Original Article

Fish consumption, long-chain omega-3 fatty acids intake and risk of stroke: An updated systematic review and meta-analysis

Chen Chen MA[1,2†], Hui Huang MA[1,2†], Qiu-qin Dai BA[1,2], Jing Ren BA[1,2], Hong-hua Cai MA[3], Wen-jia Hu MA[1,2], Xiu-juan Zhang PhD[4], Wang-tao Zhong MD[5], Xiang-yong Li MD[1,2]

[1] *Institute of Biochemistry and Molecular Biology, Guangdong Medical University, Zhanjiang, P.R. China*
[2] *Collaborative innovation center for antitumor active substance research and development, Guangdong Medical University, Zhanjiang, P.R. China*
[3] *Department Electrocardiogram, The Affiliated Hospital of Guangdong Medical University, Zhanjiang, P.R. China*
[4] *Departments of Physiology, Guangdong Medical University, Zhanjiang, P.R. China*
[5] *Department of Neurology, The Affiliated Hospital of Guangdong Medical University, Zhanjiang, P.R. China.*
[†] *Both authors contributed equally to this manuscript*

Background and Objectives: Although fish consumption or omega-3 intake is associated with cardio- cerebrovascular disease including stroke, their correlation is still controversial. Therefore, this meta-analysis is to identify the relationship between the risk of stroke and fish consumption or omega-3 intake. **Methods and Study Design:** We searched the PubMed, EMBASE and Cochrane Library databases as of May 2019. Multivariate-adjusted risk ratios (RRs) with 95% confidence interval (CI) for stroke in different level intake of fish or Long-chain omega-3 polyunsaturated fatty acids (LC ω3-PUFAs) were pooled using a random-effects meta-analysis. A dose-response analysis was conducted with the 2-stage generalized least-squares trend program. **Results:** Our meta-analysis identified a total of 17 prospective cohort studies including 14986 strokes events in 672711 individuals. Meta-analysis revealed that the higher fish consumption was significantly associated with lower risk of stroke (RR=0.871, 95% CI: 0.779-0.975, p=0.016), especially with ischemic stroke (RR=0.808, 95% CI: 0.696-0.937, p=0.005). Meantime, the combined RR of total stroke was 0.859 (95% CI: 0.769-0.959, p=0.007) for the highest versus lowest intake of LC ω3-PUFAs, and stratification analysis showed that higher LC ω3-PUFAs intake was associated with reduced stroke risk in women (RR=0.793, 95% CI: 0.706-0.891, p=0.000) but not in men. In addition, the dose-response analysis showed fish consumption with 1000g per month and LC ω3-PUFAs intake with 0.5g per month was associated with 17.3% (RR=0.927, 95% CI: 0.83-0.98) and 14% (RR=0.86, 95% CI: 0.78-0.95) lower risk of stroke, respectively. **Conclusions:** Both fish consumption and LC ω3-PUFAs intake were negatively associated with the risk of stroke, especially in women, which suggest that increased intake of fishery products and LC ω3-PUFAs may benefit primary prevention of stroke.

Key Words: fish, long-chain omega-3 fatty acids, stroke, meta-analysis

INTRODUCTION

Stroke is a medical condition in which poor blood flow to the brain results in cell death, which primarily include ischemic stroke (IS) and hemorrhagic stroke (HS). According to both epidemiological survey and the report of World Health Organization (WHO), stroke has become the second leading cause of death and a major cause of disability among adults worldwide, causing a heavy economic and social burden in most developed and developing regions.[1,2] In recent years, significant progress has been made in the diagnosis and treatment of cerebrovascular diseases. However, due to the limitation of treatment time window, most stroke patients still seldom seek medical treatment in time and their prognosis is poor.[3] Therefore, the early intervention and prevention of risk factors for cerebrovascular disease should be actively carried out to reduce the occurrence of stroke.[4]

As a widely recognized source of important nutrients, fish is rich in high quality protein, various vitamins, essential trace elements and LC ω3-PUFAs. Many epide-

Corresponding Author: Dr Xiang-yong Li, Institute of Biochemistry and Molecular Biology, Guangdong Medical University, Zhanjiang 524023, P.R. China.
Tel: +86-13828273540
Email: xyli75@126.com
Manuscript received 16 June 2020. Initial review completed 06 June 2020. Revision accepted 16 November 2020.
doi: 10.6133/apjcn.202103_30(1).0017

miological studies have reported the relationship between fish consumption and stroke, but the results are not consistent. A previous meta-analysis revealed that intake of fish is inversely related to risk of stroke, particularly ischemic stroke[5] and a meta-analysis from Cambridge University found that the pooled relative risk for cerebrovascular disease for >5 servings fish consumption/week versus 1 serving fish consumption/week was reduced by 12%.[6] However, a cohort study from Japan showed that fish consumption had no significant effect on stroke and its subtypes.[7] Prospective studies of fish consumption in relation to the risk of stroke have yielded inconsistent results.[8]

LC ω3-PUFAs, including eicosapentaenoic acid (EPA, 20:5n-3), docosapentaenoic acid (DPA, 22:5n-3), and docosahenxaenoic acid (DHA, 22:6n-3), which is primarily derived from fish or seafood consumption, may reduce the risk of developing cardiovascular disease. Previous studies have shown that omega-3 fatty acids can not only reduce the triglycerides, but also play an important role in anti-inflammation, anti-arrhythmia, anti-thrombosis and other protective effects against cardiovascular diseases.[9] Several meta-analyses found that not only dietary intake of omega-3[10] but also circulating LC ω3-PUFAs[11] are inversely associated with risk of stroke. However, a recent randomized controlled trial (RCT) study found that supplementation with ω-3 fatty acids did not result in a reduction in the incidence of major cardiovascular events or cancer than placebo.[12] In general, the role of fish consumption and LC ω3-PUFAs intake in stroke remains uncertain.

We conducted a meta-analysis to investigate the relationship between fish consumption and omega-3 intake and the risk of stroke, and if there is a significant correlation between them, we further perform a dose-response meta-analysis to quantitatively evaluate the relationship between them.

METHODS

Literature research

We followed MOOSE guidelines of observational studies for conducting and reporting the present meta-analysis.[13] Systematic literature searches were conducted to identify prospective cohort studies that reported the association between fish consumption or LC ω3-PUFAs intake and risk of stroke from PubMed, EMBASE and the Cochrane Library up to May 2019. The literature strategy was performed using a method of the key words combined with medical subject headings, and the full details are presented in supplementary method. Our research was restricted to human studies that were published in English, and duplicated studies were excluded. Meanwhile, we also searched systematic reviews from the above-mentioned databases, and checked their reference to avoid missing some original studies.

Eligibility criteria

The relevant studies were included if they meet the following inclusion criteria: (1) Participants: adults of any age located in different countries. (2) Exposure of interest: Assessment of fish or LC ω3-PUFAs intake. (3) Outcome: evaluating incidence and/or mortality of stroke or its specific subtypes (IS and HS) as an end point but excluding other similar cardiovascular and cerebrovascular diseases such as coronary heart disease, arrhythmia, myocardial infarction and so on; reporting multivariate-adjusted RR or hazard ratio (HR) with 95% CI. (4) Study design: prospective cohort study.

Data extraction

Data extraction was completed independently and performed twice by two investigators, and disagreements were reconciled by consensus. The following data was extracted from each publication: participant characteristics (baseline age range, gender and countries), duration of follow-up, baseline fish consumption or LC ω3-PUFAs intake as exposure of interest, exposure measurement (dietary estimations), exposure source (diet) and multivariate-adjusted RR with 95% CI for all categories of fish or LC ω3-PUFAs.

