Title page

Serum α-linolenic and other ω-3 fatty acids, and risk of disabling dementia: community-based nested case-control study

Running headline: α-linolenic acid and dementia

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Abstract

Background & Aims: It has been hypothesized that ω-3 polyunsaturated fatty acids have anti-atherosclerotic and neuronal protective functions and may benefit prevention of dementia, but the epidemiological evidence, especially for α-linolenic acid, is quite limited. The aim of this study was to examine whether serum ω-3 polyunsaturated fatty acids are associated with risk of dementia.

Methods: We performed an intracohort case-control study nested in a community-based cohort, the Circulatory Risk in the Community Study, involving 7586 Japanese individuals aged 40 to 74 years at the baseline period of 1984 to 1994. Omega-3 polyunsaturated fatty acid constituents (α-linolenic, eicosapentaenoic, and docosahexaenoic acids) in serum total lipid were measured in 315 cases of incident disabling dementia in the above-mentioned cohort between 1999 and 2004, and in 630 controls whose age, sex, area, and baseline year were matched with the cases.

Results: As we had postulated, serum α-linolenic acid was inversely associated with risk of disabling dementia: the multivariate odds ratios (95% confidence intervals) were 0.57 (0.39-0.85), 0.51 (0.34-0.76), and 0.61 (0.41-0.90) for persons with the second, third, and highest quartiles of serum α-linolenic acid, respectively, as compared with the lowest quartile (*P* for trend = 0.01). Associations of other ω-3 fatty

19 acids with disabling dementia were not statistically significant

20 **Conclusions:** Serum α-linolenic acid was inversely associated with risk of disabling

21 dementia. Although the causality needs to be confirmed by randomized control trials,

22 we identified serum α-linolenic acid as a biomarker that predicts future dementia.

23 **Keywords:** ω-3 (n-3) polyunsaturated fatty acids; cognitive dysfunction; follow-up

24 study; α-linolenic acid (ALA); eicosapentaenoic acid (EPA); docosahexaenoic acid

25 (DHA)

26

Introduction

Alpha-linolenic acid (ALA), a plant-derived ω-3 polyunsaturated fatty acid, is an essential fatty acid, with anti-atherosclerotic and neuronal protective functions. Several studies have reported that long-chain ω-3 polyunsaturated fatty acids of marine origin, such as eicosapentaenoic acid (EPA) and docosahexaenoic acid (DHA), may be useful in preventing dementia.[1-3] ALA, a ω-3 polyunsaturated fatty acid of plant origin, is also expected to have a similar effect, but such evidence is limited.

The association between fish and coronary heart disease was non-linear and had a threshold effect[4], whereas that between dietary ALA intake and coronary heart disease may be linear [5]. In this context, we hypothesized that serum proportions of ALA, rather than of other ω-3 fatty acids, are inversely associated with risk of incident disabling dementia among Japanese individuals, because most Japanese may consume higher amounts of fish than those recommended for prevention of coronary heart disease [6], but may not do so for ALA.

Materials and methods

The Circulatory Risk in Communities Study (CIRCS) is an ongoing dynamic community-based prospective study involving 5 communities in Japan. Details of the CIRCS protocol have been described elsewhere.[7] In the present study, we included 2 communities, Ikawa and Kyowa, where disabling dementia surveillance is being conducted and sera were stored.

We set the baseline risk set as 7,586 people aged 40 to 74 years living in these 2 communities who participated in annual health checkups in Ikawa from1989 to 1991 and 1995 and in Kyowa from 1984 to 1994 (except for 1988), in which stored sera were available. Of these, a total of 315 patients were identified who were diagnosed in Kyowa between 1999 and 2004 and in Ikawa between 1999 and 2013 as having disabling dementia between 60 and 89 years of age and who participated in annual health checkups (baseline) at least 5 years before receiving the dementia diagnosis and provided sera for storage at baseline.

The diagnosis of disabling dementia was performed by attending physicians under the National Long-Term Care Insurance System (which is a compulsory insurance for all individuals aged 40 years or over in Japan); the criteria of disabling dementia were the same as those of our previous study[8], wherein the validation of the criteria

and the details of the study protocol are also described. As supplemental analysis, we further classified the dementia cases into cases with and without history of stroke on the basis of a systematic stroke registration described elsewhere.[7]

In total, 630 randomly selected controls, whose age (± 3 years), sex, area, and baseline year were matched at a ratio of 2:1 with the cases, were also identified from the risk set by incident density methods. Venous blood was collected at baseline at the checkup sites, and sera were prepared from the blood samples as soon as possible thereafter. The serum samples were collected in 0.3-mL tubes and stored at −80°C until measurement. The extraction of total lipids and measurements of serum fatty acid compositions using gas chromatography were described in detail elsewhere.[9] Of the studied sera, 92% were drawn in the nonfasting state (<8 hours from the last meal).

