Stroke

CLINICAL AND POPULATION SCIENCES

Omega-3 Blood Levels and Stroke Risk: A Pooled and Harmonized Analysis of 183 291 Participants From 29 Prospective Studies

James H. O'Keefe, MD; Nathan L. Tintle, PhD; William S. Harris, PhD; Evan L. O'Keefe, MD; Aleix Sala-Vila, PhD; John Attia, MD, PhD; G. Manohar Garg, PhD; Alexis Hure, PhD; Christian Sørensen Bork, MD, PhD; Erik Berg Schmidt, MD, DMSc; Stine Krogh Venø, MD; Kuo-Liong Chien, MD, PhD; Yun-Yu (Amelia) Chen, MPH; Sarah Egert, PhD; Tobias Rudholm Feldreich, PhD; Johan Ärnlöv, MD, PhD; Lars Lind, MD, PhD; Nita G. Forouhi, MD, PhD; Johanna M. Geleijnse, MD, PhD; Kamalita Pertiwi, PhD; Fumiaki Imamura, PhD; Vanessa de Mello Laaksonen, PhD; W. Matti Uusitupa, MD, PhD; Jaakko Tuomilehto, MD, PhD; Markku Laakso, MD; Maria Anneli Lankinen, PhD; Danielle Laurin, PhD; Pierre-Hugues Carmichael, MSc, PhD; Joan Lindsay, PhD; Karin Leander, PhD; Federica Laguzzi, PhD; Brenton R. Swenson, PhD; William T. Longstreth, MD; JoAnn E. Manson, MD; Samia Mora, MD, Nancy R. Cook, ScD; Matti Marklund, PhD; Debora Melo van Lent, PhD; Rachel Murphy, PhD; Vilmundur Gudnason, MD; Toshihara Ninomiya, MD, PhD; Yoichiro Hirakawa, MD, PhD; Frank Qian, MD; Qi Sun, MD, ScD; Frank Hu, MD, PhD; Andres V. Ardisson Korat, PhD; Ulf Risérus, MD, PhD; Iolanda Lázaro, PhD; Cecilia Samieri, PhD; Mélanie Le Goff, MPH; Catherine Helmer, MD, PhD; Marinka Steur, PhD; Trudy Voortman, PhD; M. Kamran Ikram, MD, PhD; Toshiko Tanaka, PhD; Jayanta K. Das, PhD; Luigi Ferrucci, PhD; Stefania Bandinelli, MD; Michael Tsai, PhD; Weihua Guan, PhD; Parveen Garg, MD; W.M. Monique Verschuren, PhD; Jolanda M.A. Boer, PhD; Anneke Biokstra, MSc; Jyrki Virtanen, PhD; Michael Wagner, PhD; Jason Westra, MS; Luc Albuisson; Kazumasa Yamagishi, MD, PhD; David S. Siscovick, MD; Rozenn N. Lemaitre, PhD; Dariush Mozaffarian, MD

BACKGROUND: The effect of marine omega-3 PUFAs on risk of stroke remains unclear.

METHODS: We investigated the associations between circulating and tissue omega-3 PUFA levels and incident stroke (total, ischemic, and hemorrhagic) in 29 international prospective cohorts. Each site conducted a de novo individual-level analysis using a prespecified analytical protocol with defined exposures, covariates, analytical methods, and outcomes; the harmonized data from the studies were then centrally pooled. Multivariable-adjusted HRs and 95% CIs across omega-3 PUFA quintiles were computed for each stroke outcome.

RESULTS: Among 183 291 study participants, there were 10 561 total strokes, 8220 ischemic strokes, and 1142 hemorrhagic strokes recorded over a median of 14.3 years follow-up. For eicosapentaenoic acid, comparing quintile 5 (Q5, highest) with quintile 1 (Q1, lowest), total stroke incidence was 17% lower (HR, 0.83 [CI, 0.76–0.91]; $P<0.0001$), and ischemic stroke was 18% lower (HR, 0.82 [CI, 0.74–0.91]; $P<0.0001$). For docosahexaenoic acid, comparing Q5 with Q1, there was a 12% lower incidence of total stroke (HR, 0.88 [CI, 0.81–0.96]; $P=0.0001$) and a 14% lower incidence of ischemic stroke (HR, 0.86 [CI, 0.78–0.95]; $P=0.0001$). Neither eicosapentaenoic acid nor docosahexaenoic acid was associated with a risk for hemorrhagic stroke. These associations were not modified by either baseline history of AF or prevalent CVD.

CONCLUSIONS: Higher omega-3 PUFA levels are associated with lower risks of total and ischemic stroke but have no association with hemorrhagic stroke.

GRAPHIC ABSTRACT: A graphic abstract is available for this article.

Key Words: cerebrovascular disease ■ atrial fibrillation ■ fish ■ fish oil ■ stroke

Correspondence to: James H. O'Keefe, MD, 4321 Washington, Ste 2400, Kansas City, MO 64111. Email jokeefe@saintlukeskc.org
Supplemental Material is available at https://www.ahajournals.org/doi/suppl/10.1161/STROKEAHA.123.044281.
For Sources of Funding and Disclosures, see page 56.

Stroke is available at www.ahajournals.org/journal/str

Nonstandard Abbreviations and Acronyms

DHA	docosahexaenoic acid
DPA	docosapentaenoic acid
EPA	eicosapentaenoic acid
IQ_5R	interquintile range
UKBB	the United Kingdom Biobank

According to a 2021 worldwide analysis, 1 in 4 adults will suffer a stroke in their lifetime, and it is the second-leading cause of death and the third-leading cause of death and disability combined.[1] To reduce the risk of ASCVD and ischemic stroke, nutritional approaches have historically focused on high-fiber diets that are low in sodium, saturated fat, and cholesterol. Consumption of marine omega-3 PUFAs, however, has also shown promise in the prevention of CVD.

Docosahexaenoic acid (DHA) and eicosapentaenoic acid (EPA) have been widely studied since the 1970s when these PUFAs were reported to be inversely associated with risk of acute MI among Greenland Inuits.[2] Over the ensuing 4 decades, intensive scientific investigation established the cardioprotective effects of EPA and DHA.[3,4] A meta-analysis of 14 prospective studies used food frequency questionnaires to estimate self-reported omega-3 PUFA intake among 514 483 individuals with 9065 strokes during follow-up.[5] Compared with the lowest omega-3 intake group in each study, individuals in the highest intake group had a 13% lower risk of total stroke and a 16% lower risk of fatal stroke.[5] A widely accepted limitation of studies based on self-reporting, however, is the ability to accurately estimate exposure to omega-3 PUFAs. Recently, a meta-analysis of 38 RCTs that included 149 051 people reported that marine omega-3 PUFA supplementation was associated with statistically significant reductions in CV mortality, major adverse CV events (composite end point of nonfatal MI, nonfatal stroke, or CV death), and coronary revascularization.[6] In that metanalysis, omega-3 was not associated with reduction in risk of stroke as a separate end point; however, only 927 strokes (0.6% of the study participants) occurred during the relatively short median follow-up of 2.0 years. Meta-analyses have also reported that pharmacological intervention with marine omega-3 PUFAs may increase risk for AF.[6,7] Without question, AF increases risk of stroke,[8] but the effects of omega-3 PUFAs on stroke risk remain uncertain.

An objective measure of omega-3 PUFA exposure is the level of EPA+DHA in blood or tissues, which is largely determined by habitual dietary intake of these PUFAs.[9–12] While biomarker-based investigations have strong potential to clarify the biological relationship between omega-3 PUFAs and stroke outcomes, reports from single studies can be limited by insufficient power, selection bias, publication bias, and inconsistent definitions of exposure, outcome, and adjustment for potential confounding factors. To address these challenges, the present study pooled de novo, harmonized, individual-level analyses across 29 prospective studies in the Fatty Acid and Outcome Research Consortium[13] to investigate the associations between marine omega-3 PUFA biomarker levels and incident stroke—total, ischemic, and hemorrhagic.

