, and extracted with petroleum ether. The final product was dissolved in heptane and injected onto a capillary Varian CP7420 100-m column with a Hewlett Packard 5890 gas chromatograph (GC) equipped with a HP6890A autosampler. The GC is configured for a single capillary column with a flame ionization detector and interfaced with HP chemstation software. Separation of individual fatty acids was obtained over an 80-minute run. Individual fatty acid values are expressed as percentage of total fatty acids. Inter-assay CVs were less than 10%.

**Prospective Investigation of Vasculature in Uppsala Seniors (PIVUS), Sweden[16]**

The PIVUS study started in 2001 with the primary aim to investigate the predictive power of different measurements of endothelial function and arterial compliance in a random sample of 1000 subjects aged 70 living in the community of Uppsala. As secondary aims, the study also included measurements of cardiac function and structure by ultrasound and MRI, evaluation of atherosclerosis by ultrasound and MRI, 7 day food intake recordings, detailed ECG analysis, cardiovascular autonomic function, body composition by DXA, DNA analysis and lung function, as well as a number of biochemical markers.

Fatty acid composition in phospholipids (PL) were measured by gas chromatography. Serum (0.5 mL) was mixed with 2.5 mL methanol, 5 mL chloroform (with 0.005% added butylated hydroxytoluene, BHT) and 7.5 mL NaH2PO4 (0.2 mol/l) and stored at 4 °C overnight for lipid extraction. The chloroform phase was then removed with a syringe and evaporated to dryness on

a 30 °C heating block using nitrogen gas. The lipid residue was dissolved in chloroform and the lipid esters were separated by thin-layer chromatography (TLC); the adsorbent containing POPOP was used as fluorescent agent. The TLC plates were eluted at room temperature with the solvent system petroleum ether/diethyl ether/acetic acid (81:18:1 by volume). The lipid fraction were visualized in UV light; the spots containing phospholipids were scraped off into vials and the lipid esters were then methylated at 60 °C overnight after addition of 2 mL H2SO4 (5%) methanol. The fatty acid methyl esters were extracted into 3 mL petroleum ether (0.005% BHT) after addition of 1.5 mL distilled water. The phases were separated after thorough mixing and centrifugation at 1500 g for 10 min. The petroleum ether phase was pipetted off and the solvent was evaporated under nitrogen gas on a 30 °C heating block. The fatty acid methyl esters were dissolved in 120 µL hexane and placed in vials. The fatty acid methyl esters were separated by gas–liquid chromatography on a 30-m glass capillary column coated with Thermo TR-FAME (Thermo Electron Corporation, USA) with helium gas as a carrier gas. An Agilent Technologies system consisting of model GLC 6890 N, autosampler 7683 and Agilent ChemStation was used. The temperature was programmed to 150–260 °C. The fatty acids were identified by comparing each peak's retention time with fatty acid methyl ester standards Nu Check Prep (Elysian, MN, USA). In 20 replicates, the CV% for the included fatty acids was 0.52–1.27%. Fatty acids are presented as the relative sum of the fatty acids analyzed.

**PRE-DETERMINE Biologic Markers and Sudden Cardiac Death Study (PRE-DETERMINE), USA and Canada[17]**

The PRE-DETERMINE (ClinicalTrials.gov ID NCT01114269) and accompanying DETERMINE Registry (ClinicalTrials.gov ID NCT00487279) study populations are multicenter prospective cohort studies comprised of 5956 patients with coronary disease on angiography or documented history of myocardial infarction (MI). The PREDETERMINE study enrolled 5764 patients with documented MI and/or mild to moderate LV dysfunction (LVEF=35-50%) who did not fulfill consensus guideline criteria for ICD implantation on the basis of LVEF and NYHA class (LVEF>35% or LVEF 30%-35% with NYHA Class I HF) at study entry.[18] Exclusion criteria included a history of cardiac arrest not associated with acute MI, current or planned ICD, or life expectancy < 6 months. The accompanying DETERMINE Registry included 192 participants screened for enrollment in PREDETERMINE who did not fulfill entry criteria on the basis of having an LVEF < 30%, LVEF 30-35% with NYHA Class II-IV heart failure, or an ICD or were unwilling to participate in the biomarker component of PREDETERMINE.

Erythrocytes (red blood cells; RBC) were isolated from blood drawn. RBC fatty acid composition was analyzed by gas chromatography (GC) with flame ionization detection at OmegaQuant Analytics. RBC was transferred to a screw-cap glass vial and 14% boron trifluoride (Sigma-Aldrich, St. Louis, MO) and hexane (EMD Chemicals, USA) was added. The vial was briefly vortexed and heated in a hot bath at 100˚C for 10 minutes. After cooling, HPLC grade water was added, the tubes were recapped, vortexed and centrifuged help to separate layers. An aliquot of the hexane layer was transferred to a GC vial. GC was carried out using a GC-2010 Gas Chromatograph (Shimadzu Corporation, Columbia, MD) equipped with a SP-2560, 100-m fused silica capillary column (0.25 mm internal diameter, 0.2 um film thickness; Supelco, Bellefonte, PA).

Fatty acids were identified by comparison with a standard mixture of fatty acids (GLC OQ-A, NuCheck Prep, Elysian, MN) which was also used to determine individual fatty acid calibration curves. The following 24 fatty acids (by class) were identified: saturated (14:0, 16:0, 18:0, 20:0, 22:0 24:0); cis monounsaturated (16:1, 18:1, 20:1, 24:1); trans [16:1, 18:1*, 18:2* - see below for more details); cis n-6 polyunsaturated (18:2, 18:3, 20:2, 20:3, 20:4, 22:4, 22:5); cis n-3 polyunsaturated (18:3, 20:5, 22:5, 22:6). Fatty acid composition was expressed as a percent of total identified fatty acids. The chromatographic conditions used in this study were sufficient to isolate the C16:1trans isomers and the C18:2 9t-12c, 9t-12t, and 9c-12t isomers, which is reported as C18:2n6t. However, each individual C18:1 trans molecular species (i.e., C18:1 □6 thru □13) could not be segregated but appeared as two blended peaks that eluted just before oleic acid. The areas of these two peaks were summed and referred to a C18:1 trans.

**Rotterdam Study (RS), the Netherlands[19]**

The Rotterdam Study is a population-based cohort study including people living in the Ommoord District of Rotterdam. Details on the design of Rotterdam Study are described elsewhere. For the first cohort (RS-I), participants aged ≥55 years were enrolled in 1989-1993 for the baseline examination (n=7983). Follow-up examinations were conducted every 3 to 4 years, and serum fatty acid measurements performed in blood samples collected during the fourth visit in 2002-2004 among a subset of the participants (n=2810), which was considered the baseline for the current study. The Rotterdam Study was approved by the Medical Ethics Committee of Erasmus University Medical Centre, and all participants provided written informed consent.

Blood samples were drawn by venipuncture and serum fatty acids profile was measured using proton nuclear magnetic resonance (NMR) spectroscopy (SFA, MUFA, PUFA, n-3PUFA, n-6 PUFA, DHA, LA, CLA, total fatty acids). In brief, plasma samples were separated from cells and stored at -80°C until use; lipids were subsequently extracted by using a modified Folch protocol. A nonvolatile chemical shift and concentration reference compound octamethylcyclotetrasiloxane were added, and 1H-NMR measurements were performed on a 500-MHz spectrometer.[20] The quantification of fatty acids was performed using an automated regression-based quantification protocol.

**Can Ruti Heart Failure Cohort (RUTI-HF), Spain[21]**

The Can Ruti Heart Failure Cohort was recruited from a structured multidisciplinary HF unit of a tertiary university hospital in Barcelona (Spain) covering ~ 850,000 inhabitants in the northern Barcelona metropolitan area. We used data and samples from 905 consecutive ambulatory patients with HF who had available serum sample stored at -80ºC. Patients were referred to the HF clinic mostly by the cardiology or internal medicine departments and to a lesser extent by the emergency or other hospital departments. The criteria for referral to the HF clinic were diagnosis of HF with at least one hospitalization and/or depressed systolic function. During the baseline visit, patients provided written consent for the use of their clinical data for research purposes. All patients were seen regularly for follow-up visits at the HF clinic according to their clinical needs and treated according to a unified protocol. Follow-up visits included a minimum of quarterly visits with a nurse and one visit with a physician (cardiologist, internist, or family physician) every 6 months, as well as optional visits with specialists in geriatrics, psychiatry, rehabilitation, endocrinology, and nephrology.

Fatty acid methyl esters of serum phospholipids were measured at Institut Hospital del Mar d'Investigacions Mèdiques (IMIM, Barcelona). Sample was spiked with 10 µg of the internal standard (ISTD) 1,2-dinonadecanoyl-sn-glycero-3-phosphocholine (Sigma-Aldrich) and the lipids were extracted with chloroform/methanol (2:1 v/v). Phospholipid fraction was isolated by solid-phase extraction, and directly trans-esterified using acidified methanol to prepare fatty acid methyl esters (FAMEs), as described in Br. J. Nutr. 2000, 84, 781–787. FAMEs were analyzed by gas chromatography/electron ionization mass spectrometry (GC/MSEI), using an Agilent 6890N GC equipped with an Agilent 7683 autosampler, and an Agilent 5973N mass spectrometry detector. FAMEs were separated with a J&W DB-FastFAME capillary column (30 m × 0.2 mm × 0.25 µm film thickness) (Agilent). The injector temperature was set at 250°C, and 1 µL injections were performed (split ratio 25:1). GC was run using an optimized temperature program, as follows: the program started at 50°C, held for 0.5 min, increased to 194°C at a rate of 25°C/min, held for 1 min, increased to 245°C at a rate of 5°C/min, and held for 3 min. Helium was used as a carrier gas (14 psi, constant pressure mode). FAMEs were detected using the selected ion monitoring (SIM) mode. Based on the work of Thurnhofer and Vetter (J. Agric. Food Chem. 2005, 53, 8896–8903), several m/z ions common to saturated, monounsaturated, and polyunsaturated FAMEs were monitored. Twelve mixtures of FAME external calibration standards, spiked with C19:0-methyl ester in an equivalent amount to that included in samples, were prepared by diluting FAME mix certified reference material (Supelco 37 Component FAME Mix, Merck) in hexane. The concentration of FAMEs in the samples were calculated by linear regression of the peak area ratio relative to that of the internal standard. The amount of each omega-3 is expressed as a percentage of the total amount of 20 determined fatty acids. Intra-assay precision of the method assessed by the relative standard deviation relative to 10 replicates of sample under the same experimental conditions and carried out by the same operator. Intra-assay CV was 3.01% for EPA, 3.05% for DHA, and 3.04% for the sum of EPA and DHA.

**Uppsala Longitudinal Study of Adult Men (ULSAM), Sweden[22,23]**

ULSAM is a community-based cohort of men living in Uppsala county, Sweden. The origin of this longitudinal study was the "Uppsala Primary Preventive Study", carried out between September 1970 and September 1973. The study comprised all men living in the County of Uppsala born between 1920 and 1924 selected from the register of County Council. All men (n=2841) were invited for the investigation, 81.7% (n=2322) participated. The mean age at this baseline examination was 49.6 (SD +/- 0.6), hence this starting cohort was referred to as ULSAM-50. After this baseline examination, all men were invited to participate in follow-up investigations at the ages 70, 82 and 88. Between the age 50 and 70, 422 had died and 219 had moved out of the Uppsala region. Of the 1681 men invited, 460 did not participate in this follow up, leaving 1221 men who participated (response rate of 73%) aged around 70. The men were invited by a letter, which also explained the aim of the examination. They received the letter 7-10 days prior to the examination. Those born at the beginning of the year were called first. Six individuals were called every weekday except for the vacation period in Sweden between June 25 and August 15. A second invitation letter was sent at the end of the examination of each age class to those who had not come after the first invitation. The screening examination program included a medical questionnaire and interview, blood and urine sampling, blood pressure and anthropometric measurements, intravenous glucose tolerance test, ECG recording, chest X-ray

and pure tone audiometry. At the baseline exam, fatty acid composition was assessed in serum cholesterol, whereas at the second exam 20 years later, fatty acids were measured in both cholesterol esters and adipose tissue. Dodecapenta and dodecahepta acids were measured only in adipose tissue lipids.

For analysis of the fatty acid composition of the serum cholesterol esters in ULSAM-50, serum was extracted with a hexane-isopropanol solution (1+4).58 Cholesterol esters were separated from the extract by thin layer chromatography before inter-esterification (acidic methanol at 85°C, 2 h),59 and free cholesterol liberated in the reaction was removed by an aluminium oxide column to avoid contamination of the gas liquid chromatography column. The percentage composition of methylated fatty acids 14:0 to 22:6 was determined by gas chromatography (a 25 m NB-351 silica capillary column, i.d. 0.32 mm, phase layer 0.20 mm) with use of a flame 11onization detector and with helium as carrier gas. Every 25$^{th}$ sample was a serum control pool. The precision of the between-series analysis (n=35) varied from 2% (large peaks) to 10% (smaller peaks) and between successive gas chromatography runs (n=17). Intraassay CV: 0.2-5% depending on the fatty acid; Interassay: 2-10% depending on the fatty acid. For concentrations below detection limit, proportions were imputed as a random value between 0 and the minimum quantified proportion in the cohort.

**Women's Health Initiatives Memory Study (WHIMS), USA[24,25]**

WHI was established to examine the effects of postmenopausal hormone therapy on cognitive function in women aged 65-80 years. Recruitment began in June 1995. Of 3200 eligible women free of probable dementia enrolled in the WHI, 2947 (92.1%) were enrolled in WHIMS. Investigators of WHIMS can be found at http://www.whi.org/researchers/.

The fatty acid composition of RBC samples was analyzed by gas chromatography equipped with a SP 2560 capillary column after direct transesterification for 10 minutes in boron trifluoride/ methanol and hexane at 100 C as previously described. This technique generates fatty acids primarily from RBC glycerophospholipids. During the aliquoting phase, the RBC samples were stored improperly at -20°C for a period of approximately 2 weeks, causing oxidative degeneration of the PUFAs before measurement. The original FA levels were estimated with multiple imputations using independent data on fatty acid degradation and length of time the samples were exposed to -20°C.