If eligible studies reported HR with 95% CI, each HR was assumed to approximate RR. To standardize units of fish consumption, we first converted frequency into grams per day (g/day). The amount of fish consumption (g/day) was estimated by multiplying the frequency of consumption (servings/day) by the corresponding portion size (grams/serving). If a publication reported servings per day as unit of measure in fish consumption, we transferred the fish amount to grams according to descriptions of the publication. If no description of portion size was reported, we deemed it to be 105 grams per serving.[5]

Statistical analysis

Statistical analyses of the combined data were performed by STATA version12.0 (StataCorp, College Station, Texas, USA). We firstly performed a meta-analysis for the highest verses the bottom category of baseline fish consumption, LC ω3-PUFAs intake, respectively. Each multivariate-adjusted RR for the highest compared with the bottom category was firstly transformed to their logarithm (logRR), and the corresponding 95% CI was used to calculate the standard error (selogRR). Summary RR with corresponding 95% CI as the overall risk estimate for eligible prospective cohort studies was calculated by using a fixed effects model or random effects model described by DerSimonian and Laird,[14] which considers both within-study and between-study variability. Heterogeneity across studies was evaluated with the Q test and I^2 statistic (I^2<30%, 30%≤ I^2≤50%, I^2>50%, represented low, moderate, and extreme heterogeneity, respectively and a fixed effects model was used if there was no evidence of heterogeneity; otherwise, a random effect model was used). Sensitivity analysis was performed to evaluate the possible influence of individual study on summary results. Begg's test and Egger's test were conducted to test the possibility of publication bias.

Dose-response analyses were conducted to determine a potential curvilinear or linear association of fish and LC ω3-PUFAs intake with risk of stroke, respectively. Individual studies with three of more categories were included in the dose-response analysis. We assigned median intake of fish or LC ω3-PUFAs for each category as previously described. Restricted cubic splines with three knots (two spline transformations) at fixed percentiles

(25%, 50%, and 75%) was firstly created,[15, 16] and then a p for nonlinearity was calculated to detect potential departure from a simpler linear trend by testing the coefficient of the second spline equal to zero. A linear trend was estimated to achieve the associations of each 1000 g/month increment of fish and each 100 mg/day increment of LC ω3-PUFAs consumption with risk of stroke using a generalized least-squares regression model (two-stage GLST in Stata), respectively. Two-tailed $p<0.05$ was considered statistically significant.

RESULTS

Literature search results

The detailed flowchart of study selection is shown in Figure 1. Briefly, a total of 1878 unique citations were identified from electronic search plus 2 additional articles retrieved from reference lists. After the title and abstracts were screened, 115 articles were eligible for further full-text review, and 98 articles were further excluded for the following reasons: 27 were non-prospective studies, 32 were experimental studies, 32 were reviews or meta-analyses, and 7 were conference abstracts. Thus, 17 relevant studies were finally included in this meta-analysis.[7, 17-32]

Study characteristics

The specific characteristics of the included studies are shown in Table 1. Over the duration of follow up, which ranged from 3 to 20 years, a total of 14986 strokes events occurred among 672711 individual aged 20-98 years from US,[17,18,20,21] Europe[22-26,28,31,32] and Asia,[7,19,27,29,30] respectively. Both fish consumption and LC ω3-PUFAs intake were investigated in 5 studies, fish consumption in 5 studies only, and dietary intake of LC ω3-PUFAs in 7 studies only. Dietary data was collected by interview-administered frequency food questionnaire (FFQ) or semi quantitative food frequency questionnaire (SFFQ), using servings/week (fish) and grams/day (LC ω3-PUFAs) as unit of measure. Among these literatures,[7,17-32] 4 articles only men,[17,19,20,32] 3 study only women[18,24,28] and 10 studies included both men and women.[7,21-23,25-27,29-31] Finally, study quality assessed by the 9-star Newcastle-Ottawa Scale (NOS) ranged from 6 to 8, with a median of 7, and the specific evaluation process is shown in Supplementary Table 1.

Fish consumption and risk of stroke

In total, 10 independent cohort studies[7,17,18,20-23,26,27,31] related to elevated fish consumption were available for meta-analysis comparing the highest to the lowest category. The higher fish consumption was significantly associated with lower risk of stroke (RR=0.871, 95% CI: 0.779-0.975, p=0.016) with a low heterogeneity (I^2=0.0%) (Figure 2A). Additionally, a sensitivity analysis which tested the influence of any individual study on the overall result suggested no significant change in pooled association estimates. No possibility of publication bias was observed by visual inspection of Begg's funnel plot (p for bias=0.180) and Egger's regression test (p for bias=0.048) (Figure 2B). At the same time, we also perform the meta-analysis of the risk of fish consumption and stroke subtypes. We found a significant negative correlation between higher fish consumption and the risk of ischemic stroke (RR=0.808, 95% CI: 0.696 -0.937, p=0.005) (Figure 2C), but was not associated with the risk of hemorrhagic stroke (RR=1.006, 95% CI=0.757-1.337, p=0.968) (Figure 2D).

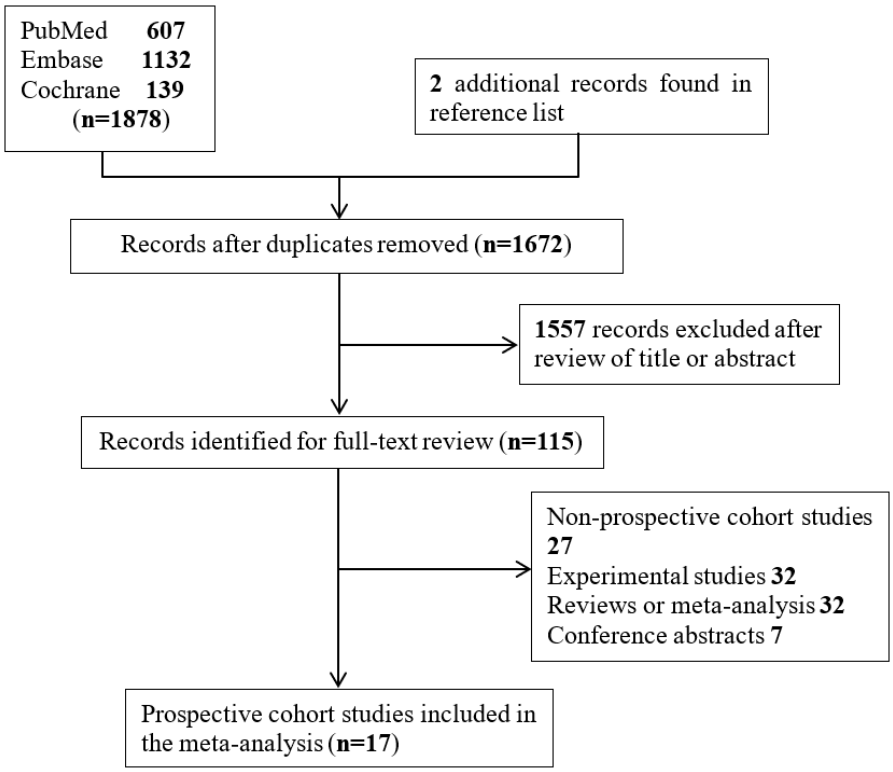


Figure 1. The flowchart of retrieval and selection of studies.

