Long-chain n-3 polyunsaturated fatty acids intake and cardiovascular disease mortality risk in Japanese: a 24-year follow-up of NIPPON DATA80

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ABSTRACT

Background: Dietary intake of long-chain n-3 PUFA (LCn3FA) among Japanese is generally higher than that in Western populations. However, little is known whether an inverse association of LCn3FA with cardiovascular disease (CVD) risk exists in a population with higher LCn3FA intake.

Objective: To investigate the association between LCn3FA intake and the long-term risk of CVDs in a Japanese general population.

Methods: We followed-up a total of 9,190 individuals (56.2% women, mean age 50.0 years) randomly selected from 300 areas across Japan and free from CVDs at baseline. Dietary LCn3FA intake was estimated using household weighed food records. Cox models were used to calculate multivariate-adjusted hazard ratios (HR) and confidence intervals (CI) according to sex specific quartiles of LCn3FA intake.

Results: During 24-year follow-up (192,897 person-years), 879 cardiovascular deaths were observed. The median daily intake of LCn3FA was 0.37%kcal (0.86 g/day). Adjusted HR for CVD mortality was lower in the highest quartile of LCn3FA intake (HR 0.80; 95%CI 0.66-0.96) compared with the lowest quartile, and the trend was statistically significant (P=0.038). The similar but statistically non-significant trends were observed for coronary heart disease death and stroke death. In analyses by age groups, the inverse associations of LCn3FA intake with the risk of total CVD death and stroke death were significant in younger individuals (30-59 years at baseline).

Conclusion: LCn3FA intake was inversely and independently associated the long-term risk of total CVD mortality in a representative sample of Japanese with high LCn3FA intake.

Keywords: long-chain n-3 polyunsaturated fatty acid; cardiovascular disease mortality; nutrition; cohort study

1. Introduction

Long-chain n-3 PUFA (LCn3FA) intake was shown to be inversely associated with the risk of cardiovascular diseases (CVD), particularly that of coronary heart disease (CHD), mainly in Western populations.[1] A protective effect of fish and LCn3FA consumption on stroke risk was also suggested from some recent meta-analyses.[2, 3] The previous studies suggested that the effect of LCn3FA on CHD death has a dose-response relationship with a ceiling effect at their dose of around 600 mg/day.[4]

Major origins of dietary LCn3FA are fish and shellfish. Many studies in Western countries showed the association of fish and LCn3FA consumption with CVD risk compared with non fish eaters. Average fish intake, therefore LCn3FA intake, of Japanese is markedly higher compared with that of Western populations - approximately 3 to 4 times higher for fish intake and 6 to 10 times higher for LCn3FA intake.[5-7] There have been only three prospective cohort studies on fish/LCn3FA intake and CVD risk from Japan,[8-10] but their dietary data were from food frequency questionnaire (FFQ); therefore, results were not controlled for sodium intake, which generally correlates with fish intake in Japan.[11] Therefore, little evidence exists whether LCn3FA intake in a higher range relates to long-term CVD risk independent from sodium intake using high-quality dietary data.

In this report, we examined the association of dietary LCn3FA intake, estimated by 3-day weighed food records in the National Nutritional Survey of Japan (NNSJ), with 24-year CVD mortality risk in a representative Japanese population where dietary intake of LCn3FA is substantially higher than typical Western diet.

2. Subjects and methods

2.1 Participants

The National Integrated Project for Prospective Observation of Non-communicable Disease And its Trends in the Aged (NIPPON DATA) is a series of cohort studies, which utilize as a baseline survey both the National Survey on Circulatory Disorders and the NNSJ conducted in 1980 and 1990 by the Ministry of Health and Welfare, Japan. We analyzed the data of NIPPON DATA80 in which the baseline survey was conducted in 1980. The details of these cohorts previously were reported elsewhere.[12, 13]

In brief, a total of 10,546 community residents (4,639 men and 5,907 women, aged 30 and greater) from 300 randomly selected districts from all-over Japan participated in the survey, with the participation rate of about 77%. Accordingly, these participants were thought to be representative of the Japanese population. A total of 1,356 men and women excluded from this analysis for the

following reasons: history of CVD (n=350), missing information (e.g., nutrition, lifestyle questionnaire) at baseline (n=124), intake of energy more than 5,000 kcal/day or less than 500 kcal/day (n=139) and lost to follow-up due to incomplete residential addresses at the baseline survey (n=1104). We included the remaining 9,190 participants (4,028 men and 5,162 women) in the analysis.

2.2 Follow-up and Outcomes

The participants were followed until November 2004, providing 24 years of follow up. Vital status of participants was followed up using registration records in local governments where they lived. National Vital Statistics were utilized to identify the causes of death with permission from the Management and Coordination Agency, Government of Japan. The underlying causes of death in the National Vital Statistics were coded according to the 9th International Classification of Disease (ICD 9) until the end of 1994, and the 10th International Classification of Disease (ICD 10) from the beginning of 1995. The details of classification were described elsewhere.[13] The corresponding ICD9 and ICD10 codes were as follows: cardiovascular mortality, 393 to 459 (ICD9), I00 to I99 (ICD10); coronary heart disease mortality, 410 to 414 (ICD9), I20 to I25 (ICD10); and stroke mortality, 430 to 438 (ICD9), I60 to I69 (ICD10). We obtained approval for the study from the Institutional review Board of Shiga University of Medical Science (No.12-18, 2000; No.17-21-1, 2010).

