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Plasma levels of n-3 fatty acids and risk of coronary heart disease among Japanese: The Japan Public Health Center-based (JPHC) study

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ABSTRACT

Background and aims: Higher intake of fish or n-3 polyunsaturated fatty acids (PUFAs) has been associated with reduced risk of coronary heart disease (CHD). However, it is unclear whether increased blood levels of n-3 PUFAs are associated with reduced risk of CHD in the Japanese population.

Methods: The relationship between circulating levels of n-3 PUFAs (eicosapentaenoic acid + docosapentaenoic acid + docosahexaenoic acid) and risk of CHD was examined in a nested case-control study among participants in the Japan Public Health Center (JPHC)-based Study Cohort. Plasma n-3 PUFA phospholipid levels were measured at baseline by gas chromatography in 209 cases with CHD and 418 controls matched for sex, age, date of blood draw, time elapsed since last meal before blood collection, and study location. The CHD cases (n = 209) comprised 168 cases of myocardial infarction and 41 of sudden cardiac death, otherwise classified as 157 non-fatal and 52 fatal coronary events, respectively. Mean duration of follow-up was 13.5 years.

Results: Multivariate conditional logistic analysis showed no significant association between n-3 PUFAs and risk of total CHD. The odds ratio (OR) for the highest *versus* lowest quartiles of plasma n-3 PUFAs was 0.79 (95% confidence interval [95% CI]: 0.41−1.51, p for trend = 0.51). Subtype analysis of CHD revealed that the multivariate ORs for the highest *versus* lowest quartiles for n-3 PUFAs were 0.91 (95% CI: 0.43−1.89, p for trend = 0.90) for myocardial infarction, 0.08 (95% CI: 0.01−0.88, p for trend = 0.04) for sudden cardiac death, 0.89 (95% CI: 0.42−1.89, p for trend = 0.97) for nonfatal coronary events, and 0.12 (95% CI: 0.02−0.75, p for trend = 0.03) for fatal coronary events.

Conclusions: Plasma n-3 PUFA levels were not associated with risk of total CHD but were inversely associated with risks of sudden cardiac death and fatal coronary events among middle-aged Japanese individuals.

1. Introduction

Some epidemiological studies and clinical trials have suggested that n-3 polyunsaturated fatty acids (PUFAs), such as eicosapentaenoic acid (EPA) and docosahexaenoic acid (DHA), may help prevent and treat coronary heart disease (CHD) [1]. A meta-analysis revealed that prospective cohort studies showing n-3 PUFAs (EPA + DHA) from both dietary intake and blood samples were associated with reduced risk of coronary disease. Randomized controlled trials showed no effect of n-3 PUFA supplementation on total coronary events [2], but an effect on cardiac death events [3].

Japanese people consume high amounts of fish and have a low prevalence of CHD compared with Western people. Three Japanese nationwide community-based large cohort studies have examined the association between fish consumption (and/or intake of n-3

Abbreviations: AA, arachidonic acid; DHA, docosahexaenoic acid; DPA, docosapentaenoic acid; EPA, eicosapentaenoic acid; PUFAs, polyunsaturated fatty acids.

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PUFAs) and risk of CHD. First, in a large cohort study using the database of NIPPON DATA80 (national representative sample of 8879 Japanese men and women aged 30 years and over, followed up for 19 years), no significant association was found between fish intake and risk of mortality from CHD [4]. Second, in the Japan Public Health Center-based (JPHC) study, Iso et al. [5] followed a total of 41,578 Japanese men and women aged 40–59 years without prior diagnosis of cardiovascular disease and cancer for 11 years and found that high fish consumption was associated with reduced risk of myocardial infarction and nonfatal CHD compared with modest fish consumption. Third, Yamagishi et al. [6] followed a total of 57,972 Japanese men and women aged 40–79 years with no history of heart disease, stroke, or cancer for 12.7 years and found no association between fish consumption (or n-3 PUFA intake) and risk of coronary death. However, they did find an inverse association between fish and n-3 PUFA intake and mortality from heart failure. The cohort study using the NIPPON DATA 80 database was extended for another 5 years (giving a total 24 years of follow-up) with 9190 subjects, and n-3 PUFA (EPA + DHA) intake was found to be inversely associated with risk of total cardiovascular disease mortality but not with CHD mortality [7].