METHODS

Study Design and Population

Authors will make data, analytic methods, and study materials available to other researchers upon request. This study was conducted within the Fatty Acid and Outcome Research Consortium[13]—an international collaboration of observational studies with baseline PUFA biomarker data and ascertainment of chronic disease events during follow-up. For the current project, all 60 prospective studies in the consortium as of November 2021 were invited to participate. Of these, 18 did not have the required PUFA and stroke data, 11 indicated a lack of funding/analyst time and 2 had too few incident stroke outcomes for adjusted statistical models to fit robustly. Thus, the current investigation comprised data from 29 studies across 15 countries. The details of each individual study are presented in Table S1, including individual cohort IRB approvals. All participating studies followed a prespecified standardized analysis protocol with harmonized inclusion and exclusion criteria, exposures, outcomes, covariates, and analytical methods inclusive of statistical models and assessment of missing covariate data. In each study, new analyses of individual data were performed according to the protocol, and study-specific results were collected using a standardized electronic form. Information regarding registration required by any of the cohorts included herein is shown in Table S1. Individual cohorts conducted their studies in accordance with the criteria set by the Declaration of Helsinki, and informed consent was obtained from all participants after IRB approval. Details of other methods including participant criteria, fatty acid analysis, covariates, and statistical analysis and pooling are given in the Supplemental Methods section.

RESULTS

Population

The pooled analyses of 29 cohorts included marine omega-3 PUFA biomarker levels from 183 291 individuals (Table S2), 67 165 excluding the the United Kingdom Biobank (UKBB). The median follow-up time was 14 years (range, 5–30 years). In total, 10 561 participants were recorded as incident stroke cases: 78% ischemic, 11% hemorrhagic, and 11% unspecified. At baseline, the mean age was 65 years, 53% were women; White adults comprised 82% of the study population. Total stroke in relation to each PUFA exposure was analyzed in 2 models—those with and without preexisting CVD (Table S3). The distribution (10th, 50th, and 90th percentiles) of PUFA levels by lipid compartment and

by cohort are shown in Table S4. In the pooled data, the estimated 10th, 50th, and 90th percentiles for RBC EPA+DHA content were 3.4%, 5.2%, and 7.9%, respectively as shown in Table S5.

Omega-3 Fatty Acid Levels and Total Stroke

When analyzing by quintile, for DHA (including the UKBB cohort), a significant inverse association was observed for total stroke, with the risk in Q4 and Q5 being 12% to 13% lower than the reference group, Q1 (Figure 1). Similar patterns were seen for EPA and EPA+DHA in analyses without the UKBB with the risk in Q5 17% lower than that in Q1 (Table). The quintile analysis of associations between docosapentaenoic acid (DPA) and total stroke (again, excluding the UKBB) suggested a threshold effect, with no associations in Q2 to Q4 but an 11% lower risk in Q5 versus Q1 (Table). These analyses showed low levels of heterogeneity (I^2<50%) in all cases. When analyzing the relations between DHA, EPA, and their sum on a per IQ_5R basis, each was associated with an 8% to 9% lower risk of total stroke (Table S6). DHA results were similar with or without inclusion of the UKBB cohort (Table S6). DPA was not significantly associated with risk of stroke in this analysis.

Omega-3 Fatty Acid Levels and Stroke Subtypes

Patterns for ischemic stroke followed those of total stroke, with risk in Q5 versus Q1 being 14% to 18% lower (Table). In contrast to ischemic stroke, results for hemorrhagic stroke showed little to no evidence of differential risk for any omega-3 PUFA tested. In IQ_5R analyses, risk for ischemic stroke was reduced by 11% to 13% for DHA and EPA+DHA (Table S6). No significant associations with DPA were observed for either stroke subtype.

Omega-3 Fatty Acid Levels and Total Stroke by Lipid Compartments

Regarding DHA, risk for total stroke comparing Q5 with Q1 ranged from 6% in RBCs to 21% for cholesteryl esters (Figure 2). Similar trends were seen for total stroke with EPA and EPA+DHA, but not with DPA

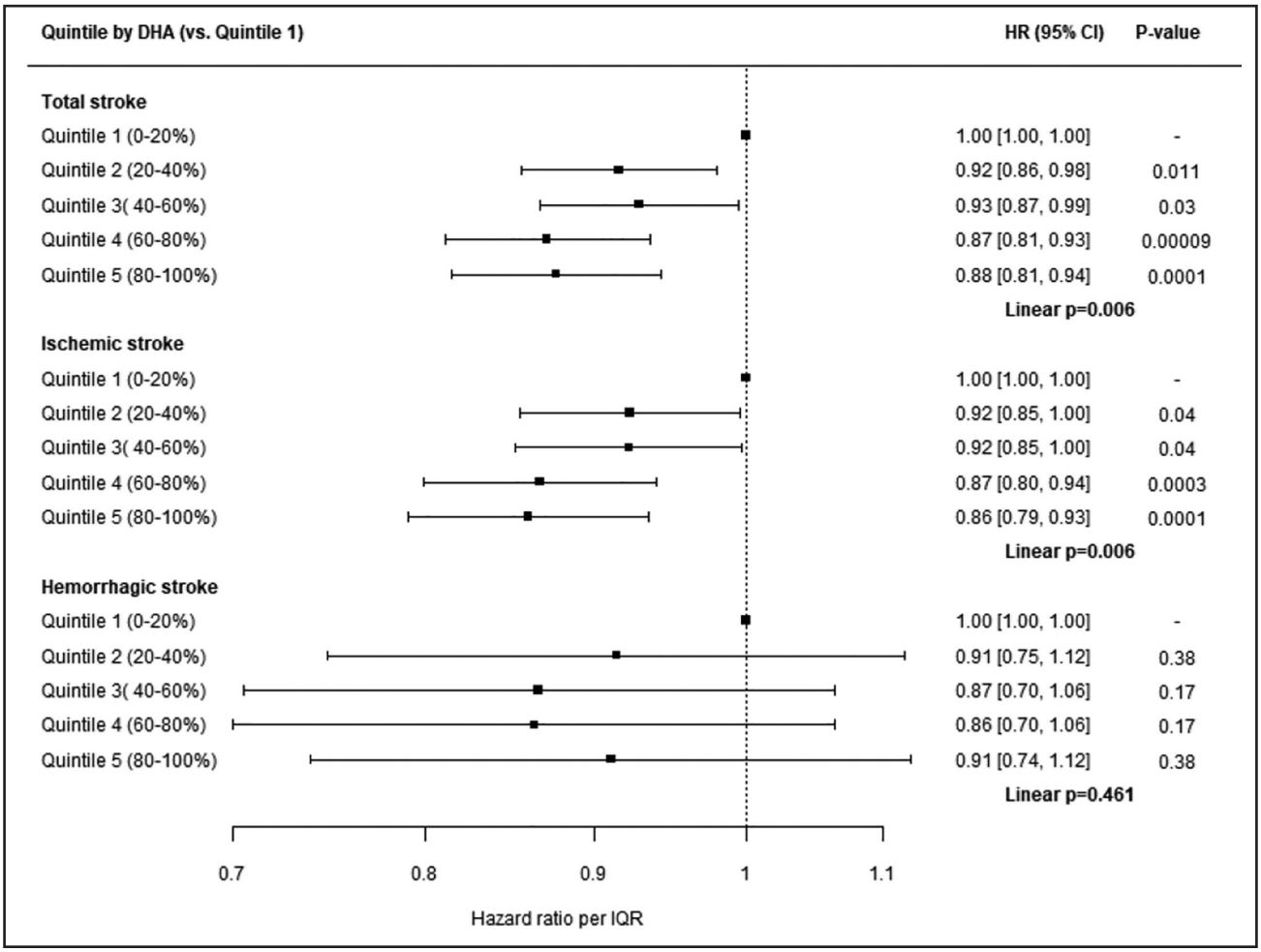

Figure 1. Association of docosahexaenoic acid (DHA) and risk of stroke.

