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Differential Association of Serum n-3 Polyunsaturated Fatty Acids with Various Cerebrovascular Lesions in Japanese Men

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Abstract
Background: An association between a high intake of marine-derived n-3 polyunsaturated fatty acids (n-3 PUFAs) with a lower risk of coronary heart disease was previously reported. However, the association between n-3 PUFAs and cerebrovascular lesions remains unclear. We evaluated this association in a general-population-based sample of Japanese men. ***Methods:*** Participants were community-dwelling men (40–79 years old) living in Kusatsu City, Shiga, Japan. Serum concentrations of n-3 PUFAs, defined as the sum of eicosapentaenoic and docosahexaenoic acids, were measured via gas-liquid chromatography between 2006 and 2008. Magnetic resonance imaging was used to assess cerebrovascular lesions (including intracerebral large-artery stenosis, lacunar infarction, and microbleeds) and white matter lesions between 2012 and 2015. Logistic regression adjusting for conventional cardiovascular risk factors was used to estimate the odds ratio of prevalent cerebrovascular lesions per 1 standard deviation higher serum concentration of n-3 PUFAs. ***Results:*** Of a total of 739 men, the numbers (crude prevalence in %) of prevalent cerebral large-artery stenoses, lacunar infarctions, microbleeds, and white matter lesions were 222 (30.0), 162 (21.9), 103 (13.9), and 164 (22.2), respectively. A 1 standard deviation higher concentration of n-3 PUFAs (30.5 μmol/L) was independently associated with lower odds of cerebral large-artery stenosis (multivariable-adjusted odds ratio, 0.80; 95% confidential interval, 0.67–0.97). There were no significant associations of *n*-3 PUFAs

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with the other types of lesions. ***Conclusions:*** n-3 PUFAs may have protective effects against large-artery stenosis, but not small vessel lesions, in the brain.

Introduction

Stroke is a leading cause of premature death and physical disability worldwide [1]. East Asian populations including Japanese people have a higher risk of lacunar infarction and intracerebral hemorrhage, and a lower risk of large-artery infarction and cardioembolic infarction, compared with Western populations, including the USA [2, 3]. This difference in major stroke subtypes between East Asian and Western populations seems to parallel the difference in the risk of coronary artery disease, a type of large-artery thrombotic disease, between these populations – low in East Asian people and high in Western people [3].

Marine-derived n-3 polyunsaturated fatty acids (n-3 PUFAs), including eicosapentaenoic acid (EPA) and docosahexaenoic acid (DHA), are essential fatty acids mainly derived from fish. Japanese population has markedly high consumption of fish and n-3 PUFAs compared to other populations, including Western populations [4]. Epidemiological studies from Japan and other countries suggest that dietary intake of n-3 PUFAs has a protective effect against atherosclerotic diseases [5, 6], including coronary heart disease [7, 8]. However, the association between n-3 PUFAs intake and stroke is controversial, with mixed or negative findings [9, 10]. These inconsistencies may relate to the heterogeneous etiology of stroke (e.g., atherothrombotic large-artery infarction, cardioembolic infarction, lacunar infarction, and intracerebral hemorrhage), and any effect of n-3 PUFAs may differ across these subtypes. Nevertheless, only a limited number of studies have evaluated the specific association between n-3 PUFAs and stroke according to its subtypes [10–12]. For example, a study of 3 cohorts in the USA showed that higher levels of plasma DHA, a subtype of n-3 PUFA, were inversely associated with atherothrombotic stroke but not with cardioembolic stroke [12].

Subclinical cerebral vascular diseases, including large-artery stenosis identified via magnetic resonance imaging (MRI) or MR angiography (MRA) involve small and/or large vessels. Such lesions play an important role in the pathogenesis of the various subtypes of stroke [13]. Therefore, examining the factors associated with each type of MR-based lesion may provide insight into the risk factors for the stroke subtypes. Herein, we separately evaluated the associations between n-3 PUFAs and various types of subclinical cerebrovascular lesions using MRI in a general population of Japanese men with high level of n-3 PUFAs consumption. We hypothesized that n-3 PUFAs are specifically associated with large-artery stenosis of the brain because of the suggested anti-atherosclerotic effect [6]. As a secondary aim, we examined the separate associations of DHA and EPA with each type of cerebral vascular lesion.

