9．日本人における長鎖 n-3 不飽和脂肪酸摂取と循環器疾患死亡リスクの関連：NIPPON DATA80 の 24 年追跡結果より

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【背景】

魚介類由来の長鎖 n-3 不飽和脂肪酸摂取と循環器疾患リスクとの負の関連が欧米諸国より報告されているが，欧米人に比して魚介類を多く摂取している日本人の一般集団を対象とした，詳細な栄養調査に基づく報告は十分とはいえない。そこで国民栄養調査対象者の長期コホート研究である NIPPON DATA80 における食事性長鎖 n-3 不飽和脂肪酸摂取と 24 年間の循環器疾患死亡リスクの関連を検討した。

【方法】

1980 年に実施された循環器疾患基礎調査と国民栄養調査の両方を受検した者のうち，脳卒中や心筋梗塞等の既往を有する者，ベースライン時のデータに欠損があった者などを除外した 9,190 人（男性 4,028 人，女性 5,162 人，平均年齢 50.0 歳）を 1980 年から 2004 年まで 24 年間追跡した。栄養素摂取量は，国民栄養調査のデータから比例案分法を用いて個人の摂取量を推定した。エイコサペンタエン酸およびドコサヘキサエン酸摂

取量を合計した量を長鎖 n-3 不飽和脂肪酸摂取量とした。Cox 比例ハザードモデルを用いて，性，年齢，生活習慣，循環器疾患の危険因子，栄養素などの交絡因子を調整し，長鎖 n-3 不飽和脂肪酸摂取量の性別四分位の循環器疾患死亡の多変量調整ハザード比を算出した。

【結果】

24 年追跡期間中，879 人の循環器疾患死亡，171 人の冠動脈疾患死亡，417 人の脳卒中死亡を認めた。本研究対象者における長鎖 n-3 不飽和脂肪酸の摂取エネルギー比率の中央値は 0.73%（0.86g/日）だった。循環器疾患死亡の多変量調整ハザード比は，第 1 四分位を基準にすると，第 2 四分位 0.85 (95%信頼区間 0.70-1.03)，第 3 四分位 0.85 (95%信頼区間 0.70-1.03)，第 4 四分位 0.80 (95%信頼区間 0.66-0.96)と，長鎖 n-3 不飽和脂肪酸摂取量が多いほど有意に低く，トレンド検定でも有意(p=0.038)であった。冠動脈疾患死亡，脳卒中死亡をアウトカムにした場合も同様の傾向を認めたが統計的には有意ではなかった。ベースラインの年齢層別に 60 歳未満，60 歳以上に分けて分析した結果，60 歳未満の対象者では，長鎖 n-3 不飽和脂肪酸摂取量と循環器疾患死亡，脳卒中死亡との関連をより強く認めた。

【結論】

日本人を代表する一般成人集団において，高い長鎖 n-3 不飽和脂肪酸摂取は長期の循環器疾患死亡リスク低下に関連しており，特に 60 歳未満においてその関連が顕著であった。

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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 9190 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.

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

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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 3–4 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 (4639 men and 5907 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 1356 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 5000 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 9190 participants (4028 men and 5162 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 (ICD10) from the beginning of 1995. The details of classification were described elsewhere [13]. The corresponding ICD9 and ICD10 codes were as follows: cardiovascular mortality, 393–459 (ICD9), I00–I99 (ICD10); coronary heart disease mortality, 410–414 (ICD9), I20–I25 (ICD10); and stroke mortality, 430–438 (ICD9), I60–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/1000 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 1672 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

Table 1
Baseline characteristics of cardiovascular risk factors and selected dietary variables in 4028 men and 5162 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/1,000 kcal	8.4 (2.0)	8.5 (2.0)	8.6 (2.1)	8.6 (2.2)	0.007
Sodium, mg/1,000 kcal	2350 (746)	2522 (790)	2631 (776)	2906 (1000)	<0.001
Fish and shellfish, g/1,000 kcal	30.9 (14.2)	44.5 (16.0)	55.0 (18.1)	75.0 (26.0)	<0.001

Values are means (standard deviation) or %.
LCn3FA, long-chain n-3 polyunsaturated fatty acids; PUFA, polyunsaturated fatty acid.
[a] Differences were evaluated using trend analysis or chi-square test.

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/1,000 kcal) and sodium (mg/1,000 kcal).
LCn3FA, long-chain n-3 polyunsaturated fatty acids; HR, hazard ratio; CI, confidence interval; PUFA, polyunsaturated fatty acid.

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 (0.66–0.96). 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 (1–6 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

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/1,000 kcal) and sodium (mg/1,000 kcal).
LCn3FA, long-chain n-3 polyunsaturated fatty acids; HR, hazard ratio; CI, confidence interval; PUFA, polyunsaturated fatty acid.

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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Appendix A. Supplementary data

Supplementary data related to this article can be found online at http://dx.doi.org/10.1016/j.atherosclerosis.2013.11.073.

