Association of Fish Consumption-Derived Ratio of Serum n-3 to n-6 Polyunsaturated Fatty Acids and Cardiovascular Risk With the Prevalence of Coronary Artery Disease

A Cross-Sectional Pilot Study

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SUMMARY

We investigated the relationships between the ratio of serum n-3 polyunsaturated fatty acids (n-3PUFAs: eicosapentaenoic acid [EPA] and docosahexaenoic acid [DHA]) to n-6PUFA (arachidonic acid [AA]) and the prevalence of coronary artery disease (CAD), and assessed the association of the ratio of serum n-3 to n-6 PUFAs with atherosclerosis-related markers.

This study was designed as a hospital-based cross-sectional study of 649 consecutive outpatients who had undergone regular examinations between April 2009 and October 2009. We divided the patients into 5 groups based on the quintiles of the EPA/AA ratio or quintiles of the DHA/AA ratio to determine independent factors for the prevalence of CAD.

In multivariate logistic regression analyses after adjustment for coronary risk factors and serum n-3PUFAs levels to minimize confounding factors to the extent possible because the serum levels of EPA and DHA showed a strong correlation ($r = 0.812$, $P < 0.0001$), the group with the highest EPA/AA ratio had a lower probability of CAD prevalence (odds ratio: 0.328, 95% confidence interval: 0.113 to 0.956, $P = 0.041$), but this was not true for the DHA/AA ratio. Multivariate analysis showed an increase in the EPA/AA ratio, but not in the DHA/AA ratio, was associated with effects on atherosclerosis-related markers, especially triglyceride-rich lipoproteins, high-density lipoprotein cholesterol (HDL-C) containing apolipoprotein A-1, and leukocyte count in an anti-atherogenic direction.

The results suggest a higher EPA/AA ratio, but not a higher DHA/AA ratio, might be associated with a lower prevalence of CAD and improvements of triglyceride metabolism and HDL metabolism, and systemic inflammation. (Int Heart J 2015; 56: 260-268)

Key words: Lipids, Prevention

Regular fish consumption has been correlated with a reduced mortality due to coronary artery disease (CAD).[1-3] This has been ascribed to the beneficial effects of two major n-3 polyunsaturated fatty acids (n-3PUFAs), eicosapentaenoic acid (EPA) and docosahexaenoic acid (DHA).[4,5]

Recently, we reported findings from a hospital-based cross-sectional study that indicated the incidence of CAD was lower in a group of individuals with high serum EPA and DHA levels.[6] Although belonging to the same n-3PUFA family, EPA and DHA differ from one another in molecular structure[4,5] and it is known that the percentage of EPA and DHA contained in each organ differs depending on the difference in the percent uptake by the respective cell membranes.[7] It seems therefore likely that *in vivo*, EPA and DHA exhibit different physiological activities.[8-11] A strong positive correlation is known to exist between serum EPA and DHA levels, and this was also shown in our study cited above.[4-6] Because serum EPA and serum DHA levels serve as confounding factors for each other, there are limits to discussing their effects on the incidence of coronary artery disease.

In the Japan EPA Lipid Intervention Study (JELIS), patients with hypercholesterolemia were randomized to treatment with a statin alone or a statin plus 1800 mg/day of highly purified EPA (EPA group). At the end of the 5-year study, those randomized to the EPA group showed a 19% reduction in the incidence of major cardiovascular (CV) events.[12] This result suggests that interventions targeting the serum EPA level or serum EPA/arachidonic acid (AA) ratio could be useful for the prevention of CAD.[11,13] Thus, the n-3/n-6 PUFA ratio may play an important role in reducing the risk of a CV event.

In recent years, several observational studies have dem-

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onstrated the existence of an association between the occurrence of coronary events and a low serum EPA/AA ratio, whereas no correlation has been observed between the occurrence of coronary events and the serum DHA/AA ratio.[14-16] However, the mechanism underlying the association remains unclear. Also, in our cross-sectional study without intervention with an n3PUFA preparation, we tested the hypothesis that correction of the serum n3PUFA level with the serum AA level (ie, n-3PUFA/n-6PUFA ratio) may clarify its relationship with the onset of CAD and atherosclerosis-related markers.

The aim of this study was to attempt stratification of the risk for the development of CAD through correction of the serum n3PUFA level with the serum AA level and to analyze the relationship between the corrected serum n3PUFA level and atherosclerosis-related factors in patients enrolled in our previously reported cross-sectional study.[6]

Methods

Study design and study population: This study was designed as a hospital-based cross-sectional study to investigate the relationships between the serum EPA/AA and DHA/AA ratios and the prevalence of CAD, and also to assess the association between the serum n-3/n-6 PUFA ratio and the serum levels of atherosclerosis-related markers in patients with one or more of the conventional risk factors for CAD.