Table 1. Characteristics of participants (men, *n* = 739; 2006–2008)

	Men (*n* = 739)
Age, years	63.7±9.4
Body mass index, kg/m^2	23.6±2.8
Systolic blood pressure, mm Hg	135.7±17.9
Diastolic blood pressure, mm Hg	80.1±10.8
Total cholesterol, mmol/L	5.44±0.85
HDL cholesterol, mmol/L	1.53±0.44
Non-HDL cholesterol, mmol/L	3.91±0.88
Triglyceride, mmol/L	1.20 (0.86–1.68)
Fasting plasma glucose, mmol/L	5.70±1.12
HbA1c, %	6.0±0.8
HbA1c, mmol/mol	42.2±8.5
n-3 PUFAs, μmol/L	75.9±30.5
n-3 PUFAs (%)	9.2±3.1
EPA, μmol/L	28.2±16.0
DHA, μmol/L	47.7±16.7
Smoking status, *n* (%)	
Current	214 (29.0)
Past	383 (51.8)
Never	142 (19.2)
Drinking status, *n* (%)	
Current	584 (79.0)
Past	38 (5.1)
Never	117 (15.8)
Diabetes mellitus, *n* (%)	164 (22.2)
Hypertension, *n* (%)	401 (54.3)
Use of antihypertensive agents, *n* (%)	226 (30.6)
Use of statin, *n* (%)	78 (10.6)
History of stroke at baseline, *n* (%)	19 (2.6)

Data are mean ± standard deviation or median (interquartile range). HDL indicates high-density lipoprotein.

Materials and Methods

Study Design and Participants

Shiga Epidemiological Study of Subclinical Atherosclerosis is a population-based prospective study constructed on a random sample from the general population of a city in Japan [14]. In brief, from 2006 to 2008, 1,094 Japanese men (40–79 years old) were

Table 2. Odds ratio for cerebral vascular lesions per 1 standard deviation[1] higher serum n-3 PUFAs, EPA, and DHA concentrations (μmol/L)

	Odds ratio (95% confidence interval)		
	n-3 PUFAs	EPA	DHA
Cerebral large-artery stenosis			
Any stenosis (>0%), *n* = 222			
Unadjusted	0.99 (0.85–1.16)	1.02 (0.88–1.19)	0.96 (0.82–1.13)
Age-adjusted	0.95 (0.81–1.12)	0.97 (0.83–1.14)	0.94 (0.80–1.11)
Multivariable-adjusted[2]	0.80 (0.67–0.97)	0.88 (0.74–1.04)	0.76 (0.62–0.92)
Severe (≥50%) stenosis, *n* = 48			
Unadjusted	1.05 (0.79–1.39)	1.15 (0.88–1.50)	0.94 (0.69–1.29)
Age-adjusted	1.03 (0.77–1.39)	1.10 (0.84–1.44)	0.96 (0.69–1.32)
Multivariable-adjusted[2]	0.88 (0.64–1.22)	1.00 (0.75–1.34)	0.78 (0.54–1.12)
Proportional odds analysis			
Unadjusted	1.00 (0.85–1.16)	1.03 (0.89–1.21)	0.96 (0.82–1.13)
Age-adjusted	0.96 (0.82–1.13)	0.98 (0.84–1.15)	0.94 (0.80–1.11)
Multivariable-adjusted[2]	0.81 (0.67–0.97)	0.89 (0.75–1.05)	0.75 (0.62–0.91)
Lacunar infarction, *n* = 162			
Unadjusted	1.17 (0.99–1.38)	1.16 (0.98–1.36)	1.16 (0.99–1.37)
Age-adjusted	1.15 (0.97–1.37)	1.10 (0.92–1.30)	1.18 (0.99–1.40)
Multivariable-adjusted[2]	1.13 (0.94–1.36)	1.08 (0.90–1.29)	1.16 (0.96–1.40)
Microbleeds, *n* = 103			
Unadjusted	1.23 (1.02–1.48)	1.16 (0.96–1.41)	1.25 (1.04–1.51)
Age-adjusted	1.21 (1.00–1.46)	1.12 (0.92–1.36)	1.26 (1.04–1.52)
Multivariable-adjusted[2]	1.13 (0.92–1.39)	1.06 (0.86–1.30)	1.18 (0.96–1.45)
White matter lesion, *n* = 164			
Unadjusted	1.08 (0.91–1.27)	1.05 (0.89–1.25)	1.09 (0.92–1.28)
Age-adjusted	1.05 (0.89–1.25)	1.02 (0.86–1.21)	1.08 (0.91–1.28)
Multivariable-adjusted[2]	1.03 (0.86–1.24)	1.00 (0.84–1.20)	1.06 (0.88–1.28)