Table 1. Baseline characteristics of included studies

Reference	Country	Average follow-up	Age range, gender	No. of case/ participants	Baseline measurement		Outcomes RR (95% CI)	Study quality
					Exposure assessment	Exposure range (H vs L)		
Morris et al., 1995[17]	USA	4 years	40-84years, Men	173/21185	Total stroke; FFQ	LC n-3 (g/week): >2.3 vs <0.5	1 (0.6-1.6)	9
					Total stroke; FFQ	Fish (servings/week): >5 vs <1	0.6 (0.3-1.6)	
Iso et al., 2001[18]	USA	14 years	34-59years, Women	574/79839	Total stroke; FFQ	LC n-3 (g/day): >0.481 vs <0.077	0.72(0.53-0.99)	8
						Fish (servings/month): >20 vs <1	0.48(0.21-1.06)	
					Ischemic stroke; FFQ	LC n-3 (g/day): >0.481 vs <0.077	0.71 (0.46-1.10)	
						Fish (servings/month): >20 vs <1	0.38 (0.12-1.19)	
					Hemorrhagic stroke; FFQ	LC n-3 (g/day): >0.481 vs <0.077	0.76 (0.43-1.37)	
						Fish (servings/month): >20 vs <1	1.02 (0.26-4.09)	
Yuan et al., 2001[19]	China	3 years	45-64 years, Men	480/18244	Total stroke; FFQ	LC n-3 (g/week): >1.10 vs <0.27	1 (0.75-1.33)	8
He et al., 2002[20]	USA	12 years	40-75 years, Men	608/43671	Total stroke; SFFQ	Fish (servings/month): >20 vs <1	0.83 (0.53-1.29)	9
					Ischemic stroke; SFFQ	Fish (servings/month): >20 vs <1	0.54 (0.31-0.94)	
					Hemorrhagic stroke; SFFQ	Fish (servings/month): >20 vs <1	1.55 (0.45-5.35)	
Mozaffarian et al., 2005[21]	USA	12 years	65-98 years, Both	626/4775	Total stroke; FFQ	Fish (servings/month): >20 vs <1	0.77 (0.56-1.07)	8
					Ischemic stroke; FFQ	Fish (servings/month): >20 vs <1	0.72 (0.51-1.03)	
					Hemorrhagic stroke; FFQ	Fish (servings/month): >20 vs <1	0.98 (0.39-2.46)	
Yamagishi et al., 2008[7]	Japan	12.7years (median)	40-79 years, Both	972/57972	Total stroke; FFQ	LC n-3: Q5 vs Q1	0.93 (0.70-1.22)	8
						Fish: Q5 vs Q1	0.91 (0.74-1.13)	
					Ischemic stroke; FFQ	LC n-3: Q5 vs Q1	1.17 (0.71-1.92)	
						Fish: Q5 vs Q1	0.93 (0.65-1.34)	
					Intraparenchymal hemorrhage stroke; FFQ	LC n-3: Q5 vs Q1	0.7 (0.40-1.24)	
						Fish: Q5 vs Q1	0.95 (0.62-1.47)	
					Subarachnoid hemorrhage stroke; FFQ	LC n-3: Q5 vs Q1	0.9 (0.44-1.81)	
						Fish: Q5 vs Q1	0.96 (0.55-1.68)	
Montonen et al., 2009[22]	Finland	5 years	40-79 years, Both	364/3958	Total stroke ;FFQ	LC n-3 (g/day): >0.655 vs <0.102	0.91 (0.66-1.26)	7
						Fish (g/day): >72 vs <6	0.99 (0.73-1.35)	
Goede et al., 2012[23]	Netherland	8-13 years	20-65 years, Both	221/20069	Total stroke; women ;FFQ	LC n-3 (g/day): >0.188 vs <0.057	0.49 (0.27-0.91)	7
						Fish (g/day): >14 vs <3	0.49 (0.26-0.94)	
					Ischemic stroke; women; FFQ	LC n-3 (g/day): >0.188 vs <0.057	0.62 (0.29-1.35)	
						Fish (g/day): >14 vs <3	0.54 (0.24-1.23)	
					Hemorrhagic stroke; women; FFQ	LC n-3 (g/day): >0.188 vs <0.057	0.45 (0.14-1.42)	
						Fish (g/day): >14 vs <3	0.67 (0.19-2.29)	
					Total stroke; men ;FFQ	LC n-3 (g/day): >0.199 vs <0.066	0.87 (0.51-1.48)	
						Fish (g/day): >14 vs <3.3	0.75 (0.44-1.26)	
					Ischemic stroke; men; FFQ	LC n-3 (g/day): >0.199 vs <0.066	0.85 (0.45-1.60)	
						Fish (g/day): >14 vs <3.3	0.79 (0.42-1.48)	
					Hemorrhagic stroke; men; FFQ	LC n-3 (g/day): >0.199 vs <0.066	0.28 (0.05-1.46)	
						Fish (g/day): >14 vs <3.3	0.17 (0.02-1.5)	

FFQ: food frequency questionnaire; SFFQ: semiquantitative food frequency questionnaire.

Table 1. Baseline characteristics of included studies (cont.)

Reference	country	Average follow-up	Age range, gender	No. of case/ participants	Baseline measurement		Outcomes RR (95% CI)	Study quality
					Exposure assessment	Exposure range (H vs L)		
Larsson et al., 2012[24]	Sweden	10.4 years (mean)	49-83 years, Women	1680/34670	Total stroke ;FFQ Ischemic stroke; FFQ Hemorrhagic stroke; FFQ	LC n-3 (g/day): >0.559 vs <0.131 LC n-3 (g/day): >0.559 vs <0.131 LC n-3 (g/day): >0.559 vs <0.131	0.84 (0.72-0.99) 0.83 (0.69-0.99) 0.68 (0.43-1.07)	7
Wallstrom et al., 2012[25]	Sweden	13.5 years (mean)	44-73 years, Both	755/20670	Ischemic stroke, men Ischemic stroke, women	LC n-3: the high vs the low category LC n-3: the high vs the low category	1.1 (0.78-1.53) 0.9 (0.65-1.25)	6
Kuhn et al., 2013[26]	German	4 years	35-65 years; Both	525/48315	Total stroke; FFQ Ischemic stroke; FFQ Hemorrhagic stroke; FFQ	Fish (g/day): >31.1 vs< 7.5 Fish (g/day): >31.1 vs <7.5 Fish (g/day): >31.1 vs <7.5	0.96 (0.73-1.26) 0.87 (0.64-1.19) 1.46 (0.77-2.78)	8
Takata et al., 2013[27]	China	12 years	40-74 years, Both	844/134296	Ischemic stroke; man; FFQ Ischemic stroke; woman; FFQ Hemorrhagic stroke; man; FFQ Hemorrhagic stroke; woman; FFQ	Fish (g/day): >107.2 vs <10.8 Fish (g/day): >105.2 vs <10.4 Fish (g/day): >107.2 vs <10.8 Fish (g/day): >105.2 vs <10.4	0.56 (0.28-1.13) 0.66 (0.4-1.1) 1.32 (0.78-2.24) 0.62 (0.4-0.96)	7
Miyagawa et al., 2014[29]	Japan	24 years	50 years(mean); Both	417/9190	Total stroke; FFQ	LC n-3 (g/day): >0.62 vs <0.15	0.75 (0.57-1)	6
Bergkvist et al., 2014[28]	Sweden	12 years	39-73 years; Women	2015/34591	Ischemic stroke; FFQ	LC n-3 (g/day): >0.52 vs <0.148	0.72 (0.54-0.96)	8
Koh et al., 2015[30]	Singapore	5 years	45-74 years; Both	1298/60298	Total stroke; SFFQ	LC n-3 (g/day): >1.26 vs <0.59	0.82 (0.66-1.01)	7
Amiano et al., 2016[31]	Spain	13.8 years (mean)	20-69 years; Both	674/41020	Total stroke; men; FFQ Total stroke; women; FFQ Ischemic stroke; man; FFQ Ischemic stroke; woman; FFQ	Fish (g/day): >111 vs <38.6 Fish (g/day): >77.8 vs <26.1 Fish (g/day): >111 vs <38.6 Fish (g/day): >77.8 vs <26.1	0.77 (0.51-1.16) 1.07 (0.68-1.69) 1.13 (0.68-1.88) 1.31 (0.69-2.47)	8
Kippler et al., 2016[32]	Sweden	12 years	45-79 years; Men	2760/39948	Ischemic stroke; FFQ Hemorrhagic; FFQ	LC n-3 (g/day): >0.73 vs <0.18 LC n-3 (g/day): >0.73 vs <0.18	1.21 (0.90-1.61) 0.42 (0.22-0.79)	7

FFQ: food frequency questionnaire; SFFQ: semiquantitative food frequency questionnaire.