2.3 Baseline examination

At baseline, non-fasting blood samples were obtained. Serum was separated and centrifuged soon after blood coagulation. Plasma samples were also obtained in a siliconized tube containing sodium fluoride. These samples were shipped to one laboratory (SRL, Tokyo) for blood measurements. Plasma glucose and serum total cholesterol were measured enzymatically. Lipid measurements were standardized by the Centers for Disease Control / National Heart, Lung, and Blood Institute (CDC-NHLBI) Lipids Standardization Program.[14]

Body mass index (BMI) was calculated as weight (kg) divided by the square of height (m). Trained observers measured baseline blood pressures by using a standard mercury sphygmomanometer on the right arm of seated participants. Public health nurses obtained information on smoking, drinking, and medical histories. We divided participants into three categories of smoking (never-smoked; ex-smoker; current smoker) and three categories of drinking (never-drinker; ex-drinker; current drinker).

2.4 Dietary Assessment

We used the data of NNSJ in 1980 (NNSJ80). Detailed methods of the dietary assessment, the estimation of individual intake of nutrients, and food groups were described elsewhere.[15-17] In brief, food intake survey by weighed food records in three consecutive representative days in each household were conducted by specially trained dietary interviewers. Dietary interviewers visited participants' house at least once during the survey, avoiding weekends and holidays. Modified Standards Tables for Food Composition in Japan, 3rd edition, with matched fatty acid values and micronutrients, were used to estimate nutrient intakes in each household for NNSJ80. Nutrient intakes of each household member were estimated by dividing household intake data of NNSJ80 proportionally using average intake by sex and age groups calculated for NNSJ conducted in 1995 (NNSJ95). The average intake in NNSJ95 were assessed by a combination method of household-based weighed food records and an approximation of proportions by which family members shared each dish or food in the household. For each person, means of the estimated individual nutrients from the three day records were used in the analyses. Dietary researchers were blinded to participant outcome status.

For energy supplying nutrients, the intake was calculated as the percent of total energy intake (%kcal). Other nutrients were calculated relative to total dietary intake (g/1,000 kcal). LCn3FA was the sum of eicosapentaenoic acid (EPA) and docosapentaenoic acid (DHA).

2.5 Statistical Analysis

Baseline characteristics and nutritional parameters of participants were presented as means and standard deviations for continuous variables and as numbers and percentages for categorical variables. Differences in baseline characteristics and nutritional parameters among sex specific quartiles of LCn3FA intake were evaluated using trend analysis or chi-square test.

Cox proportional-hazards regression models were used to estimate the multivariate-adjusted hazard ratios (HR) of death from total CVD and components of CVDs according to quartiles of LCn3FA intake. Model 1 represents the model adjusted for age and sex. Model 2 was additionally adjusted for the conventional risk factors: BMI, smoking status (never, ex, and current), drinking status (never, ex, and current), antihypertensive medication status (yes or no), systolic blood pressure (mm Hg), serum total cholesterol (mg/dl), blood glucose (mg/dl) and residential area (population size ≤10,000, 10,001-50,000, 50,001-300,000, and ≥300,001). Model 3 was further adjusted for nutritional parameters in addition to the variables adjusted in Model 2: saturated fatty acids, total n-6 polyunsaturated fatty acid (PUFA), vegetable protein, fiber and sodium. Stratified analyses were conducted by age groups (30-59 years, ≥60 years at baseline) or by sexes. Corresponding analyses were done also for total n-3 PUFA intake and for EPA and DHA intake, separately, intake instead of

LCn3FA intake. Tests for trend involved assigning participants the median value in their quartiles of fatty acids intake and evaluating this as a continuous variable.

The statistical analyses were performed by SPSS for Windows Version 18.0 (SPSS Japan Inc., Tokyo, Japan). All probability values were two-sided, and *P* values less than 0.05 were considered statistically significant.

3. **Results**

Table 1 shows the baseline characteristics of study participants according to quartiles of LCn3FA intake. The median daily intake of LCn3FA in total participants was 0.37%kcal (0.86 g/day). As the amount of LCn3FA intake increased, age, systolic blood pressure, blood glucose and dietary intake of sodium increased; in contrast, dietary energy intake, total fat intake, total n-6 PUFA intake and proportion of population resident in a metropolitan area decreased. Observed total person-years were 192,897, and the mean follow-up period was 21.0 years. During the follow-up, 879 participants died from total CVD (including 171 from CHD, 417 from stroke, 170 from heart failure) and 1,672 died from non-CVD.

Table 2 shows multivariate-adjusted HRs and 95% CIs according to sex-specific quartiles of LCn3FA intake. HRs of total CVD death were significantly lower in the highest quartile of LCn3FA intake (Q4) (HR 0.80 [95% CI 0.66-0.96]) compared with the lowest quartile (Q1) in model 3, and its inverse linear trend was statistically significant in model 3 (P =0.038). The tendency of HRs in relation to LCn3FA intake was similar for CHD death, but not statistically significant. HR of stroke in the highest quartile (Q4) was 0.75 (95%CI 0.57-1.00), although the trend was not statistically significant (P =0.115). There were no statistically significant associations between LCn3FA intake and non-CVD mortality risk.