The study was conducted on a sample of 700 consecutive outpatients who had undergone regular examinations for treatment of their own disease at the Cardiovascular Center, Surugadai Nihon University Hospital, between April 2009 and October 2009.[6] Exclusion criteria included refusal to provide consent for participation in the study, current treatment with n-3PUFAs, and/or a diagnosis of acute coronary syndrome (ACS) within 3 months prior to the study.

This study was conducted in accordance with the ethical principles of the Declaration of Helsinki. The study protocol was approved by the Institutional Review Board at our institute and written informed consent was obtained from all study participants.

The diagnostic criteria for the coronary risk factors used in this study were as follows; hypertension: systolic pressure of ≥ 140 mmHg and/or a diastolic pressure of ≥ 90 mmHg, or taking antihypertensive medication; diabetes mellitus: fasting plasma glucose ≥ 126 mg/dL and HbA1c ≥ 6.5% (according to the National Glycohemoglobin Standardisation Programme [NGSP]), or current treatment with antidiabetic agents; lipid disorder: serum low-density lipoprotein cholesterol (LDL-C) ≥ 140 mg/dL, serum triglycerides (TG) ≥ 150 mg/dL, and/or serum high-density lipoprotein cholesterol (HDL-C) less than 40 mg/dL, or current treatment with lipid-modifying medication; smoking was defined as current smoking or smoking cessation within 1 year prior to the start of the study; Chronic kidney disease (CKD): Estimated glomerular filtration rate (eGFR) < 60 mL/minute/1.73 m^2; obesity: body mass index (BMI) ≥ 25 kg/m^2.

Angiographically, CAD was defined as a history of documented myocardial infarction, prior coronary revascularization intervention (coronary artery bypass graft surgery or percutaneous coronary intervention), or the presence of ≥ 50% stenosis in 1 or more of the coronary arteries identified during cardiac catheterization.

Measurement of the laboratory parameters: Fasting blood samples were collected early in the morning after the patients had fasted for 12 hours overnight. The serum n3 PUFA level was measured by capillary gas chromatography (SRL Inc., Tokyo). The serum levels of total cholesterol (TC), HDL-C, and TG were measured using standard methods. The serum LDL-C level was estimated by the Friedewald formula.[17] The remnant-like particle cholesterol (RLP-C) level was measured by an immunoadsorption assay (SRL). The VLDL fraction was measured by polyacrylamide-gel electrophoresis using the LipoPhor system (Joko, Tokyo). The serum apolipoprotein (apo) and lipoprotein (a) (Lp[a]) levels were determined by turbidimetric latex agglutination assays (Daiichi Pure Chemicals Co., Ltd., Tokyo). The serum malondialdehyde LDL (MDA-LDL) level was measured by an enzyme-linked immunosorbent assay (SRL). The high-sensitivity C-reactive protein (hs-CRP) level was measured by a nephelometric assay (Behring Diagnostics, Marburg, Germany). The eGFR was calculated using the Japanese equation based on the serum creatinine (Cr) level, age, and gender as follows: eGFR (mL/minute/1.73 m^2) = $194 \times Cr^{-1.094} \times age^{-0.287}$ (female × 0.739).[18]

Statistical analysis: SPSS for Windows, ver 12.0 (Statistical Package for the Social Sciences, SPSS Ins., Chicago, IL) was used for all analyses. Data are expressed as the mean ± standard deviation (SD) for continuous variables and as percentages for discrete variables. The chi-square test was used to compare the number of risk factors. For cases showing significantly skewed distribution, the data are expressed as the interquartile range. The Mann-Whitney *U* test was used to evaluate differences in data between the groups, and Wilcoxon's signed-rank test was used to analyze differences in data within the same group. In the subset analysis by quintiles of the EPA/AA and DHA/AA ratios, analysis of variance (ANOVA) followed by Bonferroni's adjustment for covariates were used if differences had been detected in the patient characteristics or atherosclerosis-related markers. Multivariate logistic regression models with adjustments for age and gender (model 1), for age, gender, and traditional coronary risk factors (model 2), and for age, gender, traditional coronary risk factors, and serum n-3PUFA levels to minimize the confounding factors to the maximum extent possible, because the serum levels of EPA and DHA were strongly correlated ($r = 0.812$, $P < 0.0001$) (model 3) were used to evaluate the association between the prevalence of CAD and the quintiles of the serum EPA/AA and DHA/AA ratios. Multivariate analysis with adjustments for age, gender, coronary risk factors, and presence/absence of statin therapy was conducted to investigate the effect of the serum EPA/AA and DHA/AA ratios on the serum levels of atherosclerosis-related markers. *P* values of less than 0.05 were considered to indicate statistical significance.

