Original Article

Relationships between Plasma Fatty Acid Composition and Coronary Artery Disease

Hiroshige Itakura[1], Mitsuhiro Yokoyama[2], Masunori Matsuzaki[3], Yasushi Saito[4], Hideki Origasa[5], Yuichi Ishikawa[6], Shinichi Oikawa[7], Jun Sasaki[8], Hitoshi Hishida[9], Toru Kita[10], Akira Kitabatake[11], Noriaki Nakaya[12], Toshiie Sakata[13], Kazuyuki Shimada[14], Kunio Shirato[15], and Yuji Matsuzawa[16], for the JELIS Investigators, Japan

[1]Shinagawa East One Medical Clinic, Tokyo, Japan
[2]Hyogo Prefectural Awaji Hospital, Sumoto, Japan
[3]Division of Cardiology, Department of Medicine and Clinical Science, Yamaguchi University Graduate School of Medicine, Ube, Japan
[4]Chiba University, Chiba, Japan
[5]Division of Biostatistics and Clinical Epidemiology, University of Toyama, Toyama, Japan
[6]Kakogawa Municipal Hospital, Kakogawa, Japan
[7]Division of Endocrinology and Metabolism, Department of Medicine, Nippon Medical School, Tokyo, Japan
[8]International University of Health and Welfare Graduate School of Public Health Medicine, Fukuoka, Japan
[9]Division of Cardiology, Department of Internal Medicine, Fujita Health University School of Medicine, Toyoake, Japan
[10]Kobe City Medical Center General Hospital, Kobe, Japan
[11]Hiraoka Hospital, Osaka, Japan
[12]Nakaya Clinic, Tokyo, Japan
[13]Hisatsune Hospital, Fukuoka, Japan
[14]Division of Cardiovascular Medicine, Department of Medicine, Jichi Medical School, Tochigi, Japan
[15]Saito Hospital, Miyagi, Japan
[16]Sumitomo Hospital, Osaka, Japan

***Aim*: The Japan EPA Lipid Intervention Study (JELIS) was the first prospective randomized clinical trial to demonstrate prevention of coronary events by pure eicosapentaenoic acid (EPA). The aim of this study was to examine the relationships between various plasma fatty acid concentrations and the risk of coronary events in JELIS participants.**

***Methods*: In 15,534 participants, we calculated the hazard ratio for major coronary events (sudden cardiac death, fatal or nonfatal myocardial infarction, unstable angina pectoris, and angioplasty/stenting or coronary artery bypass grafting) relative to the on-treatment average level of plasma fatty acids with the Cox proportional hazard model.**

***Results*: As a result of EPA intervention, the plasma EPA concentration increased, but the docosahexaenoic acid (DHA) concentration did not. The other fatty acids measured decreased slightly. The higher plasma level of EPA (hazard ratio = 0.83, $p = 0.049$, in all participants and hazard ratio = 0.71, $p = 0.018$, in the EPA intervention group), but not of DHA, was inversely associated with the risk of major coronary events. The associations between other fatty acids and the risk of major coronary events were not significant. In all JELIS participants, the risk of major coronary events was significantly decreased (20%) in the group with high (150 μg/mL or more) on-treatment plasma EPA concentration compared with that in the low (less than 87 μg/mL) group.**

***Conclusion*: The risk of coronary artery disease is influenced by variations in plasma fatty acid composition. Among n-3 polyunsaturated fatty acids, EPA and DHA exhibited differences in the correlation with the risk of major coronary events.**

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Key words; Coronary heart disease, Eicosapentaenoic acid, Docosahexaenoic acid, Lipoprotein

Address for correspondence: Hiroshige Itakura, Shinagawa East One Medical Clinic, 2-16-1 Konan, Minatoku 108-0075, Tokyo, Japan
E-mail: shinagawa-east-one@jouzenkai.or.jp
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Introduction

