Original Contribution

Marine n-3 Polyunsaturated Fatty Acids and the Risk of Ischemic Stroke

Stine K. Venø, MD; Christian S. Bork, MD; Marianne U. Jakobsen, MSc, PhD; Søren Lundbye-Christensen, MSc, PhD; Peter L. McLennan, BSc, PhD; Flemming W. Bach, MD, DMSc; Kim Overvad, MD, PhD; Erik B. Schmidt, MD, DMSc

Background and Purpose—We hypothesized that total marine n-3 polyunsaturated fatty acids (PUFA), in particular eicosapentaenoic acid (EPA) and docosahexaenoic acid (DHA) in the diet and in adipose tissue (biomarkers of long-term intake and endogenous exposure) were inversely associated with the risk of ischemic stroke and its subtypes.

Methods—The Diet, Cancer and Health cohort consisted of 57 053 participants aged 50 to 65 years at enrolment. All participants filled in a food frequency questionnaire and had an adipose tissue biopsy taken at baseline. Information on ischemic stroke during follow-up was obtained from The Danish National Patient Register, and all cases were validated. Cases and a random sample of 3203 subjects from the whole cohort had their fatty acid composition of adipose tissue determined by gas chromatography.

Results—During 13.5 years of follow-up 1879 participants developed an ischemic stroke. Adipose tissue content of EPA was inversely associated with total ischemic stroke (hazard ratio [HR], 0.74; 95% CI, 0.62–0.88) when comparing the highest with the lowest quartile. Also, lower rates of large artery atherosclerosis were seen with higher intakes of total marine n-3 PUFA (HR, 0.69; 95% CI, 0.50–0.95), EPA (HR, 0.66; 95% CI, 0.48–0.91) and DHA (HR, 0.72; 95% CI, 0.53–0.99), and higher adipose tissue content of EPA (HR, 0.52; 95% CI, 0.36–0.76). Higher rates of cardioembolism were seen with higher intakes of total marine n-3 PUFA (HR, 2.50; 95% CI, 1.38–4.53) and DHA (HR, 2.12; 95% CI, 1.21–3.69) as well as with higher adipose tissue content of total marine n-3 PUFA (HR, 2.63; 95% CI, 1.33–5.19) and DHA (HR, 2.00; 95% CI, 1.04–3.84). The EPA content in adipose tissue was inversely associated with small-vessel occlusion (HR, 0.69; 95% CI, 0.55–0.88).

Conclusions—EPA was associated with lower risks of most types of ischemic stroke, apart from cardioembolism, while inconsistent findings were observed for total marine n-3 PUFA and DHA. **(*Stroke*. 2019;50:00-00. DOI: 10.1161/STROKEAHA.118.023384.)**

Key Words: adipose tissue ■ docosahexaenoic acids ■ eicosapentaenoic acid ■ fatty acids

Fish consumption may decrease cardiovascular mortality which has been attributed to the content of marine n-3 polyunsaturated fatty acids (PUFA).[1–4] However, this has mainly been shown for coronary heart disease while associations between marine n-3 PUFA and ischemic stroke have been less studied and with inconsistent findings reported.[5–13] Most studies about ischemic stroke have traditionally investigated the associations for total marine n-3 PUFA and not discriminated between eicosapentaenoic acid (EPA) and docosahexaenoic acid (DHA), which have different biological effects.[14] Furthermore, ischemic stroke is a heterogeneous condition, and the associations of EPA and DHA may differ between ischemic stroke subtypes,[15] which has not been taken into consideration in most studies. Another concern is that most observational studies on marine n-3 PUFA have only assessed self-reported dietary intakes, which are prone to measurement error.

In this study, we addressed these issues by investigating both total marine n-3 PUFA, EPA, and DHA from the diet and the contents in adipose tissue, which is a long-term biomarker of their intake and endogenous exposure. We investigated the hypothesis that intake and adipose tissue content of total marine n-3 PUFA, EPA, and DHA were inversely associated with the risk of ischemic stroke and ischemic stroke subtypes.

Methods

Study Population

The Danish Diet, Cancer and Health cohort was established between 1993 and 1997. The study design has been described previously,[16]

Received August 28, 2018; final revision received November 26, 2018; accepted December 4, 2018.

From the Department of Cardiology (S.K.V., C.S.B., K.O., E.B.S.) and Unit of Clinical Biostatistics (S.L.-C.), Aalborg University Hospital, Denmark; Department of Clinical Medicine, Aalborg University, Denmark (S.K.V., E.B.S.); Division for Diet, Disease Prevention and Toxicology, National Food Institute, Technical University of Denmark, Kongens Lyngby (M.U.J.); School of Medicine, University of Wollongong, Australia (P.L.M.); Department of Neurology, Aarhus University Hospital, Denmark (F.W.B.); and Department of Public Health, Aarhus University, Denmark (K.O.).

Presented in part at the European Atherosclerosis Society Congress, Lisbon, Portugal, May 8, 2018.

The online-only Data Supplement is available with this article at https://www.ahajournals.org/doi/suppl/10.1161/STROKEAHA.118.023384.

Correspondence to Stine Krogh Venø, MD, Department of Cardiology, Aalborg University Hospital, Søndre Skovvej 15, 9000 Aalborg, Denmark. Email s.venoe@rn.dk

but briefly, 160 725 men and women 50 to 64 years old, with no previous cancer diagnosis were invited to participate. A total of 57 053 accepted the invitation and gave written informed consent. The study was approved by the Ethics Committees and the Danish Data Protection Agency. Because of the sensitive nature of the data collected for this study, requests to access the data set from qualified researchers trained in human subject confidentiality protocols may be sent to the Diet, Cancer and Health Steering Committee at the Danish Cancer Society (contact information: louhan@cancer.dk). We used a cohort design for the analyses of dietary intake. For the adipose tissue content analyses, we used a case-cohort design where a subcohort of 3500 participants was randomly drawn from the whole cohort.