Potential risk factors for disabling dementia were measured at the baseline examination at the same time as the blood collection. Well-trained study physicians measured the arterial systolic and fifth-phase diastolic blood pressures using standard mercury sphygmomanometers on the right arm of the participants, who were quietly seated after having rested for at least 5 minutes. If the first systolic blood pressure reading was ≥140 mmHg and/or the diastolic blood pressure was ≥90 mmHg, the physicians repeated the measurement. For these cases, the second reading was used in

the analyses; otherwise, the first reading was used. Height without shoes and weight in light clothing were measured and body mass index was calculated as weight in kilograms divided by height in meters squared. Face-to-face interviews were conducted to determine drinking (non-current or current) and smoking (never, ex, or current) status, antihypertensive medication, cholesterol-lowering medication, and diabetes medication. Serum glucose and total cholesterol were measured at baseline without fasting requirement. Diabetes mellitus was defined as fasting serum glucose ≥ 126 mg/dL or nonfasting serum glucose ≥ 200 mg/dL, or being under medication for diabetes.

We conducted conditional logistic analyses using SAS 9.1.3. Service Pack 4 (SAS Institute, Cary, NC, USA) with adjustments for age, smoking status, systolic blood pressure, diabetes mellitus, and use of antihypertensive medication. For the missing values for these variables (<2% of each variable), we set dummy variables and included them in the models. All probability values for the statistical tests were 2-tailed, and probability values below 0.05 were considered significant. Informed consent was obtained from community leaders and verbally from individual participants according to the guidelines of the Council for International Organizations of Medical Science[10], which was common practice at that time in Japan. The study was approved by the institutional review boards of the Osaka Center for Cancer and Cardiovascular Disease

96 Prevention and of the University of Tsukuba.

97

Results

Systolic blood pressures and prevalence of diabetes were significantly higher in the dementia cases than in the non-cases (Table 1). Diastolic blood pressure and prevalence of current smokers were slightly higher among the cases than among the non-cases. The mean value of ALA, but not of EPA or DHA, was significantly lower among the cases than among the non-cases. The other baseline characteristics did not differ materially between them. As expected, EPA (3.6% total fatty acid) and DHA (5.5% total fatty acid) were very high among this Japanese population.

After follow-up (median, 12.5 years and maximum, 23.8 years), we found an inverse association between serum ALA and the risk of incident dementia (Table 2). The multivariate odds ratios and 95% confidence intervals for persons with the second, third, and highest quartiles of ALA were 0.57 (0.39–0.85), 0.51 (0.34–0.76), and 0.61 (0.41–0.90), respectively, compared with the lowest quartile (*P* for trend = 0.01). As for EPA and DHA, no associations with incident dementia were observed.

As supplemental analyses, we classified the dementia cases further into cases with and without history of stroke, and examined the associations of ALA, EPA and DHA with them by means of unconditional logistic regression models. The association of ALA with disabling dementia was generally similar between them: For dementia with

history of stroke (cases n=110), the multivariate odds ratios and 95% confidence intervals were 0.62 (0.35–1.11), 0.55 (0.30–1.01), and 0.70 (0.40–1.22) for persons with the second, third, and highest quartiles of ALA, respectively, as compared with the lowest quartile (*P* for trend = 0.20). For dementia without history of stroke (cases n=205), those were 0.52 (0.33–0.81), 0.48 (0.30–0.75), and 0.55 (0.35–0.85), respectively (*P* for trend = 0.009). As for EPA and DHA, no associations with dementia either with or without of history of stroke, were observed.

Discussion

A strong inverse association between serum ALA proportion and incident disabling dementia was found in the Japanese population. No such association was found for EPA or DHA. This is the first prospective study to find an inverse association of serum ALA with risk of disabling dementia.