Fish consumption (or n-3 PUFA intake) in the cohort studies mentioned above was assessed by food frequency questionnaires or weighed food records (3 consecutive representative days), both of which are self-administered dietary assessment questionnaires and records. The former is dependent on participant recall and knowledge, whereas the latter reduces the possibility of obtaining a true measure of usual intake due to the limited number of recording days. In addition, both methods are completely dependent on having accurate, up-to-date data on the n-3 PUFA content of all types of fish that Japanese eat. Measurement of specific fatty acids in the blood is more objective and better reflects tissue levels of n-3 PUFAs than food frequency questionnaires or food records [8]. A subanalysis of the control group alone (administered statin only and no EPA) in a large intervention trial in Japan showed no association of plasma n-3 PUFAs with risk of major coronary events [9]. A cohort study with patients who had undergone percutaneous coronary intervention showed that only the serum EPA/arachidonic acid (AA) ratio, but not EPA or DHA alone, was significantly associated with lower incidence of major adverse cardiac events [10]. However, these study subjects [9,10] were patients and did not belong to the general population. To the best of our knowledge, only one cohort study using blood fatty acids has been conducted in a general population in Japan. Ninomiya et al. [11], in a prospective cohort study, followed for an average of 5.1 years a total of 3103 Japanese adults aged ≥ 40 years living in the community in a suburban town and found no associations between the serum EPA/AA ratio and cardiovascular events in their overall population. However, subanalysis of participants with elevated C-reactive protein levels (≥1.0 mg/L) revealed an inverse association between the EPA/AA ratio and risk of cardiovascular events.

As far as we know, there has been no nationwide community-based prospective research on the relationship between plasma levels of n-3 PUFAs and risk of CHD in a Japanese population with high fish intake. In this nested case-control study, we investigated the association between plasma phospholipid levels of n-3 PUFAs and risk of CHD among middle-aged Japanese. We also examined the association between n-3 PUFA intake and risk of CHD.

2. Materials and methods

2.1. Study population and questionnaire

The methods of the JPHC study have been described in detail elsewhere [12]. Briefly, the study consisted of 2 cohorts: the first was initiated in 5 public health center areas in 1990 (Cohort-I) and the second in 6 public health center areas in 1993 (Cohort-II). At baseline, the study subjects comprised 116,896 residents aged 40–59 years for Cohort-I and 40–69 years for Cohort-II; they were followed until December 31, 2007.

A self-administered baseline questionnaire on lifestyle factors was presented to the residents of Cohort-I and Cohort-II at baseline. The calculation of dietary intake of n-3 PUFAs has been described in detail elsewhere [5]. This study was approved by the institutional review boards of the National Cancer Center (No. 15–24), Tokyo, and the University of Toyama, Toyama (No. 50).

2.2. Confirmation of CHD and mortality and selection of cases and controls

As is also described in the previous study [12], there were 209 cases of CHD in total, classified into two subtypes, myocardial infarction (n = 168) and sudden cardiac death (n = 41), otherwise expressed as nonfatal (n = 157) and fatal (n = 52) coronary events. Two control subjects were matched for sex, age (within 2 years), date of blood draw (within 3 months), time elapsed since the last meal and the blood draw (within 4 h), and study location (catchment areas of the participating public health centers).

2.3. Laboratory analysis

The fatty acid composition of the total phospholipid fraction was analyzed as described [13] with slight modifications. Briefly, total lipids were extracted from plasma and then the total phospholipid fraction was separated by thin-layer chromatography. Fatty acids in this fraction were transmethylated and analyzed by gas chromatography (GC-2014; Shimadzu Corporation, Kyoto, Japan) with a DB-225 capillary column (length, 30 m; internal diameter, 0.25 mm; film, 0.25 μm; J&M Scientific, Folsom, CA). The entire system was controlled with the gas chromatographic software GC solution version 2.3 (Shimadzu Corporation). The intra-assay and inter-assay coefficients of variance were 1.4% and 3.4% for EPA + DHA + docosapentaenoic acid (DPA), respectively. Fatty acids were expressed as the area percentage of total fatty acids. The fatty acid dataset was fixed before the code for cases and controls was broken.

2.4. Statistical analysis

Data are expressed as means ± SD unless described otherwise. There were 3 and 20 missing values for smoking status and alcohol consumption, respectively. For these missing data, we added an extra category for the variable indicating missingness for imputation.

Hypertension was defined as systolic blood pressure ≥160 mm Hg, diastolic blood pressure ≥95 mm Hg, and/or the use of antihypertensive medication; normotension was defined as systolic blood pressure <140 mm Hg, diastolic blood pressure <90 mm Hg, and no antihypertensive medication. All other values were classified as borderline hypertension. We added an extra category for the variable indicating missingness of the above values (n = 17).

Borderline diabetes mellitus was defined as fasting glucose 110–125 mg/dL or non-fasting glucose 140–199 mg/dL. Diabetes mellitus was defined as fasting glucose ≥126 mg/dL, non-fasting glucose ≥200 mg/dL, or use of diabetes medication. We added an extra category for the variable indicating missingness of the above values (n = 110). In descriptive analyses, differences in categorical and continuous variables were tested with the chi-square test and Student's t-test, respectively. Plasma n-3 PUFAs (EPA + DHA + DPA) were quartiled and then the categorical numbers were used in

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