The incidence of coronary artery disease (CAD) is modified by dietary fatty acid composition[1)], as is the case for other major CAD risk factors, such as

hypercholesterolemia, hypertension, type-2 diabetes[2], and probably thrombosis. Some fatty acids influence the incidence of CAD through either triglyceride accumulation, inflammation, vasodilation, or platelet aggregation[3], via mediators such as prostaglandins. As shown in the development of metabolic syndrome or insulin resistance[4], excessive accumulation of triglycerides is also a risk factor for CAD. Saturated fatty acids (SFA) and monounsaturated fatty acids (MUFA) are the principal ingredients of triglycerides, although details of the relationships between CAD and individual fatty acids remain uncertain; however, some cohort and interventional studies have reported that n-3 polyunsaturated fatty acids (PUFA) have a preventive effect on CAD. The Gruppo Italiano per lo Studio della Sopravvivenza nell'Infarto miocardico (GISSI) - Prevenzione trial, a randomized control trial using n-3 PUFAs (eicosapentaenoic acid [EPA, C20:5n-3]: docosahexaenoic acid [DHA, C22:6n-3] = 1.2:1) with a purity of ≥85%, reported that n-3 PUFA reduced coronary death in postinfarction patients[5, 6]. Harris and von Schacky found that the concentration of n-3 PUFAs (EPA and DHA) in the erythrocyte membrane (Omega-3 Index) was inversely related to the risk of death from coronary heart disease, and proposed the use of this index as a marker to prevent CAD[7]. Although several studies have examined the relationship between PUFA and the incidence of CAD, no clear consensus yet exists regarding the effects of various fatty acid compositions, including that of PUFA on the development of CAD[8-11]. We also performed the Japan EPA Lipid Intervention Study (JELIS)[12] to investigate whether long-term use of pure EPA decreases the risk of CAD in Japanese hypercholesterolemic patients treated with 3-hydroxy-3-methylglutaryl coenzyme A (HMG-CoA) reductase inhibitor (pravastatin or simvastatin). At a mean follow-up of 4.6 years, we detected the primary endpoint in 262 (2.8%) patients in the EPA group and 324 (3.5%) controls - a 19% relative reduction ($p = 0.011$)[13]. In addition, we obtained plasma fatty acid concentration data from the participants. In the present supplemental analysis of JELIS, we explored which plasma fatty acids affected the risk of CAD.

Methods

Patients

The enrollment period in JELIS was from November 1996 to November 1999. The planned duration of follow-up of the patients was 5 years, with actual monitoring for a mean of 4.6 (SD1.1) years. The institutional review boards of each facility approved the study, and all patients provided written informed consent. Eligibility criteria were a total cholesterol level of 250 mg/dL or greater, which corresponds to a low-density lipoprotein (LDL) cholesterol level of 170 mg/dL or greater at baseline. The design and inclusion and exclusion criteria were described elsewhere in detail[12]. All patients received 10 mg pravastatin or 5 mg simvastatin once daily as first-line treatment and were counseled to follow the NCEP (National Cholesterol Education Program step) I diet[14].

Study Design

The study population was randomly assigned to receive EPA (EPA group) or not (control group) after a 4- to 8-week washout of antihyperlipidemic drugs. In the EPA group, we administered a daily dose of 1800 mg EPA as 6 capsules, each containing 300 mg of pure (>98%) EPA ethyl ester. The primary endpoint of JELIS was the cumulative incidence of major coronary events (MCE), including sudden cardiac death, fatal or nonfatal myocardial infarction, unstable angina pectoris with documented myocardial ischemia, and angioplasty/stenting or coronary artery bypass grafting. Clinical endpoints were reviewed by expert cardiologists belonging to the Event Evaluation Committee and without knowledge of treatment allocation. Local physicians monitored compliance with dietary instructions and use of medications at each clinic visit.

Measurement of Plasma Fatty Acid

Plasma total fatty acid concentrations were measured annually by a central laboratory (BML Inc., Saitama, Japan) for all patients who gave informed consent for blood sampling to test them. Plasma fatty acid composition was determined by capillary gas chromatography. Briefly, plasma lipids were extracted by Folch's procedure. Then fatty acids (with tricosanoic acid, C23:0, used as the internal standard) were methylated with boron trifluoride and methanol, and methylated fatty acids were analyzed using the SHIMAZU GC-17A gas chromatograph (Shimazu Corporation, Kyoto, Japan) and a BPX70 capillary column (0.25 mm ID × 30 m; SGE International Ltd., Melbourne, Australia).