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10. 日本人一般男性において長鎖 n3 脂肪酸の高摂取は心疾患死亡リスクにおける安静時心拍数上昇の影響を減弱させる：NIPPON DATA80

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背景：長鎖 n 3 脂肪酸（LCn3FAs）は心保護効果を有する。長鎖 n3 脂肪酸の高摂取が安静時心拍数上昇に関連する循環器死亡リスクを減弱させるという仮説をたて検討した。

方法：　日本全国から無作為に抽出された 300 地区から参加した一般住民で循環器疾患の既往がなく降圧剤を内服していない 8807 人（55.7%女性；平均年齢 48.3 歳）を分析対象とした。主要エンドポイントは循環器疾患死亡とし、2 次エンドポイントは脳卒中死亡および心疾患死亡とした。長鎖 n3 脂肪酸摂取量は 3 日間秤量法を用いて評価した。また、安静時心拍数は 12 誘導心電図より算出した。Cox 比例ハザードモデルにより、交絡因子を調整し、多変量調整ハザード比 HR および 95%信頼区間 95%CI を算出した。

結果：24 年の追跡期間中 617 名の循環器疾患死亡が認められた。長鎖 n3 脂肪酸の食事摂取量の中央値は 0.37%kcal（0.86g/日）であった。循環器疾患死亡に対する長鎖

n3 脂肪酸摂取量と安静時心拍数との交互作用は統計学的に有意であった（P 値 = 0.033)。長鎖 n3 脂肪酸高摂取（0.37%kcal 以上）かつ安静時心拍数が 75bpm 未満の対象者群と比較して、長鎖 n3 脂肪酸低摂取（0.37%kcal 未満）かつ安静時心拍数が 85bpm より高値の対象者群では循環器疾患死亡リスクの有意な上昇を認めたが(HR, 1.67; 95%CI, 1.15-2.43)、長鎖 n3 脂肪酸高摂取かつ安静時心拍数が 85bpm より高値の対象者では有意なリスク上昇を認めなかった(HR, 0.92; 95%CI, 0.61-1.38)。同様の結果が脳卒中死亡についても観察されたが、心疾患死亡については認めなかった。

結論: 日本人一般住民において、安静時心拍数の上昇に関連する循環器疾患死亡リスクの上昇は、長鎖 n3 脂肪酸高摂取により減弱する可能性がある。

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Original article

High long-chain n-3 fatty acid intake attenuates the effect of high resting heart rate on cardiovascular mortality risk: A 24-year follow-up of Japanese general population

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ABSTRACT

Background: Increased resting heart rate (RHR) independently predicts cardiovascular mortality. Meanwhile, long-chain n-3 fatty acids (LCn3FAs) have a cardioprotective effect. Our aim was to evaluate whether higher LCn3FAs intake attenuates the elevated risk of cardiovascular mortality associated with increased RHR.

Methods: We conducted a population-based 24-year prospective cohort study of Japanese, whose LCn3FAs intake is relatively high. Study participants included 8807 individuals aged 30–95 years from randomly selected areas across Japan without cardiovascular diseases and anti-hypertensive drugs at baseline. The primary endpoint was cardiovascular mortality, and the secondary endpoints were cardiac and stroke mortality during 24 years of follow-up. Individual dietary LCn3FAs intake was estimated from household-based 3-day weighed food records. RHR was obtained from 3 consecutive R-wave intervals on 12-lead electrocardiography. Cox models were used to estimate the multivariable hazard ratios (HRs) and 95% confidence intervals (95% CIs) adjusting for possible confounders.

Results: During the follow-up period, 617 cardiovascular deaths were observed. The median daily intake of LCn3FAs was 0.37% kcal (0.86 g/day). The interaction between dietary LCn3FAs intake and RHR in the risk of cardiovascular mortality was statistically significant ($p = 0.033$). The risk of cardiovascular mortality was significantly higher in the low-intake group (<0.37% kcal) with an RHR >85 beats/min (bpm) [hazard ratio (HR), 1.67; 95% confidence interval (CI), 1.15–2.43], but not in the high-intake group (≥0.37% kcal) with an RHR >85 bpm (HR, 0.92; 95% CI, 0.61–1.38), compared with those in the high-intake group with an RHR <70 bpm. Similar results were observed with stroke mortality, but not with cardiac mortality.

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Conclusions: The risk of cardiovascular mortality associated with increased RHR is elevated in participants with low dietary LCn3FAs intake, but not in participants with high dietary LCn3FAs intake in a representative Japanese general population. These results suggest that high dietary LCn3FAs intake may prevent cardiovascular mortality associated with increased RHR.

Introduction

Multiple lines of evidence from epidemiological and clinical studies have shown that resting heart rate (RHR) independently predicts cardiovascular mortality [1,2] in the general population [3,4] and in patients with cardiovascular diseases [5]. Based on experimental study results, several pathophysiological plausible mechanisms between increased RHR and cardiovascular systems have been proposed, including autonomic imbalance, acceleration of atherosclerosis [6], induction of cardiac arrhythmias [7], progression of arterial stiffness [8], promotion of myocardial oxygen consumption [9], and escalation of inflammation [10].

In vitro studies and animal experiments have examined the cardioprotective effects of long-chain n-3 fatty acids (LCn3FAs) [11,12]. LCn3FAs directly reduce heart rate [13], and also favorably affect autonomic function [14], atherosclerosis [15], arrhythmogenicity [16], arterial wall compliance [17], myocardial oxygen consumption [18], and inflammatory pathways [19]. Each of these may contribute to a counteractive effect on the adverse cardiovascular influence by increased RHR. Therefore, we hypothesized that a high LCn3FAs intake attenuates the elevated risk of cardiovascular mortality associated with increased RHR in the general population free of cardiovascular diseases.

We tested this hypothesis in a 24-year prospective cohort study of a representative Japanese general population who participated in the National Survey of Circulatory Disorders and the National Nutrition Survey of Japan. This community-based Japanese population tends to have a higher LCn3FAs intake compared with other populations worldwide [20], and was therefore useful for this investigation.

Methods

Study participants

Cohort studies of the National Survey on Circulatory Disorders and the National Nutrition Survey of Japan are known as the National Integrated Project for Prospective Observation of Noncommunicable Disease And its Trends in the Aged (NIPPON DATA). We analyzed data from NIPPON DATA80 using a baseline survey conducted in 1980. The detailed methods are described elsewhere [4,21,22]. The present study was approved by the Institutional Review Board of Shiga University of Medical Science (No. 12-18, 2000; No. 17-21-1, 2010).