Stroke. 2024;55:50–58. DOI: 10.1161/STROKEAHA.123.044281

Table. Hazard Ratios (95% CI) for Stroke by FA Quintile (Versus Quintile 1) Excluding the UK Biobank

Fatty acid		Total stroke	Ischemic stroke	Hemorrhagic stroke
DHA	Quintile 1 (reference)	1.0	1.0	1.0
	Quintile 2	0.92 (0.85–1)*	0.9 (0.82–0.99)*	1.08 (0.82–1.42)
	Quintile 3	0.93 (0.86–1.02)	0.9 (0.82–0.99)*	1.03 (0.78–1.36)
	Quintile 4	0.90 (0.83–0.98)*	0.88 (0.8–0.96)†	1.02 (0.77–1.36)
	Quintile 5	0.88 (0.81–0.96)†	0.86 (0.78–0.95)†	1.09 (0.82–1.46)
	P value for trend	0.04	0.03	0.79
EPA	Quintile 1 (reference)	1.0	1.0	1.0
	Quintile 2	1.00 (0.92–1.08)	0.99 (0.9–1.08)	0.96 (0.73–1.26)
	Quintile 3	0.82 (0.75–0.89)‡	0.81 (0.74–0.9)‡	0.82 (0.62–1.09)
	Quintile 4	0.91 (0.83–0.99)*	0.91 (0.82–1.00)	0.86 (0.65–1.14)
	Quintile 5	0.83 (0.76–0.91)‡	0.82 (0.74–0.91)‡	0.9 (0.67–1.21)
	P value for trend	0.001	0.002	0.45
DPA	Quintile 1 (Reference)	1.0	1.0	1.0
	Quintile 2	1.00 (0.91–1.11)	1.04 (0.94–1.16)	0.83 (0.61–1.13)
	Quintile 3	1.03 (0.93–1.13)	1.04 (0.93–1.16)	0.64 (0.47–0.89)†
	Quintile 4	1.01 (0.91–1.12)	1.04 (0.94–1.16)	0.89 (0.66–1.22)
	Quintile 5	0.89 (0.8–0.99)*	0.93 (0.83–1.05)	0.79 (0.57–1.09)
	P value for trend	0.21	0.47	0.45
EPA+DHA	Quintile 1 (Reference)	1.0	1.0	1.0
	Quintile 2	0.94 (0.86–1.02)	0.93 (0.85–1.02)	1.14 (0.86–1.51)
	Quintile 3	0.92 (0.84–0.99)*	0.88 (0.8–0.97)*	1.00 (0.75–1.35)
	Quintile 4	0.92 (0.84–0.99)*	0.89 (0.81–0.98)*	1.17 (0.87–1.57)
	Quintile 5	0.83 (0.76–0.91)‡	0.82 (0.74–0.91)‡	1.04 (0.76–1.42)
	P value for trend	0.007	0.006	0.82

Adjusted for age (continuous), sex (men/women), race (binary: White/non-White), field center (categories), body mass index (continuous), education (less than high school graduate, high school graduate, at least some college or vocational school), occupation (if available), smoking (current, former, never), physical activity (kcal/wk, METS/wk, or h/d), alcohol intake (drinks or servings/d, g/d, or mL/d), prevalent DM (treated or physician-diagnosed), prevalent hypertension (treated or physician-diagnosed), prevalent dyslipidemia (treated or physician-diagnosed), prevalent atherosclerotic CVD, history of AF, and circulating omega-6 fatty acid levels (ie, the sum of linoleic and arachidonic acids). DHA quintiles including the UKBB data is shown in Figure 1. AF indicates atrial fibrillation; CVD, cardiovascular disease; DHA, docosahexaenoic acid; DM, diabetes; EPA, eicosapentaenoic acid; and METS, metabolic equivalents.

*$P<0.05$.

†$P<0.01$.

‡$P<0.001$.

(Figures S1 through S3). DHA levels were also inversely related to risk for ischemic stroke (Figure S4), but not with hemorrhagic stroke (Figure S5).

Nonlinearity

As shown in the Table, linear trends across quintiles showed a significant inverse relationship for each of DHA, EPA, and DHA+EPA for both total stroke and ischemic stroke ($P<0.05$) but not hemorrhagic stroke ($P>0.05$). All tests for nonlinearity (for each PUFA and stroke outcome) yielded $P>0.05$ (Table S7).

Heterogeneity and Sensitivity Analyses

In exploratory subgroup analyses, PUFA by age (<65 versus >65), sex (male versus female), and prevalent AF (yes versus no), no significant effect modification was seen for omega-3 PUFA biomarker relationships with total stroke (using Bonferroni-corrected alpha=0.004, based on 3 subgroups×4 PUFAs; Table S8). Results were also similar after excluding individuals with prevalent CVD (Table S9) and censoring all participants at 10 years of follow-up (Table S10). Because data on DPA were available from only 21 of the 29 cohorts, a post hoc sensitivity analysis was conducted to assess relationships between DHA, EPA, and their sum in only those cohorts providing data on DPA to compare the findings seen with the full dataset. In these analyses, similar significant, inverse associations with DHA, EPA, and EPA+DHA were observed, suggesting that the lack of significant associations with DPA was not simply driven by the smaller sample size (Table S11). Because of its larger N (N=116 126 participants; 2478 stroke

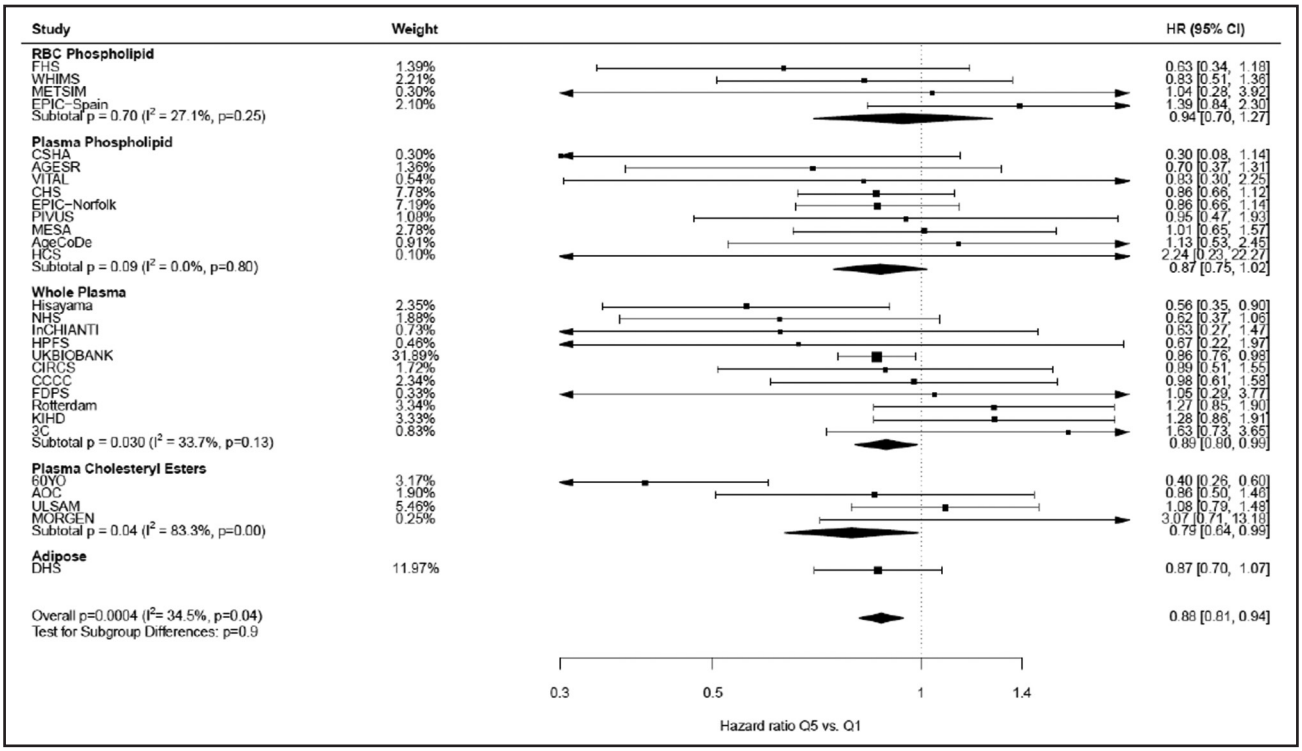

Figure 2. Association of docosahexaenoic acid (DHA) and risk of stroke by lipid compartment.

cases), Table S12 shows the results for UKBB alone. These findings are similar to those seen with the combined cohorts, with the exception that DHA levels were significantly and inversely related to risk for hemorrhagic stroke in the UKBB.

