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

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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

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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

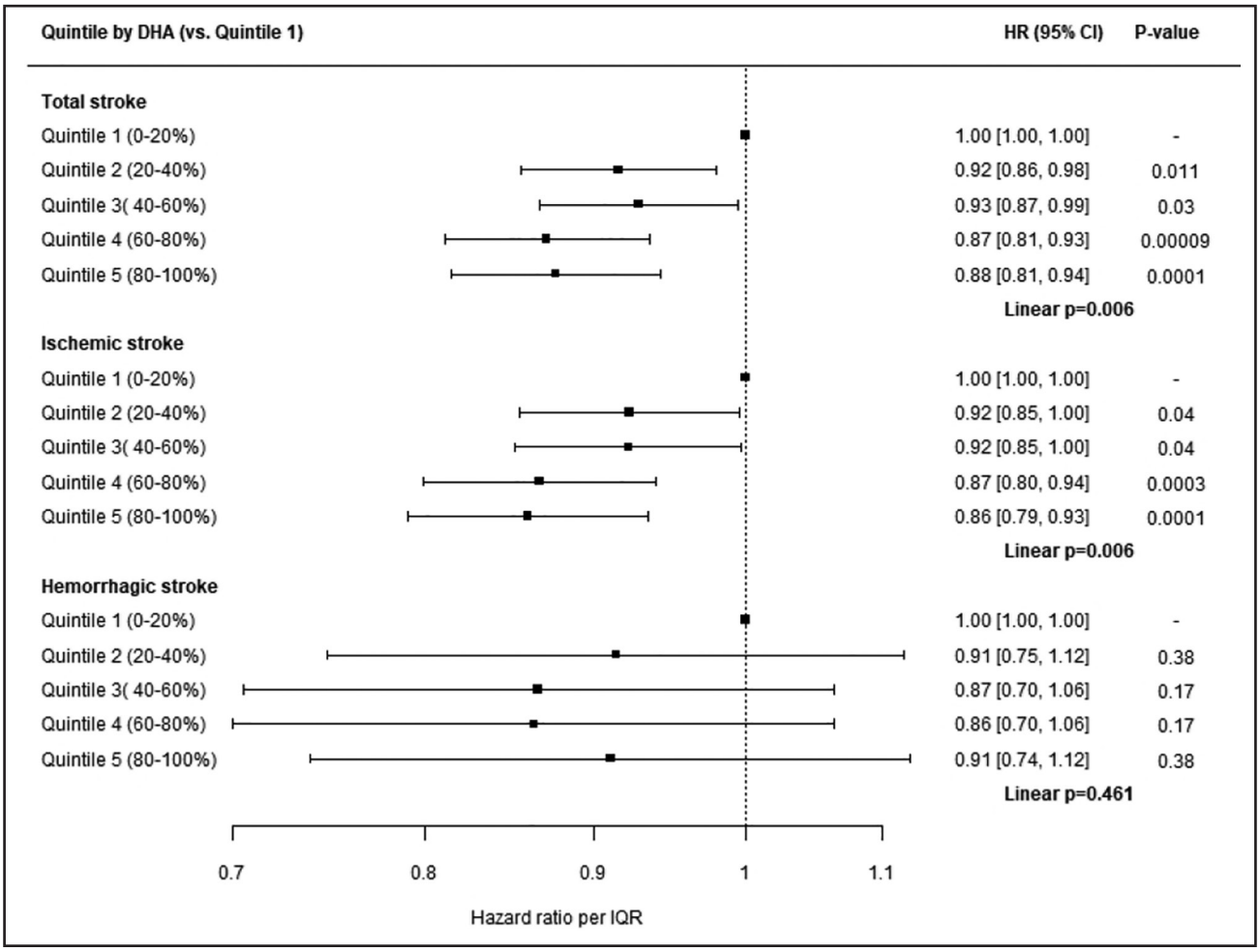

Figure 1. Association of docosahexaenoic acid (DHA) and risk of stroke.

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

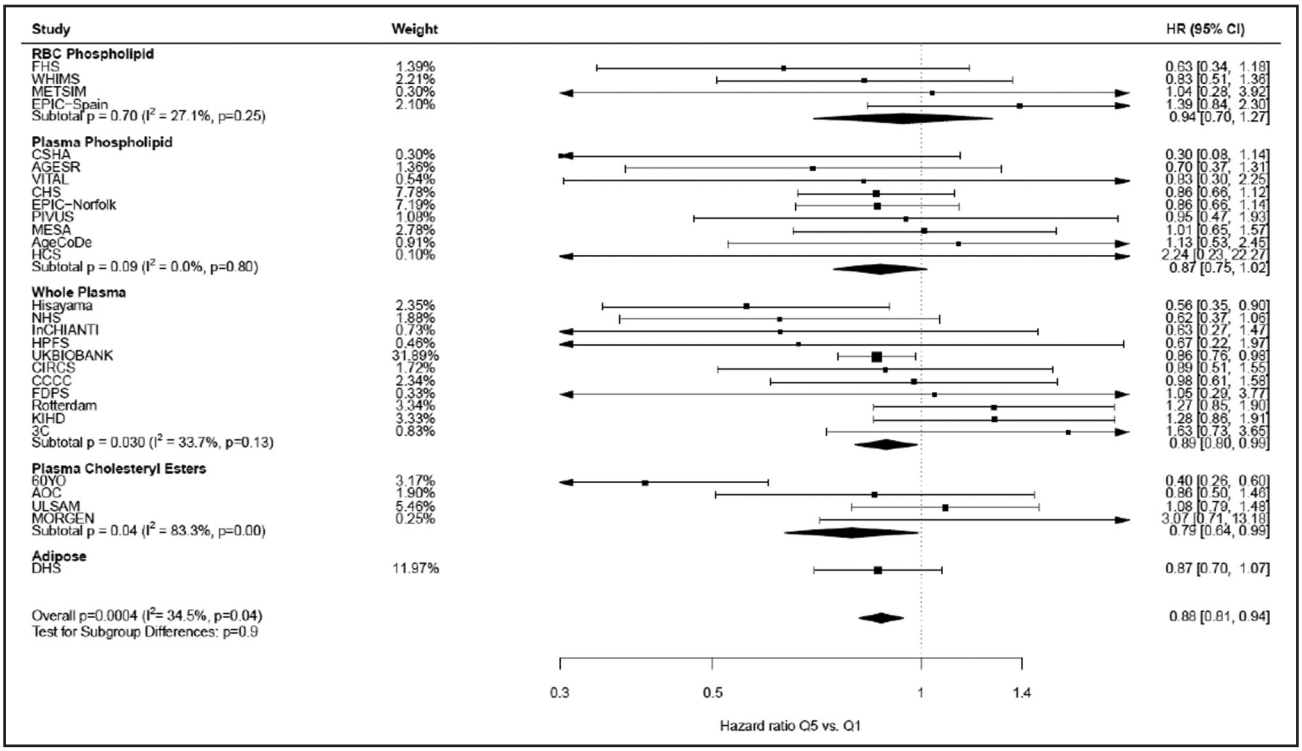

Figure 2. Association of docosahexaenoic acid (DHA) and risk of stroke by lipid compartment.

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

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

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Supplemental Material

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