**Supplementary Table 1.** Atrial fibrillation ascertainment and diagnostic criteria for the participating cohorts

| Study | AF ascertainment and diagnostic criteria |
|---|---|
| 60YO | Incident cases of atrial fibrillation were retrieved from the Swedish Hospital Discharge up to December 31st, 2017 using the International Classification of Disease 10th Revision (ICD-10), codes I48.0-I48.4 and I48.9. Both main and secondary diagnoses of atrial fibrillation were recorded. After exclusion of prior cases of atrial fibrillation (n=47) and missing on the fatty acids of interest (n=82), 595 cases of atrial fibrillation were available for analysis. |
| ARIC | AF was identified by 3 sources: ECGs from follow-up exams, hospital discharge records, and death certificates. ECGs during follow-up exams were done with MAC PC personal Cardiographs. All ECGs were transmitted to the ARIC ECG reading center for coding, interpretation, and storage. A trained cardiologist visually read recordings of ECGs automatically coded as AF for the certainty of the diagnosis. Also, the information of hospitalization was obtained during the annual follow-up interview and from surveillance of local hospitals. A trained abstractor accessed the hospital discharge records and recorded all the diagnoses according to International Classification of Diseases, Ninth Revision, Clinical Modification. AF was defined if codes 427.31 or 427.32 were present in the absence of procedure codes for open heart surgery. Finally, information on death from any cause was collected by calling participant's proxy, or from obituaries, hospital records, death certificates, or vital statistics from the National Death Index. Cases of AF were identified from death certificates if International Classification of Diseases, Tenth Revision, code I48 or International Classification of Diseases, Ninth Revision, code 427.3 were listed as one of the causes of death. |
| CHS | Incident AF was ascertained from three sources: ECGs from annual study examinations through 1999, hospital discharge diagnoses (from CHS hospitalization or Medicare data), and diagnoses of AF from outpatient or physician service claims (from Medicare data). Diagnosis of AF was based on a single hospital discharge diagnosis, inpatient, outpatient or physician claim (International Classification of Diseases, Ninth Revision, Clinical Modification (ICD-9-CM) code 427.31 or 427.32). |
| DCH | Incident cases of atrial fibrillation (AF) or flutter (AFL) was obtained by linking the cohort participants with registered diagnoses in the nationwide Danish National Patient Register. The diagnoses were recorded using the eight revision of International Classification of Disease (code 427.93 "AF" and 427.94 "AFL" in the Danish version which was equivalent to code 427.4 "AF and/or AFL" in the international version) until the end of 1993 and thereafter the tenth revision of the International Classification of Disease (code I48) was used. |
| EPIC-Norfolk | Identified as having an atrial fibrillation during follow-up if they had a hospital admission and/or died with atrial fibrillation as cause of death, encoded with ICD8 427.4, ICD9 427.3, ICD10: I48 |

| | |
|---|---|
| FHS | Atrial Fibrillation / Flutter (AF) coding forms are completed at the time of cardiovascular endpoints reviews. Framingham Heart Study electrocardiograms and/or outside medical records are reviewed for each case, and electrocardiograms are signed by a cardiologist. For Atrial Fibrillation / Flutter (AF) Follow-Up forms, the general rule is to code the date of the first & last electrocardiogram of sinus rhythm and the first & last electrocardiogram of AF. For hospitalized paroxysmal AF, the first episode of AF, the first sinus rhythm, and the final electrocardiogram rhythm are coded. If the participant is persistently in sinus rhythm or AF, one electrocardiogram per hospitalization is coded. Cardioversion is coded as unsuccessful only when clearly described as such. Time and order of electrocardiograms are filled in when there is more than one electrocardiogram per date. |
| Hisayama | The definition of AF included AF and atrial flutter (Minnesota code, 8–3–1 to 8–3–4). The primary outcome of this study was a newly diagnosed AF present at the annual health examinations, clinics, or hospitals. All events of AF were verified by ECG findings from the annual health examination and/or medical records of the clinic or hospital by the study team's cardiologists. Since it was difficult to classify AF events using a standard classification (i.e., first diagnosed, paroxysmal, persistent, long-standing persistent, and permanent AF) in our study design, new-onset AF events were classified into two subtypes. (1) Subjects were defined as having "Definite permanent AF" if they had AF on all of the 12-lead ECGs at the subsequent annual health examinations during the follow-up. (2) Subjects were defined as having "Other or indefinite subtype of AF" if they experienced sinus rhythm on ECG at least once after the onset of AF during the follow-up, or if they did not undergo ECG examinations after the AF onset during the follow-up. |
| HPFS | Participants were asked whether they had ever (or, for follow-up questionnaires, within the prior 2 years) been professionally diagnosed as having AF that lasted for more than 1 hour. All participants reporting AF were sent a supplementary questionnaire to collect additional clinical information on month and year of event, diagnosis, symptoms, and treatment. The validity of the supplementary questionnaire for diagnosing AF was assessed in a subsample of 100 participants who were asked to complete the supplementary questionnaire and consent to review of medical records documenting a physician's diagnosis of AF such as a hospital discharge summary, consultation or outpatient clinic note, or electrocardiogram. Among the 64 participants who completed the supplementary questionnaire, the supplementary questionnaire was found to be 95% accurate (61 of 64) for diagnosing AF as compared to medical records. |
| KIHD | All atrial fibrillation (AF) events that occurred between study entry and December 31, 2018, were included. Data on events were obtained by record linkage from the national computerized hospitalization registry, which covers every hospitalization in Finland. Subjects were hospitalized because of AF or had AF when they were hospitalized for other reasons. Data on vital status were obtained from Statistics Finland. Cardiovascular causes of AF were coded according to International Classification of Diseases codes ($8^{th}$ revision code 427.4, $9^{th}$ revision code 427.3, and $10^{th}$ revision code I48) and the accuracy was verified by a physician. |
| MERLIN TIMI-36 | Clinical atrial fibrillation events were identified through adverse-event reporting throughout the duration of study follow-up. |
| MESA | Incident AF through December 2014 was identified from study ECGs verified for AF at Visit 5 (2010-2012), ICD-9 hospital discharge diagnoses consistent with AF (427.31 or 427.32), and, for participants enrolled in fee-for-service Medicare, inpatient and outpatient AF claims data. |
| PRE-DETERMINE | Among patients without a prior history of AF at baseline, new inpatient and outpatient AF diagnoses (ICD-9 diagnosis code 427.31 and ICD-10 diagnosis code I48.91) and atrial flutter diagnoses (ICD-9 diagnosis code 427.32 and ICD-10 diagnosis code I48.92) were identified by linking PREDETERMINE data with inpatient and outpatient CMS claims data. |

| | |
|---|---|
| RS | Three methods were used to assess cases of AF or atrial flutter: (i) At baseline and during follow-up examinations, 10-s 12-lead ECGs were recorded at the research centre with an ACTA Gnosis IV ECG recorder (EsaOte, Florence, Italy), stored digitally and analysed with the Modular ECG Analysis System (MEANS); (ii) General practitioners participating in the Rotterdam study sent computerized information on AF, based on their own records and on hospital discharge letters, to the researchers of the Rotterdam study; (iii) Hospital discharge diagnoses were also obtained from the LMR system (de Landelijke Medische Registratie). This national registration accumulates all hospital discharge diagnoses of Dutch inhabitants. |
| RUTI-HF | Incident cases of AF, including atrial flutter, were diagnosed from annual study clinic 12-lead ECGs, read by a centralized ECG reading center, or taken from hospital discharge diagnoses (International Classification of Disease, ninth revision, code 427.3, 427.31, or 427.32) |
| WHIMS | Women were followed annually with clinic visits and with either clinic visits or telephone calls in between annual visits. Women had follow-up 12-lead ECG testing every 3 years after baseline. At each contact, women underwent a standardized interview and were asked about menopausal symptoms, adherence to medications, potential outcomes, and hospitalizations. Medical records were obtained in the event of hospitalization and the International Classification of Diseases (ICD)-9 code for AF (427.31) was extracted from these records. WHI data were linked with their Centers for Medicare & Medicaid Services (CMS) data using social security numbers, birth dates, and death dates, with 97% of Medicare-eligible WHI participants successfully linked. Among participants with Medicare coverage, incident AF was identified by first occurrence of ICD-9 code 427.31 in any diagnosis position in the inpatient Medicare Analysis and Review file [MEDPAR]), Outpatient and Carrier files during years 1993–2007 and after WHI enrollment. Medicare time eligible for analysis included those intervals where participants were enrolled in fee-for-service Medicare and not simultaneously enrolled in a Medicare managed care plan. Women with AF on follow-up ECG or any single ICD-9 code of 427.31 from review of Medicare claims or hospital records were classified as having new onset AF. |

60YO: The Stockholm Cohort of 60-year-olds; CHS: Cardiovascular Health Study; DCH: Danish Diet, Cancer and Health Study; EPIC-Norfolk: European Prospective Investigation into Cancer and Nutrition-Norfolk; FHS: Framingham Heart Study; Hisayama: Hisayama Study; HPFS: Health Professionals Follow-up Study; KIHD: Kuopio Ischaemic Heart Disease Risk Factor Study; MERLIN TIMI-36: The Metabolic Efficiency With Ranolazine for Less Ischemia in Non-ST-Elevation Acute Coronary Syndromes - Thrombolytics in Myocardial Infarction 36 Trial; MESA: Multi-Ethnic Study of Atherosclerosis; PRE-DETERMINE: PRE-DETERMINE Biologic Markers and Sudden Cardiac Death Study; RS: The Rotterdam Study; RUTI-HF: Can Ruti Heart Failure Cohort; WHIMS: Women's Health Initiative Memory Study

**Supplementary Table 2.** Spearman's correlations between omega-3 fatty acids

| Study | Number of participants | Biomarker compartment | Spearman correlation coefficients between select fatty acids | | | | | |
|---|---|---|---|---|---|---|---|---|
| | | | EPA and DPA | EPA and DHA | EPA and Sum | DPA and DHA | DPA and Sum | DHA and Sum |
| 60YO | 3987 | Cholesterol ester | - | 0.72 | 0.99 | - | - | 0.81 |
| ARIC | 3821 | Plasma phospholipid | 0.40 | 0.41 | 0.64 | 0.15 | 0.25 | 0.97 |
| ARIC | 3821 | Cholesterol ester | 0.24 | 0.49 | 0.93 | 0.12 | 0.22 | 0.77 |
| CHS | 3526 | Plasma phospholipid | 0.52 | 0.42 | 0.58 | 0.16 | 0.25 | 0.98 |
| DCH | 3187 | Adipose tissue | 0.74 | 0.84 | 0.91 | 0.81 | 0.82 | 0.99 |
| EPIC-Norfolk | 7383 | Plasma phospholipid | 0.50 | 0.57 | 0.74 | 0.53 | 0.56 | 0.97 |
| FHS | 2488 | Erythrocyte phospholipid | 0.57 | 0.66 | 0.75 | 0.33 | 0.38 | 0.99 |
| Hisayama | 3126 | Total plasma/serum | 0.69 | 0.70 | 0.78 | 0.80 | 0.84 | 1.00 |
| HPFS | 1480 | Total plasma/serum | 0.61 | 0.74 | 0.89 | 0.57 | 0.62 | 0.96 |
| HPFS | 1529 | Erythrocyte phospholipid | 0.52 | 0.72 | 0.81 | 0.51 | 0.54 | 0.99 |
| KIHD | 1703 | Total plasma/serum | 0.57 | 0.77 | 0.94 | 0.53 | 0.58 | 0.94 |
| MERLIN TIMI-36 | 1769 | Total plasma/serum | 0.67 | 0.63 | 0.81 | 0.46 | 0.56 | 0.96 |
| MESA | 6203 | Plasma phospholipid | 0.57 | 0.62 | 0.74 | 0.39 | 0.45 | 0.98 |
| PIVUS | 950 | Plasma phospholipid | 0.47 | 0.64 | 0.86 | 0.43 | 0.56 | 0.92 |
| PRE-DETERMINE | 4732 | Erythrocyte phospholipid | 0.76 | 0.72 | 0.85 | 0.54 | 0.64 | 0.98 |
| RUTI-HF | 700 | Plasma phospholipid | - | 0.57 | 0.79 | - | - | 0.95 |
| ULSAM | 2006 | Cholesterol ester | - | 0.62 | 0.97 | - | - | 0.78 |
| WHIMS | 5257 | Erythrocyte phospholipid | 0.37 | 0.43 | 0.58 | 0.22 | 0.27 | 0.98 |

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**Supplementary Table 3.** Relative risks of omega-3 fatty acid biomarkers and incident atrial fibrillation by quintiles[1]

| Exposure | RR (95% CI) fixed-effect[2] | RR (95% CI) random-effect[3] | $I^2$ (%) |
|---|---|---|---|
| **EPA** | | | |
| Q1 | 1.00 (ref.) | 1.00 (ref.) | - |
| Q2 | 0.96 (0.89, 1.04) | 0.96 (0.89, 1.04) | 0.0% |
| Q3 | 0.95 (0.88, 1.04) | 0.95 (0.84, 1.06) | 31.8% |
| Q4 | 0.97 (0.89, 1.06) | 0.98 (0.88, 1.08) | 21.9% |
| Q5 | 0.92 (0.84, 1.00) | 0.92 (0.80, 1.05) | 45.9% |
| **DPA** | | | |
| Q1 | 1.00 (ref.) | 1.00 (ref.) | - |
| Q2 | 0.96 (0.88, 1.04) | 0.96 (0.87, 1.07) | 14.2% |
| Q3 | 0.98 (0.90, 1.07) | 0.99 (0.89, 1.12) | 31.7% |
| Q4 | 0.88 (0.80, 0.97) | 0.88 (0.80, 0.97) | 0.0% |
| Q5 | 0.90 (0.82, 0.99) | 0.90 (0.82, 0.997) | 3.6% |
| **DHA** | | | |
| Q1 | 1.00 (ref.) | 1.00 (ref.) | - |
| Q2 | 0.94 (0.87, 1.01) | 0.94 (0.87, 1.01) | 0.0% |
| Q3 | 0.93 (0.86, 1.01) | 0.93 (0.84, 1.03) | 31.0% |
| Q4 | 0.90 (0.83, 0.97) | 0.90 (0.83, 0.97) | 0.0% |
| Q5 | 0.85 (0.78, 0.93) | 0.85 (0.76, 0.95) | 27.5% |
| **EPA + DHA** | | | |
| Q1 | 1.00 (ref.) | 1.00 (ref.) | - |
| Q2 | 0.92 (0.85, 0.997) | 0.92 (0.85, 0.997) | 0.0% |
| Q3 | 0.94 (0.87, 1.02) | 0.94 (0.84, 1.05) | 36.6% |
| Q4 | 0.89 (0.82, 0.96) | 0.89 (0.80, 0.98) | 25.0% |
| Q5 | 0.85 (0.78, 0.93) | 0.85 (0.74, 0.97) | 47.8% |

**Notes:**
[1] Multiple lipid fractions were available for some studies, but only one lipid fraction was used for the overall analysis.
[2] Effect estimates were pooled using inverse-variance weighted fixed-effect meta-analysis
[3] Effect estimates were pooled using random-effects meta-analysis