A few animal and human studies supported our finding that ALA may have a beneficial impact on neural protection. Rats fed a low ALA diet had inferior learning capacity [11]. Mice fed a high ALA diet had greater learning ability and less hyperactive behavior than did those fed a low ALA diet.[12] Human erythrocyte ALA, but not EPA and DHA, was correlated with cognitive decline in a cross-sectional study of 57 Korean research participants.[13] Another cross-sectional study of 1,299 Italian research participants showed that those with dementia had lower concentrations of ALA than did those with no cognitive impairment.[14] The authors of that study did not find a significant association with EPA or DHA.[14] Concordant with these animal and cross-sectional studies, the present study first showed that ALA was inversely associated with risk of dementia in a study with a prospective design and sufficient sample size. In contrast, 2 prospective studies did not find such an association. One study examined plasma fatty acid composition and risk of cognitive decline in 2,251

white research participants (aged 50–65 years) in Minnesota, USA, and found no association between plasma ALA and cognitive decline in their 9-year follow-up.[15] Another study involving 1,214 elderly research participants (≥ 65 years) in Bordeaux, France, also did not find any association of ALA with cognitive decline in their 4-year follow-up.[16] The reasons for the discrepancy between the findings of these studies and those of the present study are unknown, but the shorter follow-up (compared with the 24-year follow-up of the present study) and the smaller number of cases (140 cases in Minnesota and 65 cases in Bordeaux as compared with the 630 cases of the present study), as well as differences in ethnicity, settings, age distribution, distribution of ALA, and endpoint determination across the studies might explain the inconsistent results.

Diet studies, although showing somewhat weak association, have also reported generally similar results. The Rotterdam Study showed a non-significant inverse trend between ALA intake and risk of dementia.[1] Another study, The Chicago Health and Aging Project, showed that ALA intake was inversely and linearly associated with risk of Alzheimer disease in age-adjusted models, but not in a multivariate adjusted model including the *APOE* ε4 allele.[17] They also found an interaction of the *APOE* ε4 allele with ALA intake in relation to Alzheimer disease; that is, the dietary ALA-Alzheimer disease association was observed only among persons with the *APOE* ε4 allele. We

159 unfortunately do not have *APOE* ε4 allele information, but the *APOE* ε4 allele is less
160 prevalent among Japanese than among white individuals.[18] As for trials, Geleijnse et
161 al. performed a randomized double-blind placebo-controlled trial among 2911 coronary
162 patients. The decline in scores of the mini-mental state examination did not differ
163 significantly between the patients with 2 g/day supplementation of ALA and those
164 taking the placebo control after a 40-month intervention: the difference, however, was
165 almost marginally significant (-0.74 versus -0.60 points, p=0.12).[19] Another
166 randomized double-blind placebo-controlled trial showed that the supplementation of a
167 mixture of ALA and linolenic acids significantly improved short-term memory in
168 Alzheimer disease patients after the 4-week intervention.[20] Although their outcomes
169 were not incident dementia, the findings of those intervention studies were in line with
170 the present study.

171 The nonlinear association between fish and coronary heart disease and its
172 threshold effect have been well discussed.[4] We hypothesized that the associations of
173 EPA and DHA with risk of dementia would be weak; ie, the very high consumption of
174 fish and smaller variation in fish intake in Japanese would cause any associations to be
175 obscured. Generally, Japanese consume far higher amounts of fish than do western
176 populations.[21] In fact, in the current study's population, the EPA and DHA

177 proportions were 3.6% and 5.5% of total fatty acid, respectively, which is far higher
178 than those of Italian subjects: 0.6% for EPA and 2.3% for DHA [14]; the ALA
179 proportion was also much higher (1.0% in the present study vs 0.4% in the Italian study).
180 In addition, associations between EPA and DHA intake/supplementation and risk of
181 dementia in previous intervention trials and cohort studies were undetermined[22-24],
182 which was in line with our null results for the association between these fatty acids in
183 serum and risk of dementia. Of note, though we found a statistically significant trend for
184 linearity, the association between serum ALA and disabling dementia were virtually
185 nonlinear, which suggested the existence of a threshold effect.
186 Several limitations of this study should be noted. First, attending physicians
187 diagnosed the disabling dementia. However, we should also mention that such diagnosis
188 by attending physicians was validated in a previous study.[8] Second, we examined fatty
189 acid composition in the serum rather than in the erythrocyte membrane phospholipids
190 with an advantage of longer-term dietary consumption of fatty acids. A previous study,
191 however, showed that erythrocyte membrane, compared with plasma, reflects usual
192 dietary intakes more strongly for EPA and DHA but not for ALA[25]. Third, the sera
193 were stored over a number of years. Our previous study showed that the 8-year
194 repeatability of the proportion of ω-3 polyunsaturated fatty acids on serum total fat were

fairly good (12.8% vs 12.3%, p=0.14) [26]. Another study showed that 7 to 12 years' storage did not affect the serum ALA (reliability coefficient =0.72) [27]. Moreover in the present study, the results were not altered when we excluded 114 cases and 228 controls whose sera were collected before 1990 (not shown). Fourth, we did not have information on dementia type (ie, Alzheimer or vascular dementia). Instead, we had information on dementia with and without history of stroke. The associations seemed similar between dementia with and without history of stroke. Fifth, the generalizability to populations other than the Asian population should be cautious, especially for eicosapentaenoic and docosahexaenoic acids, since Japanese consume far higher fish than do non-Asians. Last, we did not survey prevalent dementia at baseline. Instead, we set the baseline at least 5 years before the beginning of the dementia survey, thereby reducing the possibility of reverse causation.