PUFAs, polyunsaturated fatty acids; EPA, eicosapentaenoic acid; DHA, docosahexaenoic acid; HDL, high-density lipoprotein. [1] 30.5 μmol/L for n-3 PUFAs; 16.0 μmol/L for EPA; 16.7 μmol/L for DHA. [2] Adjusted covariates included age, hypertension (yes/no), diabetes (yes/no), non-HDL cholesterol, statin use (yes/no), body mass index, smoking status (current, past, never), and drinking status (current, past, never).

randomly selected from the general population in Kusatsu City, Shiga, Japan. Participants in the baseline survey were then invited for follow-up surveys, including an MRI scan between 2012 and 2015, and 740 men participated in both the baseline and the survey of MRI scan. After excluding one man without a valid brain MRI data, a total of 739 men were included in the present study. The study was approved by the Institutional Review Board of Shiga University of Medical Science. All participants provided written informed consent.

Assessment of Cerebrovascular Lesions

All MRI and MRA scans were obtained using a 1.5-tesla MRI scanner (Signa HDxt 1.5T v16; GE Healthcare, Milwaukee, WI, USA). Two neurosurgeons (KN and AS), certified by the Japan Neurosurgery Society, independently assessed all cerebrovascular lesions in duplicate without knowledge of the participants' characteristics. Prevalent cerebral lesions were defined as follows: "any stenosis" and "severe stenosis" of the cerebral large artery as stenosis >0% and ≥50%, respectively; lacunar infarction and microbleeds as the number of respective lesions ≥1; and white matter lesion as a ≥grade 3 deep and subcortical white matter hyperintensity. Details are in the online supplemental materials (for all online suppl. material, see www.karger.com/doi/10.1159/000524243).

Measurement of Serum Fatty Acids

Serum fatty acids concentrations were measured via capillary gas chromatography and were expressed as the absolute concentration (μmol/L) and the percentage of total fatty acids (%). We defined n-3 PUFAs as the sum of EPA and DHA. The detailed measurement of fatty acids and covariates are in the online supplemental materials.

Statistical Analysis

Univariate and multivariate logistic regression models were used to obtain the odds ratio and 95% confidence interval for prevalent cerebrovascular lesions (cerebral large-artery stenosis, lacunar infarction, microbleeds, and white matter lesions) per 1 standard deviation higher serum concentration of n-3 PUFAs. Proportional odds models were also used to obtain the summary odds ratio and 95% confidence interval for cerebral large-artery stenosis, with graded outcomes as detectable stenosis, mild (1%–50%) stenosis, and severe (>50%) stenosis. For the secondary aim, we

repeated the analyses treating EPA and DHA separately. To control for potential confounders, models were adjusted for age only (in "age-adjusted model"), and further for age, hypertension (yes/no), diabetes (yes/no), non-high-density lipoprotein cholesterol, statin use (yes/no), smoking status (never/past/current), and drinking status (never/past/current) (in "multivariable-adjusted model"). All analyses were performed using statistical software (SAS v9.4; SAS Institute Inc., Cary, NC). A p value <0.05 was considered statistically significant. The details of statistical analysis are in the online supplemental materials.