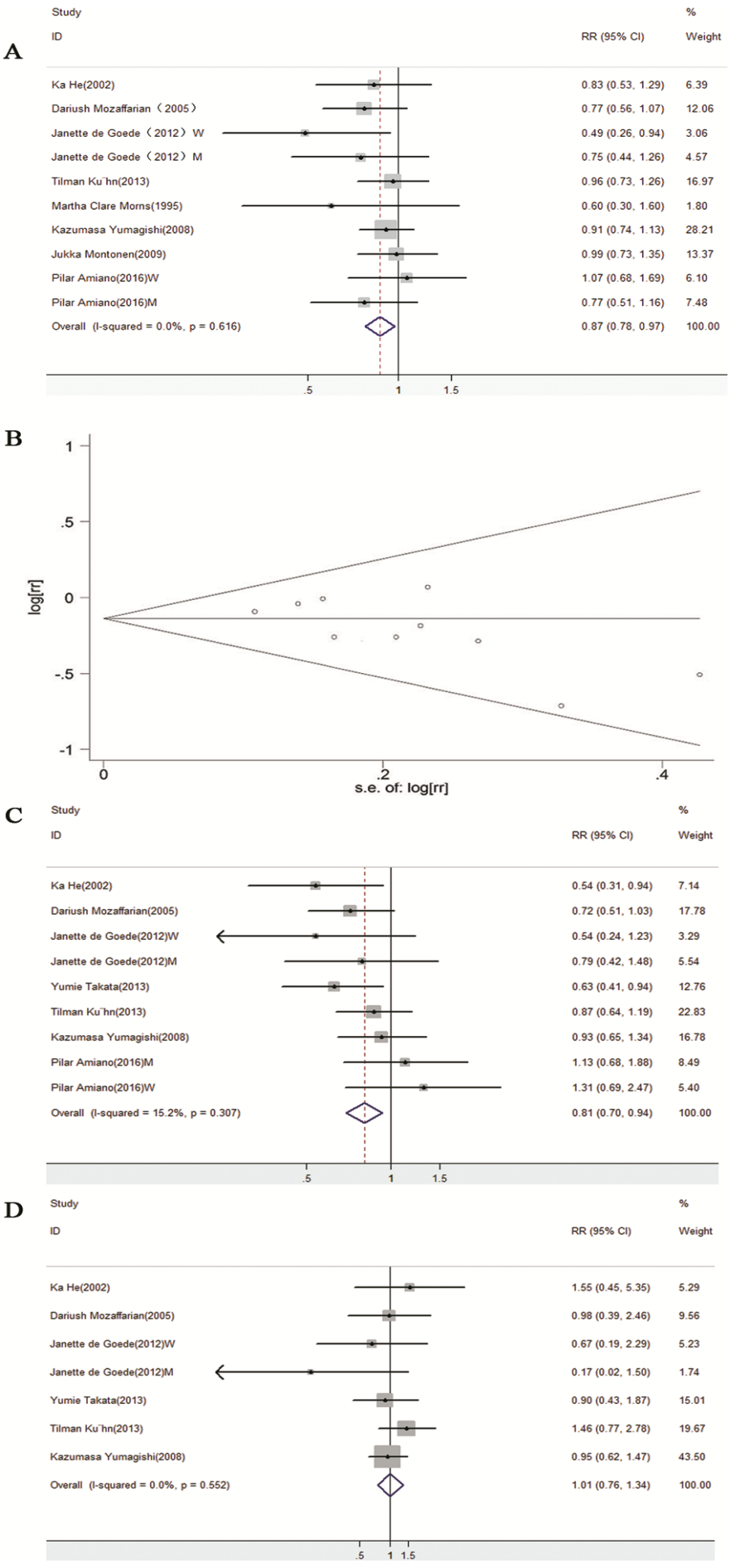


Figure 2. (A) Associations between fish consumption and risk of total stroke in the highest tertiles compared with the bottom. RR, relative risk. (B) Funnel plots in the analysis of correlation between fish consumption and risk of stroke. Associations between fish consumption and the risk of ischemic stroke (C) and hemorrhagic stroke (D) in the highest tertiles compared with the bottom. RR, relative risk

LC ω3-PUFAs and risk of stroke

In the current study, a total of 12 independent prospective cohort studies[7,17-19,22-25,28-30,32] were eligible to evaluate association between LC ω3-PUFAs and incidence of stroke. According to our results, LC ω3-PUFAs was inversely associated with incidence of stroke when comparing the highest and the lowest intake (RR=0.859, 95% CI=0.769-0.959, p=0.007) (Figure 3A). The pooled association was not significantly changed in the sensitivity analysis. Publication bias was not observed from Begg's funnel plot (p for bias=0.621) and Egger's test (p for bias=0.578) (Figure 3B)

In addition, we also performed a stratified analysis of gender and stroke subtypes based on five studies, which investigated the association between omega-3 intake and the risk of ischemic stroke and hemorrhagic stroke, respectively. Our stratification analysis showed that higher LC ω3-PUFAs intake was associated with reduced stroke risk for ischemic stroke (RR=0.876, 95% CI: 0.722, 0.994, p=0.041) and hemorrhagic stroke (RR=0.647, 95% CI: 0.706-0.891, p=0.001) with no between-study heterogeneity (I^2=0.0%) (Supplementary Figure 1A and 1B). Meanwhile, we also found that higher LC ω3-PUFAs intake was associated with reduced stroke risk for women (RR=0.793, 95% CI=0.706-0.891, p=0.000), but not for me (RR=1.012, 95% CI=0.840-1.220, p=0.898) (Supplementary figure 1C and 1D).

Dose response analyses

Among the 17 selected literature, there are three eligible studies[17-19] were available to evaluate a dose-response association between fish consumption and risk of stroke. Our results showed that the correlation between fish consumption and risk of stroke was obviously linear (p=0.015) rather than curvilinear (p=0.2023) (Figure 4A). Specifically, compared with an intake of 0g/month of fish, the risk of stroke is reduced by 17.3% for intake of 1000 grams fish per month. In addition, there are four eligible[24,29,30,32] studies can be used to evaluate a dose-response association between LC ω3-PUFAs intake and risk of stroke. According to our result of analysis, a significantly curvilinear association was observed through a test for non-linearity (p=0.0086) (Figure 4B) and the risk of stroke is reduced by 14% for intake of 0.5 grams LC ω3-PUFAs per month comparing with a minimum intake of 0 grams.