Members of an overall population (n = 13,771) aged ≥30 years from 300 randomly selected health districts throughout Japan were invited to participate in the study. Among them, 10,546 community-based individuals (76.6%) agreed to join the study. The survey consisted of a physical examination, blood test, self-administered questionnaire on lifestyle, dietary assessment, and standard 12-lead electrocardiography recording. For the present study, participants were followed up to 2004 (NIPPON DATA80, 1980–2004).

A total of 1739 participants were excluded from this analysis for the following reasons: history of cardiovascular diseases (n = 280), missing baseline information (e.g. electrocardiography, RHR, or dietary data) (n = 373), non-sinus rhythm (n = 62), and antihypertensive medication (n = 1024). Therefore, 8807 participants were finally included in the present analyses [4909 women; mean (SD) age, 48.3 (12.8) years; range, 30–95 years].

End-point determination

To determine the cause of death during the 24-year follow-up, the National Vital Statistics database of Japan was used 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 Ninth International Classification of Disease (ICD9) until the end of 1994, and according to the Tenth International Classification of Disease (ICD10) from 1995 onwards, as described elsewhere [4].

The primary endpoint was cardiovascular mortality, and the secondary endpoints were cardiac and stroke mortality occurring during the follow-up period. The corresponding ICD9 and ICD10 codes were as follows: cardiovascular mortality, 393 to 459 (ICD9) and I00 to I99 (ICD10); cardiac mortality, 393 to 429 (ICD9) and I01 to I09, I11, I13, and I20 to I52 (ICD10); and stroke mortality, 430 to 438 (ICD9) and I60 to I69 (ICD10).

Baseline dietary assessment

A dietary survey in each household was based on weighed food records for 3 consecutive representative days, avoiding weekends and holidays, by trained dietary interviewers. Dietary data were processed centrally to calculate nutrients. The nutrient intake reported for each household in the National Nutrition Survey of Japan in 1980 was proportionally allocated to each household member according to the mean consumption rate by age and sex. Dietary researchers were blinded to the participants' electrocardiographic and outcome status. For energy-supplying nutrients, the intake was calculated as the percentage of total energy intake (% kcal). Other nutrients were calculated relative to total dietary intake (g/1000 kcal).

The detailed calculation procedure and the validity of the estimation have been described elsewhere [23–26]. Furthermore, energy and macronutrient intake among Japanese individuals estimated using the same dietary assessment as in the present study were highly correlated to corresponding values with individual-based weighed food records (correlation coefficients, 0.90–0.92) [27].

Baseline RHR measurement

RHR was determined by a standardized assessment, which is the measurement from 3 consecutive intervals between R waves on 12-lead electrocardiography by trained technicians after the participant rested quietly in the supine position for 5 min. Electrocardiographic measurements are described in more detail elsewhere [4,21]. RHR and other electrocardiographic findings in accordance with the Minnesota Code (MC) were independently evaluated at a baseline survey by 2 trained researchers (blinded to participant dietary and outcome status). Findings that showed agreement between the 2 researchers' assessments were accepted,

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and findings that showed disagreement were adjudicated by a panel of epidemiologists and cardiologists.

Electrocardiographic findings included Q wave abnormality (MCs 1.1–1.3), left ventricular hypertrophy (MCs 3.1–3.3), major ST depression (MCs 4.1–4.3), major T abnormality (MC 5.1 or 5.2), complete left bundle branch block (MC 7.1), and intraventricular block with a QRS duration ≥120 ms, except for complete left or right bundle branch block (MC 7.4) [28].

Statistical analysis

First, participants were divided into 2 groups according to median dietary LCn3FAs intake (0.37% kcal: 0.86 g/day) as high (≥0.37% kcal) or low (<0.37% kcal) intake. Baseline characteristics and nutritional intake are presented as means and standard deviations for continuous variables, and frequencies and percentages for categorical variables. Differences in baseline characteristics and nutritional parameters were evaluated using the unpaired Student's *t*-test, Mann–Whitney *U*-test, or χ^2 test, as appropriate.

Cox proportional hazards models were used to estimate multivariable adjusted hazard ratios (HRs) and 95% confidence intervals (CIs) for each cardiovascular outcome according to 6 groups cross-classified by 2 categories of LCn3FAs (high- or low-intake) and 3 categories of RHR [<70 beats/min (bpm), 70–85 bpm, and >85 bpm] as follows: high-intake group with an RHR <70 bpm (the reference); high-intake group with an RHR of 70–85 bpm; high-intake group with an RHR >85 bpm; low-intake group with an RHR <70 bpm; low-intake group with an RHR of 70–85 bpm; and low-intake group with an RHR >85 bpm. The choice of cutoff points of RHR was based on identical cutoff points in previous studies of RHR and cardiovascular mortality [5,29].

To control potential confounders, multivariable models were adjusted for age, sex, body mass index, smoking status (never, ex-, and current), drinking habits (never, occasional, and daily), systolic blood pressure, total cholesterol, blood glucose, labor intensity (high, medium, and low), electrocardiographic findings [30,31] (left ventricular hypertrophy classified according to MC 3.1 or 3.3, and suspected coronary heart disease classified according to MCs 1.1–1.3, 5.1–5.2, 4.1–4.3, 7.1, or 7.4), and nutritional parameters (intake of saturated fatty acids, n-6 polyunsaturated fatty acids, sodium, and fiber). Tests for interaction between RHR and dietary LCn3FAs intake were performed by introducing a multiplicative interaction term into the main models.