Dietary Intakes of Marine n-3 PUFA

Information on habitual dietary intake was assessed by a 192-item semiquantitative food frequency questionnaire at baseline with 24 questions covering intake of seafood.[17,18] The self-administered food frequency questionnaire was validated against 2 weeks of weighed diet records. Correlations between energy-adjusted intakes from the food frequency questionnaire and the 2-week diet records for PUFAs were 0.53 for men and 0.28 for women.[18] Participants reported their average intake of different food and beverage items over the past 12 months. Calculation of average daily intakes of foods and nutrients was done using the FoodCalc program (http://www.ibt.ku.dk/jesper/foodcalc/) based on Danish food composition tables.

Adipose Tissue Biopsies

At baseline, an adipose tissue sample was taken from the subcutaneous fat from the buttocks of every participant, and fatty acid composition was determined by gas chromatography as described previously.[19] A Luer lock system (Terumo, Terumo Corp, Tokyo, Japan) was used consisting of a needle, a venoject multisample Luer adaptor, and an evacuated blood tube. Fatty acid composition was determined by gas chromatography on a CP-sil 88 60 m×0.25 mm ID capillary column, using a Varian 3900 GC with a CP-8400 autosampler (Varian, Middleburg, The Netherlands) equipped with a flame ionization detector. Commercially available standards (Nu-check-Prep, Inc, MN) were used to identify individual fatty acids. The contents of EPA, docosapentaenoic acid (DPA), and DHA were given as weight percentages of total fatty acids. The interassay coefficient of variation for determination of EPA, DPA, and DHA in adipose tissue was 6.1%, 4.2%, and 5.2 %, respectively.

Covariates

At baseline, the participants completed a lifestyle questionnaire about information on education status, smoking habits, physical activity, history of hypercholesterolemia, hypertension, and diabetes mellitus. Physical activity during the past year was reported as hours per week spent on walking, biking, housework, home maintenance, gardening, and sports during summer and winter. Smoking habits were self-reported as frequency (never, former, or current), number, and type (cigarettes, cigars, cheroots, and tobacco pipes smoked per day). Anthropometric measurements (height, weight, and waist circumference) were obtained by technicians, and waist circumference was measured in standing position using a rigid tape measure at the narrowest horizontal circumference between the lower rib and the iliac crest. Body mass index was calculated as weight (kg)/height (m).^{2} Information on alcohol intake and intake of foods was obtained from the food frequency questionnaire. Information on atrial fibrillation/atrial flutter was obtained by linkage with the Danish National Patient Register.

Outcomes

The outcomes in this study were incident ischemic stroke and subtypes of ischemic stroke. Identification of ischemic stroke cases has been described in detail elsewhere.[19,20] Briefly, participants in the Diet, Cancer and Health cohort registered with a stroke diagnosis in the Danish National Patient Register were identified. Potential stroke cases included subjects with an *International Classification of Diseases*, *Eighth Revision* (*ICD-8*) discharge code 430, 431, 433, 434, 436.01, or 436.90 or *ICD-10* discharge code I60, I61, I63, or I64. All medical records were reviewed and diagnoses validated. Ischemic stroke cases were divided into 5 subtypes according to the Trial of Org 10172 in Acute Stroke Treatment-classification system,[21] which is based on cause and includes large artery atherosclerosis, cardioembolism, small-vessel occlusion, stroke of other cause, and stroke of undetermined cause. Participants were followed from enrolment until time of ischemic stroke, death, emigration, or end of follow-up (December 2009), whichever came first.

Statistical Analyses

We used Cox proportional hazards models with age as the underlying timescale to estimate hazard ratios with 95% CI. For dietary intake analyses, total marine n-3 PUFA, EPA, DPA, and DHA were expressed as energy-adjusted intake in g/d. For the analyses of adipose tissue, we used a case-cohort approach based on weighted Cox proportional hazard regression. Cases and noncases within the subcohort were assigned the weights 1 and N/n respectively, where N was the number of noncases in the full cohort and n was the number of noncases in the subcohort.[22] Analyses were performed as sex-stratified analyses allowing for baseline hazards to differ between men and women. Exposure variables were categorized into quartiles using the lowest quartile as the reference. We did a test for trend across quartiles based on Wald's test. To address potential confounding, we adjusted for different covariates. First, we adjusted for baseline age (years, continuous) and sex (model 1A). In model 1B, we added covariates to model 1A: educational status (<7, 8–10, and >10 years), waist circumference adjusted for body mass index (cm and continuous), smoking status (noncurrent, current <15, and ≥15 g tobacco/day), physical activity (hours/wk and continuous), alcohol intake (g/d and continuous), and alcohol abstain (yes and no). The analyses were further adjusted for possible intermediate variables (model 2): self-reported hypertension or use of antihypertensive medication (yes, no, and unknown), self-reported hypercholesterolemia or use of lipid-lowering medication (yes, no, and unknown), self-reported diabetes mellitus or use of insulin (yes, no, and unknown), and atrial fibrillation/atrial flutter (yes and no). All associations were investigated for total ischemic stroke and for each of the ischemic stroke subtypes. We used restricted cubic splines with 3 knots to adjust for continuous variables. The proportional hazards assumption in the Cox regression analyses were evaluated by plotting scaled Schoenfeld residuals. We generated radar charts to illustrate possible differences in the underlying dietary patterns related to dietary intake and adipose tissue content of total marine n-3 PUFA. The statistical analyses were done using Stata 14 (StataCorp).

Results

A total of 1151 of the 57 053 participants were excluded from the study because of a diagnosis of cancer or stroke before entry into the study, and 564 participants were excluded because of missing information on covariates. Hence, a total of 55 338 participants in the full cohort and 3410 in the subcohort were available for analysis. During a median of 13.5 years of follow-up, 1879 participants developed ischemic stroke from whom 1755 adipose biopsies were available while 3201 biopsies were available from the subcohort (Figure 1). Characteristics of the cohort, subcohort and ischemic stroke cases are presented in Table 1.