Statistical Analysis

Differences between the 2 groups with or without EPA treatments were assessed with the χ^2 test for categorical variables and with the Mann-Whitney rank-sum test for continuous data in baseline characteristics. The Mann-Whitney rank-sum test was also used to compare fatty acid values of the quantity of

Table 1. Baseline characteristics of the subjects

	Control group $n=8,076$	EPA group $n=8,321$	p value
General characteristics			
Sex (male/female)	2,519/5,557	2,631/5,690	0.555
Age, years	61 ± 9	61 ± 8	0.108
BMI, kg/m^2	24.1 ± 3.3	24.0 ± 3.2	0.619
Systolic blood pressure, mmHg	134.9 ± 20.9	134.9 ± 21.4	0.658
Diastolic blood pressure, mmHg	79.2 ± 12.6	78.9 ± 12.6	0.206
Clinical history			
Coronary artery disease, n (%)	1,550 (19.2)	1,582 (19.0)	0.769
Diabetes, n (%)	1,324 (16.4)	1,357 (16.3)	0.882
Hypertension, n (%)	2,868 (35.5)	2,977 (35.8)	0.724
Smoker, n (%)	1,470 (18.2)	1,648 (19.8)	0.009
Medications, (%)			
Antiplatelet agent, n (%)	1,160 (14.4)	1,121 (13.5)	0.099
Anticoagulant agent, n (%)	246 (3.0)	250 (3.0)	0.876
Nitrate, n (%)	791 (9.8)	753 (9.0)	0.102
Calcium blocker, n (%)	2,501 (31.0)	2,511 (30.2)	0.271
Beta-blocker, n (%)	691 (8.6)	703 (8.4)	0.805

Footnotes: Values are the mean ± S.D. unless otherwise noted.

changes between 2 groups. To evaluate whether on-treatment plasma fatty acid concentrations determined the risk of coronary events in JELIS participants, we calculated the adjusted hazard ratio (HR) of MCE. The HR and its 95% confidence interval (CI) were computed with the Cox proportional hazard model adjusted for age, sex, smoking, history of coronary artery disease, history of diabetes, history of hypertension, use of drugs for coronary artery disease (nitrates, antiplatelet agents, anticoagulant agents), and on-treatment major plasma fatty acid concentrations (C16:0 palmitic acid, C18:0 stearic acid, C18:1 oleic acid, C18:2 linoleic acid, C20:4 arachidonic acid (AA), C20:5 EPA, C22:6 DHA). All probability values of 5% or less (two-sided) were considered significant. Analyses were performed using SAS statistical software (version 9.1; SAS Institute Inc., Cary, NC).

Results

Baseline Characteristics of the Subjects

Table 1 shows the characteristics of the subjects at baseline. Among 18,645 of JELIS participants, 16,397 gave informed consent to annual blood sampling to test plasma fatty acids at registration, and 15,534 during treatment. The rate of smokers was significantly higher ($p=0.009$) in the EPA group. Except for the rate of smokers, the 2 treatment groups were well-matched at baseline.

Changes in Plasma EPA Concentrations and Other Fatty Acid Profiles with EPA Treatment

Table 2 lists the mean values of serum lipid and plasma fatty acids at baseline (control group; $n=8,076$, EPA group; $n=8,321$) and the quantity of change from the baseline. Serum lipid values at baseline did not differ between the two groups. On-treatment LDL cholesterol levels were similar in both groups. Triglyceride level in the EPA group was decreased more than in the control group. Plasma SFA, MUFA, and n-6 PUFA levels in the EPA group were decreased more than in the control group. Among n-3 PUFAs, quantities of changes in plasma docosapentaenoic acid (DPA) and EPA concentrations in the EPA group were significantly higher than those in the control group; however, DHA concentrations were not increased at all by EPA treatment.