We performed the following sensitivity analysis. To take into account the possibility of an effect by an unknown preclinical disease, we conducted a separate analysis where deaths within the first 5 years of follow-up were excluded. This exclusion was performed by dealing with deaths within the first 5 years as "censored" [4]. To assess interactions between RHR and other nutritional variables, we also analyzed the risk of cardiovascular mortality according to 6 groups cross-classified by 3 categories of RHR (<70 bpm, 70–85 bpm, and >85 bpm), and 2 categories of low- or high-intake, including eicosapentaenoic acid (EPA), docosahexaenoic acid (DHA), and fish intake.

All analyses were performed with SAS version 9.1.3 (SAS Institute, Cary, NC, USA). All *p*-values were two-sided and *p*-values <0.05 were considered statistically significant.

Results

The baseline characteristics of the 2 groups of participants according to dietary LCn3FAs intake are shown in Table 1. Participants in the high-intake group tended to be older, have a higher proportion of women, were more often never smokers and never alcohol drinkers, had higher blood glucose levels, had higher systolic blood pressure, and were more likely to be labor intensive compared with those in the low-intake group. The baseline characteristics by 3 categories of RHR (<70 bpm, 70–85 bpm, and >85 bpm) are shown in Supplementary Table 1 (Supplemental Digital Content 1).

Table 1
Baseline characteristics of 3898 men and 4909 women according to dietary long-chain n-3 fatty acids intake: NIPPON DATA80, Japan, 1980–2004.

	High-intake (≥0.37% kcal) *n* = 4429	Low-intake (<0.37% kcal) *n* = 4378	*p*-Value
Age (y)[a]	49.2 (12.8)	48.1 (12.8)	<0.001
Women, *n* (%)	2581 (58.3)	2328 (53.2)	<0.001
Smoking status, *n* (%)			<0.001
Never	2625 (59.3)	2398 (54.8)	
Ex-	356 (8.0)	435 (9.9)	
Current	1448 (32.7)	1545 (35.3)	
Alcohol drinker, *n* (%)			0.007
Never	2506 (56.6)	2339 (53.4)	
Occasional	993 (22.4)	1023 (23.4)	
Daily	930 (21.0)	1016 (23.2)	
Body mass index (kg/m^2)[a]	22.6 (3.1)	22.5 (3.1)	0.056
Total cholesterol (mg/dl)[a]	187.9 (33.3)	186.8 (33.0)	0.158
Blood glucose (mg/dl)[a]	99.8 (29.5)	98.6 (29.2)	<0.001
Blood pressure (mmHg)[a]			
Systolic	133.6 (19.9)	132.8 (19.4)	0.038
Diastolic	80.3 (11.7)	80.1 (11.7)	0.340
Labor intensity, *n* (%)			0.010
Low	1672 (37.8)	1759 (40.2)	
Medium	1598 (36.1)	1587 (36.2)	
High	1159 (26.2)	1032 (23.6)	
Heart rate (beats/min)[a]	69.6 (11.1)	69.8 (11.4)	0.708
Electrocardiographic findings, *n* (%)			
Left ventricular hypertrophy[b]	654 (14.8)	639 (14.6)	0.821
Suspected coronary heart disease[c]	214 (4.8)	195 (4.5)	0.673

[a] Mean (standard deviation).
[b] Diagnosis of left ventricular hypertrophy was based on Minnesota Code 3.1 or 3.3.
[c] Suspicion of coronary heart disease was based on Minnesota Codes 1.1–1.3, 5.1–5.2, 4.1–4.3, 7.1, or 7.4.

The baseline nutritional parameters in both groups of participants are shown in Table 2. The mean dietary LCn3FAs intakes in

Table 2
Baseline nutritional variables of 3898 men and 4909 women according to dietary long-chain n-3 fatty acids intake: NIPPON DATA80, Japan, 1980–2004.

	High-intake (≥0.37% kcal) *n* = 4429	Low-intake (<0.37% kcal) *n* = 4378	*p*-Value
Total energy intake (kcal)	2121.7 (492.3)	2186.6 (486.8)	<0.001
Vegetables (g/1000 kcal)	133.9 (50.5)	126.6 (46.7)	<0.001
Meat (g/1000 kcal)	26.9 (15.9)	30.8 (15.8)	<0.001
Fruit (g/1000 kcal)	81.5 (56.2)	74.6 (51.5)	<0.001
Fish (g/1000 kcal)	63.8 (24.1)	37.6 (16.6)	<0.001
Total n-3 fatty acids (% kcal)	1.34 (0.34)	0.96 (0.27)	<0.001
Long-chain n-3 fatty acids (% kcal)	0.59 (0.22)	0.24 (0.08)	<0.001
α-Linolenic acid (% kcal)	0.68 (0.26)	0.69 (0.25)	<0.001
n-6 Polyunsaturated fatty acids (% kcal)	4.27 (1.23)	4.38 (1.20)	<0.001
Monounsaturated fatty acids (% kcal)	7.83 (2.06)	7.78 (2.08)	0.600
Saturated fatty acids (% kcal)	5.92 (1.53)	5.97 (1.60)	0.158
Total fat (% kcal)	20.6 (5.4)	21.2 (5.6)	<0.001
Trans fatty acids (% kcal)	0.31 (0.20)	0.37 (0.21)	<0.001
Sodium (g/1000 kcal)	2.74 (0.89)	2.41 (0.76)	<0.001
Fiber (g/1000 kcal)	8.53 (2.11)	8.34 (1.98)	<0.001

Values are mean (standard deviation).

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the high- and low-intake groups were 0.59% kcal (1.38 g/day) and 0.24% kcal (0.58 g/day), respectively. Participants in the high-intake group had higher intake of sodium, fiber, vegetables, fruit, fish, and total n-3 fatty acids than those in the low-intake group. In contrast, participants in the high-intake group had less total energy intake and had a lower intake of meat, α-linolenic acid, n-6 polyunsaturated fatty acids, total fat, and trans fatty acids compared with those in the low-intake group. The baseline nutritional parameters by 3 categories of RHR (<70 bpm, 70–85 bpm, and >85 bpm) are shown in Supplementary Table 2 (Supplemental Digital Content 2).