**Supplementary Table 4.** Funding information for individual cohorts

| Study | Cohort funding information |
|---|---|
| 60YO | Stockholm County Council, Swedish Heart Lung Foundation, Swedish Research Council, ALF (an agreement between central government and seven regions on physician education and clinical research), The Cardiovascular Program at Karolinska Institutet, The Strategic Research Area in Epidemiology and Biostatistics at Karolinska Institutet. |
| ARIC | The Atherosclerosis Risk in Communities Study is carried out as a collaborative study supported by National Heart, Lung, and Blood Institute contracts (HHSN268201100005C, HHSN268201100006C, HHSN268201100007C, HHSN268201100008C, HHSN268201100009C, HHSN268201100010C, HHSN268201100011C, and HHSN268201100012C), R01HL087641, R01HL59367 and R01HL086694; National Human Genome Research Institute contract U01HG004402; and National Institutes of Health contract HHSN268200625226C. The authors thank the staff and participants of the ARIC study for their important contributions. Infrastructure was partly supported by Grant Number UL1RR025005, a component of the National Institutes of Health and NIH Roadmap for Medical Research. |
| CHS | This Cardiovascular Heart Study research was supported by NHLBI contracts HHSN268201200036C, HHSN268200800007C, N01HC55222, N01HC85079, N01HC85080, N01HC85081, N01HC85082, N01HC85083, N01HC85086; and NHLBI grants HL080295, HL087652, HL105756 with additional contribution from the National Institute of Neurological Disorders and Stroke (NINDS). Additional support was provided through AG023629 from the National Institute on Aging (NIA). A full list of principal CHS investigators and institutions can be found at CHS-NHLBI.org/. The content is solely the responsibility of the authors and does not necessarily represent the official views of the National Institutes of Health. |
| DCH | The Danish Cancer Society funded the DCH cohort. |
| EPIC-Norfolk | The EPIC-Norfolk study (https://doi.org/10.22025/2019.10.105.00004) has received funding from the Medical Research Council (MR/N003284/1) and Cancer Research UK (C864/A14136). Drs Wareham, Forouhi and Imamura has received funding from the Medical Research Council (MC_UU_00006/1 and MC_UU_00006/3). Drs Wareham and Forouhi acknowledge support from National Institute for Health and Care Research (NIHR) Biomedical Research Centre, Cambridge: Nutrition, Diet, and Lifestyle Research Theme (IS-BRC-1215-20014). NGF is an NIHR Senior Investigator. |
| FHS | The Framingham Heart Study is conducted and supported by the National Heart, Lung and Blood Institute (NHLBI) and in collaboration with Boston University (Contract No. N01-HC-25195). |

| | |
|---|---|
| Hisayama | This study was supported in part by Grants-in-Aid for Scientific Research B (JP21H03200), C (JP19K07890, JP20K10503, JP20K11020, JP21K07522, JP21K11725, and JP21K10448), and Early-Career Scientists (JP18K17925) from the Ministry of Education, Culture, Sports, Science and Technology of Japan; by Health and Labour Sciences Research Grants of the Ministry of Health, Labour and Welfare of Japan (20FA1002); and by the Japan Agency for Medical Research and Development (JP21dk0207053). |
| HPFS | The Health Professionals Follow-up Study was supported by research grants U01 CA167552, R01 HL35464, AA11181, CA55075, HL60712, and P30 DK46200 from the National Institutes of Health. |
| KIHD | The KIHD study was supported mainly by funding from the Academy of Finland to Jukka T. Salonen |
| MERLIN TIMI-36 | The MERLIN-TIMI 36 trial was funded by CV Therapeutics. The protocol was developed by the TIMI Study Group in conjunction with the steering committee and review by the trial sponsor. |
| MESA | Supported by contracts HHSN268201500003I, N01-HC-95159, N01-HC-95160, N01-HC-95161, N01-HC-95162, N01-HC-95163, N01-HC-95164, N01-HC-95165, N01-HC-95166, N01-HC-95167, N01-HC-95168 and N01-HC-95169 fromthe National Heart, Lung, and Blood Institute and by grants UL1-TR-000040 and UL1-TR-001079 from NCRR. Theauthors thank the other investigators, the staff, and the participants of the MESA study for their valuable contributions. A full list of participating MESA investigators and institutions can be found at http://www.mesa-nhlbi.org. |
| PIVUS | The study was supported by Uppsala University Hospital and the Swedish Research Council for Health, Working Life and Welfare |
| PRE-DETERMINE | The PRE-DETERMINE Study has received research grant support from the National Heart, Lung, and Blood Institute (R01HL091069, R01HL165840), St Jude Medical Inc, St Jude Medical Foundation, Roche Diagnostics, and Abbott. |
| RS | The Rotterdam Study is funded by Erasmus Medical Center and Erasmus University, Rotterdam, Netherlands Organization for the Health Research and Development (ZonMw), the Research Institute for Diseases in the Elderly (RIDE), the Ministry of Education, Culture and Science, the Ministry for Health, Welfare and Sports, the European Commission (DG XII), and the Municipality of Rotterdam. The authors are grateful to the study participants, the staff from the Rotterdam Study and the participating general practitioners and pharmacists. |
| RUTI-HF | This work was supported by Instituto de Salud Carlos III (ISCIII, Spain), including CIBER Cardiovascular [CB16/11/00403] project, as a part of the National R&D&I Plan; and ISCIII-Sub-Directorate General for Research Assessment and Promotion and the European Regional Development Fund (ERDF) |
| ULSAM | The Uppsala Longitudinal Studies of Adult Men 50 and 70 were funded by Uppsala City Council; The Swedish Research Council (K2015-54X-22081-04-3); and The Swedish Research Council for Environment, Agricultural Sciences and Spatial Planning (2016-01639). |
| WHIMS | The WHI program is funded by the National Heart, Lung, and Blood Institute, National Institutes of Health, U.S. Department of Health and Human Services through contracts HHSN268201600018C, HHSN268201600001C, HHSN268201600002C, HHSN268201600003C, and HHSN268201600004C |

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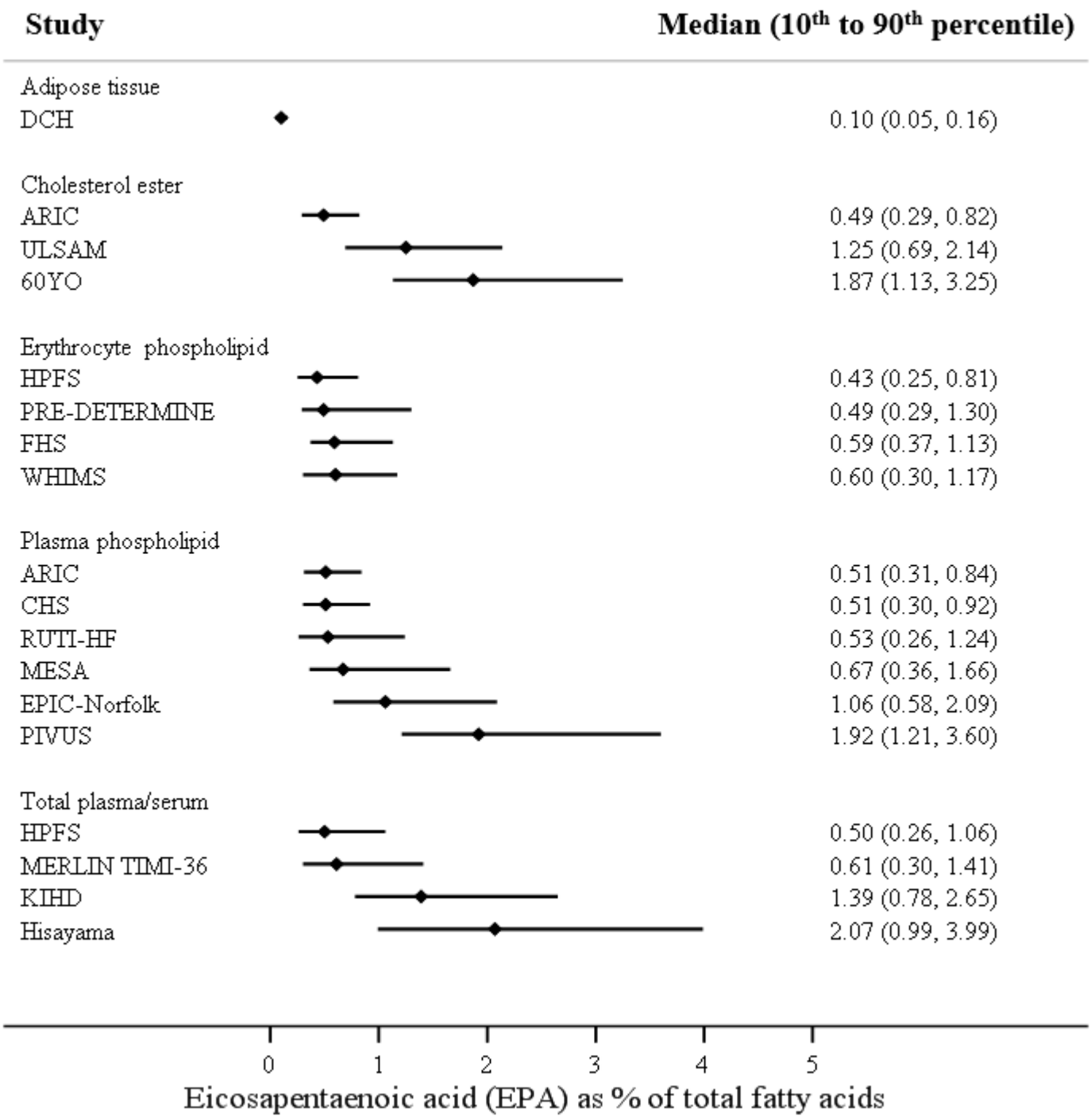
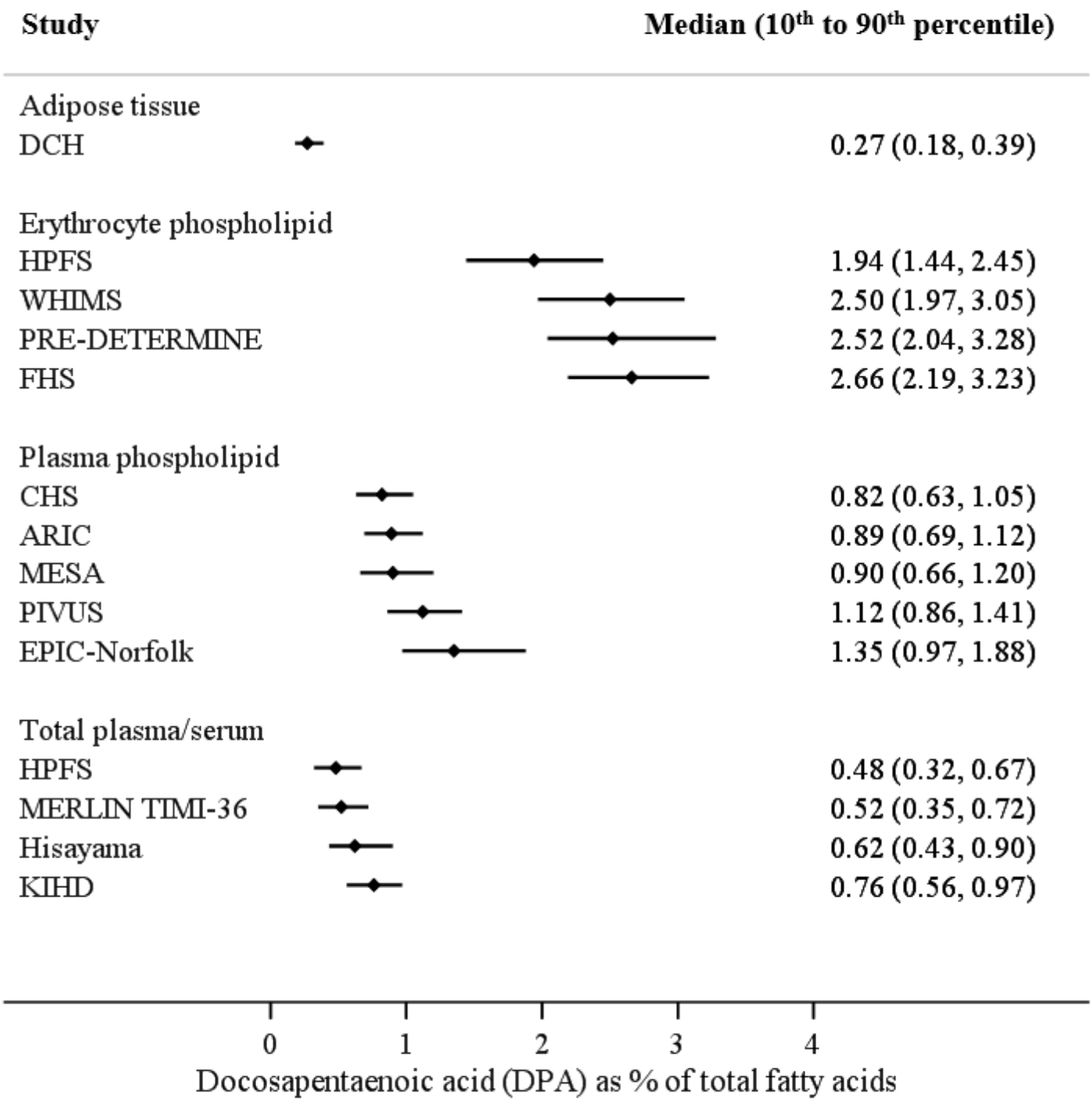
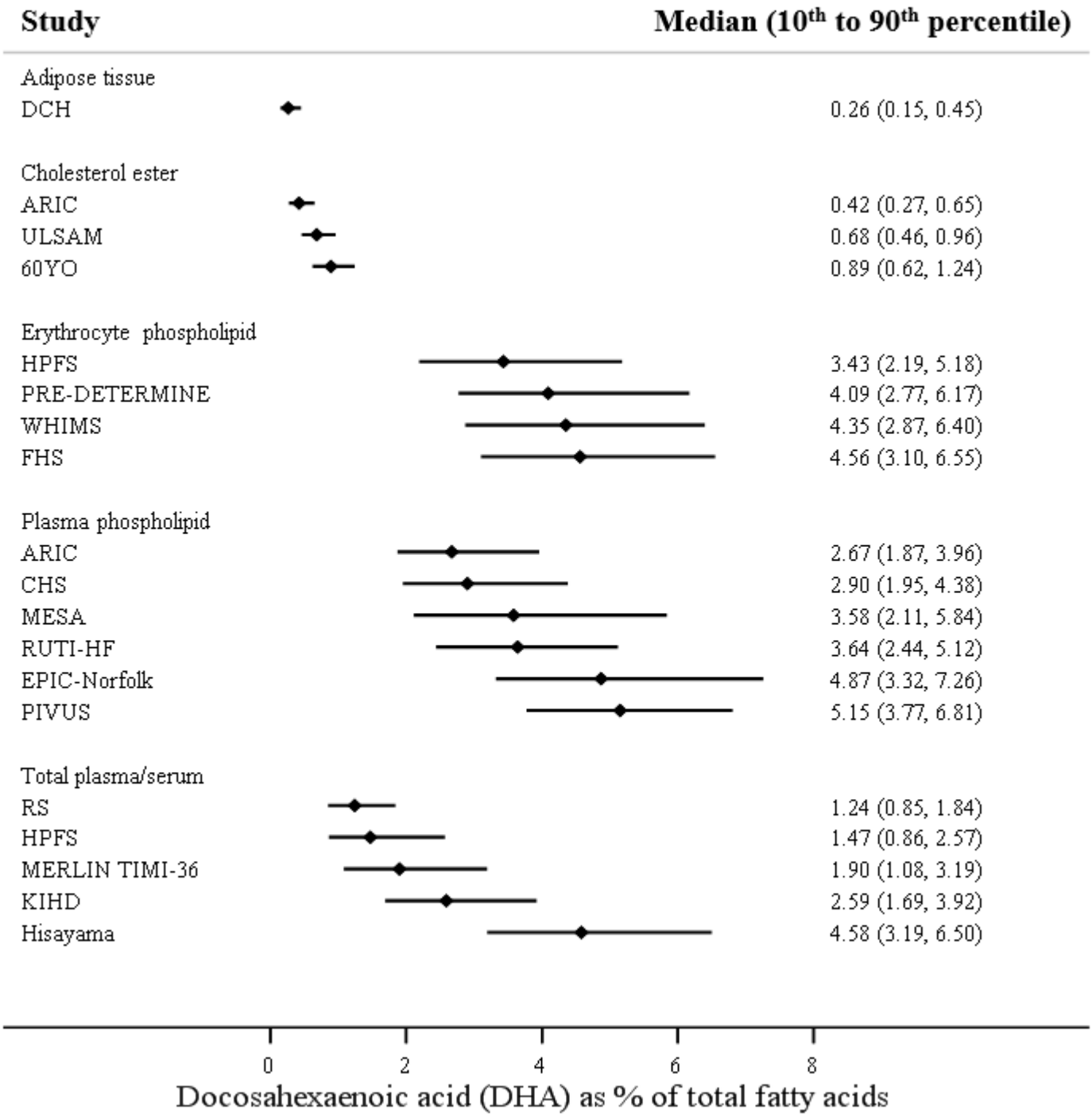