DISCUSSION

In this harmonized, pooled, de novo analysis of data from up to 183 291 people in 29 prospective studies from 15 nations, in vivo DHA, EPA, and EPA+DHA levels were inversely associated with risk of total and ischemic stroke during an average of 14 years of follow-up. Comparing Q5 with Q1, risk of ischemic stroke was 18% lower for EPA+DHA, 18% lower for EPA, and 16% lower for DHA. Omega-3 levels were not significantly associated with risk of hemorrhagic stroke. In addition, the results were robust when excluding individuals with prevalent CVD, diminishing concerns about reverse causation. DPA is a minor omega-3 PUFA that is not influenced by dietary intake of fish, seafood, or fish oil, and it was not significantly associated with stroke risk in the current study.

Scientific plausibility exists to support the hypothesis that marine omega-3 PUFA might reduce susceptibility to stroke. DHA and EPA have anti-inflammatory, antiplatelet, and hypotriglyceridemic effects, reduce arterial stiffness, improve endothelial function, and provide dose-dependent reductions in blood pressure and resting heart rate via heightened vagal tone.[3,4,14] Stroke has an ischemic etiology in about 87% of cases in the United States, generally due to atherosclerotic cerebrovascular arteries or embolic events.[15] Low blood levels of EPA and DHA have been linked with lipid-rich, inflamed plaques with thin, vulnerable, fibrous caps that are at increased risk of rupturing.[16] In a study of 188 patients awaiting carotid endarterectomy, those randomized to fish oil (providing 1.4 g of DHA+EPA per day versus placebo) before surgery had a lower prevalence of vulnerable fibrous caps, an increased thickness of fibrous caps, and reduced signs of inflammation within the carotid plaques.[17] The authors concluded, "Atherosclerotic plaques readily incorporate omega-3s from fish-oil supplementation, inducing changes that can enhance stability of atherosclerotic plaques."[17]

Because ASCVD complications such as acute coronary syndrome and ischemic stroke are triggered by inflammation, plaque erosion/rupture, and thrombosis, the disease is best described as atherothrombosis.[18] Marine omega-3 PUFAs inhibit platelet aggregation, reduce both whole blood viscosity and von Willebrand Factor, and improve RBC membrane flexibility/deformability.[19] These mechanisms of action might be playing a role in omega-3-mediated protection against ischemic stroke of atherothrombotic or cardioembolic origin.

Since stroke remains the most feared complication of AF, this study was undertaken in part due to concerns that high doses of prescription omega-3 PUFAs may increase risk of AF.[7,20] Yet, the relationship of omega-3 and AF is controversial as previous studies examining standard consumption levels of omega-3 have linked

 Stroke. 2024;55:50–58. DOI: 10.1161/STROKEAHA.123.044281

higher dietary intakes of DHA+EPA with lower incidence of AF.[21] This observation was recently supported by a biomarker-based analysis from the Fatty Acid and Outcome Research Consortium group.[22] The reduced risk of stroke demonstrated in the present study is consistent with the findings of REDUCE-IT, and neither a history of AF nor preexisting CVD confounded the inverse associations between levels of omega-3 and risk for stroke.[23]

High doses of omega-3 PUFA can inhibit platelet function and could possibly increase risk of bleeding and hemorrhagic stroke. Greenland Inuits consuming their traditional diet that was very high in marine omega-3 PUFAs (about 14 g/d DHA+EPA) had increased mortality from hemorrhagic stroke compared with Inuits living in Denmark.[24,25] In the Nurses' Health Study, women with relatively high intakes of fish, still much lower than that of the Inuits, had a lower risk of ischemic stroke with no significant association with risk of hemorrhagic stroke.[26] Very high doses (>7 g/d) of omega-3 PUFAs, especially EPA, may increase bleeding times and hemorrhagic complications.[14,19,22] In REDUCE-IT, serious bleeding events occurred in 2.7% of the people randomized to EPA 4 g/day group and in 2.1% in the placebo group (P=0.06), but the EPA group did not have an increased risk of hemorrhagic stroke.[23] Observational cohorts in the United States and Japan, using food frequency questionnaire, found no associations of fish or omega-3 intakes with risk of hemorrhagic stroke.[26,27] In the current study, individuals in the top quintile of omega-3 PUFA levels showed no signal for increased risk of hemorrhagic stroke. In another observational study, a lower prevalence of cerebral microbleeds was noted among older adults eating large amounts of oily fish (13 servings per week, equivalent to about 2 g/day of EPA+DHA per day).[28] The Omega-3 PUFAs for Prevention of Postoperative Atrial Fibrillation trial randomized 1516 patients before open-heart surgery to either 2 g/day of EPA+DHA or matching placebo capsules. Omega-3 did not increase the perioperative bleeding risk after cardiac surgery and, surprisingly, reduced the number of blood transfusions needed postoperatively.[29] The current study, which stands as the largest omega-3 biomarker study to date, showed no signal for increased risk of hemorrhagic stroke.

Our findings support the American Heart Association's Science Advisory recommendation that "1 to 2 seafood meals per week be included to reduce the risk of congestive heart failure, coronary heart disease, ischemic stroke, and sudden cardiac death." The typical US adult eats <1 serving per week of fish/seafood; accordingly, the average intake of EPA+DHA in the United States is only about 100 mg/day with a mean omega-3 index of 5.4%.[30] The median RBC EPA+DHA (ie, the omega-3 index) for Q5 in the present study was ≈8%, suggesting that reaching an omega-3 index of 8% or greater could be a cardioprotective goal—originally proposed in 2004.[31] To raise an omega-3 index of 5.4% to 8% would require intake of ≈1000 mg/day of EPA+DHA, whereas to go from the median of Q1 (about 3.5% omega-3 index) to 8% would require about 1600 mg/day of EPA+DHA.[30] These intakes are achievable from dietary fish/seafood and omega-3 supplements.

Strengths of the current study include the use of objective omega-3 PUFA biomarkers, rather than dietary questionnaires, which increases the accuracy of exposure assessment and allows for separate statistical analyses of individual omega-3 PUFA-stroke outcomes. The use of prespecified, harmonized, de novo individual-level analyses across multiple cohorts substantively increases generalizability, reduces confounding through consistent adjustment for covariates, and limits the potential for publication bias. The pooling of 29 studies including over 10 000 incident stroke cases strengthens the generalizability of the findings and allows for the statistical evaluation of stroke subtypes as well as potential heterogeneity across subgroups that may modify the observed associations.

Potential limitations of this study include limited diversity—most individuals were White, which could lower generalizability to other races/ethnicities. Despite extensive efforts to harmonize study-specific methods, moderate heterogeneity remained among studies, which may be due to study designs, unmeasured background population characteristics, differences in laboratory assessment of omega-3 PUFAs, variability in ascertainment of outcomes, chance, or any combination of these. Omega-3 PUFAs and covariates were measured once at baseline and changes over time could lead to misclassification, which could bias the results in unpredictable directions. Even so, omega-3 concentrations have shown reasonably good reproducibility over time.[32] Pooling by quintiles instead of absolute PUFA values was necessary because values physiologically differ by lipid compartment. Nevertheless, such an approach was reasonable given the observed correlations among different lipid compartments. For example, correlation coefficients were >0.9 for plasma phospholipid and RBC EPA+DHA levels,[31] for EPA and DHA in plasma CE and PL fractions[33] and for whole plasma versus RBC DHA+EPA (N=2312, WS Harris, unpublished data). Beyond classification as ischemic or hemorrhagic, we did not have specific information on whether strokes were due to emboli, large artery atherosclerosis, microganiopathy, hypertension, etc. The analysis plan did not request information from cohorts about demographics of patients with AF, so interaction analyses could not performed on this subgroup. Although we adjusted for many major demographic and socioeconomic risk factors, physical activity, smoking, and prevalent diseases, some covariates were self-reported; so residual and unmeasured confounding could bias our results in unknown directions. Still, the magnitude of the observed relationships between omega-3 PUFA levels and risk of stroke reported herein is consistent with the

known associations of EPA+DHA with CHD events, cardiac mortality, all-cause mortality, and sudden cardiac death.[6,11,34–36]

In summary, this harmonized and pooled analysis of prospective studies showed that long-chain omega-3 PUFA levels were inversely associated with risk of total and ischemic stroke but were unrelated to risk of hemorrhagic stroke. Thus, higher dietary intakes of DHA and EPA would be expected to lower risk of stroke.