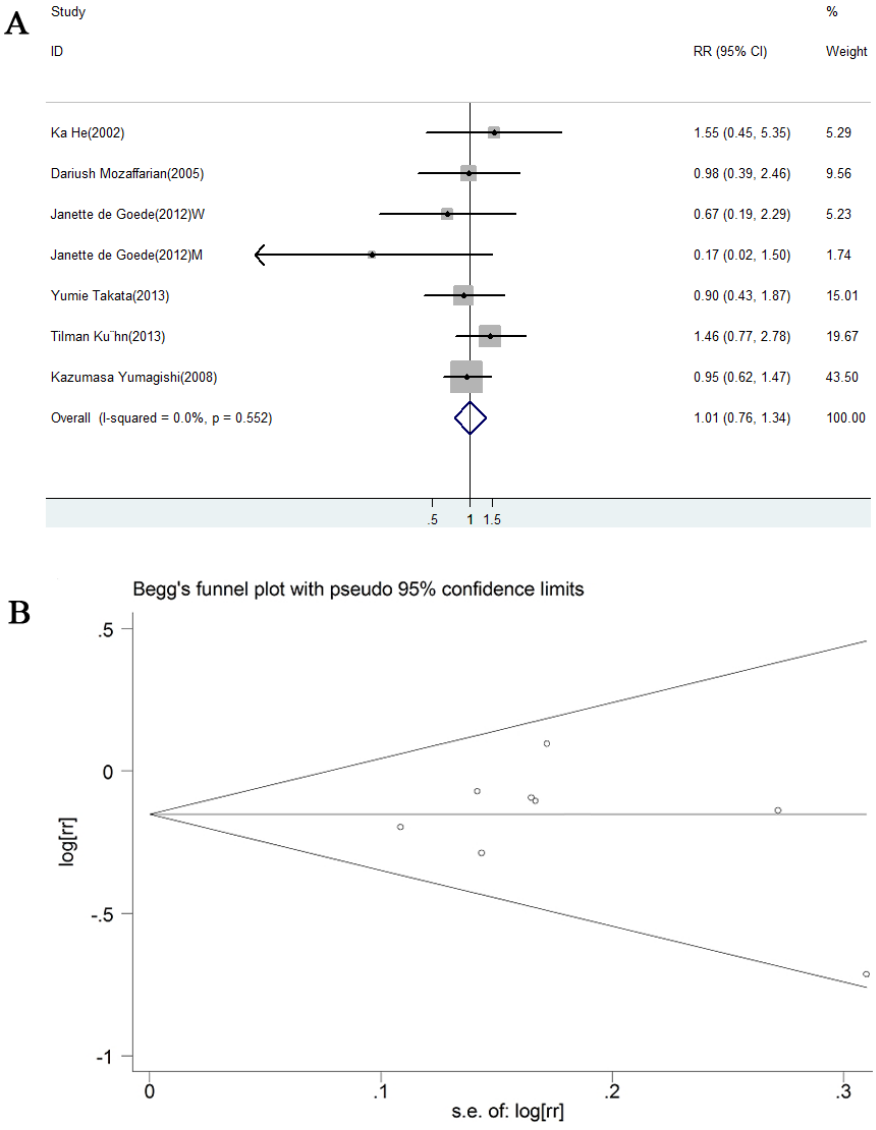


Figure 3. (A) Associations between LC ω3-PUFAs intake and risk of total stroke in the highest tertiles compared with the bottom. RR: relative risk. (B) Funnel plots in the analysis of correlation between LC ω3-PUFAs intake and risk of stroke

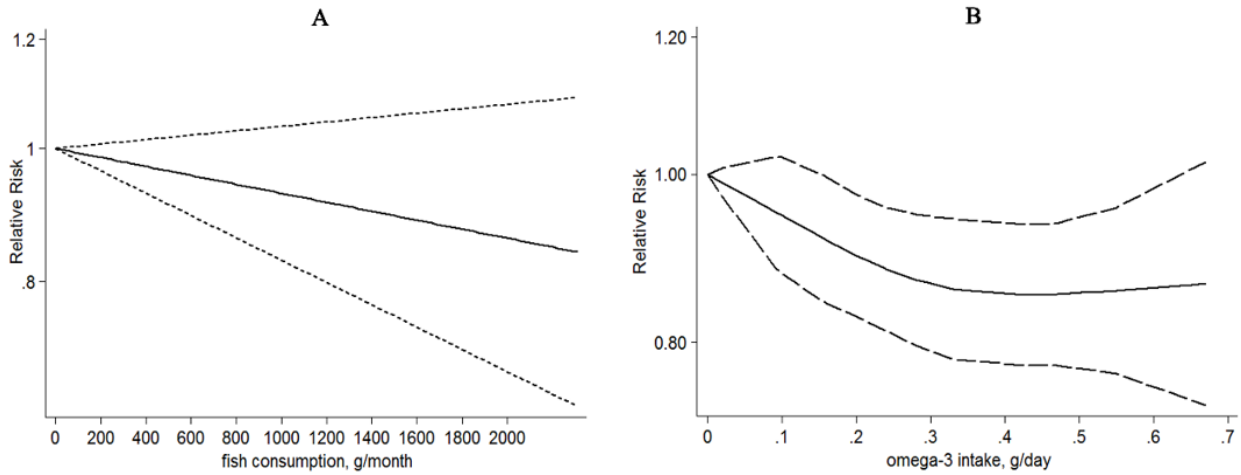


Figure 4. Dose-response association between fish consumption (A) and LC ω3-PUFAs (B) intake and risk of stroke. Solid lines represent best-fitting cubic spline models. Areas between 2 dashed lines represent the 95% CIs

DISCUSSION

In this meta-analysis, a total of 17 observational studies were included to systematically investigate the correlation between fish consumption or LC ω3-PUFAs intake and the risk of stroke, which included 10 literatures on fish consumption[7,17,18,20-23,26,27,31] and 12 literatures on LC ω3-PUFAs intake.[7,17-19,22-25,28-30,32] Our results showed that both fish consumption and omega-3 intake were significantly negatively correlated with stroke incidence, which is consistent with the previous research.[6]

More than 40 years ago, since the low incidence of cardio-cerebrovascular disease were observed in the Greenland Inuit population whose daily food mainly included seafood and was rich in omega-3, people began to pay attention to the beneficial effects of fish and omega-3 on humans.[33,34] Numerous observational studies and randomized case-control studies have focused on the risk of fish consumption and stroke. A prospective cohort study from Netherlands found that consumption of ≥1 portion/week of fish reduced the incidence of ischemic stroke.[35] However, a study from Japan showed that fish consumption had no significant correlation with stroke and its subtypes.[7] Prospective studies of fish consumption in relation to the risk of stroke have yielded inconsistent results. Our meta-analysis of prospective cohort studies found a significant negative correlation between fish consumption and stroke with a lower heterogeneity. Further subgroups found that fish consumption was significantly negatively correlated with IS but not HS, which is consistent with the study.[5] To assess the shape of the relation between fish consumption and stroke, we conducted a dose-response meta-analysis. At the same time, we conducted a dose-response analysis to describe this correlation (linear or non-linear) more specifically than simple correlation analysis. We found a linear relationship between fish consumption and stroke, and the consumption of fish twice a week was associated with 8% lower incidence of stroke. Therefore, the American Heart Association recommended that the public should eat sea food 1-2 times a week to prevent cardiovascular and cerebrovascular diseases.[36] However, our result is inconsistent with the results of Larsson's study, which suggest that fish consumption may be weakly inversely associated with the risk of stroke.[37] As far as we can speculate, this difference may be mainly due to the different sources of fish which determines the quality of the fish. Besides, the methods of cooking may also be another reason for the large difference. For example, studies have shown that fried fish will increase the risk of stroke while raw fish will not.[38,39] Candela et al[40] also found that fried cooking methods could not only cause omega-3 loss in fish but also produce some harmful substances. Overall, our study suggests that fish consumption may reduce the incidence of stroke, and this beneficial effect is more pronounced for IS.

It is well known that fish, especially deep-sea fish, are now thought to be rich in LC ω3-PUFAs. Therefore, more and more people are interested in whether the intake of omega-3 also has a significant correlation with the occurrence of cardio-cerebrovascular disease including stroke. However, the relationship between omega-3 intake and cardio-cerebrovascular disease seems controversial from current studies. For example, a previous study have shown long-term effect of high dose omega-3 fatty acid supplementation may be beneficial for the onset of cardiac death, sudden death and myocardial infarction[41] and the American Heart Association even suggested people take LC ω3-PUFAs supplements to prevent cardiovascular disease.[42] But other studies though that omega-3 fatty acid supplements had no obvious effect on cardiovascular disease.[43,44] As far as we know, this conflicting result between the various studies could be partially explained as a result of variable quality, dosage and duration of omega-3 supplementation. In addition, different criteria for inclusion and exclusion of subjects may also lead to the opposite conclusion. Therefore, considering the inconsistent effects of omega-3 on cardiovascular disease, we strictly limit the endpoints of the included articles to avoid the effects of other related cardiovascular diseases in the present study. As a result, our conclusions indicate that omega-3 intake is inversely related to the overall incidence of stroke, which is consistent with the previous study.[11] In addition, dose-response analysis of LC ω3-PUFAs intake and stroke risk showed that LC ω3-PUFAs intake was closely related to the risk of stroke and when LC ω3-PUFAs intake with 0.5 grams per month, the risk of

stroke was reduced by 14% comparing with none omega-3 intake, suggesting intake of omega-3 may be necessary to prevent stroke. In further subgroup analysis, we were surprised to find although omega-3 was negatively correlated with IS (p=0.041) and HS (p=0.001), it was more associated with hemorrhagic stroke, which didn't seem to be exactly the same as our analysis of stroke and fish consumption. However, after the sensitivity analysis for this subgroup analysis, we found that the removal of any of the studies had little effect on the final overall study. Meantime, there was no publication bias in both Begg's test and Egger's test and visual funnel plots. Therefore, we believed that the results above were credible, and the difference may be mainly due to the quality of the fish. More interestingly, our research showed that women might benefit from a higher intake of omega-3, which was also consist with other people's research.[45]

The reasons for the different results between men and women are unclear. The results of ischemic and hemorrhagic stroke are similar, so it can't be explained by the difference in the proportion of stroke subtypes between genders. It can also not be explained by differences in LC ω3-PUFAs intake because intake was similar in women and men in the same population.[25] It is possible that other potential risk factors for stroke are associated with different intakes of LC ω3-PUFAs in women and men, which could lead to either underestimation or overestimation of the true association. There may also be biological interpretations for the observed differences or be due to chance.