Neither dietary intake nor adipose tissue content of total marine n-3 PUFA showed any association with total ischemic stroke (Tables 2 and 3). Likewise, dietary intake of EPA and DHA and adipose tissue content of DHA showed no association with total ischemic stroke (Tables 2 and 3). However, adipose tissue content of EPA showed a statistically significant inverse association with total ischemic stroke with a hazard

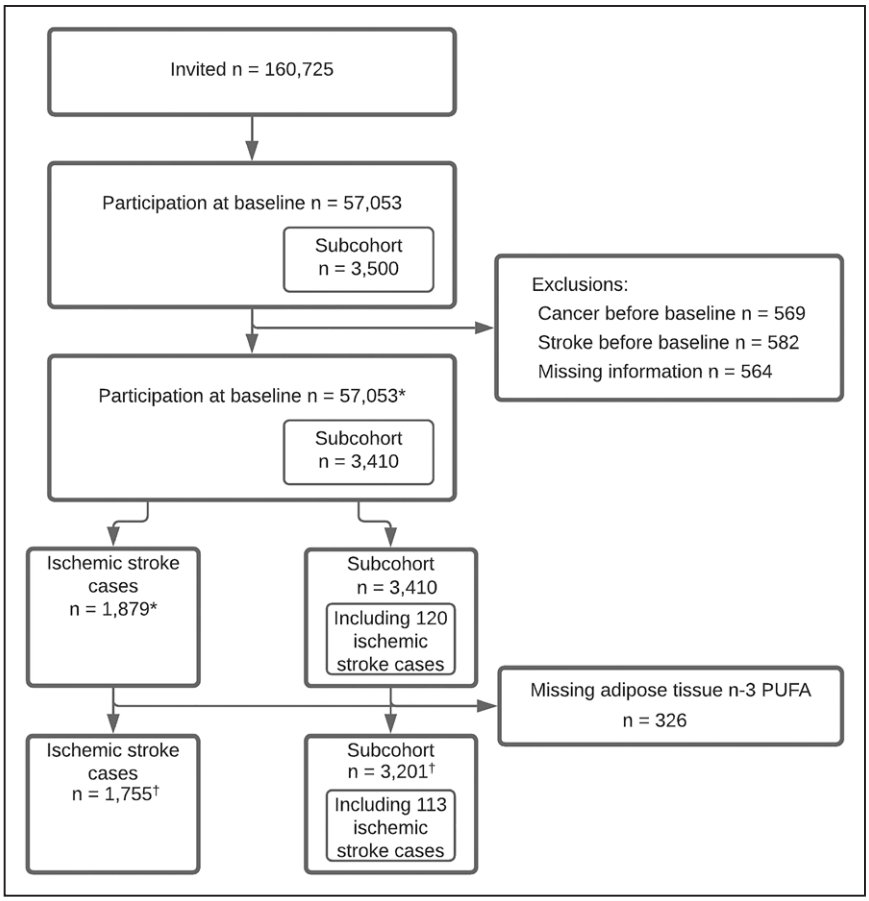

Figure 1. Flowchart of the Diet, Cancer and Health cohort, ischemic stroke cases, and the subcohort. PUFA indicates polyunsaturated fatty acid. *Included in the analyses of dietary intake. †Included in the analyses of adipose tissue content.

ratio of 0.74 (95% CI, 0.62–0.88) comparing the highest and the lowest quartile (Table 3).

Regarding subtypes of ischemic stroke, results showed statistically significant inverse associations between intake of total marine n-3 PUFA, EPA, and DHA and large artery atherosclerosis (Table 2), while only EPA in adipose tissue showed an inverse association with large artery atherosclerosis (hazard ratio, 0.52; 95% CI, 0.36–0.76) comparing the highest and the lowest quartiles, which was statistically significant (Table 3). Intake of total marine n-3 PUFA, EPA, and DHA and adipose tissue content of total marine n-3 PUFA and DHA were statistically significantly associated with higher rates of cardioembolism (Tables 2 and 3). No consistent associations were found between intake of total marine n-3 PUFA, EPA, or DHA and small-vessel occlusion (Table 2). However, a statistically significant inverse association was found between adipose tissue content of EPA and small-vessel occlusion (hazard ratio, 0.69; 95% CI, 0.55–0.88) comparing the highest and the lowest quartile; (Table 3). The results of the age- and sex-adjusted analyses (model 1A), as well as results of the analyses including possible intermediate variables (model 2), showed similar pattern of associations (Table I–IV in the online-only Data Supplement). Also, sensitivity analyses showed that the observed associations (model 1B) were robust when analyses were conducted without adjustment for smoking status (Table V in the online-only Data Supplement).

The underlying dietary patterns in relation to the intake of marine n-3 PUFA for participants in the whole cohort and content of marine n-3 PUFA in adipose tissue for participants in the subcohort are given in Figure 2. The results indicate that participants with a high intake of marine n-3 PUFA as well as a high content of marine n-3 PUFA in adipose tissue tended to consume more vegetables, vegetable oils, fish, poultry and alcohol and less, butter, sugar, sweets, and snacks.

Discussion

We found that intakes of EPA and DHA were associated with a lower risk of stroke caused by large artery atherosclerosis and a higher risk of stroke caused by cardioembolism. Adipose tissue content of EPA was associated with a lower risk of total ischemic stroke, strokes caused by large artery atherosclerosis and small-vessel occlusion, while adipose tissue content of DHA was associated with a higher risk of stroke caused by cardioembolism.

Our study had a number of strengths. First, we investigated total marine n-3 PUFA, EPA, and DHA from both dietary intake and adipose tissue content. Only a few participants were lost to follow-up (mostly because of emigration), hence the risk of selection bias was low. Furthermore, information on ischemic stroke cases was obtained by linkage with the Danish National Patient Register independently of exposure information limiting the risk of information bias. Ischemic stroke diagnoses were individually validated and classified according to the Trial of ORG 10172 in Acute Stroke Treatment-classifications,[21] which is another major strength of the study together with detailed information on potential confounders. However, the study also had some limitations. Importantly, exposures were based on baseline information and participants may have changed their diet during follow-up. Only 35% of

Table 1. Baseline Characteristics of Participants in the Cohort, Subcohort, and Ischemic Stroke Cases