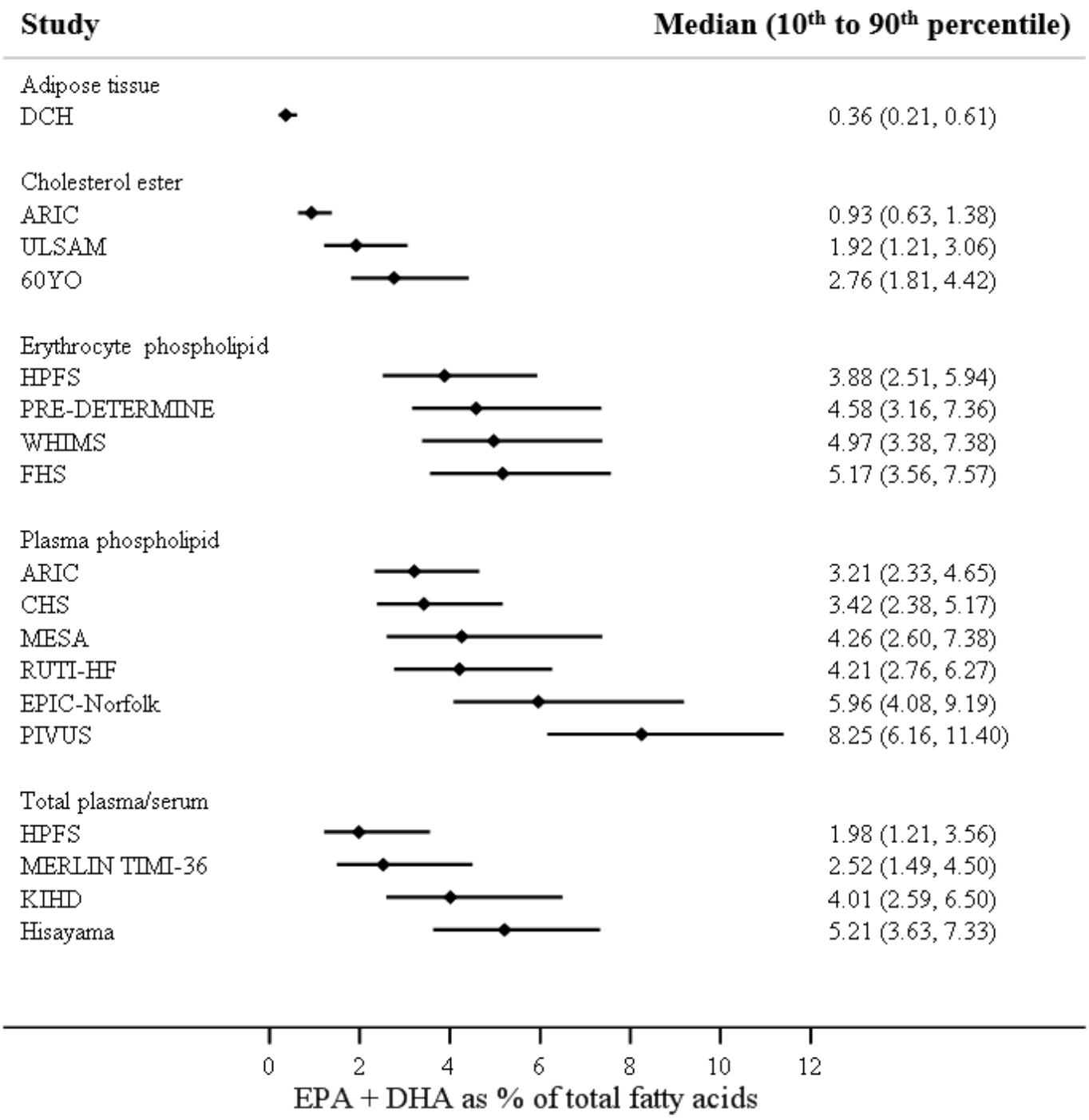

**Supplementary Figure 1(A).** Relative concentration of EPA in different lipid fractions for the 16 participating cohorts. Values in the graph represent median (point) and $10^{th}$-$90^{th}$ percentile ranges.



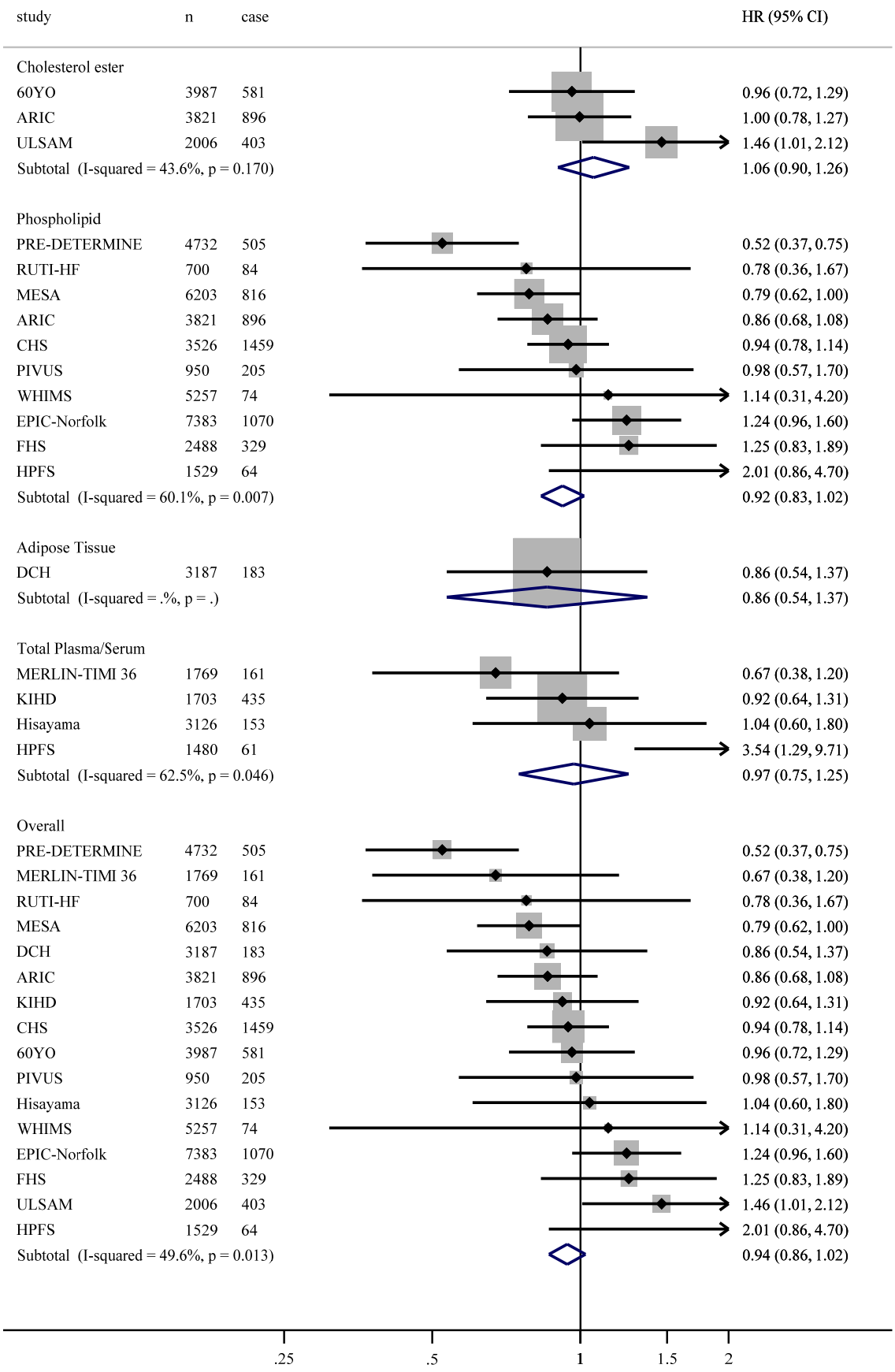

**Supplementary Figure 1(B).** Relative concentration of DPA in different lipid fractions for the 13 participating cohorts. Values in the graph represent median (point) and $10^{th}$-$90^{th}$ percentile ranges.



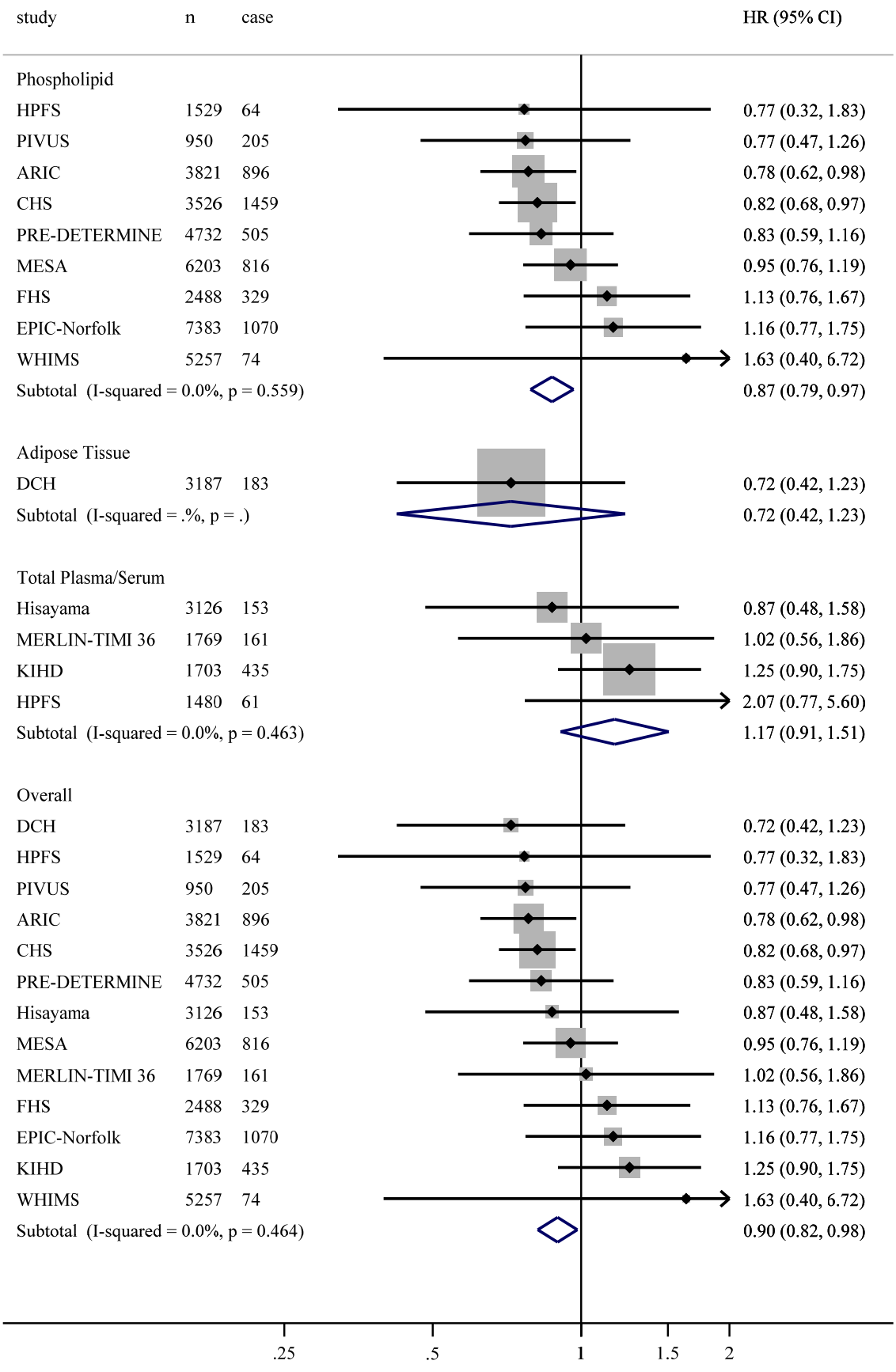

**Supplementary Figure 1(C).** Relative concentration of DHA in different lipid fractions for the 17 participating cohorts. Values in the graph represent median (point) and $10^{th}$-$90^{th}$ percentile ranges.



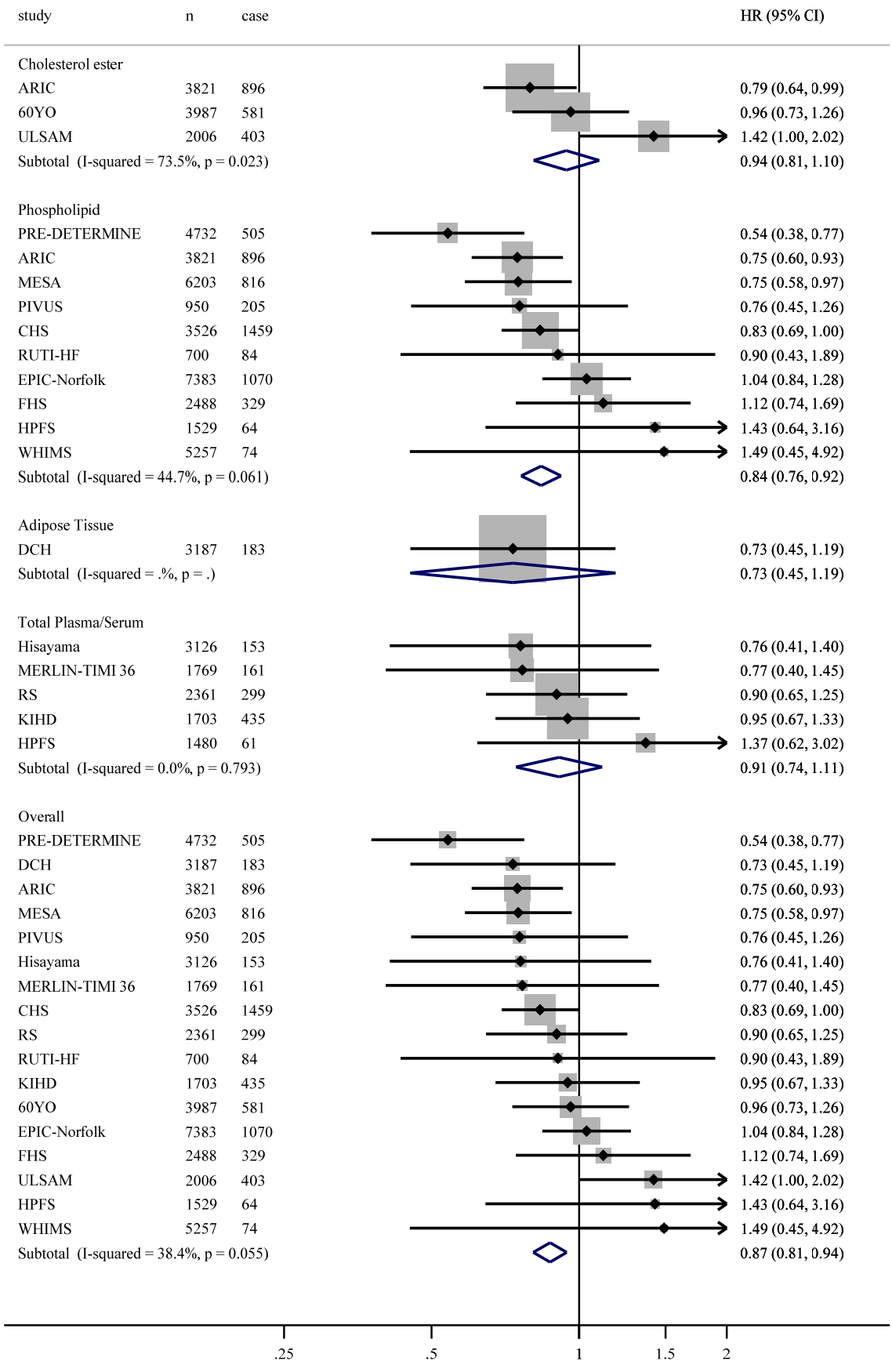

**Supplementary Figure 1(D).** Relative concentration of EPA+DHA in different lipid fractions for the 16 participating cohorts. Values in the graph represent median (point) and $10^{th}$-$90^{th}$ percentile ranges.