Fig. 1 shows histograms of baseline and on-treatment plasma concentrations of EPA and EPA/AA ratios. The mean levels of plasma EPA concentrations at baseline were 93 μg/mL in the control group and 97 μg/mL in the EPA group. On-treatment mean plasma EPA concentrations were 95 μg/mL in the control group and 170 μg/mL in the EPA group (**Fig. 1A**). The mean levels of plasma EPA/AA ratios at baseline were 0.59 in the control group and 0.62 in the EPA group. On-treatment mean plasma EPA/AA ratios were 0.60 in the control group and 1.21 in the EPA group (**Fig. 1B**).

Table 2. Mean serum LDL-cholesterol, HDL-cholesterol, triglyceride, and plasma fatty acid levels at baseline and quantity of changes

	Baseline		Quantity of change		
	Control $n=8,076$	EPA $n=8,321$	Control	EPA	p value
LDL-cholesterol (mg/dL)	182 ± 29	182 ± 29	−46 ± 36	−45 ± 38	0.156
HDL-cholesterol (mg/dL)	58 ± 17	59 ± 18	1 ± 16	0.3 ± 15	0.001
Triglyceride (mg/dL)	190 ± 154	188 ± 143	−31 ± 138	−37 ± 124	<0.001
Saturated fatty acid					
Myristic acid (C14:0, μg/mL)	34 ± 25	34 ± 26	−1 ± 30	−3 ± 26	<0.001
Palmitic acid (C16:0, μg/mL)	748 ± 313	749 ± 329	3 ± 339	−27 ± 303	<0.001
Stearic acid (C18:0, μg/mL)	228 ± 74	228 ± 77	1 ± 87	−3 ± 76	0.008
Monounsaturated fatty acid					
Palmitoleic acid (C16:1, μg/mL)	93 ± 58	93 ± 60	−6 ± 54	−13 ± 54	<0.001
Oleic acid (C18:1, μg/mL)	691 ± 370	687 ± 360	−8 ± 394	−49 ± 345	<0.001
n-6 polyunsaturated fatty acid					
Linoleic acid (C18:2, μg/mL)	835 ± 239	834 ± 246	10 ± 268	−38 ± 260	<0.001
γ-Linolenic acid (C18:3, μg/mL)	15 ± 9	15 ± 8	−2 ± 8	−4 ± 8	<0.001
Dihomo-γ-Linolenic acid (C20:3, μg/mL)	35 ± 13	35 ± 13	0.2 ± 12	−6 ± 12	<0.001
Arachidonic acid (C20:4, μg/mL)	162 ± 41	162 ± 42	8 ± 38	−9 ± 38	<0.001
n-3 polyunsaturated fatty acid					
α-Linolenic acid (C18:3, μg/mL)	20 ± 12	20 ± 13	6 ± 17	5 ± 16	<0.001
Eicosapentaenoic acid (C20:5, μg/mL)	93 ± 51	97 ± 55	2 ± 55	69 ± 83	<0.001
Docosapentaenoic acid (C22:5, μg/mL)	25 ± 11	25 ± 11	4 ± 13	17 ± 19	<0.001
Docosahexaenoic acid (C22:6, μg/mL)	169 ± 61	170 ± 61	−2 ± 58	−14 ± 56	<0.001

Footnotes: Values are the mean ± S.D.

Relationships between Fatty Acid Concentrations and Risk of MCE

The associations of on-treatment plasma fatty acid values with the risk of MCE are shown in **Fig. 2**. We divided the patients into 2 groups according to mean on-treatment major plasma fatty acid levels, and calculated the hazard ratio of MCE in the higher group relative to the lower group as a standard. In the group of all 15,534 participants (control group; $n=7,722$, EPA group; $n=7,812$), a high EPA concentration (above 133 μg/mL as mean) was significantly associated with a lower risk of MCE (HR 0.83, 95% CI: 0.68 to 0.99; $p=0.049$). Stearic acid concentration (above 225 μg/mL as mean) was also significantly associated with a lower risk of MCE (HR 0.71, 95% CI: 0.54 to 0.93; $p=0.014$). Linoleic acid exhibited a positive correlation with the risk of MCE, although the relationships were not significant. None of the other fatty acid concentrations exhibited a relationship with the risk of MCE (**Fig. 2A**). In the control group of 7,722 subjects, even a high EPA concentration (above 95 μg/mL as the mean) was not associated with the risk of MCE. Only a high linoleic acid concentration was significantly associated with a higher risk of MCE (HR 1.33, 95% CI: 1.02 to 1.74; $p=0.039$). Other fatty acids did not exhibit a significant relationship with the risk of MCE (**Fig. 2B**). In the EPA group of 7,812 subjects, a high EPA concentration (above 170 μg/mL as the mean) was significantly associated with a lower risk of MCE (HR 0.71, 95% CI: 0.54 to 0.94; $p=0.018$). Other fatty acid concentrations did not exhibit a relationship with the risk of MCE (**Fig. 2C**).