Results

Subjects: The participants consisted of 470 men (mean age, 65 ± 15 years) and 230 women (mean age, 65 ± 14 years), with or without CAD, which was defined as previous myocardial infarction ($n = 100$), coronary interventions including bypass surgery ($n = 18$), or confirmed angina pectoris ($n = 25$). We excluded 51 subjects from the analysis because of missing

laboratory data. Therefore, 649 subjects were included in the analysis.

The serum EPA level ranged from 6.2 to 373.5 μg/mL (mean ± SD, 74.7 ± 50.1 μg/mL; median, 63.0 μg/mL; interquartile range, 40.1/92.6), the serum DHA level ranged from 35.7 to 451.8 μg/mL (mean ± SD, 135.9 ± 52.8 μg/mL; median, 128.9 μg/mL; interquartile range, 100.8/60.8), and the serum AA level ranged from 67.4 to 372.3 μg/mL (mean ± SD, 163.1 ± 44.4 μg/mL; median, 157.8 μg/mL; interquartile range: 132.4/189.4). The serum EPA/AA ratio ranged from 0.056 to 2.471 (mean ± SD, 0.481 ± 0.333; median, 0.397; interquartile range, 0.246/0.610). The serum DHA/AA ratio ranged from 0.165 to 2.689 (mean ± SD, 0.870 ± 0.350; median, 0.809; interquartile range, 0.622/1051).

Patient characteristics according to serum EPA/AA and DHA/AA ratios: There were no significant differences in the BMI, percentage of patients with lipid disorder, percentage of patients with diabetes mellitus, percentage of current smokers, percentage of patients with hyperuricemia, or the serum uric acid levels among the quintiles of the serum EPA/AA ratio. On the other hand, significant differences were seen in the gender distribution, age, eGFR, percentage of patients with hypertension, and percentage of patients with CKD. Analysis of the relationships with current treatments showed that there were no significant differences among the quintiles of the serum EPA/AA ratio (Table I).

There were no significant differences in the gender distribution, BMI, percentage of patients with lipid disorder, percentage of patients with diabetes mellitus, serum HbA1c, percentage of current smokers, percentage of patients with CKD ≥ stage 3, percentage of patients with hyperuricemia, or the serum uric acid levels among the quintiles of the DHA/AA ratio. On the other hand, significant differences were seen in the age, percentage of patients with hypertension, and the eGFR. Analyses of the relationships with current treatments showed that treatment with calcium channel blockers was associated with an elevated DHA/AA ratio. There were no significant differences in the percentages of patients receiving aspirin, angiotensin-converting enzyme inhibitors (ACEIs), angiotensin receptor blockers (ARBs), β-blockers, and statins among the quintiles of the serum DHA/AA ratio (Table I).

Profile of atherogenicity-related markers according to serum EPA/AA and DHA/AA ratios: The serum apo A-II level decreased significantly with an increase in the EPA/AA ratio. No significant differences in any other atherosclerosis-related markers were noted as the quintiles of the EPA/AA ratio (Table II).

Significant differences were seen in the serum VLDL fraction, serum apoC-II and apoE levels, and the apoB/apoA-I ratio. A significant difference was also noted in the serum apo A-II level. No significant differences in any other atherosclerosis-related markers were noted among the quintiles of the DHA/AA ratio (Table II).

Multiple logistic regression analysis to determine the prevalence of CAD according to serum EPA/AA and DHA/AA ratios: In both multivariate logistic regression models 1 and 2, the EPA/AA and DHA/AA ratios in the highest quintile were shown to be independently associated with a lower prevalence of CAD. To eliminate the confounding effect stemming from the strong correlation between the serum EPA and DHA levels, model 3 incorporated correction for the serum EPA and DHA levels. After this correction, while the EPA/AA ratio in the highest quintile was still found to be associated with a low prevalence of CAD (A), the DHA/AA in the highest quintile was found to be no longer associated with the CAD prevalence (B) (Table III).