study
n
case
HR (95% CI)
Cholesterol ester
60YO 3987 581 0.96 (0.72, 1.29)
ARIC 3821 896 1.00 (0.78, 1.27)
ULSAM 2006 403 1.46 (1.01, 2.12)
Subtotal (I-squared = 43.6%, p = 0.170) 1.06 (0.90, 1.26)
Phospholipid
PRE-DETERMINE 4732 505 0.52 (0.37, 0.75)
RUTI-HF 700 84 0.78 (0.36, 1.67)
MESA 6203 816 0.79 (0.62, 1.00)
ARIC 3821 896 0.86 (0.68, 1.08)
CHS 3526 1459 0.94 (0.78, 1.14)
PIVUS 950 205 0.98 (0.57, 1.70)
WHIMS 5257 74 1.14 (0.31, 4.20)
EPIC-Norfolk 7383 1070 1.24 (0.96, 1.60)
FHS 2488 329 1.25 (0.83, 1.89)
HPFS 1529 64 2.01 (0.86, 4.70)
Subtotal (I-squared = 60.1%, p = 0.007) 0.92 (0.83, 1.02)
Adipose Tissue
DCH 3187 183 0.86 (0.54, 1.37)
Subtotal (I-squared = .%, p = .) 0.86 (0.54, 1.37)
Total Plasma/Serum
MERLIN-TIMI 36 1769 161 0.67 (0.38, 1.20)
KIHD 1703 435 0.92 (0.64, 1.31)
Hisayama 3126 153 1.04 (0.60, 1.80)
HPFS 1480 61 3.54 (1.29, 9.71)
Subtotal (I-squared = 62.5%, p = 0.046) 0.97 (0.75, 1.25)
Overall
PRE-DETERMINE 4732 505 0.52 (0.37, 0.75)
MERLIN-TIMI 36 1769 161 0.67 (0.38, 1.20)
RUTI-HF 700 84 0.78 (0.36, 1.67)
MESA 6203 816 0.79 (0.62, 1.00)
DCH 3187 183 0.86 (0.54, 1.37)
ARIC 3821 896 0.86 (0.68, 1.08)
KIHD 1703 435 0.92 (0.64, 1.31)
CHS 3526 1459 0.94 (0.78, 1.14)
60YO 3987 581 0.96 (0.72, 1.29)
PIVUS 950 205 0.98 (0.57, 1.70)
Hisayama 3126 153 1.04 (0.60, 1.80)
WHIMS 5257 74 1.14 (0.31, 4.20)
EPIC-Norfolk 7383 1070 1.24 (0.96, 1.60)
FHS 2488 329 1.25 (0.83, 1.89)
ULSAM 2006 403 1.46 (1.01, 2.12)
HPFS 1529 64 2.01 (0.86, 4.70)
Subtotal (I-squared = 49.6%, p = 0.013) 0.94 (0.86, 1.02)
.25
.5
1
1.5
2

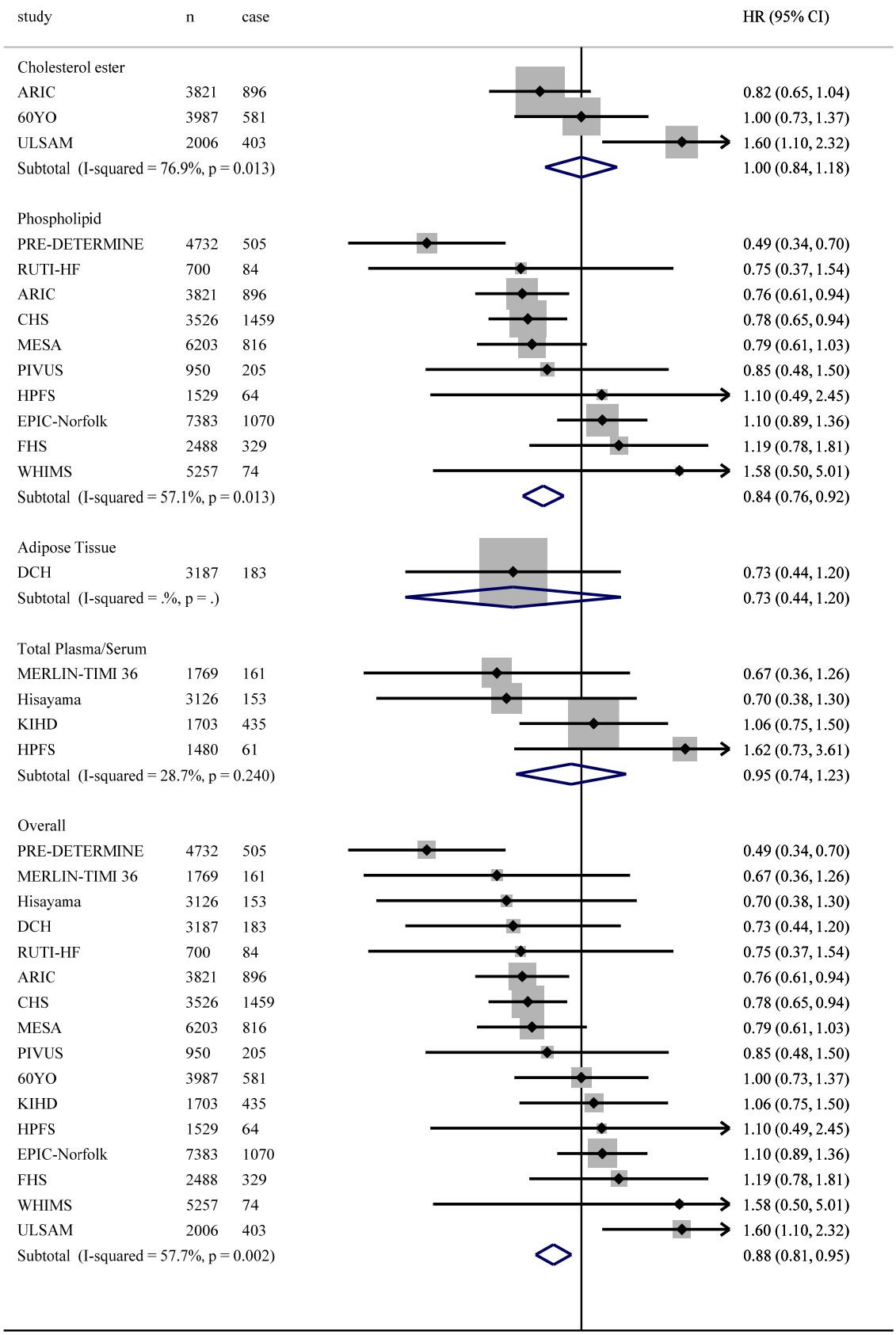
**Supplementary Figure 2.** Pooled hazard ratio of incident atrial fibrillation (AF) comparing Q5 versus Q1 for EPA biomarker. The association between EPA and AF was assessed in multivariable models for each cohort, and the results were pooled using inverse-variance weighted fixed-effect meta-analysis. In each cohort, a multivariable model was used to assess the association with adjustment for age, sex, field site (if applicable), race/ethnicity, education, smoking, alcohol use, BMI, beta blocker use, linoleic acid (LA; 18:2n-6) biomarker concentration, arachidonic acid (AA; 20:4n-6) biomarker concentration, and prevalent hypertension, dyslipidemia, diabetes mellitus, atherosclerotic cardiovascular disease (defined as the presence of one or more of the following: coronary heart disease, stroke, or peripheral artery disease), and heart failure. If multiple biomarkers were available for a study, only one was used for the overall analysis based on the best ability to reflect long-term dietary intake (in the following order of preference): adipose tissue, erythrocyte phospholipids, plasma phospholipids, total plasma/serum, and cholesterol ester. Please note that phospholipids include both erythrocyte and plasma/serum phospholipids. Abbreviations for individual cohorts are spelled out in the footnote for Table 1.


study
n
case
HR (95% CI)
Phospholipid
HPFS 1529 64 0.77 (0.32, 1.83)
PIVUS 950 205 0.77 (0.47, 1.26)
ARIC 3821 896 0.78 (0.62, 0.98)
CHS 3526 1459 0.82 (0.68, 0.97)
PRE-DETERMINE 4732 505 0.83 (0.59, 1.16)
MESA 6203 816 0.95 (0.76, 1.19)
FHS 2488 329 1.13 (0.76, 1.67)
EPIC-Norfolk 7383 1070 1.16 (0.77, 1.75)
WHIMS 5257 74 1.63 (0.40, 6.72)
Subtotal (I-squared = 0.0%, p = 0.559) 0.87 (0.79, 0.97)
Adipose Tissue
DCH 3187 183 0.72 (0.42, 1.23)
Subtotal (I-squared = .%, p = .) 0.72 (0.42, 1.23)
Total Plasma/Serum
Hisayama 3126 153 0.87 (0.48, 1.58)
MERLIN-TIMI 36 1769 161 1.02 (0.56, 1.86)
KIHD 1703 435 1.25 (0.90, 1.75)
HPFS 1480 61 2.07 (0.77, 5.60)
Subtotal (I-squared = 0.0%, p = 0.463) 1.17 (0.91, 1.51)
Overall
DCH 3187 183 0.72 (0.42, 1.23)
HPFS 1529 64 0.77 (0.32, 1.83)
PIVUS 950 205 0.77 (0.47, 1.26)
ARIC 3821 896 0.78 (0.62, 0.98)
CHS 3526 1459 0.82 (0.68, 0.97)
PRE-DETERMINE 4732 505 0.83 (0.59, 1.16)
Hisayama 3126 153 0.87 (0.48, 1.58)
MESA 6203 816 0.95 (0.76, 1.19)
MERLIN-TIMI 36 1769 161 1.02 (0.56, 1.86)
FHS 2488 329 1.13 (0.76, 1.67)
EPIC-Norfolk 7383 1070 1.16 (0.77, 1.75)
KIHD 1703 435 1.25 (0.90, 1.75)
WHIMS 5257 74 1.63 (0.40, 6.72)
Subtotal (I-squared = 0.0%, p = 0.464) 0.90 (0.82, 0.98)
.25
.5
1
1.5
2

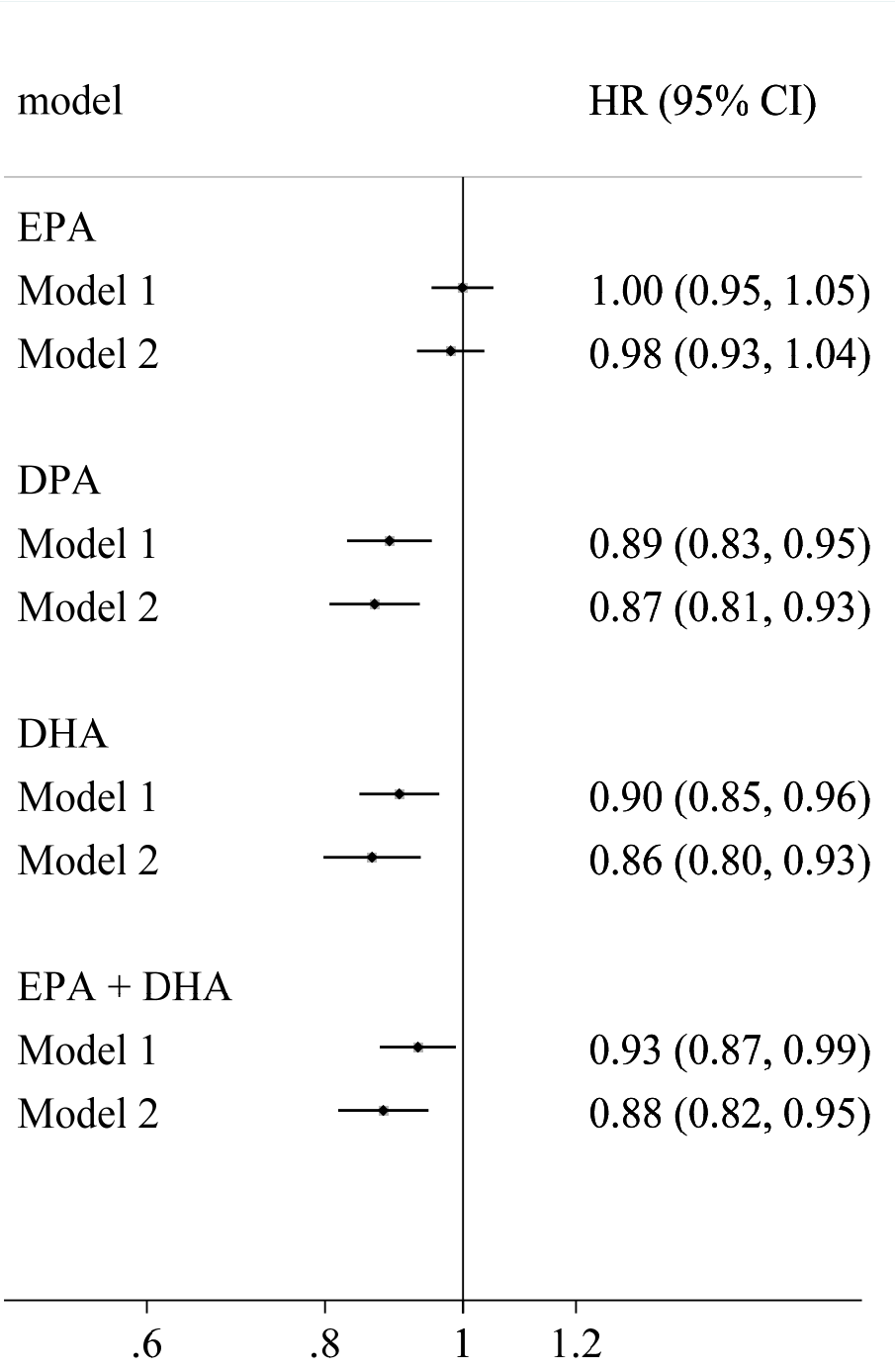
**Supplementary Figure 3.** Pooled hazard ratio of incident atrial fibrillation (AF) comparing Q5 versus Q1 for DPA biomarker. The association between EPA and AF was assessed in multivariable models for each cohort, and the results were pooled using inverse-variance weighted fixed-effect meta-analysis. In each cohort, a multivariable model was used to assess the association with adjustment for age, sex, field site (if applicable), race/ethnicity, education, smoking, alcohol use, BMI, beta blocker use, linoleic acid (LA; 18:2n-6) biomarker concentration, arachidonic acid (AA; 20:4n-6) biomarker concentration, and prevalent hypertension, dyslipidemia, diabetes mellitus, atherosclerotic cardiovascular disease (defined as the presence of one or more of the following: coronary heart disease, stroke, or peripheral artery disease), and heart failure. If multiple biomarkers were available for a study, only one was used for the overall analysis based on the best ability to reflect long-term dietary intake (in the following order of preference): adipose tissue, erythrocyte phospholipids, plasma phospholipids, total plasma/serum, and cholesterol ester. Please note that phospholipids include both erythrocyte and plasma/serum phospholipids. Abbreviations for individual cohorts are spelled out in the footnote for Table 1.