ARTICLE INFORMATION

Received June 22, 2023; final revision received October 27, 2023; accepted October 30, 2023.

Affiliations

Saint Luke's Mid America Heart Institute, University of Missouri-Kansas City (J.H.O., E.L.O.). Fatty Acid Research Institute (N.L.T., W.S.H., A.S.-V., J.W.) and University of South Dakota (W.S.H.), Sioux Falls, SD. Hospital del Mar Medical Research Institute, Barcelona, Spain (A.S.-V., I.L.). The University of Newcastle, School of Biomedical Sciences and Pharmacy, Callaghan, Australia (J. Attia, G.M.G., A.H.). Department of Cardiology, Aalborg University Hospital, Denmark (C.S.B.), Department of Clinical Medicine (E.B.S.), and Department of Clinical Biochemistry (S.K.V.), Aalborg University Hospital, Denmark. National Taiwan University, Institute of Epidemiology and Preventive Medicine, Taipei (K.-L.C.). Department of Medical Research, Taichung Veterans General Hospital, Taiwan (Y.-Y.C.). Institute of Nutrition and Food Sciences and Nutritional Physiology, University of Bonn, Germany (S.E.). Dalarna University, School of Health and Welfare, Falun, Sweden (T.R.F.). Division of Family Medicine and Primary Care, Department of Neurobiology Care Sciences and Society, Karolinska Institutet, Solna, Sweden (J. Ärnlöv). Department of Medical Sciences Cardiovascular Epidemiology, Uppsala University, Sweden (L.L.). MRC Epidemiology Unit, University of Cambridge School of Clinical Medicine, United Kingdom (N.G.F., F.I.). Division of Human Nutrition and Health, Wageningen University and Research, the Netherlands (J.M.G., K.P.). Institute of Public Health and Clinical Nutrition (V.d.M.L., W.M.U., J.T., M.A.L., J.V.) and Department of Internal Medicine (M.L.), School of Medicine, University of Eastern Finland, Kuopio, Finland. CHU de Québec-Université Laval and VITAM Research Centers, Centre d'Excellence sur le Vieillissement de Québec, Canada (D.L., P.-H.C.). School of Epidemiology and Public Health, University of Ottawa, Canada (J.L.). Karolinska Institutet, Institute of Environmental Medicine, Unit of Cardiovascular and Nutritional Epidemiology, Stockholm, Sweden (K.L., F.L.). Cardiovascular Health Research Unit (B.R.S.) and Departments of Neurology and Epidemiology (W.T.L.), University of Washington, Seattle. Department of Medicine, Harvard Medical School, Brigham and Women's Hospital, Boston, MA (J.E.M., S.M., N.R.C.). The George Institute for Global Health, University of New South Wales, Newtown, Australia (M.M.). Department of Epidemiology, Johns Hopkins Bloomberg School of Public Health, Baltimore, MD (M.M.). Department of Public Health and Caring Sciences, Uppsala University, Sweden (M.M.). Glenn Biggs Institute for Alzheimer's and Neurodegenerative Diseases, University of Texas, San Antonio (D.M.v.L.). Cancer Control Research, British Columbia Cancer, School of Population and Public Health, University of British Columbia, Vancouver, Canada (R.M.). Icelandic Heart Institute, Kopavogur, Iceland (V.G.). Department of Epidemiology and Public Health and Center for Cohort Studies, Kyushu University, Fukouka, Japan (T.N., Y.H.). Harvard Medical School, T.H. Chan School of Public Health and Beth Deaconess Medical Center, Boston, MA (F.Q.). Harvard Medical School, T.H. Chan School of Public Health and Channing Division of Network Medicine Brigham and Women's Hospital, Boston, MA (Q.S., F.H.). USDA Human Nutrition Research Center on Aging, Tufts University, Boston, MA (A.V.A.K.). Department of Public Health and Caring Sciences Clinical Nutrition and Metabolism Unit, Uppsala University, Sweden (U.R.). Bordeaux Population Health Research Centre, University of Bordeaux, France (C.S., M.L.G., C.H.). Department of Epidemiology, University Medical Center Rotterdam, the Netherlands (M.S., T.V., M.K.I.). National Institutes of Health, National Institute on Aging, Longitudinal Studies Section, Baltimore, MD (T.T., L.F., J.K.D.). Azienda Sanitaria Firenze, Geriatric Unit, Florence, Italy (S.B.). Department of Laboratory Medicine and Pathology, University of Minnesota, Minneapolis (M.T.). Division of Biostatistics, University of Minnesota, Minneapolis (W.G.). Department of Medicine, Cardiology, University of Southern California, Los Angeles (P.G.). National Institute for Public Health and the Environment Bilthoven, the Netherlands (W.M.M.V.). Julius Center for Health Sciences and Primary Care and Centre for Nutrition, Prevention and Health Services, Utrecht, the Netherlands (W.M.M.V.). National Institute for Public Health and the Environment Bilthoven, the Netherlands (J.M.A.B., A.B.). Departments of Neurodegenerative Diseases and Geriatric Psychiatry and German Center for Neurodegenerative Diseases, University Hospital, Bonn, Germany (M.W.). University of Denver, CO (L.A.). Department of Public Health Medicine, University of Tsukubu, Japan (K.Y.). Department of Epidemiology, New York Academy of Medicine (D.S.S.). Department of Medicine, University of Washington, Seattle (R.N.L.). Division of Cardiology, Tufts University, Boston, MA (D.M.).

Sources of Funding

Dr Korat was supported by the United States Department of Agriculture (USDA) Agricultural Research Service under Cooperative Agreement No. 58 to 1950 to 7 to 707 and National Center for Advancing Translational Sciences (NCATS) 1KL2TR002545T32. Dr O'Keefe was supported by Position with Saint Luke's Mid America Heart Institute is paid with a grant from The National Heart, Lung, and Blood Institute of the National Institutes of Health (NIH) under Award Number T32HL110837 supported the research reported in this publication. The content is solely the responsibility of the authors and does not necessarily represent the official views of the NIH. NIH era commons number 79260132. Dr Bork received funding from Karen Elise Jensens Foundation. Dr Forouhi received funding from Medical Research Council Epidemiology Unit; National Institutes of Health Research Cambridge Biomedical Research center.

Disclosures

Dr O'Keefe reports major ownership interest in Cardiotabs. Dr Harris reports minor ownership interest in Omegaquant Analytics, LLC. Dr Sala-Vila reports travel and grant support from California Walnut Commission. Dr Mora reports compensation for consultant services from Quest Diagnostics. Dr Murphy reports compensation from Pharmavite for consultant services. Dr Bork reports grants from Karen Elise Jensens Fond. Dr Ärnlöv reports compensation from Astellas Pharma for other services; compensation from Boehringer Ingelheim for consultant services; compensation from AstraZeneca for other services; and compensation from Novartis for other services. Dr Geleijnse reports grants from National Institutes of Health to other; grants from Upfield Europe BV to other; and grants from The Dutch Heart Foundation to other. Dr Manson reports grants from National Institutes of Health and compensation from National Institutes of Health for other services. Dr Mora reports compensation from Pfizer for consultant services; employment by Brigham and Women's Hospital; grants from National Institute of Diabetes and Digestive and Kidney Diseases; and grants from National Heart, Lung, and Blood Institute. Dr Melo van Lent reports grants from National Institutes of Health and grants from Alzheimer's Association. Dr Mozaffarian reports grants from Rockefeller Foundation; compensation from Beren Therapeutics for other services; stock holdings in HumanCo; compensation from January Inc. for other services; compensation from Filtricine for other services; compensation from Perfect Day for other services; compensation from Tiny Organics for other services; grants from Bill and Melinda Gates Foundation; compensation from Calibrate for other services; grants from Vail Innovative Global Research; grants from Kaiser Permanente Fund at East Bay Community Foundation; compensation from DayTwo for other services; compensation from Instacart health for other services; compensation from Season Health for other services; grants from National Institutes of Health; compensation from Barilla for consultant services; stock holdings in Calibrate; compensation from Kaiser Permanente Fund for other services; compensation from HumanCo for other services