During the 24-year follow-up period [mean, 21.4 (5.5) years], 617 participants (7.0%) died from cardiovascular causes. Of these, 314 (3.6%) were from cardiac causes (121 from coronary heart disease, 1.4%) and 285 (3.2%) were from stroke (162 from ischemic stroke, 1.8%).

Fig. 1 shows the multivariable adjusted HRs and 95% CIs of cardiovascular outcomes among the groups according to the 6 categories cross-classified by LCn3FAs (high- or low-intake) and RHR (<70 bpm, 70–85 bpm, and >85 bpm). The risk of cardiovascular and stroke mortality were significantly elevated (adjusted HR, 1.67; 95% CI: 1.15–2.43, and adjusted HR, 2.05; 95% CI: 1.24–3.37, respectively), but the risk of cardiac mortality was not statistically significant (adjusted HR, 1.32; 95% CI: 0.74–2.38) in the low-intake group with an RHR >85 bpm compared with those in the high-intake group with an RHR <70 bpm. In contrast, in the high-intake group with an RHR >85 bpm, the risk of cardiovascular outcomes was not significantly elevated (adjusted HR, 0.92 for cardiovascular, 0.89 for stroke, and 0.98 for cardiac mortality). A multiplicative interaction between RHR and dietary LCn3FAs intake in the risk of cardiovascular and stroke mortality was statistically significant (p for interaction = 0.033 and 0.026, respectively), but not in the risk of cardiac mortality (p for interaction = 0.410). Because further adjustment for other nutritional parameters (e.g. total energy intake, and intake of vegetables, fruit, meat, α-linolenic acid, monounsaturated fatty acids, total fat, and trans fatty acids) did not appreciably change the results, we did not include these factors in the final model. Additionally, there was no evidence of a sex interaction with 6 categories cross-classified by LCn3FAs and RHR for any of the outcomes (p for interaction >0.2).

Results were not substantially altered in the sensitivity analysis that excluded deaths within the first 5 years of follow-up for the risk of cardiovascular mortality (see Supplementary figure legends and Supplementary Figure 1, Supplemental Digital Content 3 and 4, respectively). In another sensitivity analysis according to EPA or DHA intake, participants in the low-intake group with an RHR >85 bpm had a significantly elevated risk of cardiovascular

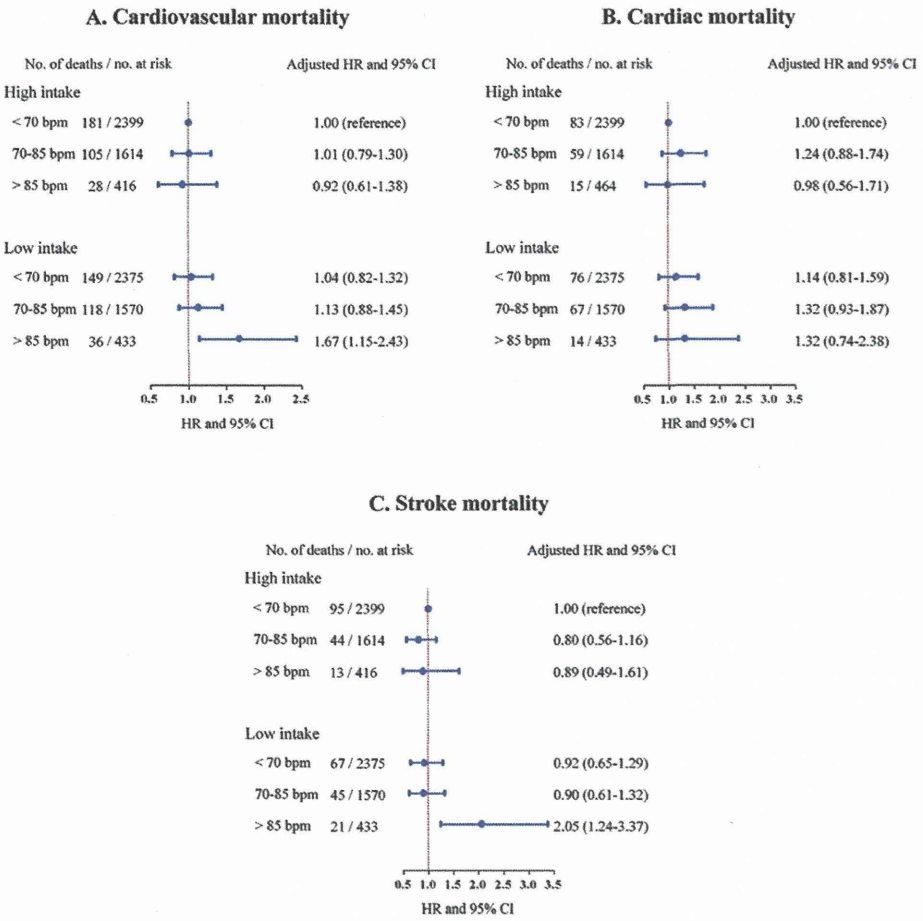

Fig. 1. Hazard ratios and 95% confidence intervals of cardiovascular outcomes among groups according to 6 categories cross-classified by long-chain n-3 fatty acids and resting heart rate in 3898 men and 4909 women: NIPPON DATA80, Japan, 1980–2004. Adjusted hazard ratios (HRs) and 95% confidence intervals (CIs) for cardiovascular mortality (A), cardiac mortality (B), and stroke mortality (C) across 6 categories are shown. The high-intake group with a resting heart rate (RHR) <70 beats/min (bpm) served as the reference category. Filled circles and horizontal bars represent HRs and 95% CIs, respectively. HRs were adjusted for age, sex, body mass index, smoking status (never, ex-, and current), drinking habits (never, occasional, and daily), systolic blood pressure, total cholesterol, blood glucose, labor intensity (high, medium, and low), electrocardiographic findings (left ventricular hypertrophy and suspected coronary heart disease), and nutritional parameters (intake of saturated fatty acids, n-6 polyunsaturated fatty acids, sodium, and fiber).