Characteristics	Cohort	Subcohort	Ischemic Stroke Cases
Age, y	56.1 (50.7–64.2)	56.3 (50.7–64.2)	58.9 (51.0–64.6)
Sex, %			
Male	47.6	54.1	61.9
Female	52.4	45.9	38.1
Education, %			
<7 y	32.9	32.9	41.0
8–10 y	46.1	45.0	42.6
>10 y	21.1	22.2	16.5
BMI, kg/m^2	25.5 (20.5–33.3)	25.8 (20.7–33.4)	26.3 (21.0–34.9)
Waist circumference, cm	88.8 (69.0–110.0)	90.0 (69.5–111.0)	93.0 (72.0–116.0)
Smoking status, %			
Noncurrent	64.1	63.9	50.5
Current <15 g/d	13.0	13.6	15.4
Current ≥15 g/d	22.9	22.5	34.0
Physical activity (h/wk)	2.5 (0.0–11.0)	2.5 (0.0–10.5)	2.0 (0.0–11.0)
Alcohol intake, g/d	12.9 (0.7–64.6)	13.8 (0.8–65.4)	14.5 (0.5–79.4)
Alcohol abstain, %			
Yes	2.3	2.1	3.0
No	97.7	97.9	97.0
Hypercholesterolemia, %			
Yes	7.4	7.9	10.8
No	50.3	49.3	48.4
Unknown	42.4	42.8	40.8
Hypertension, %			
Yes	16.0	15.7	28.8
No	70.9	71.7	57.7
Unknown	13.4	12.6	13.6
Diabetes mellitus, %			
Yes	2.0	2.0	4.3
No	93.4	92.9	89.6
Unknown	4.6	5.1	6.1
Atrial fibrillation/atrial flutter, %			
Yes	0.8	0.9	1.4
No	99.2	99.1	98.6

Values are medians (5th–95th percentile) for continuous variables and percent for categorical variables. BMI indicates body mass index.

those invited agreed to participate in the study and subjects with a higher socioeconomic status were slightly over-represented in this cohort.[16] However, for the given exposure range the associations between dietary intake and adipose tissue content of marine n-3 PUFA and risk of ischemic stroke are believed not to differ systematically between participants and nonparticipants. Also, the study only included middle-aged white subjects who had survived without a diagnosis of ischemic stroke or cancer before inclusion which limits the generalizability of our results. In addition, 30% of cases were classified as having stroke of undetermined cause which included cases with 2 or more potential causes identified and those with incomplete evaluation. Further, the radar charts illustrated that, apart from a higher alcohol intake, healthier eating habits were associated with a higher dietary intake of marine n-3 PUFA as well as with a high content of marine n-3 PUFA in adipose tissue. Thus, results from the study cannot be interpreted as associations for marine n-3 PUFA independently of the dietary pattern associated with a high fish and seafood intake. Finally, it should be mentioned that fish and seafood also contain vitamin D, selenium, and other substances that might confer benefit.[23] Smoking is an important risk factor for the development of cardiovascular diseases. Proper control for confounding is, therefore, an important issue. To explore potential residual confounding, we conducted the analyses both with and without control for smoking. No major differences in the associations were observed and we, therefore, considered residual confounding an unlikely explanation for the results. Previous studies also indicated that interaction between smoking and marine n-3 PUFA may be of concern.[24] In this study, we did not have the power to explore associations between marine n-3 PUFA and ischemic stroke according to smoking status. It is, therefore, possible that the observed association between marine n-3 PUFA and stroke could be modified by smoking status as indicated by previous studies.[24]

Other prospective cohort studies[5–8] have also investigated the relation between intake of marine n-3 PUFA and risk of ischemic stroke. Only one of the studies,[8] including 142 ischemic stroke cases, found an inverse association, however, not statistically significant. Other studies[9–11] using biomarkers of marine n-3 PUFA have investigated serum levels of n-3 PUFA, EPA, and DHA in relation to ischemic stroke subtypes. While serum levels of marine n-3 PUFA reflect dietary intake during the preceding weeks, adipose tissue contents as used in our study is a biomarker of the intake of marine n-3 PUFA during the previous 1 to 2 years.[25]

A recent prospective cohort study,[9] including 953 ischemic stroke cases found an inverse association between serum levels of DHA and total ischemic stroke and atherothrombotic stroke while there was no association between EPA and total ischemic stroke, atherothrombotic stroke, or strokes caused by cardioembolism. In another nested case-control study, including 964 female ischemic stroke cases,[10] serum levels of total marine n-3 PUFA were associated with a lower risk of total ischemic stroke, large artery atherosclerosis, and small-vessel occlusion. Also, DHA (but not EPA) levels were inversely associated with all subtypes of ischemic stroke in that study. In contrast, another nested case-control study, including 114 ischemic stroke cases,[11] reported no association between serum levels of total or individual marine n-3 PUFA and the risk of ischemic stroke. Two case-control studies reported inconsistent results using erythrocyte membrane content of

Table 2. Risk of Ischemic Stroke and Ischemic Stroke Subtypes by Quartiles of Marine n-3 PUFA, EPA, DPA, and DHA Intake