Results

From a total of 739 men analyzed, the mean (standard deviation) age was 63.7 (9.4) years, and the mean body mass index was 23.6 (2.8) kg/m^2 (Table 1). Compared to the participants excluded from our analysis (n = 355), those analyzed had higher levels of total cholesterol, non-high-density lipoprotein cholesterol, n-3 PUFAs, and EPA, but had a lower proportion of current smoking with nonsignificant trends of lower blood pressure (online suppl. Table 5). The number (crude prevalence) of participants with subclinical vessel diseases was 174 (23.5%), 48 (6.5%), 162 (21.9%), 103 (13.9%), and 164 (22.2%) for mild stenosis (<50%), severe stenosis (≥50%), lacunar infarction, microbleeds, and white matter lesions (≥grade 3), respectively. After adjusting for conventional cardiovascular risk factors including hypertension and statin use, a 1 standard deviation higher n-3 PUFAs concentration was inversely associated with prevalent any stenosis (>0%) (odds ratio, 0.80; 95% confidence interval, 0.67–0.97) (Table 2 and online suppl. Table 1). Analysis via the proportional odds model showed a similar and independent association. The corresponding multivariable-adjusted odds ratio (95% confidence interval) of being in a higher degree of stenosis was 0.81 (0.67–0.97). By contrast, there was no significant association of n-3 PUFAs with any other lesion type. Replacing the concentration unit (μmol/L) of n-3 PUFAs with its proportion to total fatty acids (%) yielded comparable results (online suppl. Table 2). Exclusion of participants with a history of stroke at baseline did not change the results (online suppl. Table 3). Similar findings were found when the association of cerebrovascular lesions was analyzed separately with EPA and DHA, although DHA (but not EPA) had a significant inverse association with stenosis in the multivariable model (Table 2). Age-stratified analyses showed no significant differences between the age groups for the associations of n-3 PUFAs with cerebrovascular lesions (all p > 0.2 for interaction; online suppl. Table 4).

Discussion

In this population-based study of Japanese men, the serum concentration of n-3 PUFAs was inversely associated with the presence and severity of cerebral large-artery stenosis, but not with other cerebrovascular lesions (lacunar infarction, microbleeds, or white matter lesions). Of note, this inverse association was statistically independent of conventional vascular risk factors, including hypertension, serum lipids, and statin use. The observed relationships were similar across the age-strata and in the subgroup of participants without a history of stroke. To our knowledge, this is the first population-based study examining the association between serum n-3 PUFAs concentrations and various types of cerebral vascular lesions identified by MRI/MRA, and the first to find an independent association of large-artery stenosis only. This differential association may be explained by differences in the pathogenesis of large-artery stenosis and the remaining cerebrovascular lesions examined. Cerebral large-artery stenosis is a large vessel disease [15], in which atherosclerotic processes, including the accumulation of lipid-rich plaques, play a major role [16]. By contrast, lacunar infarction, microbleeds, and white matter lesions identified by MRI are small vessel diseases [17], in which arteriosclerotic processes, including exposure to hypertension and aging, play a major role [18].

The protective effect of n-3 PUFAs against all cardiovascular diseases remains controversial. A recent meta-analysis by the Cochrane Library on randomized controlled trials did not support a protective effect for combined cardiovascular events [19]. However, the meta-analysis also suggested that n-3 PUFAs may be specifically protective for coronary heart disease [19]. Because coronary artery disease is a major atherosclerotic disease, the potential benefits of n-3 PUFAs against atherosclerosis are of particular interest. For example, randomized clinical studies examining the effect of n-3 PUFAs supplementation showed an increase in the n-3 PUFAs concentration in the atherosclerotic plaque lesion, which was associated with a more stabilized plaque morphology in the carotid artery [20, 21] or in the coronary artery [22], independent of statin use and/or low-density cholesterol. A recent meta-analysis of randomized controlled trials examining the anti-atherosclerotic effect of high-dose n-3 PUFAs (defined as ≥3.0 g/day in general, or ≥1.8 g/day in Japanese people, and with a purity ≥90%) reported a similar effect [6]. In addition, a recently published randomized placebo-controlled trial showed pro-

tective effect of high-dose EPA against atherosclerotic plaque progression [23]. Consistent with those studies, our findings provide further evidence that high-dose n-3 PUFAs may be protective against cerebral large-artery stenosis.

Only a few observational studies have examined the association between fish consumption and subtypes of incident stroke. One meta-analysis of 3 cohort studies (two from the USA, one from Japan) reported an inverse association with ischemic stroke, but no association with hemorrhagic stroke [24]. Consistent with our findings, another meta-analysis (five studies in the USA, five studies in Europe) reported an inverse association between circulating n-3 PUFAs and ischemic stroke, but no association with hemorrhagic stroke [25].