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mortality (adjusted HR, 1.57 for EPA; 1.64 for DHA) compared with those in the high-intake group with an RHR <70 bpm. In contrast, participants in the high-intake group with an RHR >85 bpm did not have a significantly elevated risk (adjusted HR, 1.00 for EPA; 0.96 for DHA) (p for interaction between RHR and LCn3FAs intake: 0.034 for both) (see Supplementary figure legends and Supplementary Figure 2, Supplemental Digital Content 3 and 5, respectively). Furthermore, we observed a statistically marginal interaction between RHR and fish intake in the risk of cardiovascular mortality ($p = 0.046$), but not between RHR and the intake of α-linolenic acid or other nutritional parameters (data not shown).

Discussion

In this 24-year prospective study of a representative Japanese general population without known cardiovascular diseases and anti-hypertensive medications, the elevated risk of cardiovascular mortality associated with increased RHR was attenuated by a higher dietary intake of LCn3FAs, which remained after adjustment for possible confounding factors. This association persisted in sensitivity analyses. To the best of our knowledge, this is the first study to show a significant interaction between dietary LCn3FAs intake and RHR in the risk of cardiovascular mortality.

In the high-intake group of LCn3FAs, increased RHR was not associated with an elevated risk of cardiovascular mortality. Notably, the consumption of fish (63.8 g/1000 kcal: 135.3 g/day) and LCn3FAs (0.59% kcal: 1.38 g/day) in the high-intake group was markedly higher than that of most Western populations [20]. Accordingly, our findings that cardiovascular mortality associated with an increased RHR was attenuated by a higher dietary intake of LCn3FAs may not be observed in Western countries. This community-based Japanese population with a remarkably higher LCn3FAs consumption than that worldwide is the ideal cohort in which investigation for cardioprotective evidence of LCn3FAs can extend to much higher ranges of LCn3FAs intake.

Consistent with previous studies [32,33], in the current study, increased RHR was associated with an elevated risk of metabolic abnormalities, including hyperglycemia and hypercholesterolemia, most likely via high sympathetic tone. The Japan EPA lipid intervention study (JELIS) reported that EPA treatment reduces the risk of major coronary events for patients with abnormal metabolic profiles more than for those without abnormal metabolic profiles [34,35]. LCn3FAs may affect the cardiovascular system in a favorable manner, particularly when there is a disturbance in glucose or lipid metabolism as RHR increases.

In the low-intake LCn3FAs group in our study, increased RHR was associated with a higher risk of stroke mortality, but not cardiac mortality. This observation is not surprising. In the Asia-Pacific region, the association between increased RHR and stroke is stronger than that between increased RHR and coronary heart diseases [36]. Moreover, the incidence and mortality rate from stroke are higher than those from myocardial infarction in Japan [37,38]. In fact, in our study, there were more deaths from stroke than from coronary heart diseases. Such characteristics of our study population may explain why an increased risk by higher RHR was observed for stroke, but not for cardiac diseases.

In our study, there were no significant differences in RHR between categories of dietary LCn3FAs intake. Intake of n-3 fatty acids, particularly from fish oil, can contribute to a reduction in heart rate in a dose-dependent fashion, although additional effects appear small at higher levels of intake [11]. Even in the low-intake groups of our study, the mean value of LCn3FAs intake (0.24% kcal: 0.58 g/day) was equivalent to required and acceptable levels for LCn3FAs [39,40]. However, the exact mechanisms of no significant difference in the RHR between high- and low-intake groups remain unclear and deserve further investigation.

The strengths of our study include its prospective design and 24-year follow-up of participants of the national circulatory and nutritional surveys from randomly selected health districts in Japan. Our dietary assessment using weighed food records during 3 days also appears to be well suited for a large-scale prospective study, in which a broad evaluation of diet is usually desirable [41]. Although recent meta-analysis, including 20 randomized clinical trials, the majority of which were short-term studies ranging from 1 to 6.2 years, reported that n-3 fatty acids supplementation was not associated with a lower risk of cardiovascular diseases [42], we observed a long-term effect of dietary LCn3FAs intake against the cardiovascular risk by increased RHR. Furthermore, although RHR is a highly variable cardiovascular parameter because of the effects of physical, psychological, and environmental factors, our standardized measurement for RHR, assessed by electrocardiography, is more accurate than pulse palpitation for a reliable estimate [1].

There are some limitations to our findings. First, our analyses were based on a single baseline measurement and may not have accurately reflected long-term LCn3FAs intake and RHR in the follow-up period. Our analyses were also limited to the general population without known cardiovascular disease, and our results should not be generalized to patients with various cardiovascular diseases. Finally, although we carefully controlled for the major known confounders and intake of other fatty acids, we cannot deny the possibility of residual confounding by other unmeasured dietary and/or lifestyle factors, such as mental stress, physical activity, pulmonary disease, and hormonal function.

We used mortality data as endpoints, which might have led to misclassification of the causes of death. However, it has been reported that the death-certificate diagnosis of stroke in Japan is quite accurate [43]. Furthermore, mortality statistics for coronary heart disease by the end of 1994 may have been underestimated using the ICD9, because deaths coded as "heart failure" may hide certain coronary events [44]. However, in our study, we addressed those problems by death from all heart diseases by classifying them as "cardiac mortality".