Table 3 shows results by age groups at baseline. The associations of LCn3FA intake with total CVD death and stroke death were significant for younger group (30-59 years at baseline). Inverse associations were observed both in men and women (Online Supplement Table 1).

When the analyses were done according to the quartiles of total n-3 PUFA intake, the results were similar; however, the associations were weaker than those for LCn3FA (Online Supplement Table 2). The association of alpha linoleic acid, the main part of non-LCn3FA, with CVD death was not significant (data not shown). HRs according to the quartiles of EPA or DHA intake showed similar inverse associations; although the trend was somewhat stronger for DHA intake (Online Supplement Tables 3 and 4).

Based on an examination of more complex models that include tests of interaction and

non-linearity, we did not detect any significant departures from the assumption of proportionality.

4. Discussion

In this 24-year, community-based, prospective cohort study of representative Japanese, we observed an inverse association between LCn3FA intake and risk of CVD mortality after adjustment for cardiovascular risk factors and nutritional factors including sodium intake. The association was significant for younger individuals aged 30-59 years at baseline. The association was not significant for older individuals aged 60 years or over.

The relative strength of effect is estimated from effects of LCn3FA on each risk factor and on the corresponding impact on cardiovascular risk. For example, dose- response for antiarrhythmic, blood pressure lowering and heart rate lowering effects are initially steep with a subsequent plateau at 0.5 g/day, 0.75 g/day and 0.75 g/day, respectively.[4] In our study, 30% of participants ingested LCn3FA 1 g/day or over and 90% of participants ingested 0.5 g/day or over; almost all participants in the present study have ingested high amount of LCn3FA. We observed dose-dependently lower risk of CVD mortality, and multivariate-adjusted HRs (95% CI) for the highest (median intake of LCn3FA was 0.68% energy; 1.6 g/day) versus lowest (median intake of LCn3FA was 0.18% energy; 0.4 g/day) quartiles of LCn3FA intake was 0.80 (0.66 to 0.96). Median value of the lowest quartile of LCn3FA intake in the present study was twice as high as the average intake in U.S. population.[18] Namely, our long-term cohort study indicated that a higher intake of LCn3FA was associated with reduced long-term risk of CVD compared with a modest LCn3FA intake. Recent studies show that LCn3FA exert many actions on cell physiology and functions, including assembly of signaling platform, i.e., lipid rafts, and intracellular signaling cascades leading to gene expression.[19] These actions most likely require intakes of LCn3FA above 1 g/day, which many Japanese in Japan regularly consumed. Thus, these effects might be associated with our observation. Moreover, our results suggested that other n-3 PUFAs, mainly alpha linoleic acid from vegetable sources, do not have a protective effect for CVD.

Only three prospective cohort studies reported the association of dietary fish intake or LCn3FA with CVD in Japanese. Two of the studies evaluated food and nutrient intake using FFQ, and their follow-up period was shorter (about 10 years) than ours. Although the previous three studies in Japan showed no significant associations for stroke risk, we observed a significant inverse relationship of LCn3FA to the risk of stroke mortality in younger age group. In addition, no previous studies in Japan showed HRs adjusted for sodium intake. Higher fish intake would coexist with higher sodium intake due to traditional Japanese dietary pattern; therefore, the inverse association of LCn3FA with

CVD risk could be under-estimated without adjustment of sodium intake. Our study would be the first to show the relationship between n-3 PUFA intake and stroke risk with adjustment of sodium intake.

There have been several randomized controlled trials supplementing 0.4-0.9 g/day EPA and DHA in Western populations.[1] The Japan EPA Lipid Intervention Study [20] demonstrated a protective effect of EPA supplementation (1.8 g/day) on nonfatal coronary events for 4.6 years, but effect on fatal coronary events was not confirmed. In addition, almost all trials were for secondary prevention or for primary prevention in high risk individuals, and their follow-up period was shorter (1 to 6 years).[20, 21] Long-term effect of n-3 PUFA on primary prevention of CVD in general population may only be investigated by long-term observational studies. In our study, a significant association between LCn3FA intake and reduce mortality from total CVD and stroke was observed in individuals that were 30-59 years at baseline but not in individuals who were 60 years or older at baseline. The lack of association who were older individuals at baseline may due to a variety of factors such as survivor effect, poorer nutrition in their younger age, or dietary modification or medical treatment in high risk individuals.

There have been few previous large-scale cohort studies using weighed food records for dietary assessment to investigate LCn3FA intake and CVD risk. It was reported that correlations of plasma phospholipid fatty acids with intake of total n-3 PUFA, EPA and DHA by weighed food records were stronger than those by FFQ.[22] Furthermore, studies in Japanese showed that correlations between serum fatty acids and intake of EPA, DHA and total n-3 PUFA evaluated by seven-days weighed food records were high (correlation coefficients 0.47-0.86).[23, 24]

There are several limitations in this study. First, the follow-up period of our study was long, and dietary exposure may have changed over time.[25] Also, we assumed that smoking habit and other confounding factors, adjusted in multivariate-adjusted models, did not change during the follow-up period. Second, information on fish oil supplementation was not available in the baseline survey, although supplement use was not common among Japanese in 1980. Third, we cannot exclude residual confounding from unmeasured or unknown factors. For example, physical activity, socioeconomic status and amount of alcohol consumption were not considered in this analysis. Fourth, we did not have data on CVD incidence; therefore, we could not examine the relationships to CVD incidence. Fifth, we used non-fasting blood glucose for adjustment, although we reported a positive relationship of non-fasting blood glucose to CVD risk from this cohort study.[26] Finally, there may be potential misclassifications in causes of deaths from vital statistics, although data on total CVD mortality would be more reliable.