Supplemental Material

Tables S1–S12
Figures S1–S5
References 37–90

REFERENCES

1. Katan M, Luft A. Global burden of stroke. *Semin Neurol.* 2018;38:208–211. doi: 10.1055/s-0038-1649503
2. Dyerberg J, Bang HO, Stoffersen E, Moncada S, Vane JR. Eicosapentaenoic acid and prevention of thrombosis and atherosclerosis? *Lancet.* 1978;2:117–119. doi: 10.1016/s0140-6736(78)91505-2
3. DiNicolantonio JJ, O'Keefe JH. The benefits of omega-3 fats for stabilizing and remodeling atherosclerosis. *Mo Med.* 2020;117:65–69. doi: 10.4172/2153-0637.1000e112
4. O'Keefe EL, Harris WS, DiNicolantonio JJ, Elagizi A, Milani RV, Lavie CJ, O'Keefe JH. Sea change for marine omega-3s: randomized trials show fish oil reduces cardiovascular events. *Mayo Clin Proc.* 2019;94:2524–2533. doi: 10.1016/j.mayocp.2019.04.027

5. Cheng P, Huang W, Bai S, Wu Y, Yu J, Zhu X, Qi Z, Shao W, Xie P. BMI affects the relationship between long chain N-3 polyunsaturated fatty acid intake and stroke risk: a meta-analysis. *Sci Rep*. 2015;5:14161. doi: 10.1038/srep14161
6. Khan SU, Lone AN, Khan MS, Virani SS, Blumenthal RS, Nasir K, Miller M, Michos ED, Ballantyne CM, Boden WE, et al. Effect of omega-3 fatty acids on cardiovascular outcomes: a systematic review and meta-analysis. *EClinicalMedicine*. 2021;38:100997. doi: 10.1016/j.eclinm.2021.100997
7. Gencer B, Djousse L, Al-Ramady OT, Cook NR, Manson JE, Albert CM. Effect of long-term marine ω-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;144:1981–1990. doi: 10.1161/CIRCULATIONAHA.121.055654
8. Wolf PA, Abbott RD, Kannel WB. Atrial fibrillation as an independent risk factor for stroke: the Framingham Study. *Stroke*. 1991;22:983–988. doi: 10.1161/01.str.22.8.983
9. Svensson BG, Akesson B, Nilsson A, Skerfving S. Fatty acid composition of serum phosphatidylcholine in healthy subjects consuming varying amounts of fish. *Eur J Clin Nutr*. 1993;47:132–140.
10. Jackson KH, Polreis JM, Tintle NL, Kris-Etherton PM, Harris WS. Association of reported fish intake and supplementation status with the omega-3 index. *Prostaglandins Leukot Essent Fatty Acids*. 2019;142:4–10. doi: 10.1016/j.plefa.2019.01.002
11. Siscovick DS, Raghunathan TE, King I, Weinmann S, Wicklund KG, Albright J, Bovbjerg V, Arbogast P, Smith H, Kushi LH. Dietary intake and cell membrane levels of long-chain n-3 polyunsaturated fatty acids and the risk of primary cardiac arrest. *JAMA*. 1995;274:1363–1367. doi: 10.1001/jama.1995.03530170043030
12. Katan MB, Deslypere JP, van Birgelen AP, Penders M, Zegwaard M. Kinetics of the incorporation of dietary fatty acids into serum cholesteryl esters, erythrocyte membranes, and adipose tissue: an 18-month controlled study. *J Lipid Res*. 1997;38:2012–2022. doi: 10.1016/S0022-2275(20)37132-7
13. Fatty Acids and Outcomes Research Consortium. FORCE: Fatty Acids and Outcomes Research Consortium. Accessed August 1, 2023. https://sites.tufts.edu/force/. 2020.
14. Zhang X, Ritonja JA, Zhou N, Chen BE, Li X. Omega-3 polyunsaturated fatty acids intake and blood pressure: a dose-response meta-analysis of randomized controlled trials. *J Am Heart Assoc*. 2022;11:e025071. doi: 10.1161/JAHA.121.025071
15. CDC. Stroke Facts. Accessed August 1, 2023. https://www.cdc.gov/stroke/facts.htm. Atlanta, Georgia: Prevention CfDCa; 2022.
16. Nishio R, Shinke T, Otake H, Nakagawa M, Nagoshi R, Inoue T, Kozuki A, Hariki H, Osue T, Taniguchi Y, et al. Stabilizing effect of combined eicosapentaenoic acid and statin therapy on coronary thin-cap fibroatheroma. *Atherosclerosis*. 2014;234:114–119. doi: 10.1016/j.atherosclerosis.2014.02.025
17. Thies F, Garry JM, Yaqoob P, Rerkasem K, Williams J, Shearman CP, Gallagher PJ, Calder PC, Grimble RF. Association of n-3 polyunsaturated fatty acids with stability of atherosclerotic plaques: a randomised controlled trial. *Lancet*. 2003;361:477–485. doi: 10.1016/S0140-6736(03)12468-3
18. Simon DI, Silverstein RL. Atherothrombosis: seeing red? *Circulation*. 2015;132:1860–1862. doi: 10.1161/CIRCULATIONAHA.115.019259
19. DiNicolantonio JJ, OKeefe J. Importance of maintaining a low omega-6/omega-3 ratio for reducing platelet aggregation, coagulation and thrombosis. *Open Heart*. 2019;6:e001011. doi: 10.1136/openhrt-2019-001011
20. Olshansky B, Bhatt DL, Miller M, Steg PG, Brinton EA, Jacobson TA, Ketchum SB, Doyle RT Jr, Juliano RA, Jiao L, et al; REDUCE-IT Investigators. Cardiovascular benefits of icosapent ethyl in patients with and without atrial fibrillation in REDUCE-IT. *J Am Heart Assoc*. 2023;12:e026756. doi: 10.1161/JAHA.121.026756
21. Mozaffarian D, Psaty BM, Rimm EB, Lemaitre RN, Burke GL, Lyles MF, Lefkowitz D, Siscovick DS. Fish intake and risk for incident atrial fibrillation. *Circulation*. 2004;110:368–373. doi: 10.1161/01.CIR.0000138154.00779.A5
22. Qian F, Tintle NL, Jensen PN, Lemaitre RN, Imamura F, Feldreich TR, Nomura SO, Guan W, Laguzzi F, Kim E, et al. Omega-3 fatty acids biomarkers and incident atrial fibrillation. *JACC*. 2023;82:336–349. doi: 10.1016/j.jacc.2023.05.024
23. Bhatt DL, Steg PG, Miller M, Brinton EA, Jacobson TA, Ketchum SB, Doyle RT Jr, Juliano RA, Jiao L, Granowitz C, et al; REDUCE-IT Investigators. Cardiovascular risk reduction with icosapent ethyl for hypertriglyceridemia. *N Engl J Med*. 2019;380:11–22. doi: 10.1056/NEJMoa1812792