Multivariate regression analyses to determine the relationships between serum EPA/AA and DHA/AA ratios and serum levels of atherosclerosis-related markers: The serum EPA/AA ratio was an independent negative predictor of the serum TG, non-HDL-C, MDA-LDL, and RLP-C levels, and VLDL fraction, the LDL-C/HDL-C and apo B/apo A-I ratios, and WBC count, and a positive predictor of the serum HDL-C and apo A-I levels. The serum DHA/AA ratio was a negative predictor that was independent of the serum HDL-C, apo A-I, and apo E levels, and a positive predictor of the serum TG, non-HDL-C, MDA-LDL, RLP-C, apo B, apo C-II apo, hs-CRP levels, and the LDL-C/HDL-C and apo B/apo A-I ratios. The serum TC, LDL-C, Lp(a) and apo A-II levels were not predictive of either the serum EPA/AA ratio or the serum DHA/AA ratio (Table IV).

Discussion

The main findings of the present study can be summarized as follows: 1) the results of multivariate logistic regression analyses after adjustments for coronary risk factors and n-3PUFAs levels to minimize confounding factors, because the serum levels of EPA and DHA correlated strongly, revealed that the highest EPA/AA, but not the highest DHA/AA, was associated with the lowest CAD prevalence; 2) We found that the serum EPA/AA and DHA/AA ratios had opposite effects on almost all of the serum lipid markers. In short, we may say that elevation of the serum EPA/AA ratio is generally associated more closely with improvement of the HDL and TG metabolism and amelioration of systemic inflammatory reactions than elevation of the serum DHA/AA ratio, suggesting the possible anti-atherosclerotic effects of elevation of the serum EPA/AA ratio (Table V).

Comparison among the quintiles of the serum EPA/AA ratio revealed no significant differences among the groups in terms of the association with atherosclerosis-related markers except the serum apoA-2 level. However, the results of multivariate analysis with correction for the serum EPA/AA and DHA/AA ratios revealed that the populations with high serum EPA/AA ratios showed decreases in the VLDL fraction, and in RLP-C, which are strongly atherogenous triglyceride-rich lipoproteins (TRLs) produced during the process of TG metabolism. This may lead to a decrease of the sd-LDL, which also has powerful atherogenous activity.[19,20]

Our cross-sectional study, reported recently, demonstrated an association between elevated serum EPA and a decrease of serum TRLs.[6] Linoleic acid, which is a precursor of AA, is abundantly contained in the TG fraction, and it seems likely that the effect of EPA in lowering the serum TG may lead to a decrease of the serum AA, and consequently, elevation of the serum EPA/AA ratio.[13] For this reason, evaluation of the extent of influence of the EPA/AA ratio on atherosclerosis may be more accurate than the evaluation based on the serum EPA level alone. In addition, we previously reported increasing activation of the triglyceride metabolism as the EPA/AA ratio in-