There are several limitations in the present study. Firstly, despite the usage of improved FFQ or SFFQ for surveys, there was an inherent risk of bias in our results during to the diversity and uncertainty of diet. Secondly, since dietary data is usually collected through dietary questionnaires or weighted food records, measurement errors and biases may underestimate or overestimate the true correlation. Thirdly, although such known risk factors of stroke as age, smoking, body mass index, physical activity, history of hypertension, alcohol intake and other dietary factors had been adjusted in most studies, there might still have residual confounding and unknown risk factors. Finally, the potential publication bias might be considered because all of the included studies in our research were limited to English publications.

In conclusion, the present meta-analysis identified that the reduced risk of stroke may not only be linearly associated with fish consumption, especially ischemic stroke, but also be nonlinearly associated with LC ω3-PUFAs intake. Moreover, omega-3 intake might be more beneficial for women to prevent stroke than men. When this article analyzes the relationship between Omega-3 and food intake, this will further deepen and improve the study of supplementary fish consumption and stroke. Therefore, we recommend that appropriate fish consumption and dietary omega-3 intake (especially in women) have a positive effect on reducing the incidence of stroke. Nevertheless, there are many factors that affect stroke besides fish consumption and omega-3 intake, and the mechanism is not entirely clear, both eligible studies for targeted research with large sample sizes and basic mechanism research are necessary to clarify the exact role of fish and omega-3 in the prevention of stroke.

AUTHOR DISCLOSURES

The author(s) declared no potential conflicts of interest with respect to the research, authorship, and/or publication of this article.

This study was supported by the open program of Guangdong provincial key laboratory of medical molecular diagnostics (FZZD201605), Guangdong sails Project High level Talent Project (4YF17001G) and Special funds for Science and Technology Innovation Strategy of Guangdong Province (2018A030310155).

REFERENCES

1. Katan M, Luft A. Global burden of stroke. Semin Neurol. 2018;38:208-11. doi: 10.1055/s-0038-1649503.
2. Bennett DA, Krishnamurthi RV, Barker-Collo S, Forouzanfar MH, Naghavi M, Connor M et al. The global burden of ischemic stroke: findings of the GBD 2010 study. Global Heart. 2014;9:107-12. doi: 10.1016/j.gheart.2014.01.001.
3. Zhang J. Advances in surgical treatment of ischemic cerebrovascular disease. Zhejiang Da Xue Xue Bao Yi Xue Ban. 2019;48:233-40. (In Chinese)
4. Wang Y, Liao X, Zhao X, Wang D, Wang C, Nguyen-Huynh M et al. Using recombinant tissue plasminogen activator to treat acute ischemic stroke in China: analysis of the results from the Chinese National Stroke Registry (CNSR). Stroke. 2011;42:1658-64. doi: 10.1161/STROKEAHA.110.604249.
5. He K, Song Y, Daviglus ML, Liu K, Horn L, Dyer AR, Goldbourt U, Greenland P. Fish consumption and incidence of stroke: a meta-analysis of cohort studies. Stroke. 2004;35:1538-42. doi: 10.1161/01.STR.0000130856.31468.47.
6. Chowdhury R, Stevens S, Gorman D, Pan A, Warnakula S, Chowdhury S, Ward H, Johnson L, Crowe F, Hu FB, Franco OH. Association between fish consumption, long chain omega 3 fatty acids, and risk of cerebrovascular disease: systematic review and meta-analysis. BMJ. 2012;345:e6698. doi: 10.1136/bmj.e6698.
7. Yamagishi K, Iso H, Date C, Fukui M, Wakai K, Kikuchi S, Inaba Y, Tanabe N, Tamakoshi A. 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;52:988-96. doi: 10.1016/j.jacc.2008.06.018.
8. Ward RE, Cho K, Nguyen XT, Vassy JL, Ho Y, Quaden RM, Gagnon DR, Wilson PWF, Gaziano JM, Djoussé L. Omega-3 supplement use, fish intake, and risk of non-fatal coronary artery disease and ischemic stroke in the Million Veteran Program. Clin Nutr. 2020;39:574-9. doi: 10.1016/j.clnu.2019.03.005.
9. Kar S. Omacor and omega-3 fatty acids for treatment of coronary artery disease and the pleiotropic effects. Am J Ther. 2014;21:56-66. doi: 10.1097/MJT.0b013e31822b5603.
10. 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.
11. Yang B, Ren XL, Huang H, Guo XJ, Ma AG, Li D. Circulating long-chain n-3 polyunsaturated fatty acid and incidence of stroke: a meta-analysis of prospective cohort