study
n
case
HR (95% CI)
Cholesterol ester
ARIC 3821 896 0.79 (0.64, 0.99)
60YO 3987 581 0.96 (0.73, 1.26)
ULSAM 2006 403 1.42 (1.00, 2.02)
Subtotal (I-squared = 73.5%, p = 0.023) 0.94 (0.81, 1.10)
Phospholipid
PRE-DETERMINE 4732 505 0.54 (0.38, 0.77)
ARIC 3821 896 0.75 (0.60, 0.93)
MESA 6203 816 0.75 (0.58, 0.97)
PIVUS 950 205 0.76 (0.45, 1.26)
CHS 3526 1459 0.83 (0.69, 1.00)
RUTI-HF 700 84 0.90 (0.43, 1.89)
EPIC-Norfolk 7383 1070 1.04 (0.84, 1.28)
FHS 2488 329 1.12 (0.74, 1.69)
HPFS 1529 64 1.43 (0.64, 3.16)
WHIMS 5257 74 1.49 (0.45, 4.92)
Subtotal (I-squared = 44.7%, p = 0.061) 0.84 (0.76, 0.92)
Adipose Tissue
DCH 3187 183 0.73 (0.45, 1.19)
Subtotal (I-squared = .%, p = .) 0.73 (0.45, 1.19)
Total Plasma/Serum
Hisayama 3126 153 0.76 (0.41, 1.40)
MERLIN-TIMI 36 1769 161 0.77 (0.40, 1.45)
RS 2361 299 0.90 (0.65, 1.25)
KIHD 1703 435 0.95 (0.67, 1.33)
HPFS 1480 61 1.37 (0.62, 3.02)
Subtotal (I-squared = 0.0%, p = 0.793) 0.91 (0.74, 1.11)
Overall
PRE-DETERMINE 4732 505 0.54 (0.38, 0.77)
DCH 3187 183 0.73 (0.45, 1.19)
ARIC 3821 896 0.75 (0.60, 0.93)
MESA 6203 816 0.75 (0.58, 0.97)
PIVUS 950 205 0.76 (0.45, 1.26)
Hisayama 3126 153 0.76 (0.41, 1.40)
MERLIN-TIMI 36 1769 161 0.77 (0.40, 1.45)
CHS 3526 1459 0.83 (0.69, 1.00)
RS 2361 299 0.90 (0.65, 1.25)
RUTI-HF 700 84 0.90 (0.43, 1.89)
KIHD 1703 435 0.95 (0.67, 1.33)
60YO 3987 581 0.96 (0.73, 1.26)
EPIC-Norfolk 7383 1070 1.04 (0.84, 1.28)
FHS 2488 329 1.12 (0.74, 1.69)
ULSAM 2006 403 1.42 (1.00, 2.02)
HPFS 1529 64 1.43 (0.64, 3.16)
WHIMS 5257 74 1.49 (0.45, 4.92)
Subtotal (I-squared = 38.4%, p = 0.055) 0.87 (0.81, 0.94)
.25
.5
1
1.5
2

**Supplementary Figure 4.** Pooled hazard ratio of incident atrial fibrillation (AF) comparing Q5 versus Q1 for DHA biomarker. The association between EPA and AF was assessed in multivariable models for each cohort, and the results were pooled using inverse-variance weighted fixed-effect meta-analysis. In each cohort, a multivariable model was used to assess the association with adjustment for age, sex, field site (if applicable), race/ethnicity, education, smoking, alcohol use, BMI, beta blocker use, linoleic acid (LA; 18:2n-6) biomarker concentration, arachidonic acid (AA; 20:4n-6) biomarker concentration, and prevalent hypertension, dyslipidemia, diabetes mellitus, atherosclerotic cardiovascular disease (defined as the presence of one or more of the following: coronary heart disease, stroke, or peripheral artery disease), and heart failure. If multiple biomarkers were available for a study, only one was used for the overall analysis based on the best ability to reflect long-term dietary intake (in the following order of preference): adipose tissue, erythrocyte phospholipids, plasma phospholipids, total plasma/serum, and cholesterol ester. Please note that phospholipids include both erythrocyte and plasma/serum phospholipids. Abbreviations for individual cohorts are spelled out in the footnote for Table 1.

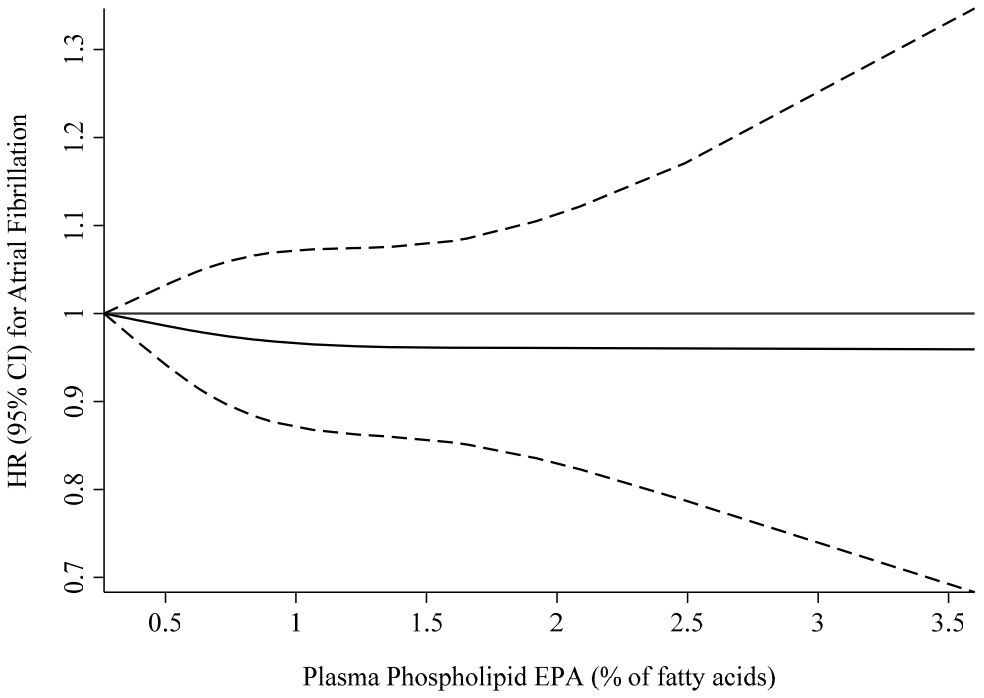

study
n
case
HR (95% CI)
Cholesterol ester
ARIC 3821 896 0.82 (0.65, 1.04)
60YO 3987 581 1.00 (0.73, 1.37)
ULSAM 2006 403 1.60 (1.10, 2.32)
Subtotal (I-squared = 76.9%, p = 0.013) 1.00 (0.84, 1.18)
Phospholipid
PRE-DETERMINE 4732 505 0.49 (0.34, 0.70)
RUTI-HF 700 84 0.75 (0.37, 1.54)
ARIC 3821 896 0.76 (0.61, 0.94)
CHS 3526 1459 0.78 (0.65, 0.94)
MESA 6203 816 0.79 (0.61, 1.03)
PIVUS 950 205 0.85 (0.48, 1.50)
HPFS 1529 64 1.10 (0.49, 2.45)
EPIC-Norfolk 7383 1070 1.10 (0.89, 1.36)
FHS 2488 329 1.19 (0.78, 1.81)
WHIMS 5257 74 1.58 (0.50, 5.01)
Subtotal (I-squared = 57.1%, p = 0.013) 0.84 (0.76, 0.92)
Adipose Tissue
DCH 3187 183 0.73 (0.44, 1.20)
Subtotal (I-squared = .%, p = .) 0.73 (0.44, 1.20)
Total Plasma/Serum
MERLIN-TIMI 36 1769 161 0.67 (0.36, 1.26)
Hisayama 3126 153 0.70 (0.38, 1.30)
KIHD 1703 435 1.06 (0.75, 1.50)
HPFS 1480 61 1.62 (0.73, 3.61)
Subtotal (I-squared = 28.7%, p = 0.240) 0.95 (0.74, 1.23)
Overall
PRE-DETERMINE 4732 505 0.49 (0.34, 0.70)
MERLIN-TIMI 36 1769 161 0.67 (0.36, 1.26)
Hisayama 3126 153 0.70 (0.38, 1.30)
DCH 3187 183 0.73 (0.44, 1.20)
RUTI-HF 700 84 0.75 (0.37, 1.54)
ARIC 3821 896 0.76 (0.61, 0.94)
CHS 3526 1459 0.78 (0.65, 0.94)
MESA 6203 816 0.79 (0.61, 1.03)
PIVUS 950 205 0.85 (0.48, 1.50)
60YO 3987 581 1.00 (0.73, 1.37)
KIHD 1703 435 1.06 (0.75, 1.50)
HPFS 1529 64 1.10 (0.49, 2.45)
EPIC-Norfolk 7383 1070 1.10 (0.89, 1.36)
FHS 2488 329 1.19 (0.78, 1.81)
WHIMS 5257 74 1.58 (0.50, 5.01)
ULSAM 2006 403 1.60 (1.10, 2.32)
Subtotal (I-squared = 57.7%, p = 0.002) 0.88 (0.81, 0.95)

**Supplementary Figure 5.** Pooled hazard ratio of incident atrial fibrillation (AF) comparing Q5 versus Q1 for EPA+DHA biomarker. The association between EPA and AF was assessed in multivariable models for each cohort, and the results were pooled using inverse-variance weighted fixed-effect meta-analysis. In each cohort, a multivariable model was used to assess the association with adjustment for age, sex, field site (if applicable), race/ethnicity, education, smoking, alcohol use, BMI, beta blocker use, linoleic acid (LA; 18:2n-6) biomarker concentration, arachidonic acid (AA; 20:4n-6) biomarker concentration, and prevalent hypertension, dyslipidemia, diabetes mellitus, atherosclerotic cardiovascular disease (defined as the presence of one or more of the following: coronary heart disease, stroke, or peripheral artery disease), and heart failure. If multiple biomarkers were available for a study, only one was used for the overall analysis based on the best ability to reflect long-term dietary intake (in the following order of preference): adipose tissue, erythrocyte phospholipids, plasma phospholipids, total plasma/serum, and cholesterol ester. Please note that phospholipids include both erythrocyte and plasma/serum phospholipids. Abbreviations for individual cohorts are spelled out in the footnote for Table 1.

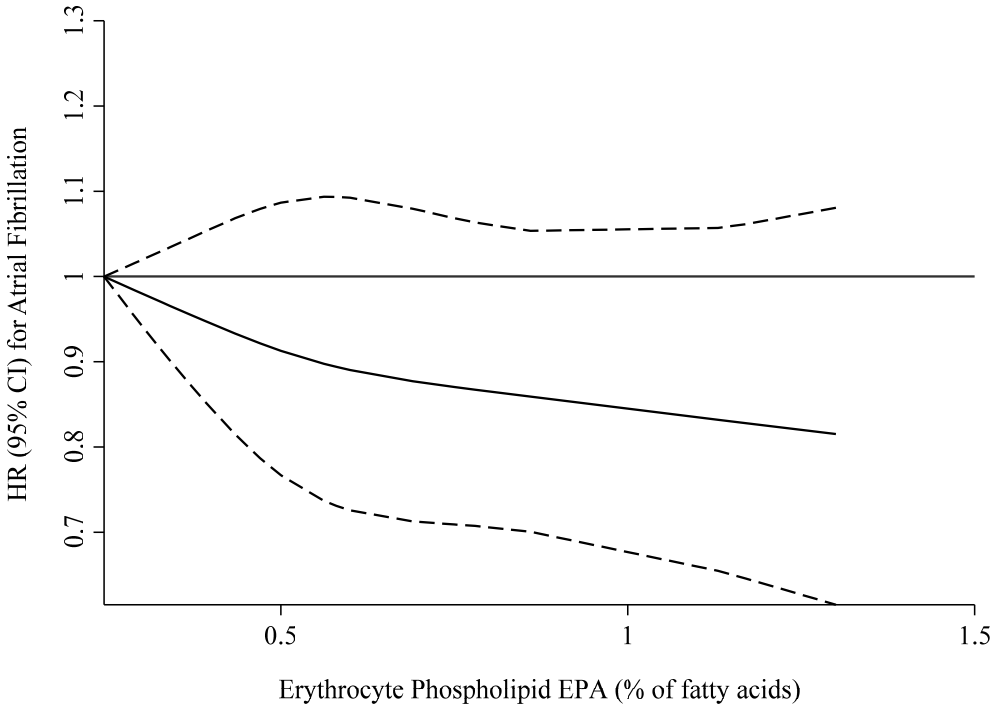



**Supplementary Figure 6.** Sensitivity analysis of omega-3 fatty acid biomarkers and incident atrial fibrillation (AF) with or without additional adjustment for dietary intakes. The association between each omega-3 fatty acid and atrial fibrillation was assessed in multivariable models for each cohort per interquintile range (difference between the $90^{th}$ and $10^{th}$ percentiles for each fatty acid), and the results were pooled using inverse-variance weighted fixed effects meta-analysis. In each cohort, multivariate RR was assessed adjusting for – Model 1: age, sex, field site (if applicable), race/ethnicity, education, smoking, alcohol use, BMI, beta blocker use, linoleic acid (LA; 18:2n-6) biomarker concentration, arachidonic acid (AA; 20:4n-6) biomarker concentration, and prevalent hypertension, dyslipidemia, diabetes mellitus, atherosclerotic cardiovascular disease (defined as the presence of one or more of the following: coronary heart disease, stroke, or peripheral artery disease), and heart failure. Model 2 additionally adjusted for intakes of fish/seafood, fruits, vegetables, and coffee/tea (servings/day or grams/day) (13 cohorts had available dietary information).

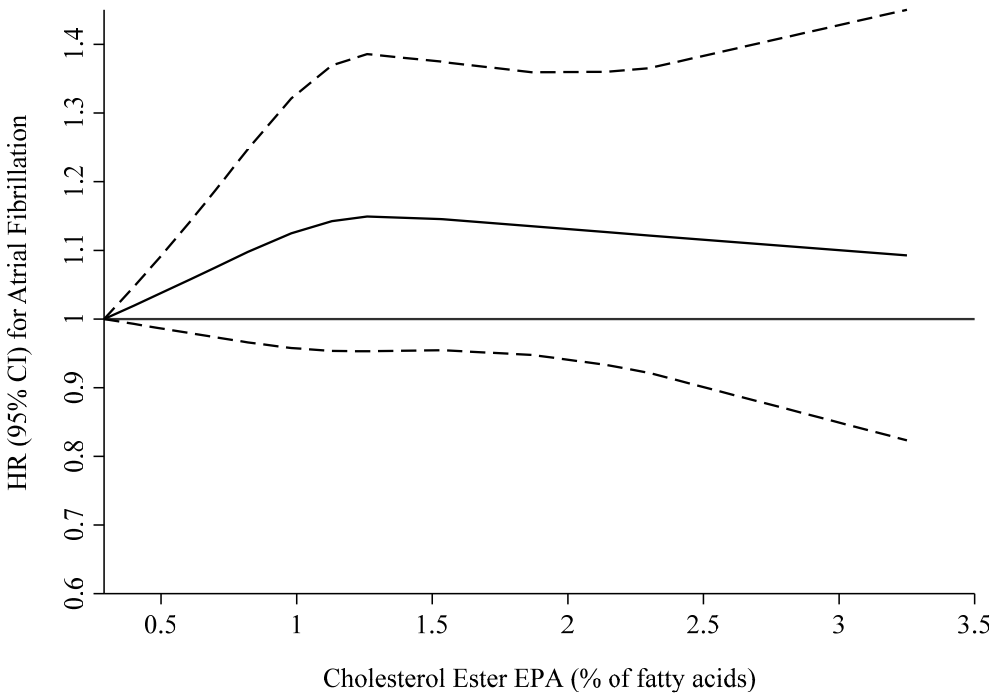
**Supplementary Figure 7(A-N).** Restricted cubic splines for individual omega-3 fatty acid biomarkers and incident atrial fibrillation (AF) by lipid fraction.