	Total Marine n-3 PUFA		EPA		DPA		DHA	
	HR	95% CI	HR	95% CI	HR	95% CI	HR	95% CI
Total ischemic stroke								
n=1879								
Q1	1		1		1		1	
Q2	1.06	(0.93–1.21)	1.05	(0.92–1.20)	1.10	(0.96–1.26)	1.08	(0.95–1.23)
Q3	1.06	(0.93–1.21)	1.09	(0.96–1.24)	1.10	(0.96–1.26)	1.02	(0.90–1.67)
Q4	1.06	(0.93–1.20)	1.01	(0.89–1.15)	1.16	(1.02–1.33)	1.06	(0.94–1.21)
P_{trend}	0.458		0.732		0.033		0.513	
Large artery atherosclerosis								
n=319								
Q1	1		1		1		1	
Q2	0.97	(0.72–1.30)	0.86	(0.64–1.16)	1.01	(0.74–1.38)	0.90	(0.67–1.22)
Q3	0.88	(0.65–1.19)	0.82	(0.60–1.11)	0.91	(0.67–1.25)	0.86	(0.63–1.16)
Q4	0.69	(0.50–0.95)	0.66	(0.48–0.91)	0.88	(0.64–1.21)	0.72	(0.53–0.99)
P_{trend}	0.020		0.012		0.353		0.043	
Cardioembolism								
n=102								
Q1	1		1		1		1	
Q2	1.36	(0.69–2.66)	1.17	(0.58–2.35)	0.80	(0.41–1.56)	0.97	(0.50–1.89)
Q3	1.49	(0.78–2.88)	2.34	(1.27–4.30)	1.14	(0.63–2.08)	1.27	(0.68–2.36)
Q4	2.50	(1.38–4.53)	2.02	(1.09–3.73)	1.68	(0.96–2.91)	2.12	(1.21–3.69)
P_{trend}	0.001		0.005		0.020		0.002	
Small-vessel occlusion								
n=844								
Q1	1		1		1		1	
Q2	1.15	(0.94–1.40)	1.14	(0.94–1.38)	1.27	(1.04–1.55)	1.26	(1.04–1.54)
Q3	1.20	(0.98–1.45)	1.16	(0.96–1.41)	1.21	(0.99–1.48)	1.17	(0.96–1.43)
Q4	1.06	(0.87–1.30)	1.05	(0.86–1.28)	1.19	(0.98–1.46)	1.13	(0.93–1.38)
P_{trend}	0.518		0.647		0.179		0.411	
Stroke of other cause								
n=98								
Q1	1		1		1		1	
Q2	1.15	(0.67–1.98)	1.29	(0.75–2.21)	1.65	(0.93–2.94)	0.95	(0.55–1.65)
Q3	0.89	(0.50–1.61)	0.85	(0.46–1.58)	1.08	(0.57–2.03)	0.83	(0.47–1.49)
Q4	1.05	(0.60–1.84)	1.17	(0.67–2.05)	1.51	(0.84–2.71)	1.02	(0.59–1.75)
P_{trend}	0.926		0.905		0.419		0.937	
Stroke of undetermined cause								
n=516								
Q1	1		1		1		1	
Q2	0.95	(0.74–1.22)	1.00	(0.78–1.29)	0.87	(0.67–1.14)	0.98	(0.76–1.26)
Q3	0.96	(0.74–1.23)	1.08	(0.84–1.39)	1.06	(0.82–1.36)	0.93	(0.72–1.20)
Q4	1.12	(0.89–1.43)	1.08	(0.84–1.37)	1.16	(0.91–1.48)	1.07	(0.84–1.36)
P_{trend}	0.321		0.459		0.091		0.661	

Adjusted for baseline age, sex, education, waist circumference adjusted for BMI, smoking, physical activity, alcohol intake, and alcohol abstain (Model 1B). BMI indicates body mass index; DHA, docosahexaenoic acid; DPA, docosapentaenoic acid; EPA, eicosapentaenoic acid; HR, hazard ratio; and PUFA, polyunsaturated fatty acid.

Table 3. Risk of Ischemic Stroke and Ischemic Stroke Subtypes by Quartiles of Marine n-3 PUFA, EPA, DPA, and DHA Adipose Tissue Content

	Total Marine n-3 PUFA		EPA		DPA		DHA	
	HR	95% CI	HR	95% CI	HR	95% CI	HR	95% CI
Total ischemic stroke								
n=1755								
Q1	1		1		1		1	
Q2	0.98	(0.82–1.17)	0.91	(0.77–1.07)	1.45	(1.21–1.74)	0.92	(0.77–1.10)
Q3	1.12	(0.94–1.33)	0.66	(0.55–0.81)	1.37	(1.40–1.65)	1.09	(0.91–1.30)
Q4	1.08	(0.90–1.30)	0.74	(0.62–0.88)	1.36	(1.13–1.65)	1.00	(0.83–1.20)
P_{trend}	0.213		<0.001		0.006		0.580	
Large artery atherosclerosis								
n=300								
Q1	1		1		1		1	
Q2	0.86	(0.61–1.22)	0.96	(0.70–1.32)	1.46	(1.04–2.07)	0.94	(0.67–1.32)
Q3	1.09	(0.78–1.52)	0.64	(0.43–0.94)	1.23	(0.86–1.76)	1.08	(0.77–1.52)
Q4	0.78	(0.53–1.13)	0.52	(0.36–0.76)	1.02	(0.69–1.51)	0.79	(0.54–1.16)
P_{trend}	0.404		<0.001		0.845		0.386	
Cardioembolism								
n=99								
Q1	1		1		1		1	
Q2	2.08	(1.04–4.15)	1.13	(0.61–2.11)	2.45	(1.19–5.05)	1.37	(0.71–2.64)
Q3	2.04	(1.03–4.04)	1.06	(0.52–2.14)	2.61	(1.30–5.25)	1.64	(0.87–3.10)
Q4	2.63	(1.33–5.19)	1.52	(0.82–2.81)	3.06	(1.50–6.24)	2.00	(1.04–3.84)
P_{trend}	0.007		0.183		0.003		0.030	
Small-vessel occlusion								
n=781								
Q1	1		1		1		1	
Q2	0.91	(0.72–1.15)	0.84	(0.68–1.04)	1.37	(1.08–1.74)	0.86	(0.68–1.09)
Q3	1.05	(0.83–1.32)	0.61	(0.47–0.79)	1.29	(1.01–1.64)	1.07	(0.85–1.34)
Q4	0.99	(0.79–1.26)	0.69	(0.55–0.88)	1.19	(0.93–1.52)	0.92	(0.72–1.17)
P_{trend}	0.768		<0.001		0.283		0.916	
Stroke of other cause								
n=91								
Q1	1		1		1		1	
Q2	1.51	(0.84–2.72)	0.91	(0.54–1.53)	1.37	(0.73–2.56)	1.13	(0.64–2.00)
Q3	0.96	(0.51–1.83)	0.62	(0.32–1.19)	1.45	(0.78–2.69)	1.07	(0.59–1.94)
Q4	1.40	(0.77–2.52)	0.52	(0.27–0.98)	1.28	0.68–2.41)	1.07	(0.58–1.98)
P_{trend}	0.538		0.019		0.383		0.869	
Stroke of undetermined cause								
n=484								
Q1	1		1		1		1	
Q2	0.99	(0.73–1.32)	0.95	(0.73–1.24)	1.53	(1.13–2.07)	0.91	(0.68–1.21)
Q3	1.19	(0.89–1.59)	0.72	(0.53–1.00)	1.46	(1.07–1.98)	1.06	(0.79–1.42)
Q4	1.22	(0.92–1.64)	0.87	(0.65–1.16)	1.76	(1.29–2.41)	1.12	(0.83–1.50)
P_{trend}	0.093		0.176		0.001		0.312	