In conclusion, this long-term cohort study of representative Japanese showed that a higher

amount of long-chain n-3 PUFA intake would lower the long-term risk of cardiovascular disease mortality, especially in younger adults.

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Conflict of interest

There is no potential conflict of interest that relates to the manuscript.

Contribution

The author's responsibilities were as follows; NM, KM, and UH: study concept and design; NO, TK, NT, SN, YM, AH, AF, KY, T Okamura, AO and HU: acquisition of data; NM, KM, AS, AF and T Ohkubo: analysis and interpretation of data; NM: drafting of the manuscript, NM, KM, SN, YN, AH, AF, TH, AS, T Ohkubo, RDA and HU: critical revision of the manuscript for important intellectual content; NM and KM: statistical analysis; and T Okamura, AO and HU: study supervisor. All authors read and approved the final manuscript. None of the authors declared a conflict of interest.

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Table 1. Baseline characteristics of cardiovascular risk factors and selected dietary variables in 4,028 men and 5,162 women by the quartiles of LCn3FA intake: NIPPON DATA80, 1980

	Quartiles of LCn3FA intake, %kcal				*P* value [a]
	Q1 (Low)	Q2	Q3	Q4 (High)	
Range of LCn3FA intake (median), %kcal					
Men	0.00-0.23 (0.18)	0.24-0.35 (0.29)	0.36-0.51 (0.43)	0.52-2.34 (0.65)	
Women	0.02-0.25 (0.19)	0.26-0.38 (0.32)	0.39-0.55 (0.46)	0.56-2.43 (0.70)	
Mean intake of LCn3FA, g/day	0.42 (0.15)	0.74 (0.17)	1.06 (0.25)	1.72 (0.62)	< 0.001
Number at risk	2263	2374	2257	2296	
Number of women, %	56.1	56.6	56.0	56.0	0.975
Age at baseline, years	49.4 (13.3)	49.4 (13.0)	50.5 (13.1)	50.8 (13.3)	< 0.001
Body mass index, kg/m^2	22.6 (3.2)	22.7 (3.1)	22.6 (3.1)	22.9 (3.2)	0.005
Systolic blood pressure, mm Hg	134.9 (20.7)	135.5 (21.5)	136.1 (21.4)	136.7 (21.2)	0.002
Diastolic blood pressure, mm Hg	81.2 (12.3)	81.1 (12.1)	81.4 (12.3)	81.5 (12.2)	0.229
Antihypertensive medication, %	9.9	12.1	12.0	13.3	0.024
Casual blood glucose, mg/dL	98.3 (27.5)	100.4 (31.3)	100.6 (28.5)	101.0 (32.1)	0.003
Serum total cholesterol, mg/dL	188.0 (33.5)	188.7 (33.8)	190.5 (34.1)	187.9 (33.2)	0.602
Current smoker, %	33.7	31.8	32.0	33.7	0.141
Current drinker, %	43.0	44.6	43.3	44.9	0.789
Resident in metropolitan area, %	30.4	28.4	27.9	22.6	< 0.001
Intake of nutrients and foods					

Total energy, kcal/day	2155 (500)	2167 (468)	2132 (478)	2100 (510)	< 0.001
Total fat, %kcal	21.2 (5.7)	21.0 (5.4)	20.4 (5.3)	20.3 (5.4)	< 0.001
Saturated fatty acids, %kcal	6.0 (1.7)	5.9 (1.5)	5.8 (1.5)	5.9 (1.5)	0.026
Total n-6 PUFA, %kcal	4.4 (1.2)	4.3 (1.2)	4.3 (1.2)	4.2 (1.2)	< 0.001
Total n-3 PUFA , %kcal	0.9 (0.3)	1.0 (0.3)	1.2 (0.3)	1.5 (0.4)	< 0.001
Eicosapentaenoic acid, %kcal	0.06 (0.02)	0.11 (0.02)	0.17 (0.02)	0.29 (0.09)	< 0.001
Docosahexaenoic acid, %kcal	0.11 (0.03)	0.19 (0.02)	0.28 (0.03)	0.46 (0.13)	< 0.001
Vegetable protein, %kcal	7.4 (1.0)	7.4 (0.9)	7.4 (0.9)	7.5 (1.1)	0.059
Total dietary fiber, g/1,000kcal	8.4 (2.0)	8.5 (2.0)	8.6 (2.1)	8.6 (2.2)	0.007
Sodium, mg/1,000kcal	2350 (746)	2522 (790)	2631 (776)	2906 (1000)	< 0.001
Fish and shellfish, g/1,000kcal	30.9 (14.2)	44.5 (16.0)	55.0 (18.1)	75.0 (26.0)	< 0.001

Values are means (standard deviation) or %. [a] Differences were evaluated using trend analysis or chi-square test.