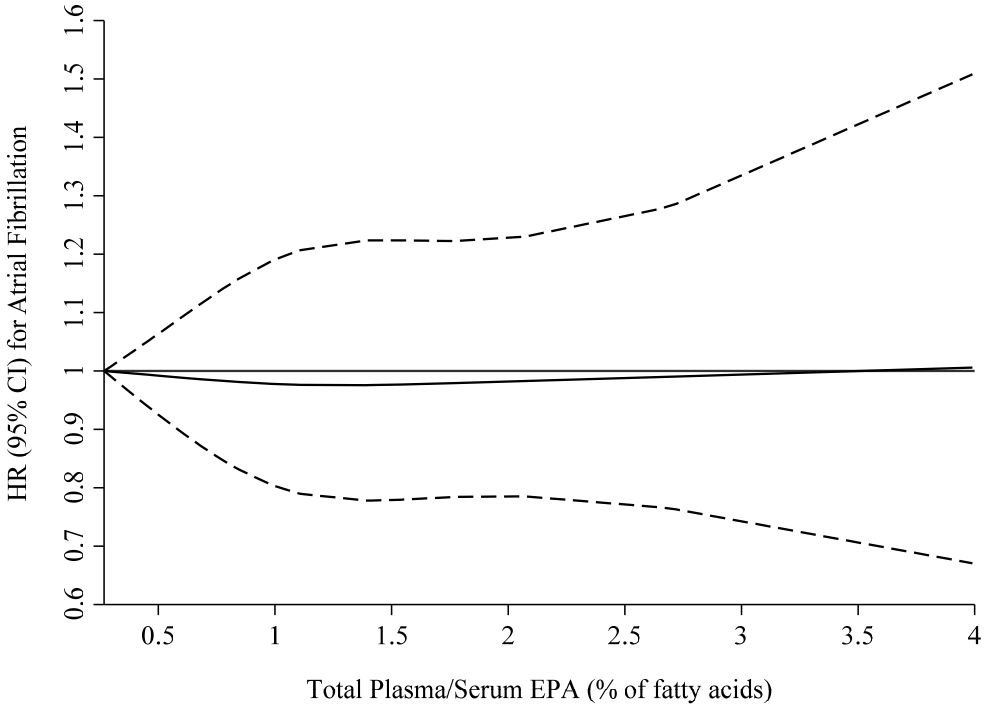

**(A)** EPA – Plasma Phospholipids ($P_{linear}$ = 0.56, $P_{non\text{-}linear}$ = 0.68)



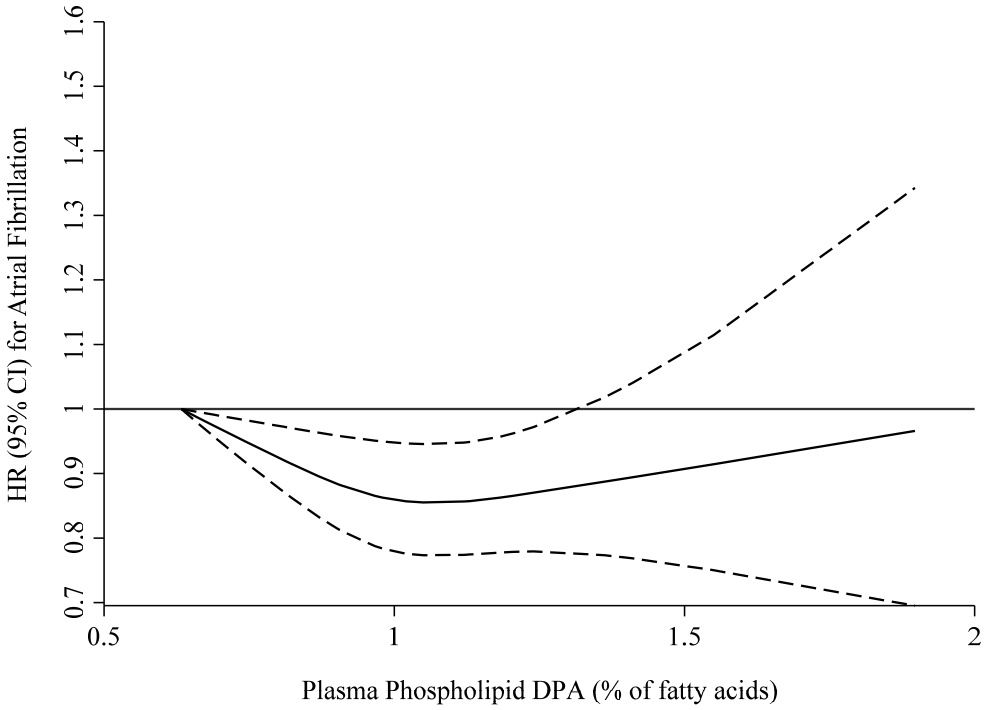

**(B)** EPA – Erythrocyte Phospholipids ($P_{linear}$ = 0.12, $P_{non\text{-}linear}$ = 0.59)



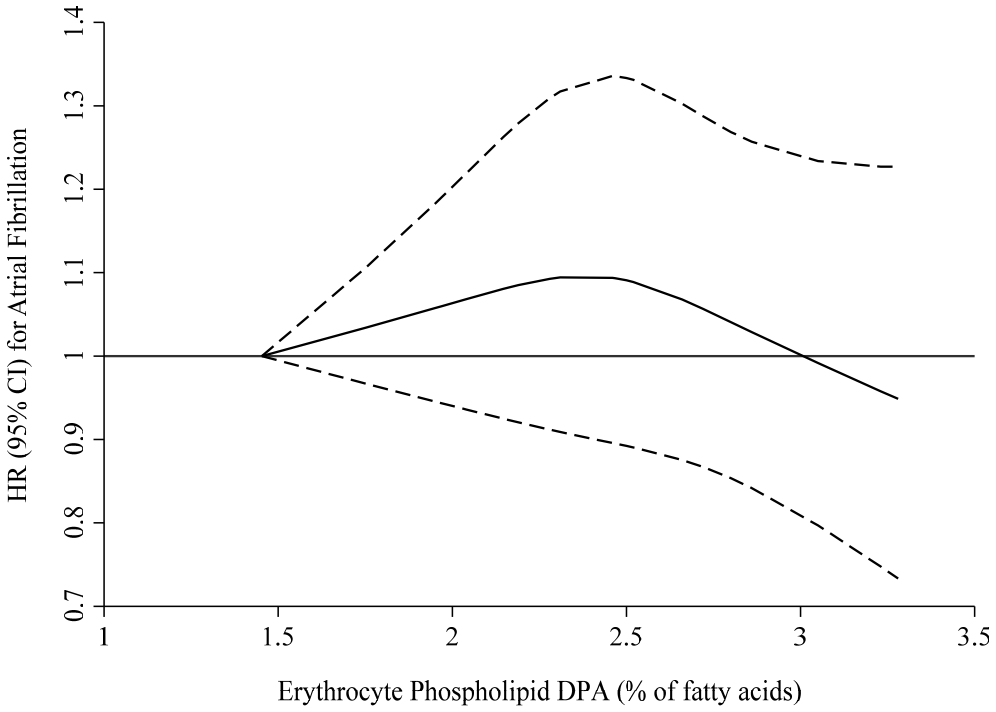

**(C)** EPA, Cholesterol Esters ($P_{linear}$ = 0.35, $P_{non\text{-}linear}$ = 0.18)



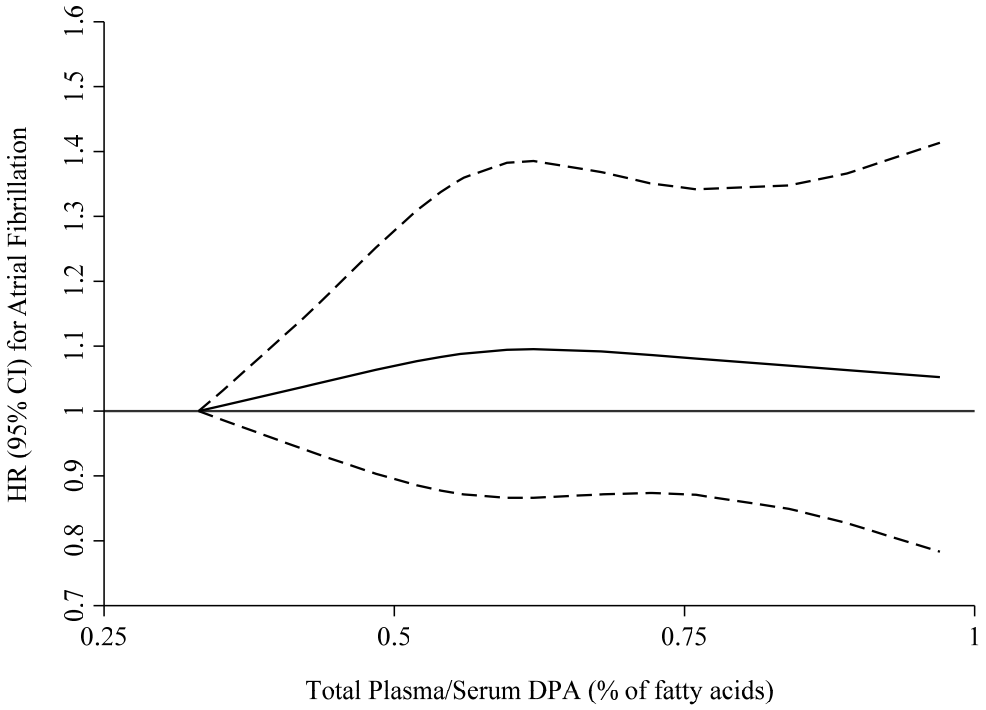

**(D)** EPA – Total Plasma/Serum ($P_{linear}$ = 0.97, $P_{non\text{-}linear}$ = 0.80)



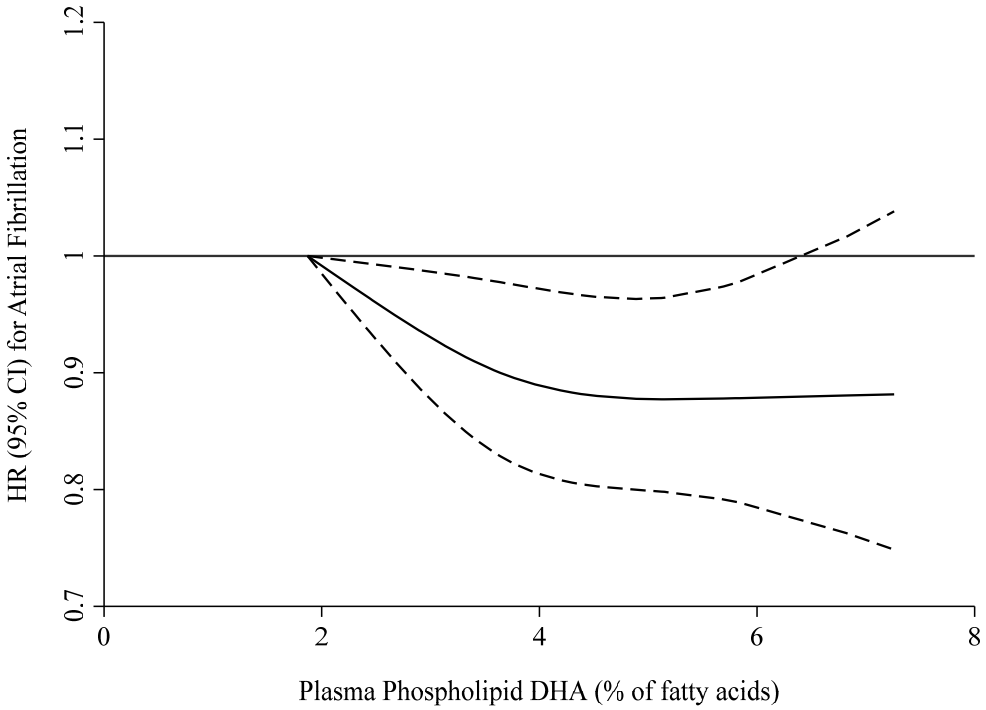

**(E)** DPA – Plasma Phospholipids ($P_{linear}$ = 0.01, $P_{non\text{-}linear}$ = 0.04)



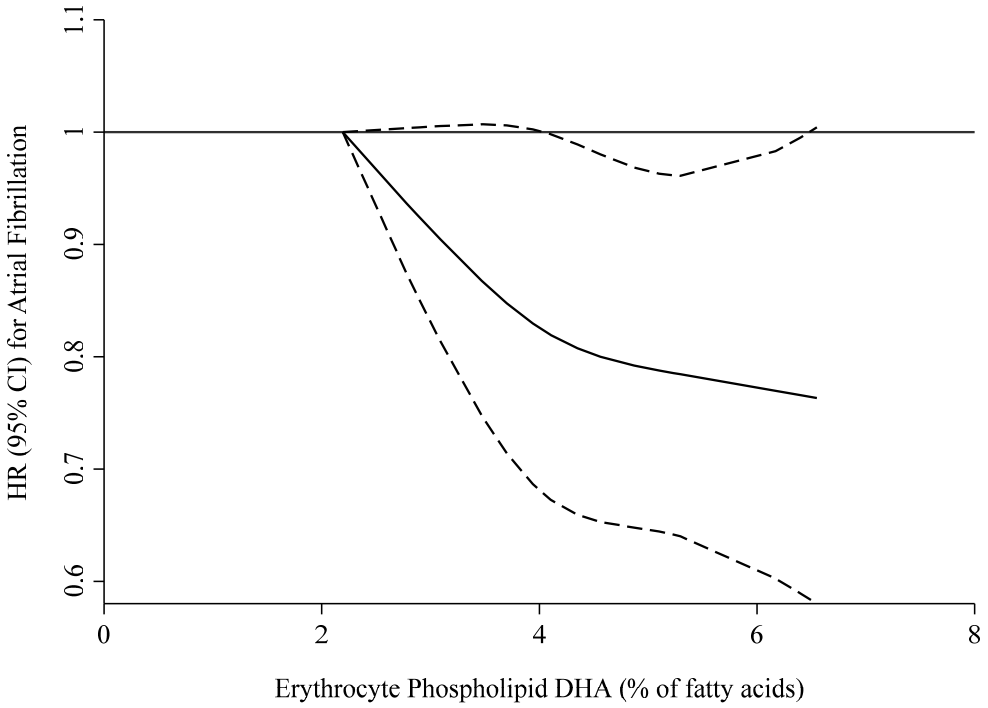

**(F)** DPA – Erythrocyte Phospholipids ($P_{linear}$ = 0.92, $P_{non\text{-}linear}$ = 0.16)



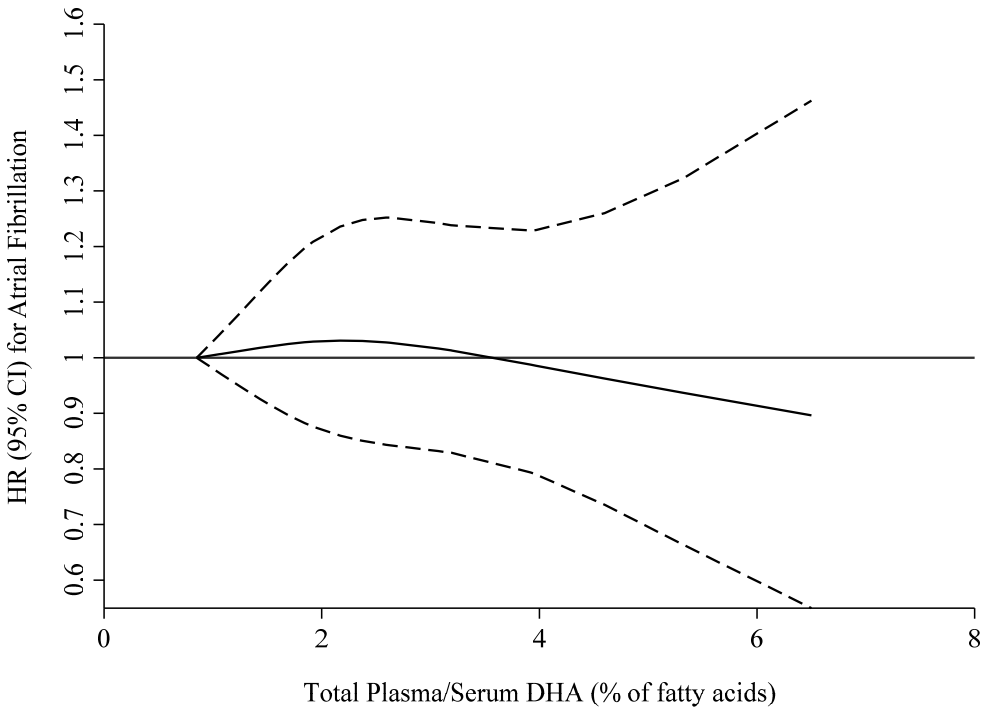

**(G)** DPA – Total Plasma/Serum ($P_{linear}$ = 0.60, $P_{non\text{-}linear}$ = 0.53)



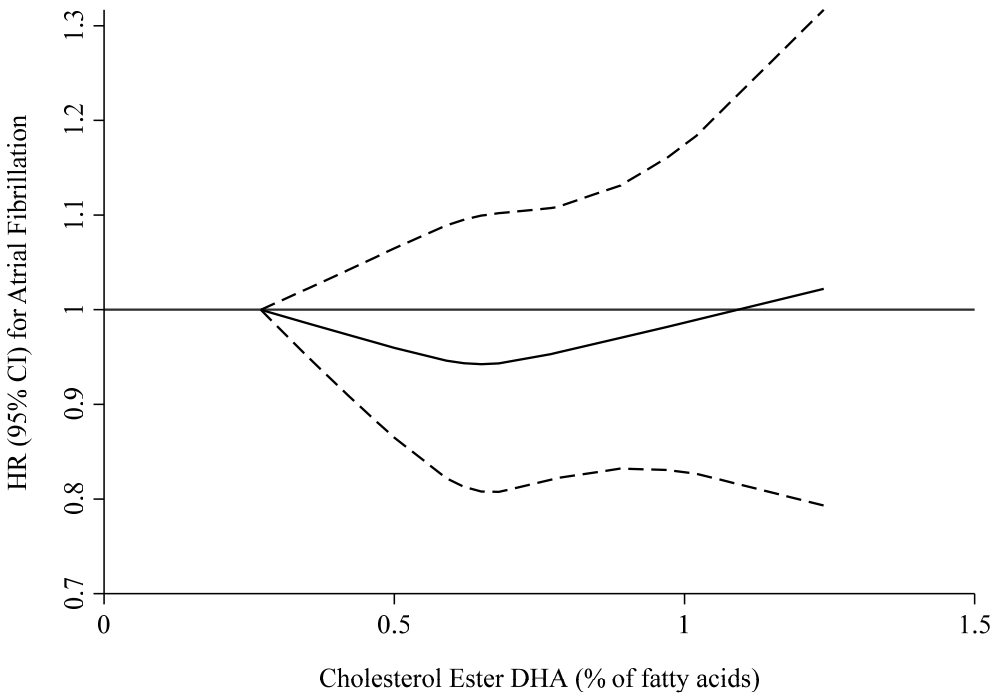

**(H)** DHA – Plasma Phospholipids ($P_{linear}$ = 0.01, $P_{non\text{-}linear}$ = 0.15)



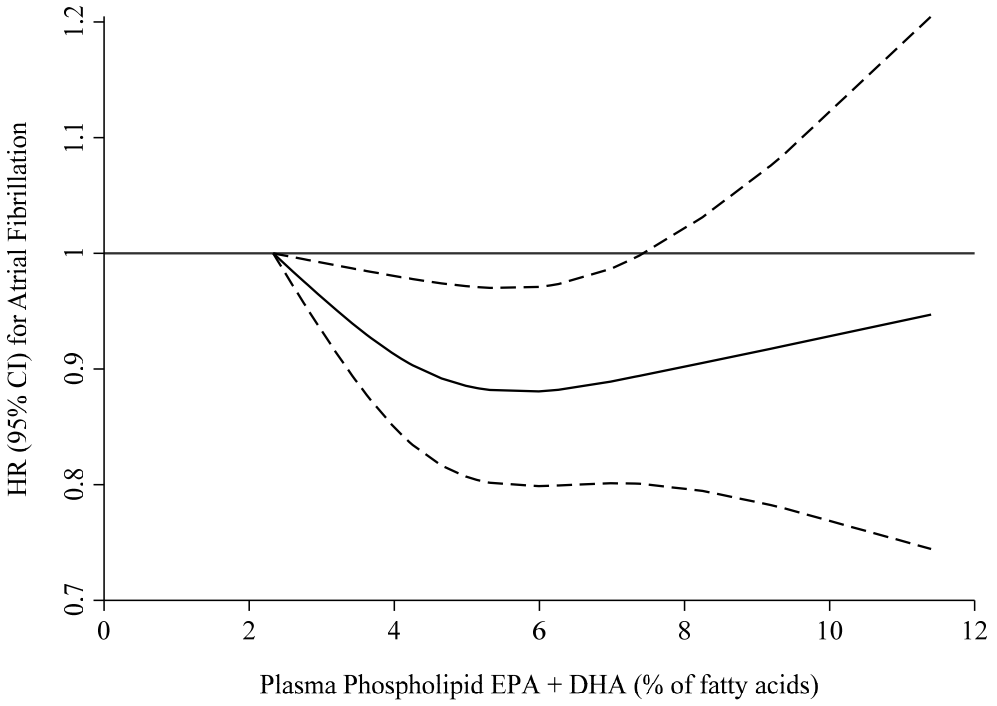

**(I)** DHA – Erythrocyte Phospholipids ($P_{linear}$ = 0.02, $P_{non\text{-}linear}$ = 0.36)



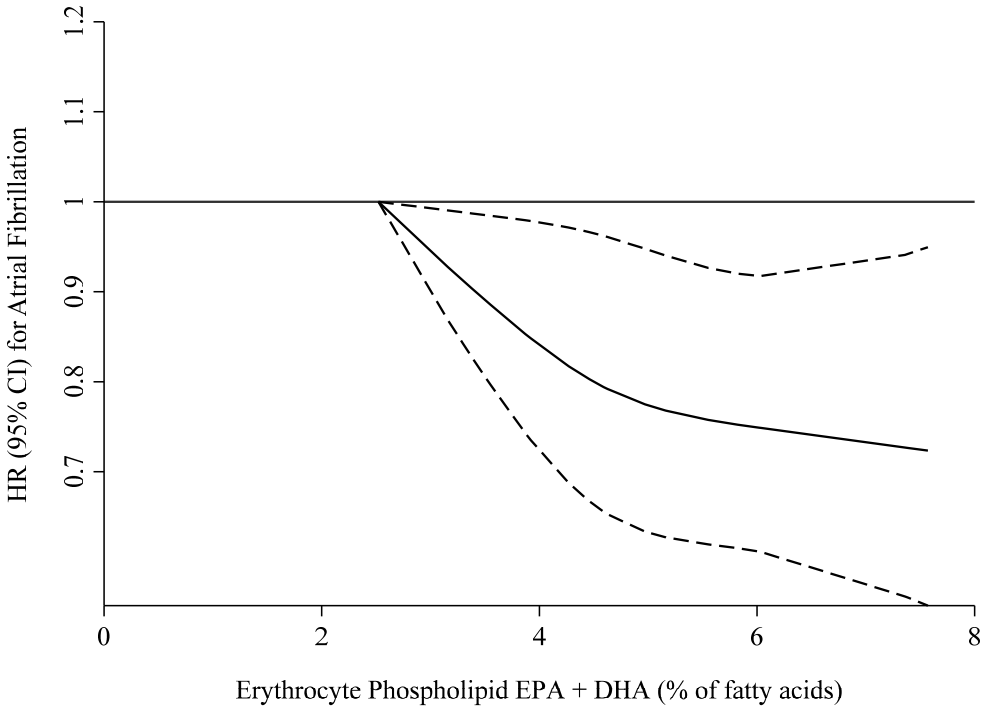

**(J)** DHA – Total Plasma/Serum ($P_{linear}$ = 0.84, $P_{non\text{-}linear}$ = 0.60)



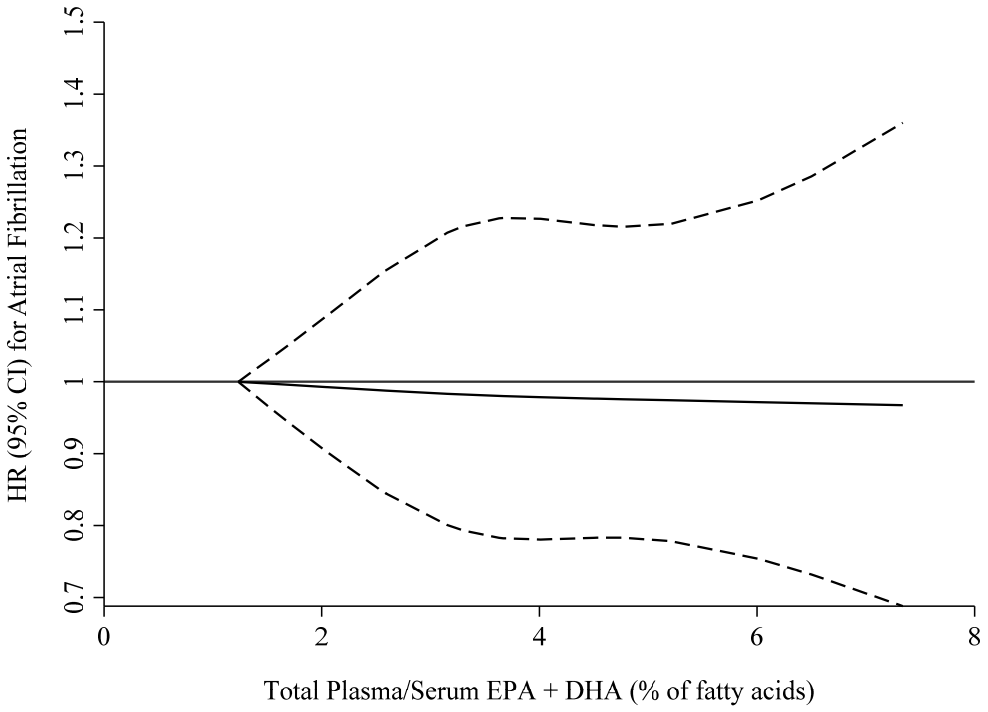

**(K)** DHA – Cholesterol Ester ($P_{linear}$ = 0.85, $P_{non\text{-}linear}$ = 0.37)




**(L)** EPA + DHA – Plasma Phospholipid ($P_{linear}$ = 0.06, $P_{non\text{-}linear}$ = 0.06)




**(M)** EPA + DHA – Erythrocyte Phospholipid ($P_{linear}$ = 0.006, $P_{non\text{-}linear}$ = 0.25)




**(N)** EPA + DHA – Total Plasma/Serum ($P_{linear}$ = 0.81, $P_{non\text{-}linear}$ = 0.95)

**References**


1. Halldin M, Rosell M, de Faire U, Hellenius ML. The metabolic syndrome: prevalence and association to leisure-time and work-related physical activity in 60-year-old men and women. Nutr Metab Cardiovasc Dis 2007;17:349-57.
2. The Atherosclerosis Risk in Communities (ARIC) Study: design and objectives. The ARIC investigators. Am J Epidemiol 1989;129:687-702.
3. Ma J, Folsom AR, Eckfeldt JH, Lewis L, Chambless LE. Short- and long-term repeatability of fatty acid composition of human plasma phospholipids and cholesterol esters. The Atherosclerosis Risk in Communities (ARIC) Study Investigators. Am J Clin Nutr 1995;62:572-8.
4. Fried LP, Borhani NO, Enright P et al. The Cardiovascular Health Study: design and rationale. Ann Epidemiol 1991;1:263-76.
5. Tjonneland A, Olsen A, Boll K et al. Study design, exposure variables, and socioeconomic determinants of participation in Diet, Cancer and Health: a population-based prospective cohort study of 57,053 men and women in Denmark. Scand J Public Health 2007;35:432-41.
6. Khaw KT, Friesen MD, Riboli E, Luben R, Wareham N. Plasma phospholipid fatty acid concentration and incident coronary heart disease in men and women: the EPIC-Norfolk prospective study. PLoS Med 2012;9:e1001255.
7. Kannel WB, Feinleib M, McNamara PM, Garrison RJ, Castelli WP. An investigation of coronary heart disease in families. The Framingham offspring study. Am J Epidemiol 1979;110:281-90.
8. Harris WS, Pottala JV, Vasan RS, Larson MG, Robins SJ. Changes in erythrocyte membrane trans and marine fatty acids between 1999 and 2006 in older Americans. J Nutr 2012;142:1297-303.