Adjusted for baseline age, sex, education, waist circumference adjusted for BMI, smoking, physical activity, alcohol intake, and alcohol abstain (Model 1B). BMI indicates body mass index; DHA, docosahexaenoic acid; DPA, docosapentaenoic acid; EPA, eicosapentaenoic acid; HR, hazard ratio; and PUFA, polyunsaturated fatty acid.

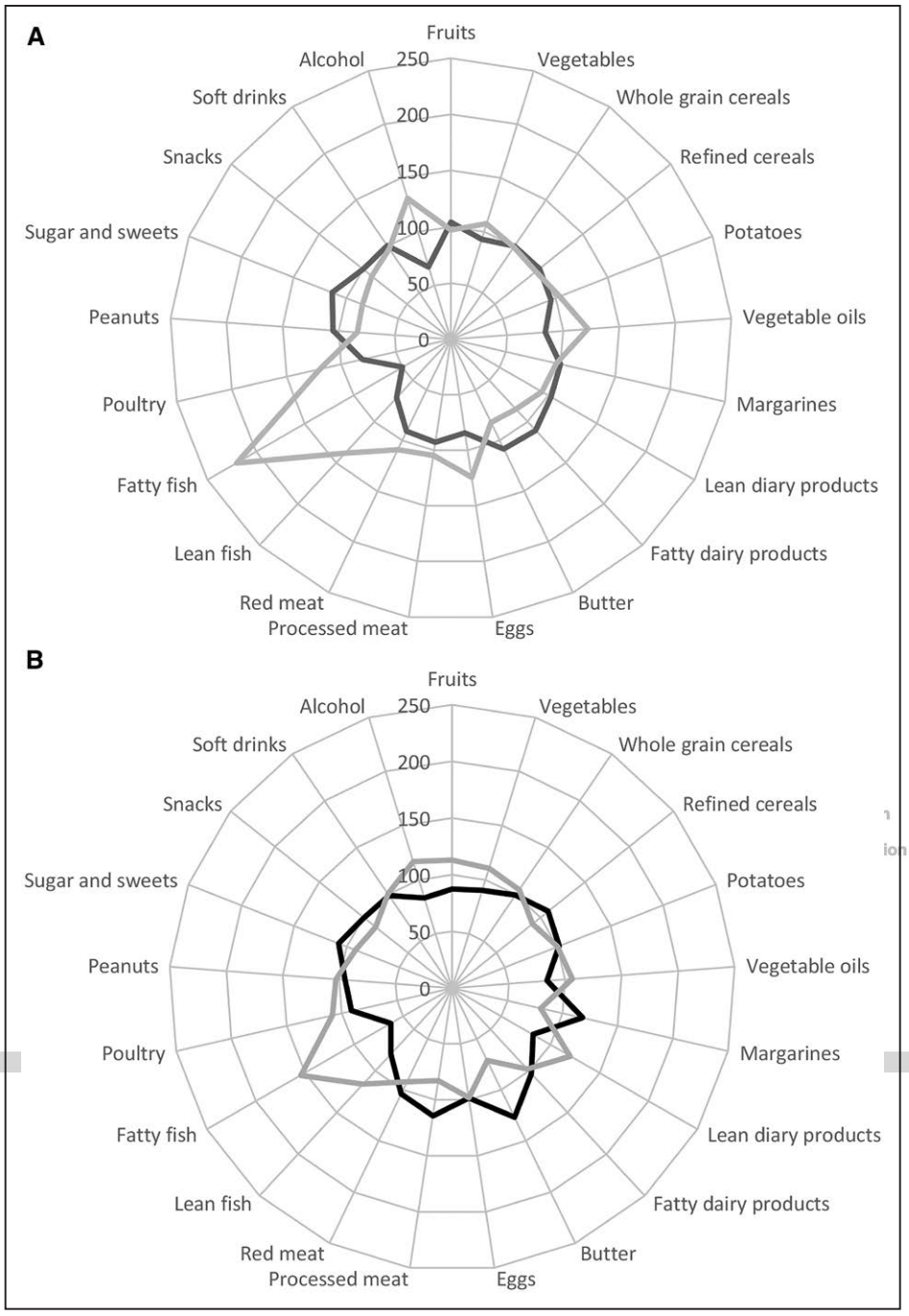

Figure 2. Radar charts illustrating the percentage-wise difference in median intake of different foods and beverages for (**A**) participants in the cohort with the lowest and the highest intake of marine n-3 polyunsaturated fatty acid (PUFA) and for (**B**) participants in the subcohort with the lowest and the highest adipose tissue content of marine n-3 PUFA. The black line equals the first quartile, and the gray line equals the fourth quartile.

marine n-3 PUFA as a biomarker.[12,13] Thus, results have not been consistent, but 3 of the studies[9,10,12] found associations between DHA and ischemic stroke and ischemic stroke subtypes. This contrasts our findings of an association between adipose tissue content of EPA and total ischemic stroke, large artery atherosclerosis, and small-vessel occlusion strokes with less consistent findings for DHA.

DHA and in particular EPA are known to possess anti-inflammatory effects, which could protect against atherosclerosis.[26] In line with this we found a lower risk of stroke from large artery atherosclerosis with a high EPA intake, as well as a high adipose tissue content of EPA. Also, a lower risk of large artery atherosclerosis was seen with intake of DHA, while no clear association was found between DHA in adipose tissue and stroke because of large artery atherosclerosis. While atherosclerosis in the smaller intracerebral arteries is mostly affected by blood pressure, atherosclerosis in the larger arteries is also related to blood lipids.[15,27] Both EPA and DHA lower plasma triglycerides, but DHA may raise low-density lipoprotein cholesterol,[14,28–30] which could be an explanation for the difference between associations of EPA and DHA in relation to large artery atherosclerosis stroke.