LCn3FA, long-chain n-3 polyunsaturated fatty acids**;** PUFA, polyunsaturated fatty acid.

Table 2. Multivariate-adjusted HRs and 95% CIs of mortality from total cardiovascular diseases, coronary heart disease, stroke, and total non-cardiovascular diseases according to quartiles of LCn3FA intake: NIPPON DATA80

	Quartiles of LCn3FA intake				*P* value for trend
	Q1 (Low)	Q2	Q3	Q4 (High)	
Person-years	47402	50196	47359	47940	
Total cardiovascular disease deaths, n	222	210	216	231	
Model 1	1	0.90 (0.75-1.09)	0.90 (0.74-1.08)	0.90 (0.75-1.09)	0.358
Model 2	1	0.87 (0.72-1.05)	0.88 (0.73-1.06)	0.85 (0.70-1.02)	0.144
Model 3	1	0.85 (0.70-1.03)	0.85 (0.70-1.03)	0.80 (0.66-0.96)	0.038
Coronary heart disease deaths, n	39	43	43	46	
Model 1	1	1.04 (0.68-1.61)	1.00 (0.65-1.55)	1.02 (0.66-1.56)	0.994
Model 2	1	1.01 (0.66-1.57)	0.95 (0.61-1.46)	0.94 (0.61-1.45)	0.713
Model 3	1	0.95 (0.61-1.47)	0.88 (0.57-1.36)	0.82 (0.53-1.29)	0.363
Stroke death, n	104	95	112	106	
Model 1	1	0.87 (0.66-1.15)	0.99 (0.76-1.30)	0.89 (0.68-1.17)	0.583
Model 2	1	0.82 (0.62-1.08)	0.97 (0.74-1.27)	0.81 (0.62-1.06)	0.267
Model 3	1	0.81 (0.61-1.07)	0.94 (0.72-1.24)	0.75 (0.57-1.00)	0.115
Total non-cardiovascular disease deaths, n	392	421	416	443	
Model 1	1	1.00 (0.87-1.15)	0.96 (0.84-1.10)	0.98 (0.86-1.13)	0.714
Model 2	1	1.00 (0.87-1.14)	0.97 (0.84-1.11)	0.97 (0.85-1.12)	0.637

Model 3	1	1.00 (0.87-1.15)	0.97 (0.85-1.12)	0.97 (0.84-1.12)	0.562

Model 1 was adjusted for age and sex. Model 2 was adjusted for variables in Model 1 plus smoking status, drinking status, systolic blood pressure, blood glucose, serum total cholesterol, body mass index, antihypertensive medication status and residential area. Model 3 was adjusted for variables in Model 2 plus dietary intakes of saturated fatty acids (%kcal), total n-6 PUFA (%kcal), vegetable protein (%kcal), total dietary fiber (g/1,000kcal) and sodium (mg/1,000kcal).

LCn3FA, long-chain n-3 polyunsaturated fatty acids; HR, hazard ratio; CI, confidence interval**;** PUFA, polyunsaturated fatty acid.

Table 3. Multivariate-adjusted HRs and 95% CIs of mortality from total cardiovascular diseases, coronary heart disease and stroke according to quartiles of LCn3FA intake by age groups: NIPPON DATA80

	Quartiles of LCn3FA intake				*P* value for trend
	Q1 (Low)	Q2	Q3	Q4 (High)	
Total cardiovascular disease deaths					
30-59 years at baseline					
Number of deaths	57	62	56	59	
Model 1	1	0.95 (0.66-1.36)	0.82 (0.57-1.19)	0.83 (0.58-1.20)	0.271
Model 2	1	0.94 (0.65-1.35)	0.77 (0.53-1.12)	0.75 (0.51-1.08)	0.085
Model 3	1	0.92 (0.64-1.32)	0.75 (0.51-1.09)	0.68 (0.46-1.00)	0.031
≥60 years at baseline					
Number of deaths	165	148	160	172	
Model 1	1	0.89 (0.71-1.11)	0.93 (0.75-1.15)	0.93 (0.75-1.15)	0.665
Model 2	1	0.87 (0.70-1.09)	0.93 (0.75-1.16)	0.91 (0.73-1.13)	0.564
Model 3	1	0.86 (0.68-1.07)	0.91 (0.73-1.13)	0.86 (0.69-1.08)	0.323
Coronary heart disease deaths					
30-59 years at baseline					
Number of deaths	13	15	13	13	
Model 1	1	1.00 (0.47-2.10)	0.83 (0.38-1.79)	0.79 (0.36-1.71)	0.472
Model 2	1	0.98 (0.46-2.08)	0.79 (0.36-1.72)	0.77 (0.35-1.69)	0.440
Model 3	1	0.94 (0.44-1.99)	0.75 (0.34-1.66)	0.70 (0.31-1.60)	0.341