24. Dyerberg J. Linolenate-derived polyunsaturated fatty acids and prevention of atherosclerosis. *Nutr Rev*. 1986;44:125–134. doi: 10.1111/j.1753-4887.1986.tb07603.x
25. Kromann N, Green A. Epidemiological studies in the Upernavik district, Greenland Incidence of some chronic diseases 1950-1974. *Acta Med Scand*. 1980;208:401–406.
26. Iso H, Rexrode KM, Stampfer MJ, Manson JE, Colditz GA, Speizer FE, Hennekens CH, Willett WC. Intake of fish and omega-3 fatty acids and risk of stroke in women. *JAMA*. 2001;285:304–312. doi: 10.1001/jama.285.3.304
27. Yamagishi K, Iso H, Date C, Fukui M, Wakai K, Kikuchi S, Inaba Y, Tanabe N, Tamakoshi A; Japan Collaborative Cohort Study for Evaluation of Cancer Risk Study Group. Fish, omega-3 polyunsaturated fatty acids, and mortality from cardiovascular diseases in a nationwide community-based cohort of Japanese men and women the JACC (Japan Collaborative Cohort Study for Evaluation of Cancer Risk) Study. *J Am Coll Cardiol*. 2008;52:988–996. doi: 10.1016/j.jacc.2008.06.018
28. Del Brutto OH, Mera RM, Ha JE, Del Brutto VJ, Castillo PR, Zambrano M, Gillman J. Oily fish consumption is inversely correlated with cerebral microbleeds in community-dwelling older adults: results from the Atahualpa Project. *Aging Clin Exp Res*. 2016;28:737–743. doi: 10.1007/s40520-015-0473-6
29. Akintoye E, Sethi P, Harris WS, Thompson PA, Marchioli R, Tavazzi L, Latini R, Pretorius M, Brown NJ, Libby P, et al. Fish oil and perioperative bleeding. *Circ Cardiovasc Qual Outcomes*. 2018;11:e004584. doi: 10.1161/CIRCOUTCOMES.118.004584
30. Walker RE, Jackson KH, Tintle NL, Shearer GC, Bernasconi A, Masson S, Latini R, Heydari B, Kwong RY, Flock M, et al. Predicting the effects of supplemental EPA and DHA on the omega-3 index. *Am J Clin Nutr*. 2019;110:1034–1040. doi: 10.1093/ajcn/nqz161
31. Harris WS, Von Schacky C. The Omega-3 Index: a new risk factor for death from coronary heart disease? *Prev Med*. 2004;39:212–220. doi: 10.1016/j.ypmed.2004.02.030
32. 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;142:1297–1303. doi: 10.3945/jn.112.158295
33. Marklund M, Pingel R, Rosqvist F, Lindroos AK, Eriksson JW, Vessby B, Oscarsson J, Lind L, Risérus U. Fatty acid proportions in plasma cholesterol esters and phospholipids are positively correlated in various Swedish populations. *J Nutr*. 2017;147:2118–2125. doi: 10.3945/jn.117.254250
34. Bernasconi AA, Wiest MM, Lavie CJ, Milani RV, Laukkanen JA. Effect of omega-3 dosage on cardiovascular outcomes: an updated meta-analysis and meta-regression of interventional trials. *Mayo Clin Proc*. 2021;96:304–313. doi: 10.1016/j.mayocp.2020.08.034
35. Harris WS, Tintle NL, Imamura F, Qian F, Korat AVA, Marklund M, Djousse L, Bassett JK, Carmichael PH, Chen YY, et al; Fatty Acids and Outcomes Research Consortium (FORCE). Blood n-3 fatty acid levels and total and cause-specific mortality from 17 prospective studies. *Nat Commun*. 2021;12:2329. doi: 10.1038/s41467-021-22370-2
36. O'Keefe EL, O'Keefe JH, Tintle N, Westra J, Albuisson L, Harris WS. Circulating docosahexaenoic acid and risk of all-cause and cause-specific mortality. *Lancet*. 2023
37. 3C Study Group. Vascular factors and risk of dementia: design of the Three-City Study and baseline characteristics of the study population. *Neuroepidemiology*. 2003;22:316–325. doi: 10.1159/000072920
38. Jiang C, Lei SF, Liu MY, Xiao SM, Chen XD, Deng FY, Xu H, Tan LJ, Yang YJ, Wang YB, et al. Evaluating the correlation and prediction of trunk fat mass with five anthropometric indices in Chinese females aged 20-40 years. *Nutr Metab Cardiovasc Dis*. 2007;17:676–683. doi: 10.1016/j.numecd.2006.04.007
39. Jessen F, Wiese B, Bickel H, Eifflander-Gorfer S, Fuchs A, Kaduszkiewicz H, Kohler M, Luck T, Mosch E, Pentzek M, et al; AgeCoDe Study Group. Prediction of dementia in primary care patients. *PLoS One*. 2011;6:e16852. doi: 10.1371/journal.pone.0016852
40. Luck T, Riedel-Heller SG, Kaduszkiewicz H, Bickel H, Jessen F, Pentzek M, Wiese B, Koelsch H, van den Bussche H, Abholz HH, et al; AgeCoDe group. Mild cognitive impairment in general practice: age-specific prevalence and correlate results from the German study on ageing, cognition and dementia in primary care patients (AgeCoDe). *Dement Geriatr Cogn Disord*. 2007;24:307–316. doi: 10.1159/000108099
41. Harris TB, Launer LJ, Eiriksdottir G, Kjartansson O, Jonsson PV, Sigurdsson G, Thorgeirsson G, Aspelund T, Garcia ME, Cotch MF, et al. Age, Gene/ Environment Susceptibility-Reykjavik Study: multidisciplinary applied phenomics. *Am J Epidemiol*. 2007;165:1076–1087. doi: 10.1093/aje/kwk115
42. Kromhout D, Giltay EJ, Geleijnse JM; Alpha Omega Trial Group. n-3 fatty acids and cardiovascular events after myocardial infarction. *N Engl J Med*. 2010;363:2015–2026. doi: 10.1056/NEJMoa1003603
43. Geleijnse JM, Giltay EJ, Schouten EG, de Goede J, Oude Griep LM, Teitsma-Jansen AM, Katan MB, Kromhout D; Alpha Omega Trial Group. Effect of low doses of n-3 fatty acids on cardiovascular diseases in 4,837 post-myocardial infarction patients: design and baseline characteristics of the Alpha Omega Trial. *Am Heart J*. 2010;159:539–546.e2. doi: 10.1016/j.ahj.2009.12.033