We examined in detail the relationship between plasma EPA concentration and the risk of MCE to provide a target level of EPA for the prevention of MCE. As shown in **Fig. 3**, the risk of MCE in all 15,534 participants was significantly lower in the group with the highest plasma EPA concentration (≥150 μg/mL) than in the group with the lowest concentration (<87 μg/mL, median plasma EPA concentration in the control group during the follow-up period) (adjusted HR 0.80, $p=0.042$). In addition, the risk of MCE in the group with a plasma EPA concentration above 100 μg/mL was lower, although not significantly so, than in the group with a concentration of less than 100 μg/mL. The risk of MCE in groups with a concentration above 150 μg/mL and

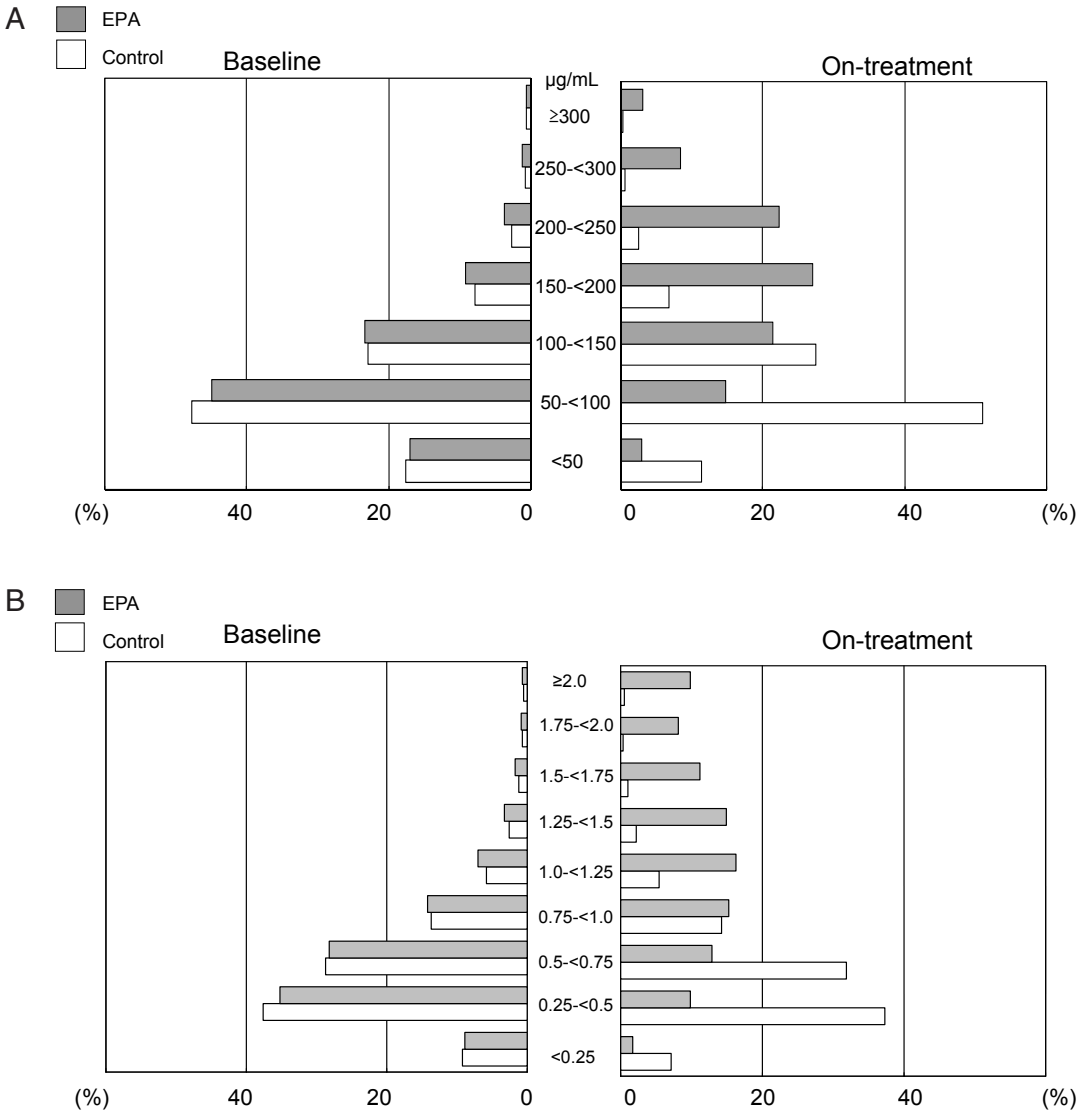

Fig. 1. Distribution of plasma EPA and EPA/AA ratio concentrations.

Footnotes: A) EPA concentrations; B) EPA/AA ratios
Columns show frequencies of patients.

200 μg/mL was significantly lower than in groups with a concentration less than 150 μg/mL (HR=0.82, p=0.032) and 200 μg/mL (HR=0.78, p=0.043), respectively (**Table 3**). Ten percent of patients in the control group and 61% of those in the EPA group attained 150 μg/mL or higher plasma EPA concentration. In addition, the risk of MCE in groups with EPA/AA ratio above 0.75 and 1 was significantly lower than in groups with the ratio less than 0.75 (HR=0.83, p=0.031) and 1 (HR=0.80, p= 0.021), respectively (**Table 3**).

Discussion

Fatty acids are classified as saturated fatty acids, monounsaturated fatty acids, and the n-6 and n-3 polyunsaturated fatty acids. All these fatty acids are known to have different effects. SFA and MUFA are the principal ingredients of triglycerides, although details of the relationships between CAD and individual fatty acids remain uncertain; however, some cohort and interventional studies have reported that n-3PUFA has a preventive effect on CAD. In contrast, oversupply of n-6 PUFA increases the risk of CAD, because of the inflammatory and thrombogenetic