Table I. Patient Characteristics According to the Serum EPA/AA and DHA/AA Ratios

	All cases (n = 649)	EPA/AA Quintile					P	DHA /AA Quintile					P
		1Q (n = 130)	2Q (n = 130)	3Q (n = 130)	4Q (n = 130)	5Q (n = 129)		1Q (n = 130)	2Q (n = 130)	3Q (n = 130)	4Q (n = 130)	5Q (n = 129)	
Male/female, n (%)	441 (68) / 208 (32)	78 (60) / 52 (40)	87 (67) / 43 (33)	88 (68) / 42 (32)	86 (66) / 44 (34)	101 (78) / 28 (22)	0.026	82 (63) / 48 (33)	87 (67) / 43 (33)	86 (68) / 44 (32)	93 (72) / 37 (28)	92 (71) / 37 (29)	0.500
Age (years)	62 ± 14	53 ± 19	62 ± 15*	65 ± 13*	67 ± 11*,†	66 ± 9.0*,†	< 0.0001	53 ± 17	60 ± 15†	65 ± 12†	66 ± 11*,†	68 ± 9.0*,†	< 0.0001
BMI (kg/m^2)	24.0 ± 3.9	23.9 ± 4.9	24.0 ± 4.2	23.9 ± 3.4	24.0 ± 3.9	23.9 ± 3.1	0.989	23.6 ± 4.5	24.4 ± 4.4	23.6 ± 3.7	24.1 ± 3.7	24.1 ± 3.1	0.999
Hypertension, n (%)	454 (70)	74 (57)	96 (74)	91 (70)	99 (76)	98 (76)	0.003	79 (61)	84 (65)	78 (60)	82 (63)	80 (62)	0.951
Lipid disorder, n (%)	403 (62)	73 (56)	82 (63)	78 (60)	92 (71)	78 (60)	0.123	75 (58)	91 (70)	91 (70)	99 (76)	104 (80)	0.002
Diabetes mellitus, n (%)	175 (27)	26 (20)	36 (28)	33 (25)	44 (34)	41 (32)	0.089	30 (23)	38 (29)	36 (28)	36 (28)	39 (30)	0.703
HbA1c (%)	6.0 ± 0.8	6.0 ± 0.7	6.1 ± 1.0	6.0 ± 0.8	6.0 ± 0.6	6.0 ± 0.6	0.058	5.9 ± 1.0	6.0 ± 0.7	5.9 ± 0.6	6.1 ± 0.8	6.0 ± 0.6	0.687
Current smoking, n (%)	91 (14)	26 (20)	14 (11)	17 (13)	18 (14)	17 (13)	0.247	23 (18)	22 (17)	13 (10)	17 (13)	17 (13)	0.306
eGFR (mL/minute/1.73 m^2)	72 ± 18	75 ± 21	68 ± 20†	69 ± 18†	68 ± 16†	72 ± 16	0.012	76 ± 21	71 ± 19	68 ± 18†	68 ± 16†	69 ± 16†	0.006
CKD ≥ Stage 3, n (%)	175 (27)	34 (26)	44 (34)	39 (30)	42 (32)	28 (22)	0.014	27 (21)	30 (23)	42 (32)	39 (30)	39 (30)	0.184
Hyperuricemia, n (%)	117 (18)	23 (18)	29 (22)	25 (19)	22 (17)	22 (17)	0.517	20 (15)	38 (19)	23 (18)	23 (18)	23 (18)	0.915
Uric acid (mg/dL)	5.8 ± 1.4	5.7 ± 1.4	5.8 ± 1.4	5.8 ± 1.4	5.8 ± 1.4	5.9 ± 1.1	0.823	5.7 ± 1.4	5.6 ± 1.3	5.7 ± 1.4	6.0 ± 1.5	5.8 ± 1.1	0.132
Concomitant drugs, n (%)													
Aspirin	175 (27)	25 (19)	34 (26)	39 (30)	46 (35)	35 (27)	0.081	31 (24)	38 (29)	33 (25)	42 (32)	35 (27)	0.627
ACEIs/ARBs	305 (47)	49 (38)	63 (50)	69 (53)	62 (48)	63 (49)	0.169	52 (40)	64 (49)	62 (48)	62 (48)	68 (52)	0.400
β Blockers	136 (21)	20 (15)	38 (29)	23 (18)	29 (22)	28 (22)	0.059	38 (19)	31 (24)	27 (21)	30 (23)	26 (20)	0.882
Calcium channel blockers	331 (51)	52 (40)	55 (42)	56 (43)	69 (53)	70 (54)	0.056	55 (42)	59 (45)	66 (51)	72 (55)	79 (61)	0.013
Statins	305 (47)	49 (38)	34 (50)	61 (47)	69 (53)	65 (50)	0.126	57 (44)	69 (53)	64 (49)	61 (47)	65 (50)	0.675

BMI indicates body mass index; Hb, hemoglobin; eGFR, estimated glomerular filtration rate; CKD, chronic kidney disease; ACE, angiotensin converting enzyme; ARB, angiotensin receptor blocker; and Hyperuricemia, serum uric acid ≥ 7.0 mg/dL or treatment with antihyperuricemic medication. The patients were divided into quintiles of the EPA/AA and DHA/AA ratios. The ranges according to the quintile of the EPA/AA ratio were as follows: 0.056-0.211 (quintile 1), 0.213-0.340 (quintile 2), 0.341-0.478 (quintile 3), 0.478-0.668 (quintile 4), and 0.669-2.470 (quintile 5), and the ranges according to the quintile of the DHA/AA ratio were as follows: 0.165-0.574 (quintile 1), 0.576-0.737 (quintile 2), 0.739-0.893 (quintile 3), 0.896-1.117 (quintile 4), and 1.121-2.689 (quintile 5), respectively. ANOVA and post hoc tests with Bonferroni correction were performed to test between-group differences. *$P < 0.01$, †$P < 0.0001$ versus Q1.