9. Ninomiya T, Nagata M, Hata J et al. Association between ratio of serum eicosapentaenoic acid to arachidonic acid and risk of cardiovascular disease: the Hisayama Study. Atherosclerosis 2013;231:261-7.
10. Nagata T, Hata J, Sakata S et al. Serum N-terminal pro-B-type natriuretic peptide as a predictor for future development of atrial fibrillation in a general population: the Hisayama Study. Int J Cardiol 2020;320:90-96.
11. Malik VS, Chiuve SE, Campos H et al. Circulating Very-Long-Chain Saturated Fatty Acids and Incident Coronary Heart Disease in US Men and Women. Circulation 2015;132:260-8.
12. Yakoob MY, Shi P, Willett WC et al. Circulating Biomarkers of Dairy Fat and Risk of Incident Diabetes Mellitus Among Men and Women in the United States in Two Large Prospective Cohorts. Circulation 2016;133:1645-54.
13. Salonen JT. Is there a continuing need for longitudinal epidemiologic research? The Kuopio Ischaemic Heart Disease Risk Factor Study. Ann Clin Res 1988;20:46-50.
14. Morrow DA, Scirica BM, Karwatowska-Prokopczuk E et al. Effects of ranolazine on recurrent cardiovascular events in patients with non-ST-elevation acute coronary syndromes: the MERLIN-TIMI 36 randomized trial. JAMA 2007;297:1775-83.
15. Bild DE, Bluemke DA, Burke GL et al. Multi-Ethnic Study of Atherosclerosis: objectives and design. Am J Epidemiol 2002;156:871-81.
16. Rosqvist F, Bjermo H, Kullberg J et al. Fatty acid composition in serum cholesterol esters and phospholipids is linked to visceral and subcutaneous adipose tissue content in elderly individuals: a cross-sectional study. Lipids Health Dis 2017;16:68.
17. Chatterjee NA, Tikkanen JT, Panicker GK et al. Simple electrocardiographic measures improve sudden arrhythmic death prediction in coronary disease. Eur Heart J 2020;41:1988-1999.
18. Chatterjee NA, Moorthy MV, Pester J et al. Sudden Death in Patients With Coronary Heart Disease Without Severe Systolic Dysfunction. JAMA cardiology 2018;3:591-600.
19. Ikram MA, Brusselle G, Ghanbari M et al. Objectives, design and main findings until 2020 from the Rotterdam Study. Eur J Epidemiol 2020;35:483-517.

20. Soininen P, Kangas AJ, Wurtz P, Suna T, Ala-Korpela M. Quantitative serum nuclear magnetic resonance metabolomics in cardiovascular epidemiology and genetics. Circ Cardiovasc Genet 2015;8:192-206.
21. Lupon J, Gavidia-Bovadilla G, Ferrer E et al. Dynamic Trajectories of Left Ventricular Ejection Fraction in Heart Failure. J Am Coll Cardiol 2018;72:591-601.
22. Hedstrand H. A study of middle-aged men with particular reference to risk factors for cardiovascular disease. Ups J Med Sci Suppl 1975;19:1-61.
23. Huang X, Sjogren P, Arnlov J et al. Serum fatty acid patterns, insulin sensitivity and the metabolic syndrome in individuals with chronic kidney disease. J Intern Med 2014;275:71-83.
24. Shumaker SA, Reboussin BA, Espeland MA et al. The Women's Health Initiative Memory Study (WHIMS): a trial of the effect of estrogen therapy in preventing and slowing the progression of dementia. Control Clin Trials 1998;19:604-21.
25. Espeland MA, Rapp SR, Shumaker SA et al. Conjugated equine estrogens and global cognitive function in postmenopausal women: Women's Health Initiative Memory Study. JAMA 2004;291:2959-68.