≥60 years at baseline					
Number of deaths	26	28	30	33	
Model 1	1	1.05 (0.61-1.79)	1.07 (0.63-1.82)	1.11 (0.66-1.86)	0.685
Model 2	1	1.07 (0.63-1.84)	1.06 (0.62-1.80)	1.10 (0.65-1.85)	0.758
Model 3	1	0.99 (0.57-1.70)	0.97 (0.57-1.66)	0.95 (0.55-1.63)	0.827
Stroke deaths					
30-59 years at baseline					
Number of deaths	24	32	31	25	
Model 1	1	1.17 (0.69-1.98)	1.10 (0.64-1.87)	0.85 (0.48-1.49)	0.416
Model 2	1	1.15 (0.68-1.96)	1.02 (0.60-1.75)	0.71 (0.40-1.25)	0.133
Model 3	1	1.10 (0.64-1.87)	0.95 (0.55-1.64)	0.59 (0.33-1.08)	0.043
≥60 years at baseline					
Number of deaths	80	63	81	81	
Model 1	1	0.78 (0.56-1.09)	0.97 (0.71-1.32)	0.91 (0.66-1.23)	0.878
Model 2	1	0.73 (0.52-1.02)	0.95 (0.70-1.30)	0.85 (0.62-1.16)	0.665
Model 3	1	0.73 (0.52-1.03)	0.95 (0.69-1.30)	0.81 (0.59-1.13)	0.497

Model 1 was adjusted for age and sex. Model 2 was adjusted for variables in Model 1 plus smoking status, drinking status, systolic blood pressure, blood glucose, serum total cholesterol, body mass index, antihypertensive medication status and residential area. Model 3 was adjusted for variables in Model 2 plus dietary intakes of saturated fatty acids (%kcal), total n-6 PUFA (%kcal), vegetable protein (%kcal), total dietary fiber (g/1,000kcal) and sodium (mg/1,000kcal).

LCn3FA, long-chain n-3 polyunsaturated fatty acids; HR, hazard ratio; CI, confidence interval; PUFA, polyunsaturated fatty acid.

Online Supplement Table 1. Multivariate-adjusted HRs and 95% CIs of mortality from total cardiovascular diseases, coronary heart disease and stroke according to quartiles of LCn3FA intake by sexes: NIPPON DATA80

	Quartiles of LCn3FA intake				*P* value for trend
	Q1 (Low)	Q2	Q3	Q4 (High)	
Total cardiovascular disease deaths					
Men					
Model 1	1	0.82 (0.63-1.08)	0.87 (0.67-1.14)	0.89 (0.68-1.15)	0.599
Model 2	1	0.80 (0.61-1.05)	0.87 (0.67-1.13)	0.87 (0.66-1.13)	0.537
Model 3	1	0.79 (0.60-1.04)	0.84 (0.64-1.10)	0.79 (0.60-1.04)	0.204
Women					
Model 1	1	0.99 (0.76-1.29)	0.91 (0.70-1.19)	0.89 (0.69-1.16)	0.322
Model 2	1	0.95 (0.73-1.25)	0.88 (0.67-1.15)	0.82 (0.63-1.07)	0.113
Model 3	1	0.94 (0.72-1.23)	0.86 (0.66-1.13)	0.78 (0.60-1.03)	0.066
Coronary heart disease deaths					
Men					
Model 1	1	1.02 (0.55-1.86)	0.84 (0.45-1.57)	1.01 (0.55-1.84)	0.949
Model 2	1	1.00 (0.54-1.83)	0.78 (0.41-1.47)	0.98 (0.53-1.80)	0.862
Model 3	1	0.93 (0.50-1.73)	0.73 (0.39-1.38)	0.86 (0.46-1.62)	0.582
Women					
Model 1	1	1.10 (0.59-2.04)	1.18 (0.65-2.16)	1.02 (0.55-1.87)	0.999
Model 2	1	1.02 (0.55-1.92)	1.11 (0.61-2.04)	0.92 (0.50-1.71)	0.783

Model 3	1	0.98 (0.52-1.85)	1.04 (0.57-1.91)	0.81 (0.43-1.54)	0.505
Stroke deaths					
Men					
Model 1	1	0.80 (0.54-1.18)	0.94 (0.65-1.36)	0.90 (0.62-1.30)	0.839
Model 2	1	0.76 (0.51-1.12)	0.94 (0.65-1.36)	0.84 (0.57-1.23)	0.667
Model 3	1	0.76 (0.51-1.13)	0.92 (0.63-1.34)	0.78 (0.53-1.16)	0.408
Women					
Model 1	1	0.94 (0.63-1.40)	1.04 (0.70-1.53)	0.85 (0.57-1.26)	0.467
Model 2	1	0.90 (0.60-1.34)	1.01 (0.68-1.49)	0.76 (0.51-1.13)	0.203
Model 3	1	0.89 (0.59-1.33)	0.99 (0.67-1.47)	0.71 (0.47-1.07)	0.124

Model 1 was adjusted for age. Model 2 was adjusted for variables in Model 1 plus smoking status, drinking status, systolic blood pressure, blood glucose, serum total cholesterol, body mass index, antihypertensive medication status and residential area. Model 3 was adjusted for variables in Model 2 plus dietary intakes of saturated fatty acids (%kcal), total n-6 PUFA (%kcal), vegetable protein (%kcal), total dietary fiber (g/1,000kcal) and sodium (mg/1,000kcal).

LCn3FA, long-chain n-3 polyunsaturated fatty acids; HR, hazard ratio; CI, confidence interval; PUFA, polyunsaturated fatty acid.