Table II. Atherogenic-related Marker Profile According to the Serum EPA/AA and DHA/AA Ratios

	All cases (n = 650)	EPA/AA Ratio Quintile					P	DHA/ AA Ratio Quintile					P
		1Q (n = 130)	2Q (n = 130)	3Q (n = 130)	4Q (n = 130)	5Q (n = 129)		1Q (n = 130)	2Q (n = 130)	3Q (n = 130)	4Q (n = 130)	5Q (n = 129)	
Lpid-related													
TC (mg/dL)	195 ± 37	198 ± 36	201 ± 39	191 ± 39	194 ± 42	194 ± 32	0.273	195 ± 37	192 ± 35	199 ± 48	196 ± 39	192 ± 31	0.521
LDL-C (mg/dL)	110 ± 30	112 ± 29	114 ± 33	108 ± 30	109 ± 36	106 ± 26	0.235	108 ± 29	109 ± 28	113 ± 36	111 ± 31	105 ± 25	0.323
HDL-C (mg/dL)	58 ± 17	60 ± 17	57 ± 18	56 ± 16	59 ± 15	61 ± 18	0.203	61 ± 18	56 ± 17	59 ± 16	57 ± 16	67 ± 15	0.089
TG (mg/dL)*	115 (86 / 149)	115 (85 / 160)	122 (94 / 164)	118 (86 / 145)	116 (90 / 144)	105 (80 / 145)	0.306	112 (79 / 147)	115 (83 / 147)	108 (84 / 140)	123 (88 / 160)	128 (92 / 126)	0.643
non HDL-C (mg/dL)	137 ± 34	133 ± 39	144 ± 36	135 ± 35	135 ± 39	133 ± 32	0.160	134 ± 34	136 ± 31	140 ± 40	139 ± 37	135 ± 32	0.541
MDA-LDL (U/L)	109 ± 44	110 ± 47	114 ± 45	103 ± 42	107 ± 43	110 ± 43	0.367	103 ± 44	107 ± 40	112 ± 49	110 ± 45	111 ± 39	0.492
Lp (a) (mg/dL)*	11 (5 / 24)	11 (7 / 23)	13 (6 / 23)	10 (5 / 27)	12 (6 / 26)	10 (5 / 25)	0.924	12 (7 / 26)	13 (5 / 25)	12 (5 / 27)	15 (10 / 21)	11 (5 / 21)	0.432
RLP-C (mg/dL)*	5.4 (4.0 / 7.7)	5.6 (4.0 / 8.2)	5.7 (4.2 / 8.4)	5.1 (3.9 / 7.0)	5.2 (3.8 / 7.9)	5.0 (3.7 / 7.1)	0.119	5.2 (3.6 0 / 8.0)	5.2 (4.0 / 6.7)	5.0 (3.7 / 7.6)	6.0 (4.2 / 8.0)	5.2 (4.1 / 8.2)	0.759
VLDL (%)	12.8 ± 6.4	13.5 ± 7.0	13.7 ± 7.1	12.5 ± 6.2	12.4 ± 5.4	11.7 ± 6.0	0.080	12.0 ± 6.2	13.4 ± 6.8	11.6 ± 6.3$^{\#}$	13.1 ± 5.7	13.6 ± 6.7†,¶	0.039
Apolipoprotein													
apo A-I (mg/dL)	147 ± 30	149 ± 32	145 ± 32	143 ± 30	146 ± 27	150 ± 29	0.273	152 ± 6.3	142 ± 31	148 ± 38	145 ± 29	146 ± 29	0.108
apo A-II (mg/dL)	30 ± 6.1	32 ± 6.3	30 ± 6.4†	30 ± 6.7†	29 ± 5.8†	29 ± 5.8†	0.023	32 ± 6.5	29 ± 6.3†	30 ± 6.0	29 ± 5.6‡	29 ± 5.3§	0.010
apo B (mg/dL)	89 ± 21	89 ± 20	92 ± 23	88 ± 21	89 ± 24	90 ± 19	0.672	86 ± 20	89 ± 18	90 ± 24	92 ± 24	89 ± 18	0.179
apo C-II (mg/dL)	4.5 ± 1.9	4.3 ± 2.0	4.8 ± 2.2	4.5 ± 2.0	4.5 ± 1.7	4.6 ± 1.6	0.502	4.2 ± 1.8	4.4 ± 1.8	4.6 ± 2.1	4.8 ± 2.0$^{\ddagger,\#}$	4.6 ± 1.6	0.046
apo C-III (mg/dL)	9.9 ± 3.3	9.7 ± 3.7	10.5 ± 3.7	9.8 ± 3.4	9.7 ± 2.8	10.3 ± 2.9	0.256	9.8 ± 3.7	9.6 ± 3.0	9.8 ± 3.3	10.3 ± 3.3	10.1 ± 3.1	0.380
apo E (mg/dL)	4.2 ± 1.2	4.0 ± 1.1	4.3 ± 1.6	4.1 ± 1.3	4.1 ± 1.1	4.2 ± 1.1	0.449	3.9 ± 1.0	4.0 ± 1.1	4.7 ± 1.7†	4.3 ± 1.1$^{\dagger,\#}$	4.2 ± 1.1†	0.024
Lipid, apo ratio													
LDL-C/HDL-C	2.0 ± 0.8	2.02 ± 0.74	2.16 ± 0.86	2.06 ± 0.74	1.96 ± 0.78	1.89 ± 0.68	0.082	1.91 ± 0.73	2.11 ± 0.81	2.03 ± 0.75	2.12 ± 0.84	1.96 ± 0.66	0.116
apo B/apo A-1	0.63 ± 0.20	0.66 ± 0.21	0.63 ± 0.19	0.63 ± 0.20	0.63 ± 0.20	0.62 ± 0.18	0.536	0.59 ± 0.19	0.66 ± 0.20‡	0.60 ± 0.20	0.66 ± 0.22‡	0.63 ± 0.17	0.030
Inflammatory markers													
leukocyte count (mm^{-1})	6032 ± 1537	6227 ± 1632	6193 ± 1714	6060 ± 1477	5815 ± 1404	5868 ± 1415	0.108	6078 ± 1606	6188 ± 1722	6094 ± 1592	5881 ± 1394	5918 ± 1344	0.464
hs-CRP (mg/L)	0.5 (0.3 / 1.1)	0.5 (0.2 / 1.1)	0.5 (0.2 / 1.1)	0.4 (0.2 / 1.1)	0.5 (0.3 / 0.9)	0.6 (0.3 / 1.2)	0.633	0.4 (0.2 / 1.0)	0.5 (0.3 / 1.2)	0.5 (0.2 / 1.1)	0.5 (0.3 / 0.9)	0.6 (0.3 / 1.2)	0.204