In conclusion, in this long-term prospective observation of a representative sample of the Japanese general population, the risk of cardiovascular mortality associated with increased RHR is elevated in participants with low dietary LCn3FAs intake, but not in participants with high dietary LCn3FAs intake. These results suggest that high dietary LCn3FAs intake may prevent cardiovascular mortality associated with increased RHR as a primary prevention. Whether the predictive role of increased RHR on cardiovascular diseases represents a causal relationship remains unclear, although it should not detract from the concept of RHR as a simple predictor of cardiovascular risk [36]. If our observed association is indeed causal, a higher intake of LCn3FAs by eating more fish may be a useful, low-cost intervention in primary prevention for the apparently healthy general population with a higher RHR.

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Conflict of interest statement

All authors disclose no financial conflicts of interest. The sponsors did not participate in the design or conduct of the study; the collection; management, analysis, and interpretation of the study; or the preparation, review, or approval of the manuscript.

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Appendix A. Supplementary data

Supplementary data associated with this article can be found, in the online version, at http://dx.doi.org/10.1016/j.jjcc.2014.01.005.

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11.

日本から発信する
血管病の EBM 第26回

NIPPON DATA80/90/2010

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NIPPON DATA80/90/2010 は，国による循環器疾患基礎調査，国民栄養調査，国民健康・栄養調査のコホート研究であり，厚生労働省研究班として実施されている．一般国民代表集団のコホート研究に位置付けられる本研究からは，血清コレステロール，血圧，喫煙など代表的危険因子と循環器疾患死亡リスクとの明確な関連が明らかにされた．NIPPON DATA リスク評価チャートは，日本人のための絶対リスク評価ツールとして，新しい『動脈硬化性疾患予防ガイドライン』にも活用されることになった．近年，心電図所見のリスクに関する新たな知見も報告されており，さらに新たなコホートである NIPPON DATA2010 からのエビデンス創出も期待される．

KEY WORDS
疫学　コホート研究　循環器疾患基礎調査　国民健康・栄養調査

はじめに

NIPPON DATA（National Integrated Project for Prospective Observation of Non-communicable Disease And its Trends in the Aged）は国による循環器疾患基礎調査対象者のコホート研究である．1980 年の第 3 次循環器疾患基礎調査の追跡研究が NIPPON DATA80，1990 年の第 4 次の追跡研究が NIPPON DATA90 であり，それぞれ 29 年，20 年の長期にわたる追跡がおこなわれてきた．また，2010 年に実施された循環器疾患基礎調査後継調査（厚労省研究班が実施）の追跡研究 NIPPON DATA2010 も開始された．全国から無作為抽出された 300 地区の地域住民を対象としたこの研究は，日本を代表する一般国民のコホート研究に位置付けられる．

NIPPON DATA の最大の目的の一つは，脳卒中・心筋梗塞など循環器疾患による死亡の危険因子を，わが国の国民代表集団において明らかにすることである．これまでの知見は日本人を代表するエビデンスとして健康日本 21 策定や各種診療ガイドライン策定に活用された．コホート研究のエビデンスは，無作為化比較試験（RCT）のエビデンスにくらべて一段下と思われがちであるが，ある疾患の危険因子や原因を明らかにするためには最善の疫学研究手法であり，RCT が不可能な有害因子（たとえば喫煙）の健康影響については最高のエビデンスを提供する．

本稿では NIPPON DATA の方法と，これまでに明らかに

された重要なエビデンスや最新エビデンスを紹介する．

NIPPON DATA の研究方法

米国の国民健康栄養調査である NHANES など欧米の同様の調査でも対象者を長期間追跡するコホート研究をおこなっているが，わが国でも国が実施した全国調査である 1980 年循環器疾患基礎調査の追跡調査をおこなう旧厚生省研究班（班長：上島弘嗣）が 1994 年に組織されたのが本研究のはじめである．

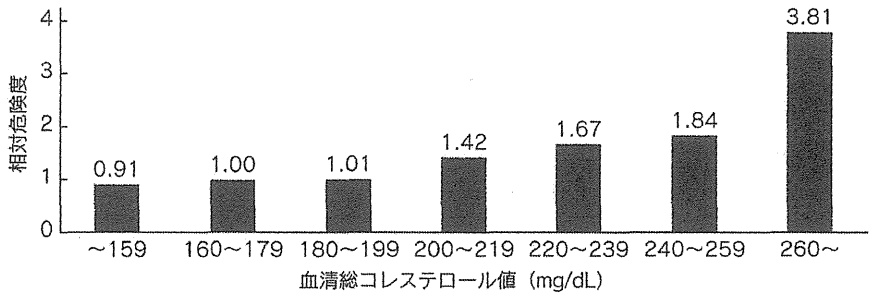
NIPPON DATA80 では，無作為抽出された全国 300 地区（**図❶**）の 1980 年国民栄養調査対象者のうち，30 歳以上の約 1 万人について，総務庁（当時）から目的外使用許可を受け，各市町村に住民票を請求し，生存の有無，死亡者について死亡地と死亡年月日を同定した．死亡者については，総務庁の許可を得て人口動態統計テープを使用して死因の同定作業をおこなった[1]．NIPPON DATA90 は 1990 年に調査を受けた約 8,000 人であり，ともに以後 5 年ごとに住民票請求による生死確認と死因同定作業をおこない，現在までに NIPPON DATA80 は 2009 年までの 29 年，NIPPON DATA90 は 2010 年までの 20 年間の追跡を完了している．また全国の保健所の協力を得て，約 5 年ごとに高齢者における日常生活動作（ADL）と生活の質（QOL）の追跡調査もおこなっている．