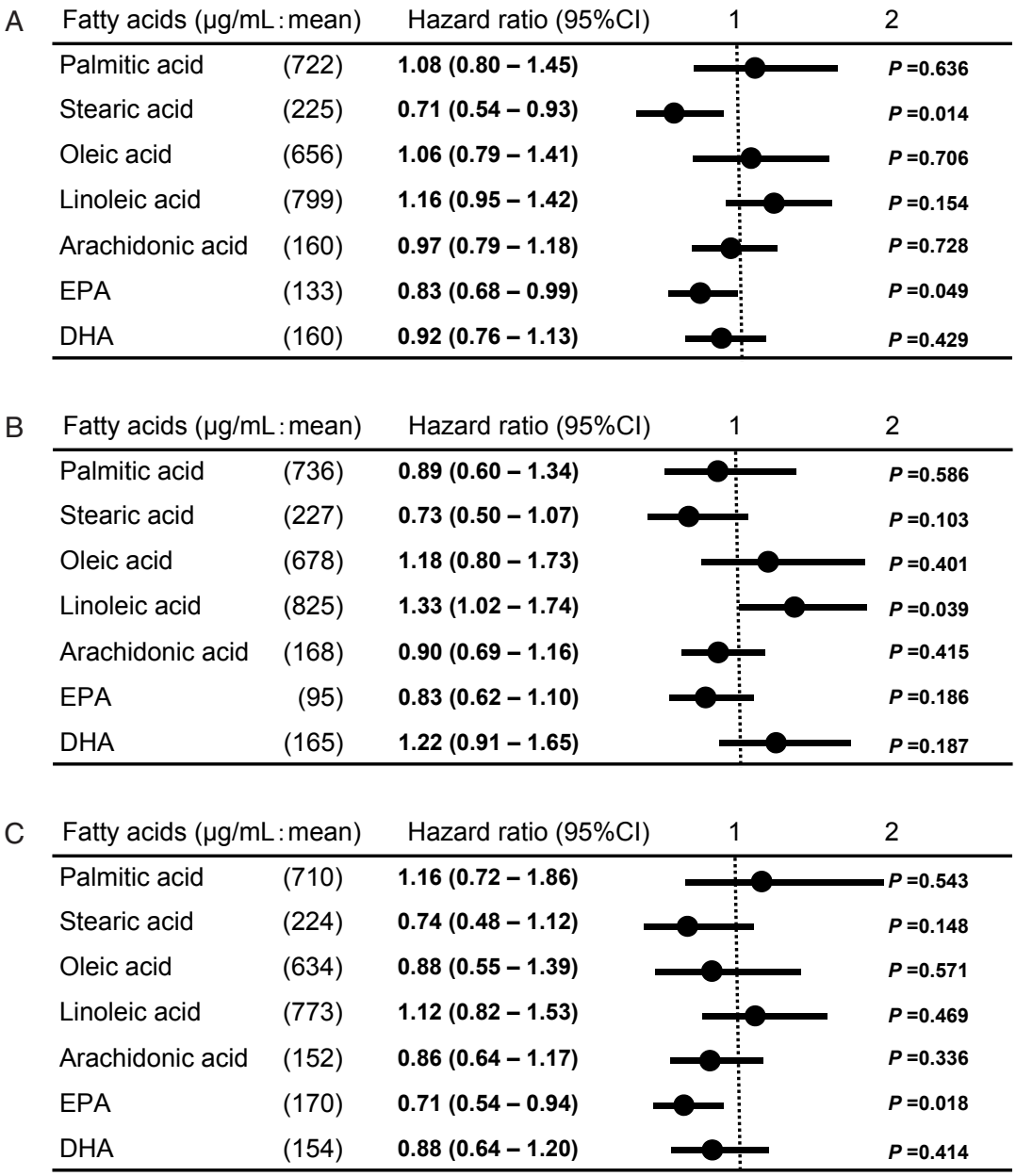

Fig. 2. Relationships between fatty acid levels and major coronary events.

Footnotes: A) All participants; B) Control group; C)EPA group
Risks of major coronary events in the higher groups were calculated relative to the standard in the lower group.
The unit for plasma fatty acids is μg/mL.

effects of metabolites of AA[15].

We had already found that EPA reduced MCE by 19% in a previous study[13], and the result of this sub-analysis of all participants confirmed the findings of JELIS with regard to the composition of fatty acids. In the control group, neither EPA nor DHA concentration exhibited a significant relationship with the risk of coronary events. NIPPON DATA80, which observed 8,879 Japanese men and women from 1980 to 1999, reported that the frequency of fish consumption was associated, although not to a significant extent, with decreased risk of death due to coronary artery disease. The authors speculated that the reason for this was that the majority of Japanese subjects in their study ate fish in amounts above the threshold level shown to be beneficial in previous studies[16]. The

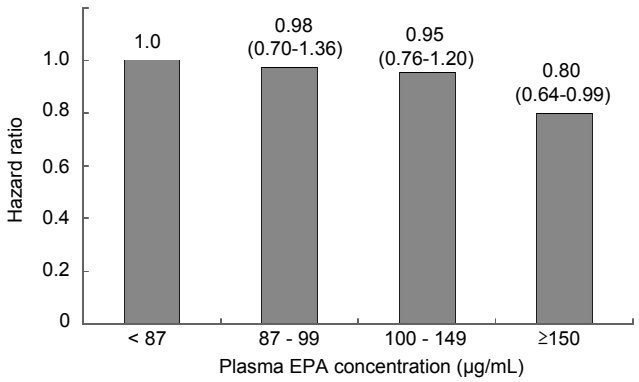

Fig. 3. Relationship between on-treatment EPA concentration and adjusted risk of major coronary events.