studies. Oncotarget. 2017;8:83781-91. doi: 10.18632/oncotarget.19530.
12. Manson JE, Cook NR, Lee IM, Christen W, Bassuk S, Mora S et al. Marine n-3 fatty acids and prevention of cardiovascular disease and cancer. N Engl J Med. 2019;380: 23-32. doi: 10.1056/NEJMoa1811403.
13. Stroup DF, Berlin JA, Morton SC, Olkin I, Williamson GD, Rennie D, Moher D, Becker BJ, Sipe TA, Thacker SB. Meta-analysis of observational studies in epidemiology: a proposal for reporting. Meta-analysis Of Observational Studies in Epidemiology (MOOSE) group. JAMA. 2000;283: 2008-12. doi: 10.1001/jama.283.15.2008.
14. Dersimonian R, Laird N. Meta-analysis in clinical trials. Controlled Clinical Trials. 1986;7:177. doi: 10.1016/j.cct.2015.09.002.
15. Greenland S, Longnecker MP. Methods for trend estimation from summarized dose-response data, with applications to meta-analysis. Am J Epidemiol. 1992;135:1301-9. doi: 10.1093/oxfordjournals.aje.a116237.
16. Orsini N, Bellocco R, Greenland S. Generalized least squares for trend estimation of summarized dose–response data. Stata Journal. 2006;6:40-57.
17. Morris MC, Manson JE, Rosner B, Buring JE, Willett WC, Hennekens CH. Fish consumption and cardiovascular disease in the physicians' health study: a prospective study. Am J Epidemiol. 1995;142:166-75. doi: 10.1093/oxfordjournals.aje.a117615.
18. 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;285:304-12. doi: 10.1001/jama.285.3.304.
19. Yuan JM, Ross RK, Gao YT, Yu MC. Fish and shellfish consumption in relation to death from myocardial infarction among men in Shanghai, China. Am J Epidemiol. 2001; 154:809-16. doi: 10.1093/aje/154.9.809.
20. He K, Rimm EB, Merchant A, Rosner BA, Stampfer MJ, Willett WC, Ascherio A. Fish consumption and risk of stroke in men. JAMA. 2002;288:3130-6. doi: 10.1001/jama.288.24.3130.
21. Mozaffarian D, Longstreth Jr WT, Lemaitre RN, Manolio TA, Kuller LH, Burke GL, Siscovick DS. Fish consumption and stroke risk in elderly individuals: The Cardiovascular Health Study. Arch Intern Med. 2005;165:200-6. doi: 10.1001/archinte.165.2.200.
22. Montonen J, Jarvinen R, Reunanen A, Knekt P. Fish consumption and the incidence of cerebrovascular disease. Br J Nutr. 2009;102:750-6. doi: 10.1017/S0007114509274782.
23. de Goede J, Verschuren WMM, Boer JMA, 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.
24. Larsson SC, Virtamo J, Wolk A. Dietary fats and dietary cholesterol and risk of stroke in women. Atherosclerosis, 2012;221:282-6. doi: 10.1016/j.atherosclerosis.2011.12.043.
25. Wallstrom P, Sonestedt E, Hlebowicz J, Ericson U, Drake I, Persson M, Gullberg B, Hedblad B, Wirfält E. Dietary fiber and saturated fat intake associations with cardiovascular disease differ by sex in the Malmo Diet and Cancer Cohort: a prospective study. PLoS One. 2012;7:e31637. doi: 10.1371/journal.pone.0031637.
26. Kuhn T, Teucher B, Kaaks R, Boeing H, Weikert C, Buijsse B. Fish consumption and the risk of myocardial infarction and stroke in the German arm of the European Prospective Investigation into Cancer and Nutrition (EPIC-Germany). Br J Nutr. 2013;110:1118-25. doi: 10.1017/S0007114513000202.
27. Takata Y, Zhang X, Li H, Gao YT, Yang G, Gao J, Cai H, Xiang YB, Zheng W, Shu XO. Fish intake and risks of total and cause-specific mortality in 2 population-based cohort studies of 134,296 men and women. Am J Epidemiol. 2013; 178:46-57. doi: 10.1093/aje/kws584.
28. Bergkvist C, Kippler M, Larsson SC, Berglund M, Glynn A, Wolk A, Åkesson A. Dietary exposure to polychlorinated biphenyls is associated with increased risk of stroke in women. J Inter Med. 2014;276:248-59. doi: 10.1111/joim.12194.
29. Miyagawa N, Miura K, Okuda N, Kadowaki T, Takashima N, Nagasawa SY et al. Long-chain n-3 polyunsaturated fatty acids intake and cardiovascular disease mortality risk in Japanese: a 24-year follow-up of NIPPON DATA80. Atherosclerosis. 2014;232:384-9. doi: 10.1016/j.atherosclerosis.2013.11.073.
30. Koh AS, Pan A, Wang R, Odegaard AO, Pereira MA, Yuan JM, Koh WP. The association between dietary omega-3 fatty acids and cardiovascular death: The Singapore Chinese Health Study. Eur J Prev Cardiol. 2015;22:364-72. doi: 10.1177/2047487313517576.
31. Amiano P, Chamosa S, Etxezarreta N, Arriola L, Moreno-Iribas C, Huerta JM et al. No association between fish consumption and risk of stroke in the Spanish cohort of the European Prospective Investigation into Cancer and Nutrition (EPIC-Spain): a 13.8-year follow-up study. Public Health Nutr. 2016;19:674-81. doi: 10.1017/S1368980015001792.
32. Kippler M, Larsson SC, Berglund M, Glynn A, Wolk A, Åkesson A. Associations of dietary polychlorinated biphenyls and long-chain omega-3 fatty acids with stroke risk. Environ Int. 2016;94:706-11. doi: 10.1016/j.envint.2016.07.012.
33. Dyerberg J, Bang HO, Stoffersen E, Moncada S, Vane JR. Eicosapentaenoic acid and prevention of thrombosis and atherosclerosis?. Lancet. 1978;2(8081):117-9. doi: 10.1016/s0140-6736(78)91505-2.
34. Dyerberg J, Bang HO. Haemostatic function and platelet polyunsaturated fatty acids in Eskimos. Lancet. 1979; 2(8140):433-5. doi: 10.1016/s0140-6736(79)91490-9.
35. Hengeveld LM, Praagman J, Beulens JWJ, Brouwer IA, Schouw YT, Sluijs I. Fish consumption and risk of stroke, coronary heart disease, and cardiovascular mortality in a Dutch population with low fish intake. Eur J Clin Nutr. 2018; 72:942-50. doi: 10.1038/s41430-018-0190-2.
36. Rimm EB, Appel LJ, Chiuve SE, Djoussé L, Engler MB, Kris-Etherton PM, Mozaffarian D, Siscovick DS, Lichtenstein AH. 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.
37. Larsson SC, Orsini N. Fish consumption and the risk of stroke: a dose-response meta-analysis. Stroke. 2011;42: 3621-3. doi: 10.1161/STROKEAHA.111.630319.
38. Nahab F, Pearson K, Frankel MR, Ard J, Safford MM, Kleindorfer D, Howard VJ, Judd S. Dietary fried fish intake increases risk of CVD: the REasons for Geographic And Racial Differences in Stroke (REGARDS) study. Public Health Nutr. 2016;19:3327-36. doi: 10.1017/S136898001600152X.
39. Mozaffarian D, Lemaitre RN, Kuller LH, Burke GL, Tracy RP, Siscovick DS. Cardiac benefits of fish consumption may depend on the type of fish meal consumed: the Cardiovascular Health Study. Circulation. 2003;107:1372-7. doi: 10.1161/01.cir.0000055315.79177.16.

40. Candela M, Astiasarán I, Bello J. Deep-fat frying modifies high-fat fish lipid fraction. J Agric Food Chem. 1998;46: 2793-6.
41. Casula M, Soranna D, Catapano AL, Corrao G. Long-term effect of high dose omega-3 fatty acid supplementation for secondary prevention of cardiovascular outcomes: A meta-analysis of randomized, placebo controlled trials [corrected]. Atheroscler Suppl. 2013;14:243-51. doi: 10.1016/S1567-5688(13)70005-9.
42. Siscovick DS, Barringer TA, Fretts AM, Wu JHY, Lichtenstein AH, Costello RB et al. Omega-3 polyunsaturated fatty acid (Fish Oil) supplementation and the prevention of clinical cardiovascular disease: A science advisory from the American Heart Association. Circulation. 2017;135:e867-e84. doi: 10.1161/CIR.0000000000000482.
43. Aung T, Halsey J, Kromhout D, Gerstein HC, Marchioli R, Tavazzi L et al. Associations of omega-3 fatty acid supplement use with cardiovascular disease risks: Meta-analysis of 10 trials involving 77917 individuals. JAMA Cardiol. 2018;3:225-34. doi: 10.1001/jamacardio.2017.5205.
44. Burke MF, Burke FM, Soffer DE. Review of cardiometabolic effects of prescription omega-3 fatty acids. Curr Atheroscler Rep. 2017;19:60. doi: 10.1007/s11883-017-0700-z.
45. Larsson SC, Orsini N, Wolk A. Long-chain omega-3 polyunsaturated fatty acids and risk of stroke: a meta-analysis. Eur J Epidemiol. 2012;27:895-901. doi: 10.1007/s10654-012-9748-9.