TC indicates total cholesterol; LDL, low-density lipoprotein; HDL, high-density lipoprotein; TG, triglyceride; MDA, malondealdehyde-modified; Lp, lipoprotein; RLP, remnant–like particle; VLDL, very low-density; apo, apolipoprotein; and hs-CRP, high-sensitivity c-reactive protein. ANOVA and post hoc tests with Bonferroni correction were performed to test between-group differences. $^{\dagger}P < 0.05$, $^{\ddagger}P < 0.01$, $^{\S}P < 0.001$ versus Q1, $^{\#}P < 0.05$ versus Q2, $^{\P}P < 0.05$ versus Q3. *Median; interquartile range in parentheses.

Table III. Multi-logistic Regression Analysis of Observed Risk of CAD

	Quintile of EPA/AA	Odds ratio (95%CI) Model 1	*P*	Odds ratio (95%CI) Model 2	*P*	Odds ratio (95%CI) Model 3	*P*
A	1Q*						
	2Q	0.748 (0.392-1.430)	0.380	1.005 (0.460-2.194)	0.991	0.944 (0.423-2.160)	0.888
	3Q	1.003 (0.533-1.887)	0.993	1.259 (0.585-2.708)	0.552	1.142 (0.506-2.578)	0.750
	4Q	0.841 (0.440-1.608)	0.600	0.778 (0.354-1.710)	0.531	0.679 (0.283-1.629)	0.386
	5Q	0.482 (0.245-0.946)	0.034	0.418 (0.185-0.947)	0.037	0.328 (0.113-0.956)	0.041
B	1Q*						
	2Q	0.828 (0.444-1.523)	0.534	0.699 (0.332-1.471)	0.346	0.758 (0.349-1.645)	0.483
	3Q	0.671 (0.354-1.274)	0.223	0.771 (0.357-1.666)	0.508	0.870 (0.376-2.016)	0.746
	4Q	0.714 (0.380-1.340)	0.294	0.915 (0.426-1.964)	0.819	1.078 (0.444-2.621)	0.868
	5Q	0.437 (0.223-0.856)	0.016	0.390 (0.175-0.870)	0.021	0.490 (0.178-1.353)	0.169
	2Q	0.828 (0.444-1.523)	0.534	0.699 (0.332-1.471)	0.346	0.758 (0.349-1.645)	0.483

