

Serum  $\alpha$ -linolenic and other  $n-3$  fatty acids,  
and risk of disabling dementia:  
Community-based nested case control study

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**Title page****Serum  $\alpha$ -linolenic and other  $\omega$ -3 fatty acids, and risk of disabling dementia:  
community-based nested case-control study****Running headline:**  $\alpha$ -linolenic acid and dementia

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1 **Abstract**

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

7 **Methods:** We performed an intracohort case-control study nested in a  
8 community-based cohort, the Circulatory Risk in the Community Study, involving  
9 7586 Japanese individuals aged 40 to 74 years at the baseline period of 1984 to 1994.  
10 Omega-3 polyunsaturated fatty acid constituents ( $\alpha$ -linolenic, eicosapentaenoic, and  
11 docosahexaenoic acids) in serum total lipid were measured in 315 cases of incident  
12 disabling dementia in the above-mentioned cohort between 1999 and 2004, and in 630  
13 controls whose age, sex, area, and baseline year were matched with the cases.

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

19 acids with disabling dementia were not statistically significant

20 **Conclusions:** Serum  $\alpha$ -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  $\alpha$ -linolenic acid as a biomarker that predicts future dementia.

23 **Keywords:**  $\omega$ -3 (n-3) polyunsaturated fatty acids; cognitive dysfunction; follow-up  
24 study;  $\alpha$ -linolenic acid (ALA); eicosapentaenoic acid (EPA); docosahexaenoic acid  
25 (DHA)

26

## 27 **Introduction**

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

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

41

## 42 **Materials and methods**

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

48 We set the baseline risk set as 7,586 people aged 40 to 74 years living in these  
49 2 communities who participated in annual health checkups in Ikawa from 1989 to 1991  
50 and 1995 and in Kyowa from 1984 to 1994 (except for 1988), in which stored sera were  
51 available. Of these, a total of 315 patients were identified who were diagnosed in  
52 Kyowa between 1999 and 2004 and in Ikawa between 1999 and 2013 as having  
53 disabling dementia between 60 and 89 years of age and who participated in annual  
54 health checkups (baseline) at least 5 years before receiving the dementia diagnosis and  
55 provided sera for storage at baseline.

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

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

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

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



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

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

96 Prevention and of the University of Tsukuba.

97

## 98 **Results**

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

106         After follow-up (median, 12.5 years and maximum, 23.8 years), we found an  
107 inverse association between serum ALA and the risk of incident dementia (Table 2). The  
108 multivariate odds ratios and 95% confidence intervals for persons with the second, third,  
109 and highest quartiles of ALA were 0.57 (0.39–0.85), 0.51 (0.34–0.76), and 0.61  
110 (0.41–0.90), respectively, compared with the lowest quartile ( $P$  for trend = 0.01). As for  
111 EPA and DHA, no associations with incident dementia were observed.

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

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

## 123 **Discussion**

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

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

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

151 Diet studies, although showing somewhat weak association, have also reported  
152 generally similar results. The Rotterdam Study showed a non-significant inverse trend  
153 between ALA intake and risk of dementia.[1] Another study, The Chicago Health and  
154 Aging Project, showed that ALA intake was inversely and linearly associated with risk  
155 of Alzheimer disease in age-adjusted models, but not in a multivariate adjusted model  
156 including the *APOE*  $\epsilon 4$  allele.[17] They also found an interaction of the *APOE*  $\epsilon 4$  allele  
157 with ALA intake in relation to Alzheimer disease; that is, the dietary ALA-Alzheimer  
158 disease association was observed only among persons with the *APOE*  $\epsilon 4$  allele. We

159 unfortunately do not have *APOE*  $\epsilon$ 4 allele information, but the *APOE*  $\epsilon$ 4 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  $\omega$ -3 polyunsaturated fatty acids on serum total fat were



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

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

212

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219

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225 **Conflict of interest:** None declared.

226

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228 data: KY, AI, H Iso; Analysis of data: KY; Acquisition of data and final approval of the  
229 version to submitted: all authors; drafting the article: KY and H Iso; revising the article  
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- 304

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

	Non-cases	Dementia cases	<i>p</i> for difference
Number	630	315	
Age, y	64.2	64.6	0.37
Male gender, %	34	34	1.00
Body mass index, kg/m <sup>2</sup>	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.

<b>Men and Women</b>					
<b>Alpha-linolenic acid (%total fatty acid)</b>					
	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
<b>Eicosapentaenoic acid (%total fatty acid)</b>					
	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
<b>Docosahexaenoic acid (%total fatty acid)</b>					
	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 ( $\pm 3$  years), sex, area and baseline-year

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