さらに，2010 年には循環器疾患基礎調査後継調査として「循環器病の予防に関する調査（NIPPON DATA2010）」を筆者らの研究班が厚労省指定研究として実施した．2010 年国民健康・栄養調査に参加した全国 300 地区の 20 歳以上の成人約 3,000 人から同意を得て，循環器関連の問診と検査を実施した．対象者には将来にわたって年 1 回の健康状態調査をおこない，脳卒中，冠動脈疾患，心不全，糖尿病の発症も把握しており，現時点で追跡 2 年目である．

血清総コレステロールと冠動脈疾患リスク

血清総コレステロール高値による心筋梗塞リスク上昇については，欧米諸国からの多くのエビデンスがあったが，日本人におけるエビデンスは乏しかった．しかし，NIPPON DATA80 の 19 年追跡データの解析において，**図❷**に示すように，ベースラインの血清総コレステロール値とその後 19 年間の冠動脈疾患死亡リスクとの段階

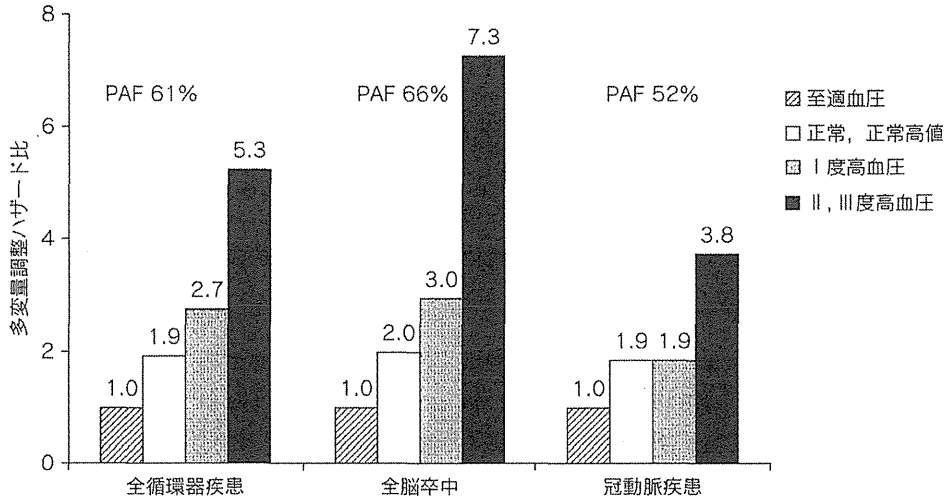
図❶ NIPPON DATA80 の調査地区（全国 300 地区）



相対危険度は性，年齢，血清アルブミン，BMI，高血圧，糖尿病，喫煙，飲酒を調整

図❷ 血清総コレステロール値と虚血性心疾患死亡との関連（NIPPO DATA80, 19 年追跡, 男女計）

（Okamura T *et al*, 2007[2] より引用）



ハザード比は，性，年齢，総コレステロール，BMI，糖尿病有無，喫煙，飲酒歴を調整．
PAF：血圧高値による人口寄与危険割合

図❸ 30～59 歳男女における血圧レベルと 24 年間の循環器疾患死亡ハザード比，および至適血圧を超える血圧による循環器疾患死亡の人口寄与危険割合（PAF）（NIPPON DATA80, 24 年追跡, 30～59 歳男女計）

（Takashima N *et al*, 2012[5] より引用）

的・連続的な関連が明らかとなり，日本人での強力なエビデンスとなった[2]．ベースラインの 1980 年当時はスタチン発売以前であり，血清総コレステロールと冠動脈疾患の因果関係の観察がより自然に近い形で可能であった．

近年，血清コレステロールが高いほうが，総死亡率が低く長生きすると主張する一部の学者がおり，マスコミも巻き込んで国民を混乱させている．本来，血清総コレステロールは栄養指標の一つであるので，総死亡に関する疫学的検討は慎重におこなう必要がある[3]．血清総コレステロールが低い者のなかには，がん・肝臓病などの患者や，加齢による衰弱や低栄養の者が含まれ，これを考慮しない分析では「因果の逆転」が含まれる．NIPPON DATA80 で肝臓病死亡とベースラインから 5 年以内の早期死亡を除いた解析をすると，血清総コレステロールの低いグループでの総死亡リスク上昇はなく，260 mg/dL 以上のグループでは総死亡リスクにおいても上昇が認められた[2]．

血圧と循環器疾患リスクの強い関連

NIPPON DATA80 では従来，血圧レベルと循環器疾患死亡リスクとの関連を明確に示し，その関連がどの年齢層でも the lower, the better であることを示してきた[4]．NIPPON DATA80 は追跡期間が 20 年を超えたが，20 年以上の長期にわたる循環器疾患リスクを検討した報告はわが国ではほとんどない．そこでベースラインの血圧レベルとその後 24 年間の循環器疾患死亡リスクとの関連を検討した[5]．24 年という長期の追跡により，ベースラインで 30～59 歳と比較的若年だった成人の長期リスクの解析が可能となった（図❸）[5]．その結果，全循環器疾患死亡，脳卒中死亡，冠動脈疾患死亡のリスクも至適血圧レベルで最も低く，正常・正常高値レベルでもその 2 倍のリスクを示した．また，至適血圧を超える血圧による循環器疾患死亡の人口寄与危険割合（population attributable fraction：PAF）を算出したところ，全循環器疾患死亡の 61%，脳卒中死亡の 66%，冠動脈疾患死亡の 52%が血圧高値によって説明できることが明らかになった．この結果は，若い年代から正常血圧を維持する重要性，また，そのための高血圧発症予防の重要性を示すものである．

メタボリックシンドロームと循環器疾患リスク

メタボリックシンドロームの構成要素である高血圧，