In conclusion, serum proportions of ALA were inversely associated with risk of disabling dementia among Japanese. This suggests that low ALA intake may carry a potential risk of dementia. Although the causality needs to be confirmed by randomized control trials, we identified serum α-linolenic acid as a biomarker that predicts future dementia.

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231 TO, TT, AK, MK.

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Table 1. Baseline characteristics of dementia cases and non-cases, CIRCS aged 40-74 years.

	Non-cases	Dementia cases	*p* for difference
Number	630	315	
Age, y	64.2	64.6	0.37
Male gender, %	34	34	1.00
Body mass index, kg/m^2	24.0	23.7	0.27
Current smokers, %	19	24	0.07
Current drinkers, %	27	27	0.85
Systolic blood pressure, mmHg	133	137	0.002
Diastolic blood pressure, mmHg	78	79	0.02
Antihypertensive medication, %	31	34	0.43
Diabetes mellitus, %	5	11	0.004
Serum total cholesterol, mg/dL	199	201	0.37
Cholesterol-lowering medication, %	3	2	0.48
Saturated fatty acids, % total fatty acids	34.6	34.8	0.34
Monounsaturated fatty acids, % total fatty acids	22.4	22.7	0.13
Omega-6 polyunsaturated fatty acids, % total fatty acids	32.0	31.6	0.23
Alpha-linolenic acid, % total fatty acids	0.97	0.90	0.002
Eicosapentaenoic acid, % total fatty acids	3.63	3.55	0.57
Docosahexaenoic acid, % total fatty acids	5.50	5.53	0.82

Table 2. Age and sex-matched and multivariate adjusted conditional odds ratios and 95% confidence intervals of incident dementia according to quartiles of serum alpha-linolenic, eicosapentaenoic and docosahexaenoic acids.

	Men and Women				
	Alpha-linolenic acid (%total fatty acid)				
	Q1	Q2	Q3	Q4	Trend p
	0.30-0.74	0.75-0.90	0.91-1.11	1.12-2.26	
Median, % total fatty acid	0.64	0.82	1.00	1.32	
Number of non-cases	157	155	156	162	
Number of cases	115	64	61	75	
Matched OR (95%CI)*	1.0	0.56 (0.38-0.82)	0.52 (0.35-0.77)	0.60 (0.41-0.88)	0.01
Multivariable OR (95%CI)†	1.0	0.57 (0.39-0.85)	0.51 (0.34-0.76)	0.61 (0.41-0.90)	0.01
	Eicosapentaenoic acid (%total fatty acid)				
	Q1	Q2	Q3	Q4	Trend p
	0.50-2.14	2.15-3.23	3.24-4.66	4.67-12.8	
Median, % total fatty acid	1.62	2.70	3.89	6.02	
Number of non-cases	157	158	157	158	
Number of cases	82	85	77	71	
Matched OR (95%CI)*	1.0	1.00 (0.68-1.48)	0.90 (0.59-1.37)	0.81 (0.52-1.27)	0.32
Multivariable OR (95%CI)†	1.0	0.99 (0.67-1.49)	0.90 (0.58-1.39)	0.79 (0.49-1.26)	0.28
	Docosahexaenoic acid (%total fatty acid)				
	Q1	Q2	Q3	Q4	Trend p
	1.78-4.19	4.20-5.23	5.24-6.52	6.53-11.1	
Median, % total fatty acid	3.55	4.77	5.80	7.69	
Number of non-cases	157	158	156	159	
Number of cases	81	69	81	84	
Matched OR (95%CI)*	1.0	0.86 (0.58-1.27)	1.05 (0.68-1.60)	1.10 (0.68-1.78)	0.54
Multivariable OR (95%CI)†	1.0	0.88 (0.58-1.31)	1.20 (0.77-1.86)	1.18 (0.72-1.94)	0.34

*Matched with age (±3 years), sex, area and baseline-year

†Multivariable model further includes age, smoking status, systolic blood pressure, diabetes mellitus and medication of hypertension.