Of interest, many epidemiological studies in Japan have reported no association between circulating n-3 PUFAs and ischemic stroke and/or an inverse association with hemorrhagic stroke. These inconsistencies between USA/Western populations and the Japanese population may relate to a number of factors. First, the majority of relevant Japanese studies performed the baseline assessments in the 1980s or earlier [11, 26–28], a period when blood pressure levels were higher and cholesterol levels were lower than typical USA and Western populations [3, 29]. As such, in Japan, arteriolosclerotic vascular disease, particularly lacunar stroke, was the major subtype of ischemic stroke, while large-artery atherosclerosis was much less common [30], in contrast to USA/Western countries [3, 30]. Thus, any effect of n-3 PUFAs on large-artery atherosclerotic lesions may have been obscured in the Japanese population but observed in the USA and Western populations. Additionally, the nutritional status associated with low serum cholesterol in combination with increased blood pressure in the Japanese population during that period may have increased the susceptibility to hemorrhagic stroke [3]. For example, a lower risk of hemorrhagic stroke was associated with a high fish intake, as well as a high intake of combined animal products [27]; the authors attributed these findings to a low cholesterol level associated with less consumption of animal products. Alongside recent trends of improved blood pressure control and more prevalent hypercholesterolemia in Japan [29], the relative incidence of large-artery infarction and other ischemic stroke subtypes has also increased in modern Japanese populations [31]. As such, the anti-atherosclerotic effect of n-3 PUFAs on large-artery disease may be more evident.

For our secondary aim, we observed an inverse association between DHA, but not EPA, and large-artery stenosis. These findings are consistent with a meta-analysis of 10 prospective cohort studies showing that circulating DHA, but not EPA, was significantly associated with reduced risk of ischemic stroke [25]. Nevertheless, further studies are required to confirm our secondary findings.

This study has several limitations. First, considering its observational nature, a causal relationship cannot be determined. Second, n-3 PUFAs concentrations were measured 5 years prior to assessment of the cerebrovascular lesions, and we did not have information on cerebral lesions at baseline. Therefore, our analyses could not account for potential changes in the level of n-3 PUFAs among participants. However, it is unlikely that a reverse causation between n-3 PUFA and cerebral lesion (i.e., the latter being a cause of the former) plays a role because participants were not aware of their brain lesions at baseline. Third, it seems difficult to infer whether or not our exclusion of participants resulted in bias in our main estimates, because blood pressure tended to be higher in the excluded group whereas lipid levels were higher in the analyzed group, and we previously reported that both risk factors, blood pressure, and lipids, were important determinants of cerebrovascular lesions in this sample population [32]. Fourth, we did not perform a dietary survey, and could not account for confounders such as total energy, total fat, or other fatty acids. Fifth, although we statistically adjusted for major known confounders, our results may not have excluded unknown and residual confounders. Finally, because only Japanese men were included in our study, the findings may not be applicable to women or to other ethnic groups.

Conclusions

Serum concentrations of n-3 PUFAs in Japanese men were inversely and independently associated with cerebral large-artery stenosis, but not with other cerebrovascular lesions. This differential association suggests that n-3 PUFAs may be protective against cerebral large-artery stenosis, but not other types of cerebrovascular lesions.

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Statement of Ethics

The study was approved by the Institutional Review Board of the Shiga University of Medical Science, approval number G2008-061. All participants provided written informed consent.

Conflict of Interest Statement

Hisatomi Arima is one of the journal's Associate Editors. The other authors have no conflicts of interest to declare.

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Author Contributions

Conceptualization: Keiko Kondo and Hisatomi Arima; methodology: Keiko Kondo, Hisatomi Arima, and Akira Fujiyoshi; formal analysis: Keiko Kondo; investigation: Akira Fujiyoshi, Aya Kadota, Takashi Hisamatsu, Sayuki Torii, Akira Sekikawa, Naoko Miyagawa, Kazuhiko Nozaki, Katsuyuki Miura, and Hirotsugu Ueshima; writing-original draft preparation: Keiko Kondo; writing-review and editing: Hisatomi Arima, Akira Fujiyoshi, Akira Sekikawa, Aya Kadota, Takashi Hisamatsu, Sayuki Torii, Akihiko Shiino, Katsutaro Morino, Naoko Miyagawa, Hiroyoshi Segawa, Yoshiyuki Watanabe, Hiroshi Maegawa, Kazuhiko Nozaki, Katsuyuki Miura, and Hirotsugu Ueshima; supervision: Hisatomi Arima, Akira Fujiyoshi, and Akira Sekikawa; project administration: Akira Fujiyoshi, Takashi Hisamatsu, Katsuyuki Miura, and Hirotsugu Ueshima; funding acquisition: Katsuyuki Miura and Hirotsugu Ueshima. All authors have read and agreed to the published version of the manuscript.

Data Availability Statement

Research data are not publicly available on ethical grounds.

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