44. Lee Y, Lin RS, Sung FC, Yang C, Chien K, Chen W, Su T, Hsu H, Huang Y. Chin-Shan Community Cardiovascular Cohort in Taiwan-baseline data and five-year follow-up morbidity and mortality. *J Clin Epidemiol*. 2000;53:838–846. doi: 10.1016/s0895-4356(00)00198-0
45. Fried LP, Borhani NO, Enright P, Furberg CD, Gardin JM, Kronmal RA, Kuller LH, Manolio TA, Mittelmark MB, Newman A. The Cardiovascular Health Study: design and rationale. *Ann Epidemiol*. 1991;1:263–276. doi: 10.1016/1047-2797(91)90005-w
46. Yamagishi K, Muraki I, Kubota Y, Hayama-Terada M, Imano H, Cui R, Umesawa M, Shimizu Y, Sankai T, Okada T, et al. The Circulatory Risk in Communities Study (CIRCS): a long-term epidemiological study for lifestyle-related disease among Japanese men and women living in communities. *J Epidemiol*. 2019;29:83–91. doi: 10.2188/jea.JE20180196
47. Canadian study of health and aging: study methods and prevalence of dementia. *CMAJ*. 1994;150:899–913.
48. Kroger E, Verreault R, Carmichael PH, Lindsay J, Julien P, Dewailly E, Ayotte P, Laurin D. Omega-3 fatty acids and risk of dementia: the Canadian Study of Health and Aging. *Am J Clin Nutr*. 2009;90:184–192. doi: 10.3945/ajcn.2008.26987
49. Tjonneland A, Olsen A, Boll K, Stripp C, Christensen J, Engholm G, Overvad K. 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;35:432–441. doi: 10.1080/14034940601047986
50. 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. doi: 10.1371/journal.pmed.1001255
51. Buckland G, Mayen AL, Agudo A, Travier N, Navarro C, Huerta JM, Chirlaque MD, Barricarte A, Ardanaz E, Moreno-Iribas C, et al. Olive oil intake and mortality within the Spanish population (EPIC-Spain). *Am J Clin Nutr*. 2012;96:142–149. doi: 10.3945/ajcn.111.024216
52. Tuomilehto J, Lindstrom J, Eriksson JG, Valle TT, Hamalainen H, Ilanne-Parikka P, Keinanen-Kiukaanniemi S, Laakso M, Louheranta A, Rastas M, et al; Finnish Diabetes Prevention Study Group. Prevention of type 2 diabetes mellitus by changes in lifestyle among subjects with impaired glucose tolerance. *N Engl J Med*. 2001;344:1343–1350. doi: 10.1056/NEJM200105033441801
53. Lindstrom J, Peltonen M, Eriksson JG, Ilanne-Parikka P, Aunola S, Keinanen-Kiukaanniemi S, Uusitupa M, Tuomilehto J; Finnish Diabetes Prevention Study (DPS). Improved lifestyle and decreased diabetes risk over 13 years: long-term follow-up of the randomised Finnish Diabetes Prevention Study (DPS). *Diabetologia*. 2013;56:284–293. doi: 10.1007/s00125-012-2752-5
54. 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;110:281–290. doi: 10.1093/oxfordjournals.aje.a112813
55. McEvoy M, Smith W, D'Este C, Duke J, Peel R, Schofield P, Scott R, Byles J, Henry D, Ewald B, et al. Cohort profile: the Hunter Community Study. *Int J Epidemiol*. 2010;39:1452–1463. doi: 10.1093/ije/dyp343
56. Ninomiya T, Nagata M, Hata J, Hirakawa Y, Ozawa M, Yoshida D, Ohara T, Kishimoto H, Mukai N, Fukuhara M, et al. Association between ratio of serum eicosapentaenoic acid to arachidonic acid and risk of cardiovascular disease: the Hisayama Study. *Atherosclerosis*. 2013;231:261–267. doi: 10.1016/j.atherosclerosis.2013.09.023
57. Malik VS, Chiuve SE, Campos H, Rimm EB, Mozaffarian D, Hu FB, Sun Q. Circulating very-long-chain saturated fatty acids and incident coronary heart disease in US men and women. *Circulation*. 2015;132:260–268. doi: 10.1161/CIRCULATIONAHA.114.014911
58. Yakoob MY, Shi P, Willett WC, Rexrode KM, Campos H, Orav EJ, Hu FB, Mozaffarian D. 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;133:1645–1654. doi: 10.1161/CIRCULATIONAHA.115.018410
59. Ferrucci L, Bandinelli S, Benvenuti E, Di Iorio A, Macchi C, Harris TB, Guralnik JM. Subsystems contributing to the decline in ability to walk: bridging the gap between epidemiology and geriatric practice in the InCHIANTI study. *J Am Geriatr Soc*. 2000;48:1618–1625. doi: 10.1111/j.1532-5415.2000.tb03873.x
60. 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.
61. Bild DE, Bluemke DA, Burke GL, Detrano R, Diez Roux AV, Folsom AR, Greenland P, Jacob DR Jr, Kronmal R, Liu K, et al. Multi-Ethnic Study of Atherosclerosis: objectives and design. *Am J Epidemiol*. 2002;156:871–881. doi: 10.1093/aje/kwf113
62. Laakso M, Kuusisto J, Stancakova A, Kuulasmaa T, Pajukanta P, Lusis AJ, Collins FS, Mohlke KL, Boehnke M. The Metabolic Syndrome in Men study: a resource for studies of metabolic and cardiovascular diseases. *J Lipid Res*. 2017;58:481–493. doi: 10.1194/jlr.O072629
63. Lankinen MA, Stancakova A, Uusitupa M, Agren J, Pihlajamaki J, Kuusisto J, Schwab U, Laakso M. Plasma fatty acids as predictors of glycaemia and type 2 diabetes. *Diabetologia*. 2015;58:2533–2544. doi: 10.1007/s00125-015-3730-5
64. Mahendran Y, Agren J, Uusitupa M, Cederberg H, Vangipurapu J, Stancakova A, Schwab U, Kuusisto J, Laakso M. Association of erythrocyte membrane fatty acids with changes in glycemia and risk of type 2 diabetes. *Am J Clin Nutr*. 2014;99:79–85. doi: 10.3945/ajcn.113.069740
65. Colditz GA, Manson JE, Hankinson SE. The Nurses' Health Study: 20-year contribution to the understanding of health among women. *J Womens Health*. 1997;6:49–62. doi: 10.1089/jwh.1997.6.49
66. Sun Q, Ma J, Campos H, Hu FB. Plasma and erythrocyte biomarkers of dairy fat intake and risk of ischemic heart disease. *Am J Clin Nutr*. 2007;86:929–937. doi: 10.1093/ajcn/86.4.929
67. Baylin A, Kabagambe EK, Siles X, Campos H. Adipose tissue biomarkers of fatty acid intake. *Am J Clin Nutr*. 2002;76:750–757. doi: 10.1093/ajcn/76.4.750
68. Rosqvist F, Bjermo H, Kullberg J, Johansson L, Michaelsson K, Ahlstrom H, Lind L, Riserus U. 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. doi: 10.1186/s12944-017-0445-2
69. Ikram MA, Brusselle G, Ghanbari M, Goedegebure A, Ikram MK, Kavousi M, Kieboom BCT, Klaver CCW, de Knegt RJ, Luik AI, et al. Objectives, design and main findings until 2020 from the Rotterdam Study. *Eur J Epidemiol*. 2020;35:483–517. doi: 10.1007/s10654-020-00640-5
70. Sudlow C, Gallacher J, Allen N, Beral V, Burton P, Danesh J, Downey P, Elliott P, Green J, Landray M, et al. UK biobank: an open access resource for identifying the causes of a wide range of complex diseases of middle and old age. *PLoS Med*. 2015;12:e1001779. doi: 10.1371/journal.pmed.1001779
71. Centre UBC. UK Biobank: Protocol for a large-scale prospective epidemiological resource (Amendment One Final). Accessed August 1, 2023. https://www.ukbiobank.ac.uk/media/gnkeyh2q/study-rationale.pdf. Adswood, Stockport, Cheshire, England: UK Biobank Coordinating Centre; 2007.
72. Julkunen H, Cichonska A, Tiainen M, Koskela H, Nybo K, Makela V, Nokso-Koivisto J, Kristiansson K, Perola M, Salomaa V, et al. Atlas of plasma NMR biomarkers for health and disease in 118,461 individuals from the UK Biobank. *Nat Commun*. 2023;14:604. doi: 10.1038/s41467-023-36231-7
73. Hagstrom E, Kilander L, Nylander R, Larsson EM, Michaelsson K, Melhus H, Ahlstrom H, Johansson L, Lind L, Arnlov J. Plasma parathyroid hormone is associated with vascular dementia and cerebral hyperintensities in two community-based cohorts. *J Clin Endocrinol Metab*. 2014;99:4181–4189. doi: 10.1210/jc.2014-1736
74. Huang X, Sjogren P, Arnlov J, Cederholm T, Lind L, Stenvinkel P, Lindholm B, Riserus U, Carrero JJ. Serum fatty acid patterns, insulin sensitivity and the metabolic syndrome in individuals with chronic kidney disease. *J Intern Med*. 2014;275:71–83. doi: 10.1111/joim.12130
75. 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.
76. Manson JE, Buring JE, Directors VS. VITamin D and OmegA-3 TriaL (VITAL). Accessed August 1, 2023. https://vitalstudy.org/index.html. Harvard Medical School; 2010.
77. Espeland MA, Rapp SR, Shumaker SA, Brunner R, Manson JE, Sherwin BB, Hsia J, Margolis KL, Hogan PE, Wallace R, et al; Women's Health Initiative Memory Study. Conjugated equine estrogens and global cognitive function in postmenopausal women: Women's Health Initiative Memory Study. *JAMA*. 2004;291:2959–2968. doi: 10.1001/jama.291.24.2959
78. Shumaker SA, Reboussin BA, Espeland MA, Rapp SR, McBee WL, Dailey M, Bowen D, Terrell T, Jones BN. 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;19:604–621. doi: 10.1016/s0197-2456(98)00038-5
79. Hu XF, Sandhu SK, Harris WS, Chan HM. Conversion ratios of n-3 fatty acids between plasma and erythrocytes: a systematic review and meta-regression. *Br J Nutr*. 2017;117:1162–1173. doi: 10.1017/S0007114517001052
80. Schuchardt JP, Tintle N, Westra J, Harris WS. Estimation and predictors of the Omega-3 Index in the UK Biobank. *Br J Nutr*. 2023;130:312–322. doi: 10.1017/S0007114522003282