Online Supplement Table 2. Multivariate-adjusted HRs and 95% CIs of mortality from total cardiovascular diseases, coronary heart disease and stroke according to quartiles of total n-3 fatty acid intake: NIPPON DATA80

	Quartiles of total n-3 fatty acid intake				*P* value for trend
	Q1 (Low)	Q2	Q3	Q4 (High)	
Range of total n-3 fatty acid intake, %kcal					
Men	0.20-0.85	0.86-1.05	1.06-1.28	1.29-3.92	
Women	0.21-0.93	0.94-1.15	1.16-1.39	1.40-3.66	
Person-years	45771	49814	48873	48438	
Total cardiovascular disease deaths, n	281	222	179	197	
Model 1	1	0.95 (0.79-1.13)	0.87 (0.72-1.05)	0.92 (0.77-1.11)	0.265
Model 2	1	0.95 (0.80-1.14)	0.85 (0.71-1.03)	0.91 (0.76-1.09)	0.187
Model 3	1	0.94 (0.78-1.13)	0.83 (0.68-1.01)	0.85 (0.68-1.06)	0.092
Coronary heart disease deaths, n	52	47	34	38	
Model 1	1	1.05 (0.71-1.56)	0.85 (0.55-1.32)	0.92 (0.60-1.40)	0.518
Model 2	1	1.06 (0.71-1.57)	0.81 (0.52-1.25)	0.89 (0.58-1.36)	0.395
Model 3	1	1.12 (0.75-1.70)	0.89 (0.56-1.41)	0.99 (0.60-1.65)	0.759
Stroke deaths, n	127	107	92	91	

Model 1	1	1.01 (0.78-1.31)	0.99 (0.76-1.29)	0.95 (0.72-1.24)	0.638
Model 2	1	1.02 (0.79-1.32)	0.99 (0.75-1.29)	0.91 (0.69-1.20)	0.449
Model 3	1	0.96 (0.73-1.25)	0.89 (0.67-1.19)	0.74 (0.53-1.04)	0.064

Model 1 was adjusted for age and sex. Model 2 was adjusted for variables in Model 1 plus smoking status, drinking status, systolic blood pressure, blood glucose, serum total cholesterol, body mass index, antihypertensive medication status and residential area. Model 3 was adjusted for variables in Model 2 plus dietary intakes of saturated fatty acids (%kcal), total n-6 PUFA (%kcal), vegetable protein (%kcal), total dietary fiber (g/1,000kcal) and sodium (mg/1,000kcal).

HR, hazard ratio; CI, confidence interval; PUFA, polyunsaturated fatty acid.

Online Supplement Table 3. Multivariate-adjusted HRs and 95% CIs of mortality from total cardiovascular diseases, coronary heart disease and stroke according to quartiles of eicosapentaenoic acid: NIPPON DATA80

	Quartiles of EPA intake				*P* value for trend
	Q1 (Low)	Q2	Q3	Q4 (High)	
Range of EPA intake, %kcal					
Men	0.00-0.08	0.09-0.13	0.14-0.19	0.20-0.95	
Women	0.00-0.09	0.10-0.14	0.15-0.21	0.22-0.98	
Person-years	49312	49840	45546	48200	
Total cardiovascular disease deaths, n	221	220	201	237	
Model 1	1	0.93 (0.77-1.12)	0.90 (0.74-1.09)	0.93 (0.77-1.11)	0.454
Model 2	1	0.92 (0.76-1.11)	0.88 (0.72-1.06)	0.89 (0.74-1.07)	0.209
Model 3	1	0.90 (0.75-1.09)	0.85 (0.70-1.03)	0.83 (0.69-1.00)	0.057
Coronary heart disease deaths, n	41	44	41	45	
Model 1	1	1.00 (0.65-1.53)	0.98 (0.63-1.50)	0.95 (0.62-1.45)	0.773
Model 2	1	1.01 (0.66-1.54)	0.92 (0.59-1.42)	0.89 (0.58-1.36)	0.518
Model 3	1	0.95 (0.62-1.45)	0.84 (0.54-1.30)	0.77 (0.50-1.20)	0.208
Stroke deaths, n	102	102	103	110	

Model 1	1	0.93 (0.71-1.23)	1.00 (0.76-1.31)	0.93 (0.71-1.22)	0.734
Model 2	1	0.91 (0.69-1.20)	0.97 (0.74-1.28)	0.87 (0.66-1.14)	0.394
Model 3	1	0.90 (0.68-1.18)	0.95 (0.72-1.26)	0.81 (0.61-1.07)	0.184

Model 1 was adjusted for age and sex. Model 2 was adjusted for variables in Model 1 plus smoking status, drinking status, systolic blood pressure, blood glucose, serum total cholesterol, body mass index, antihypertensive medication status and residential area. Model 3 was adjusted for variables in Model 2 plus dietary intakes of saturated fatty acids (%kcal), total n-6 PUFA (%kcal), vegetable protein (%kcal), total dietary fiber (g/1,000kcal) and sodium (mg/1,000kcal).

EPA, eicosapentaenoic acid; HR, hazard ratio; CI, confidence interval; PUFA, polyunsaturated fatty acid.