Japan Public Health Center-Based (JPHC) Study Cohort I examined the association between a high intake of fish and n-3 PUFAs and the risk of CAD in 41,578 Japanese men and women aged 40 to 59 years who were free of a prior diagnosis of cardiovascular diseases. The study group reported a strong inverse association between dietary intake of n-3 PUFA and the risk of definite myocardial infarction and nonfatal coronary events[17]. In the EPA group in the present study, a higher plasma EPA concentration was associated with a significant reduction of 29% ($p=0.018$) in the risk of MCE. Our findings suggested that larger samples or stronger intervention with EPA agents may be needed to detect significant benefits of EPA in Japanese clinical trials or surveillance. In this analysis, we found that increasing plasma EPA concentration reduced the risk of coronary events. On the other hand, DHA might not play a role in the prevention of coronary events by EPA since the plasma DHA concentration was not increased. We therefore could not determine whether the increase in DHA concentration did not decrease the risk of MCE.

We found that the risk of MCE in all participants was significantly lower in the group with a high plasma EPA concentration (150 μg/mL or more) than in the group with a low concentration. Furthermore, we found that the risk of MCE was significantly lower in the group with a high plasma EPA/AA ratio (0.75 or more) than in the group with a low ratio. It was noted that AA accelerates platelet aggregation and inflammatory reactions, and EPA works as an antagonistic regulator of AA. Recently, a significant positive correlation between EPA/AA ratios and insulin sensitivity as well as a negative correlation between those ratios and insulin resistance were observed in a subject with metabolic syndrome[18]. In fact, we expect that the EPA/AA ratio, which shows the balance of each PUFA concentration, may be used as a precise biomarker for arteriosclerotic disease; however, our results cannot provide definitive target levels since they were obtained through a comparison of control patients with a relatively low EPA concentration in the Japanese population. The desirable EPA value for a much greater decrease in the incidence of coronary artery disease may differ depending on eating habits and risks. Large-scale clinical studies and analyses from more perspectives should be conducted to specify the desirable EPA level for lowering the risk of coronary events. Additionally, among saturated fatty acids, stearic acid concentration, but not palmitic acid concentration was associated with a lower risk of MCE. Many clinical studies have shown the heterogeneity of the effect of saturated fatty acids on risk factors. Lauric, myristic, and palmitic acids are known to be hypercholesterolemic; however, stearic acid is not hypercholesterolemic, and so its intake might beneficially affect CVD risk reduction. The P/S (polyunsaturated/saturated) ratio has long been used as an index in dietary counseling to prevent CAD, but its composition might need to be reconsidered; however, it is unclear whether stearic acid itself protects against cardiovascular disease.

Table 3. Hazard ratios of major coronary events by cut-off point of on-treatment plasma EPA concentration and EPA/AA ratio

Plasma EPA concentration (μg/mL)	Hazard ratio	95% CI	*p* value
Low (<100) vs High (≧100)	0.87	0.72-1.03	0.110
Low (<150) vs High (≧150)	0.82	0.68-0.98	0.032
Low (<200) vs High (≧200)	0.78	0.62-0.99	0.043
Plasma EPA/AA ratio	Hazard ratio	95% CI	*p* value
Low (<0.50) vs High (≧0.50)	0.94	0.77-1.14	0.519
Low (<0.75) vs High (≧0.75)	0.83	0.69-0.98	0.031
Low (<1) vs High (≧1)	0.80	0.67-0.97	0.021

JELIS reported that the differences in changes in HDL cholesterol and LDL cholesterol between the two treatment groups were very small[13]. The question thus arises how EPA exhibited its beneficial effects. We speculate that anti-inflammatory effects of EPA might be principally responsible for reducing atherosclerotic lesions and prevent cardiovascular events[15, 19-25]. Concerning the prevention of CAD by EPA as well as its anti-inflammatory effect, it should be noted that EPA exhibits an antiarrhythmic effect[26], inhibits platelet aggregation[27, 28], exhibits vasodilatory activity[29], increases the circulating adiponectin level[30], has a triglyceride-lowering effect[31], and induces plaque stabilization[24, 32]. These effects may function synergistically to prevent CAD. Further study of the relationship between atherosclerosis and these fatty acids is warranted.

In conclusion, plasma fatty acid concentrations were found to be correlated with the risk of coronary events, and administration of pure EPA was found to affect this relationship. Plasma EPA concentration and the EPA/AA ratio may be used as biomarkers of CAD.

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