We found a higher risk of cardioembolism with a high intake of EPA and DHA as well as for a high adipose tissue content of DHA. This was unexpected as EPA and DHA are generally believed to have antithrombotic effects.[2] Some studies have reported a higher risk of atrial fibrillation with high intakes of marine n-3 PUFA[31,32] and because atrial fibrillation is a risk factor of cardioembolism,[33] this could provide a possible explanation for our findings. However, due to a small number of cases with cardioembolism, these results should be interpreted with caution. The major risk factor for small-vessel occlusion is hypertension.[34,35] Although, both EPA and DHA have been associated with mild antihypertensive effects, we only found a clear inverse association with adipose tissue content of EPA.

Little is known about biological effects of DPA, and apart from in fish, DPA is also contained in meat.[36,37] Further, DPA levels in human tissue are presumable influenced significantly by endogenous metabolism.[3] We, therefore, reported the results of intake and content of DPA in adipose tissue without discussing them.

We used complementary measurements n-3 PUFA, EPA, and DHA from both dietary intake and adipose tissue content. However, consistencies between dietary intake and adipose tissue content were only found for the association between EPA and large artery atherosclerosis and between total marine n-3 PUFA and DHA in relation to cardioembolism.

Our study suggests that intake of marine n-3 PUFAs may protect middle-aged subjects against the development of ischemic stroke of atherosclerotic origin, which supports recent guidelines.[4] Furthermore, the ischemic stroke should not be seen isolated but viewed together with other atherosclerotic vascular events. Thus, there is solid epidemiological evidence that fish consumption protects against ischemic heart disease[4] and we recently reported that marine n-3 PUFA may also lower the risk of the third major atherosclerotic disorder, peripheral arterial disease.[38]

Conclusions

High intake and high adipose tissue levels of EPA were associated with a lower risk of most types of ischemic stroke, apart from cardioembolism, while inconsistent findings were observed for total marine n-3 PUFA and DHA.

Sources of Funding

The research reported in this article was supported by a research grant from the Danish Heart Foundation, grant number 16-R107-A6620. The primary data collection for the Diet, Cancer and Health was funded by the Danish Cancer Society.

Disclosures

None.

References

1. Dyerberg J, Bang HO, Stoffersen E, Moncada S, Vane JR. Eicosapentaenoic acid and prevention of thrombosis and atherosclerosis? *Lancet*. 1978;2:117–119.
2. De Caterina R. n-3 fatty acids in cardiovascular disease. *N Engl J Med*. 2011;364:2439–2450. doi: 10.1056/NEJMra1008153
3. Mozaffarian D, Wu JH. Omega-3 fatty acids and cardiovascular disease: effects on risk factors, molecular pathways, and clinical events. *J Am Coll Cardiol*. 2011;58:2047–2067. doi: 10.1016/j.jacc.2011.06.063
4. Rimm EB, Appel LJ, Chiuve SE, Djoussé L, Engler MB, Kris-Etherton PM, et al; American Heart Association Nutrition Committee of the Council on Lifestyle and Cardiometabolic Health; Council on Epidemiology and Prevention; Council on Cardiovascular Disease in the Young; Council on Cardiovascular and Stroke Nursing; and Council on Clinical Cardiology. Seafood long-chain n-3 polyunsaturated fatty acids and cardiovascular disease: a science advisory from the American Heart Association. *Circulation*. 2018;138:e35–e47. doi: 10.1161/CIR.0000000000000574
5. He K, Rimm EB, Merchant A, Rosner BA, Stampfer MJ, Willett WC, et al. Fish consumption and risk of stroke in men. *JAMA*. 2002;288:3130–3136.
6. Montonen J, Järvinen R, Reunanen A, Knekt P. Fish consumption and the incidence of cerebrovascular disease. *Br J Nutr*. 2009;102:750–756. doi: 10.1017/S0007114509274782
7. Iso H, Rexrode KM, Stampfer MJ, Manson JE, Colditz GA, Speizer FE, et al. Intake of fish and omega-3 fatty acids and risk of stroke in women. *JAMA*. 2001;285:304–312.
8. de Goede J, Verschuren WM, Boer JM, Kromhout D, Geleijnse JM. Gender-specific associations of marine n-3 fatty acids and fish consumption with 10-year incidence of stroke. *PLoS One*. 2012;7:e33866. doi: 10.1371/journal.pone.0033866
9. Saber H, Yakoob MY, Shi P, Longstreth WT Jr, Lemaitre RN, Siscovick D, et al. Omega-3 fatty acids and incident ischemic stroke and its atherothrombotic and cardioembolic subtypes in 3 US cohorts. *Stroke*. 2017;48:2678–2685. doi: 10.1161/STROKEAHA.117.018235
10. Yaemsiri S, Sen S, Tinker LF, Robinson WR, Evans RW, Rosamond W, et al. Serum fatty acids and incidence of ischemic stroke among postmenopausal women. *Stroke*. 2013;44:2710–2717. doi: 10.1161/STROKEAHA.111.000834
11. Iso H, Sato S, Umemura U, Kudo M, Koike K, Kitamura A, et al. Linoleic acid, other fatty acids, and the risk of stroke. *Stroke*. 2002;33:2086–2093.
12. Ricci S, Patoia L, Berrettini M, Binaglia L, Scarcella MG, Bucaneve G, et al. Fatty acid pattern of red blood cell membranes and risk of ischemic brain infarction: a case-control study. *Stroke*. 1987;18:575–578.
13. Ricci S, Celani MG, Righetti E, Caruso A, De Medio G, Trovarelli G, et al. Fatty acid dietary intake and the risk of ischaemic stroke: a multicentre case-control study. UFA Study Group. *J Neurol*. 1997;244:360–364.
14. Mozaffarian D, Wu JH. (n-3) fatty acids and cardiovascular health: are effects of EPA and DHA shared or complementary? *J Nutr*. 2012;142:614S–625S. doi: 10.3945/jn.111.149633
15. He K, Xu Y, Van Horn L. The puzzle of dietary fat intake and risk of ischemic stroke: a brief review of epidemiologic data. *J Am Diet Assoc*. 2007;107:287–295. doi: 10.1016/j.jada.2006.11.010
16. Tjønneland A, Olsen A, Boll K, Stripp C, Christensen J, Engholm G, 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;35:432–441. doi: 10.1080/14034940601047986
17. Overvad K, Tjønneland A, Haraldsdóttir J, Ewertz M, Jensen OM. Development of a semiquantitative food frequency questionnaire to assess food, energy and nutrient intake in Denmark. *Int J Epidemiol*. 1991;20:900–905.
18. Tjønneland A, Overvad K, Haraldsdóttir J, Bang S, Ewertz M, Jensen OM. Validation of a semiquantitative food frequency questionnaire developed in Denmark. *Int J Epidemiol*. 1991;20:906–912.
19. Venø SK, Bork CS, Jakobsen MU, Lundbye-Christensen S, Bach FW, Overvad K, et al. Linoleic acid in adipose tissue and development of ischemic stroke: a Danish Case-Cohort study. *J Am Heart Assoc*. 2018;7:e009820. doi: 10.1161/JAHA.118.009820
20. Lühdorf P, Overvad K, Schmidt EB, Johnsen SP, Bach FW. Predictive value of stroke discharge diagnoses in the Danish National Patient Register. *Scand J Public Health*. 2017:1403494817716582.
21. Adams HP Jr, Bendixen BH, Kappelle LJ, Biller J, Love BB, Gordon DL, et al. Classification of subtype of acute ischemic stroke. Definitions for use in a multicenter clinical trial. TOAST. Trial of Org 10172 in Acute Stroke Treatment. *Stroke*. 1993;24:35–41.
22. Kalbfleisch JD, Lawless JF. Likelihood analysis of multi-state models for disease incidence and mortality. *Stat Med*. 1988;7:149–160.
23. Roos N, Wahab MA, Chamnan C, Thilsted SH. The role of fish in food-based strategies to combat vitamin A and mineral deficiencies in developing countries. *J Nutr*. 2007;137:1106–1109. doi: 10.1093/jn/137.4.1106
24. Eshak ES, Iso H, Yamagishi K, Kokubo Y, Saito I, Yatsuya H, et al; JPHC Study Group. Modification of the excess risk of coronary heart disease