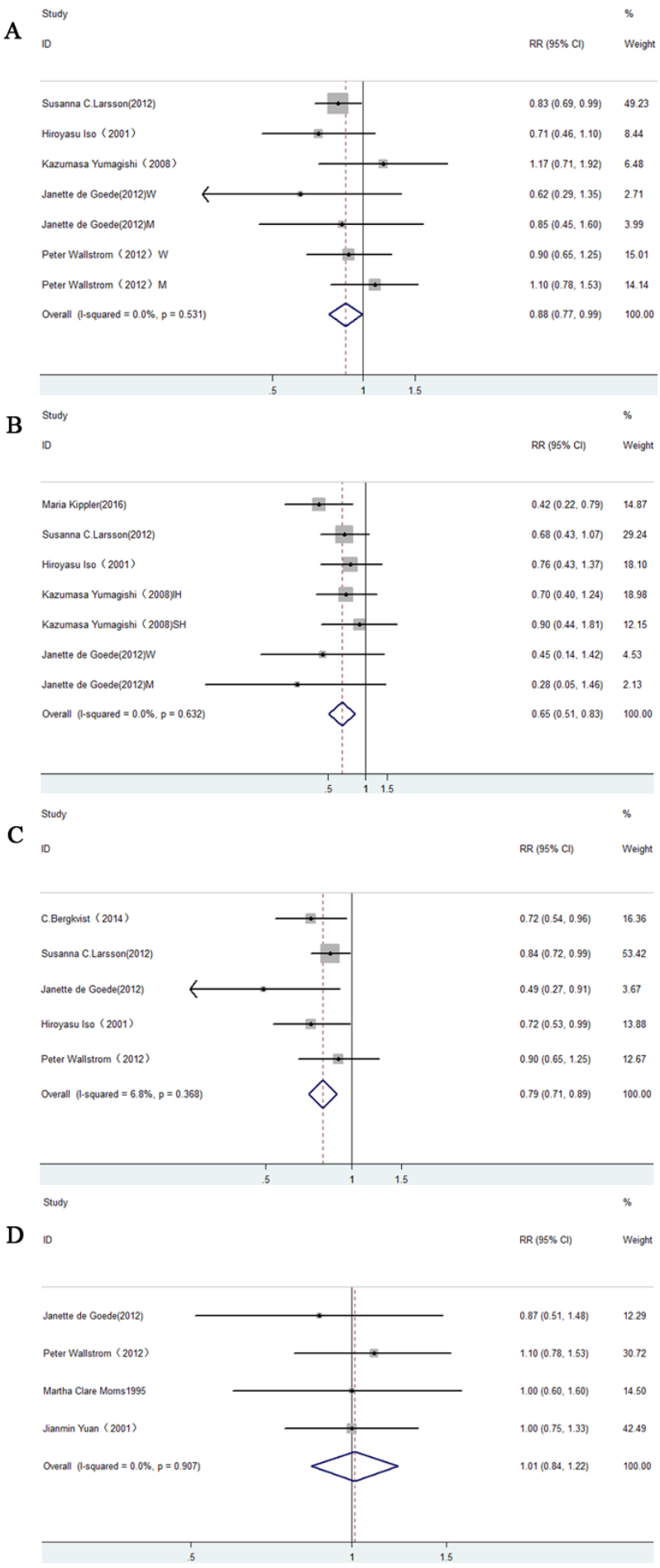
Supplementary method

1)"Fatty Acids, Omega-3" OR n-3 Fatty Acids OR n-3 Polyunsaturated Fatty Acid OR n-3 PUFA OR PUFA, n-3 OR Omega 3 Fatty Acids OR n3 Polyunsaturated Fatty Acid OR n-3 Oils OR Omega-3 Fatty Acids OR n3 Fatty Acid OR Fatty Acid, n3 OR fatty acid OR Fish Oil OR fish OR alpha-Linolenic Acid OR Linolenic Acid OR "Docosahexaenoic Acids" OR Acids, Docosahexaenoic OR Docosahexenoic Acids OR DHA OR Docosahexaenoate OR "Eicosapentaenoic Acid" OR Eicosapentanoic Acid OR EPA OR Acid, Eicosapentanoic OR omega-3-Eicosapentaenoic Acid OR Timnodonic Acid OR "Fish Oils" OR Fish Oil OR fish OR "Dietary Fats, Unsaturated" OR Unsaturated Dietary Fats OR Dietary Fat, Unsaturated OR Fat, Unsaturated Dietary OR Fats, Unsaturated Dietary OR Unsaturated Dietary Fat OR Dietary Oils OR Dietary Oil OR Oil, Dietary OR Oils, Dietary

2) "Stroke" OR Strokes OR Cerebrovascular Accident OR brain vascular accident OR Cerebrovascular Apoplexy OR Apoplexy, Cerebrovascular OR Vascular Accident, Brain OR Cerebrovascular Stroke OR Apoplexy OR Cerebral Stroke OR Stroke, Acute OR Cerebrovascular Accident, Acute

3) human OR humans OR men OR women

4)1) and 2) and 3)



Supplementary figure 1. Forest plot of relative risk for LC n-3 PUFA intake and stroke risk for stroke subgroup (A) ischemic stroke, (B) hemorrhagic stroke and sex subgroups(C) female, (D) male. RR: relative risk

Supplementary table 1. Quality assessment of included prospective cohort studies by Newcastle-Ottawa Scale (√)

Study design	Selection ()	Comparability ()	Exposure or outcome ()	Stars	Quality scores
Cohort studies	1) Representativeness of the exposed cohort? √ 2) Selection of the non exposed cohort? √ 3) Evaluating exposure? √ 4) Outcomes of interest were not present at study start? √	1) Study controls for the most important factor? √ 2) Study controls for any additional factors? √	1) How to ascertain outcome? √ a) Independent blindness b) record linkage 2) Follow-up till outcomes happened? √ 3) Adequacy of follow up? √	√√√√√√√√ (9)	High quality: 8–9√ Moderate quality: 6–7√ Low quality: 1–5√
Included cohort studies					
Morris et al., 1995 (American)	1) √, 2) √, 3) √ 4) √	1) √, 2) √	1) √a, 2) √, 3) √	√√√√√√√√ (9)	High
Iso et al., 2001 (American)	1) √, 2) √, 3) √,4) ×: no statement	1) √, 2) √	1) √a, 2) √, 3) √	√√√√√√√ (8)	High
Yuan et al, 2001 (China)	1) √Men cohort 2) √, 3) √, 4) √	1) √; 2) ×: no control for additional factors	1) √b 2) √, 3) ×√	√√√√√√√ (8)	High
He et al., 2002 (American)	1) √ Men cohort, 2) √, 3) √, 4) √	1) √; 2) √	1) √ b, 2) √, 3) √	√√√√√√√√ (9)	High
Mozaffarian et al., 2005 (American)	1) √, 2) √, 3) √ 4) √	1) √; 2) √	1) √ a, 2) √, 3) ×: no statement	√√√√√√√ (8)	High
Yamagishi et al., 2008 (Japan)	1) √, 2) √, 3) √ 4) √	1) √; 2) √	1) √ a, 2) √, 3) ×: no statement	√√√√√√√ (8)	High
Montonen et al., 2009 (Finland)	1) √, 2) √, 3) √ 4) √	1) √, 2) ×: without covariates adjust	1) √ a, 2) √, 3) ×: no statement	√√√√√√ (7)	Moderate
Goede et al., 2012 (Netherlands)	1) √, 2) √, 3) √ 4) ×: no mention	1) √; 2) √	1) √ a, 2) √, 3) ×: no statement	√√√√√√ (7)	Moderate
Larsson et al., 2012 (Sweden)	1) × :no mention, 2) √ 3) √ 4) ×: no mention	1) √; 2) √	1) √ b, 2) √, 3) √	√√√√√√ (7)	Moderate
Wallstrom et al., 2012 (Sweden)	1) √; 2) √, 3) √, 4) ×: no mention	1) √, 2) ×: without covariates adjusted	1) √ a, 2) √, 3) ×: no statement	√√√√√ (6)	Moderate
Kuhn et al., 2013 (Germany)	1) √; 2) √, 3) √, 4) ×: no mention	1) √; 2) √	1) √ b, 2) √, 3) √	√√√√√√√ (8)	High
Takata et al., 2013 (China)	1) √; 2) √, 3) √, 4) ×: no mention	1) √, 2) ×: without covariates adjusted	1) √ b, 2) √, 3) √	√√√√√√ (7)	Moderate
Bergkvist et al., 2014 (Sweden)	1) √ Women cohort, 2) √, 3) √, 4) √	1) √; 2) √	1) √ a, 2) √, 3) ×: no statement	√√√√√√√ (8)	High
Miyagawa et al., 2014 (Japan)	1) √; 2) √, 3) √, 4) ×: no mention	1) √, 2) ×: without covariates adjusted	1) √ a, 2) √, 3) ×: no statement	√√√√√ (6)	Moderate
Koh et al., 2015 (Singapore)	1) √; 2) √, 3) √, 4) ×: no mention	1) √; 2) √	1) √ a, 2) √, 3) ×: no statement	√√√√√√ (7)	High
Pilar et al., 2016 (Spain)	1) √, 2) √, 3) √ 4) √	1) √; 2) √	1) √ a, 2) √, 3) ×: no statement	√√√√√√√ (8)	High
Maria et al., 2016 (Sweden)	1) √, 2) √, 3) √ 4) √	1) √, 2) ×: without covariates adjusted	1) √ a, 2) √, 3) ×: no statement	√√√√√√ (7)	High