Online Supplement Table 4. Multivariate-adjusted HRs and 95% CIs of mortality from total cardiovascular diseases, coronary heart disease and stroke according to quartiles of docosahexaenoic acid: NIPPON DATA80

	Quartiles of DHA intake				*P* value for trend
	Q1 (Low)	Q2	Q3	Q4 (High)	
Range of DHA intake, %kcal					
Men	0.00-0.15	0.16-0.22	0.23-0.31	0.32-1.39	
Women	0.01-0.16	0.17-0.23	0.24-0.34	0.35-1.45	
Person-years	49413	46366	50022	47097	
Total cardiovascular disease deaths, n	234	198	221	226	
Model 1	1	0.91 (0.75-1.10)	0.88 (0.73-1.06)	0.90 (0.75-1.07)	0.267
Model 2	1	0.88 (0.73-1.07)	0.86 (0.72-1.04)	0.85 (0.70-1.02)	0.099
Model 3	1	0.87 (0.72-1.05)	0.84 (0.69-1.01)	0.79 (0.65-0.96)	0.023
Coronary heart disease deaths, n	40	43	44	44	
Model 1	1	1.15 (0.74-1.76)	1.01 (0.66-1.55)	1.01 (0.66-1.55)	0.842
Model 2	1	1.11 (0.72-1.71)	0.96 (0.63-1.48)	0.94 (0.61-1.44)	0.565
Model 3	1	1.06 (0.68-1.63)	0.90 (0.58-1.39)	0.82 (0.53-1.29)	0.261
Stroke deaths, n	109	89	113	106	

Model 1	1	0.88 (0.66-1.16)	0.97 (0.75-1.27)	0.91 (0.69-1.18)	0.626
Model 2	1	0.83 (0.63-1.11)	0.94 (0.72-1.23)	0.83 (0.63-1.09)	0.292
Model 3	1	0.82 (0.62-1.09)	0.91 (0.70-1.19)	0.77 (0.58-1.02)	0.121

Model 1 was adjusted for age and sex. Model 2 was adjusted for variables in Model 1 plus smoking status, drinking status, systolic blood pressure, blood glucose, serum total cholesterol, body mass index, antihypertensive medication status and residential area. Model 3 was adjusted for variables in Model 2 plus dietary intake of saturated fatty acids (%kcal), total n-6 PUFA (%kcal), vegetable protein (%kcal), total dietary fiber (g/1,000kcal) and sodium (mg/1,000kcal). DHA, docosahexaenoic acid; HR, hazard ratio; CI, confidence interval; PUFA, polyunsaturated fatty acid.

Appendix. The Members of the NIPPON DATA80/90 Research Group.

- *Chairperson*: Hirotsugu Ueshima (Department of Health Science, Shiga University of Medical Science, Otsu, Shiga).
- *Co-Chairpersons*: Akira Okayama (The First Institute for Health Promotion and Health Care, Japan Anti-Tuberculosis Association, Tokyo) for the NIPPON DATA80, and Tomonori Okamura (Department of Preventive Medicine and Public Health, Keio University, Tokyo) for the NIPPON DATA90.
- *Research members*: Shigeyuki Saitoh (Sapporo Medical University School of Health Science, Sapporo, Hokkaido), Kiyomi Sakata (Department of Hygiene and Preventive Medicine, Iwate Medical University, Morioka, Iwate), Atsushi Hozawa (Department of Preventive Medicine and Epidemiology, Tohoku Medical Megabank Organization, Tohoku University, Sendai, Miyagi), Takehito Hayakawa (Department of Hygiene and Preventive Medicine, Fukushima Medical University, Fukushima), Yosikazu Nakamura (Department of Public Health, Jichi Medical University, Shimotsuke, Tochigi), Yasuhiro Matsumura (Faculty of Health and Nutrition, Bunkyo University, Chigasaki, Kanagawa), Nobuo Nishi (Project for the National Health and Nutrition Survey, National Institute of Health and Nutrition, Tokyo), Nagako Okuda (Section of the National Health and Nutrition Survey, National Institute of Health and Nutrition, Tokyo), Fumiyoshi Kasagi (Institute of Radiation Epidemiology, Radiation Effects Association, Tokyo), Toru Izumi (Faculty of Medicine, Kitasato University, Sagamihara, Kanagawa), Toshiyuki Ojima (Department of Community Health and Preventive Medicine, Hamamatsu University School of Medicine, Hamamatsu, Shizuoka), Koji Tamakoshi (Department of Public Health and Health Information Dynamics, Nagoya University Graduate School of Medicine, Nagoya, Aichi), Hideaki Nakagawa (Department of Epidemiology and Public Health, Kanazawa Medical University, Kanazawa, Ishikawa), Katsuyuki Miura, Takayoshi Ohkubo, Yoshikuni Kita (Department of Health Science, Shiga University of Medical Science, Otsu, Shiga), Yasuyuki Nakamura (Cardiovascular Epidemiology, Kyoto Women's University, Kyoto), Katsushi Yoshita (Osaka City University Graduate School of human life science, Osaka), Aya Kadota (Department of School Nursing and Health Education, Osaka Kyoiku University, Kashiwara, Osaka), Kazunori Kodama (Radiation Effects Research Foundation, Hiroshima) and Yutaka Kiyohara (Department of Environmental Medicine, Kyushu University, Fukuoka).