due to smoking by seafood/fish intake. *Am J Epidemiol*. 2014;179:1173–1181. doi: 10.1093/aje/kwu030
25. Hodson L, Skeaff CM, Fielding BA. Fatty acid composition of adipose tissue and blood in humans and its use as a biomarker of dietary intake. *Prog Lipid Res*. 2008;47:348–380. doi: 10.1016/j.plipres.2008.03.003
26. Calder PC. Very long-chain n-3 fatty acids and human health: fact, fiction and the future. *Proc Nutr Soc*. 2018;77:52–72. doi: 10.1017/S0029665117003950
27. Kuller L, Reisler DM. An explanation for variations in distribution of stroke and arteriosclerotic heart disease among populations and racial groups. *Am J Epidemiol*. 1971;93:1–9.
28. Jacobson TA, Glickstein SB, Rowe JD, Soni PN. Effects of eicosapentaenoic acid and docosahexaenoic acid on low-density lipoprotein cholesterol and other lipids: a review. *J Clin Lipidol*. 2012;6:5–18. doi: 10.1016/j.jacl.2011.10.018
29. Mori TA, Burke V, Puddey IB, Watts GF, O'Neal DN, Best JD, et al. Purified eicosapentaenoic and docosahexaenoic acids have differential effects on serum lipids and lipoproteins, LDL particle size, glucose, and insulin in mildly hyperlipidemic men. *Am J Clin Nutr*. 2000;71:1085–1094. doi: 10.1093/ajcn/71.5.1085
30. Maki KC, Van Elswyk ME, McCarthy D, Hess SP, Veith PE, Bell M, et al. Lipid responses to a dietary docosahexaenoic acid supplement in men and women with below average levels of high density lipoprotein cholesterol. *J Am Coll Nutr*. 2005;24:189–199.
31. Shen J, Johnson VM, Sullivan LM, Jacques PF, Magnani JW, Lubitz SA, et al. Dietary factors and incident atrial fibrillation: the Framingham Heart Study. *Am J Clin Nutr*. 2011;93:261–266. doi: 10.3945/ajcn.110.001305
32. Tomita T, Hata T, Takeuchi T, Oguchi Y, Okada A, Aizawa K, et al. High concentrations of omega-3 fatty acids are associated with the development of atrial fibrillation in the Japanese population. *Heart Vessels*. 2013;28:497–504. doi: 10.1007/s00380-012-0264-3
33. Hart RG, Halperin JL. Atrial fibrillation and stroke: concepts and controversies. *Stroke*. 2001;32:803–808.
34. Lammie GA. Pathology of small vessel stroke. *Br Med Bull*. 2000;56:296–306.
35. You R, McNeil JJ, O'Malley HM, Davis SM, Donnan GA. Risk factors for lacunar infarction syndromes. *Neurology*. 1995;45:1483–1487.
36. Rahmawaty S, Charlton K, Lyons-Wall P, Meyer BJ. Dietary intake and food sources of EPA, DPA and DHA in Australian children. *Lipids*. 2013;48:869–877. doi: 10.1007/s11745-013-3812-4
37. Byelashov OA, Sinclair AJ, Kaur G. Dietary sources, current intakes, and nutritional role of omega-3 docosapentaenoic acid. *Lipid Technol*. 2015;27:79–82. doi: 10.1002/lite.201500013
38. Lasota AN, Grønholdt MM, Bork CS, Lundbye-Christensen S, Overvad K, Schmidt EB. Marine n-3 fatty acids and the risk of peripheral arterial disease. *J Am Coll Cardiol*. 2018;72:1576–1584. doi: 10.1016/j.jacc.2018.07.045