CAD indicates coronary artery disease; EPA, eicosapentaenoic acid; DHA, docosahexaenoic acid; AA, arachidonic acid; and CI, confidence interval. Model 1: adjusted for the age and gender. Model 2: adjusted for the age, gender, body mass index, hypertension (+/-), diabetes mellitus (+/-), smoking (+/-), CKD (+/-), serum levels of LDL-C, HDL-C, and log TG, and statin use. Model 3: A: adjusted for the same variables as model 2 + the serum DHA level, B : adjusted for the same variables as model 2 + the serum EPA level. EPA/AA: Q 1 (CAD: *n* = 26, 20.0%), Q 2 (CAD: *n* = 27, 21.0%), Q 3 (CAD: *n* = 35, 24.5%), Q 4 (CAD: *n* = 31, 21.7%), Q 5 (CAD: *n* = 31, 19.0%); DHA/AA: Q 1 (CAD: *n* = 29, 22.3%), Q 2 (CAD: *n* = 30, 23.1%), Q 3 (CAD: *n* = 29, 22.3%), Q 4 (CAD: *n* = 32, 24.6%), Q 5 (CAD: *n* = 24, 18.0%) *Reference.

creased following treatment with EPA preparations, resulting in a greater increase of the LDL particle size.[21)]

To date, however, very few reports have been published concerning the relationship between elevation of the EPA/AA ratio and the improvement of HDL metabolism. As stated above, we cannot rule out the possibility that elevation of the serum EPA/AA ratio may indirectly improve the HDL metabolism, mediated by improvement of the TG metabolism.[19)] Among others, the serum level of apo-A-1, a major HDL carrier protein, has attracted close attention as a functional HDL on the grounds that the antiatherosclerotic activity of HDL-C is enhanced in the presence of high serum apo-A-1 levels.[22)] No consensus has been reached in regard to the effects on the serum HDL-C in intervention trials using EPA and/or DHA preparations.[7,8)] However, some of the trials reported an increase of the serum level of HDL_3, which is said to be an HDL subclass with a potent anti-atherogenic effect. Moreover, n-3PUFAs have the effect of reducing the catabolism of an HDL-apoA-1 complex, and their anti-atherogenic effect may be promoted by the prolonged presence of HDL-apoA-1 complexes in the blood.[23-25)] These are some of the speculations to explain the mechanism of improvement of HDL metabolism in individuals with high EPA/AA ratios.

EPA has anti-inflammatory activity, while AA has pro-inflammatory activity. Therefore, elevation of the EPA/AA ratio may result in a greater degree of amelioration of systemic inflammation.[13)] Although leukocyte count does not reflect all inflammatory reactions, the results of this study may be interpreted as suggesting the existence of a relationship between a high EPA/AA ratio and anti-inflammatory activity. Also, a meta-analysis of prospective cohort studies designed to analyze the onset of CAD in relation to the peripheral leukocyte count revealed the existence of a positive correlation between elevation of the leukocyte count and the incidence of CAD.[26)] Progression of atherosclerosis may involve white blood cells. Conversely, we reported previously that a decrease of the leukocyte count was involved in the regression of coronary artery plaques.[27)] Furthermore, in an American Heart Association (AHA) Scientific Statement, leukocyte count is proposed as a marker of inflammation that is predictive of cardiovascular risk.[28)]

EPA intake is associated with anti-inflammatory and anti-platelet effects; conversely, a high AA intake leads to a high content of AA in phospholipids, which are components of cell membranes, inducing the production of strong pro-inflammatory substances and biologically active substances with strong platelet aggregation activity, although the precise mechanisms involved have not been clarified. This indicates the possible existence of an antagonistic relationship between the physiological activities of EPA and AA,[13)] and it is speculated that a high serum EPA/AA ratio may have a protective effect against the development of CAD, based on its effect on the rate of progression of arteriosclerosis. According to recent reports, a low EPA/AA ratio is involved somehow in the mechanism of onset of ACS and coronary stent thrombosis.[14,16,29,30)]

The subjects of the present study were patients with stable CAD, however, the results suggest the possibility that a high EPA/AA ratio may protect against the development of CAD. In a recent report, we indicated that the prevalence of CAD was lower in a population with higher serum EPA and DHA levels, although it was not possible in that study to stratify the risk for CAD according to the EPA and DHA levels.[6)] However, we may now say that the n-3 PUFA/AA ratio is probably useful in stratification of the risk for CAD.

Study limitations: First, this was a cross-sectional study, which made it difficult to establish a cause-effect relationship. Second, we must say that this was designed as a cross-sectional study, with the timing of onset of CAD varying from case to case. Therefore, it must be borne in mind that there would have been patients who improved their lifestyle (primarily dietary style) after onset of CAD. Thus, we cannot arrive at any conclusion from this study, except to state that the prevalence of CAD is probably lower in populations with a high EPA/AA ratio. Third, because determination of the presence of CAD in this study population relied on the findings of coronary angiography, the existence of subjects in the study population of

Table IV. Multi-regression Analysis of the Relationship between n-3 PUFAs/A Ratios and Atherogenic-related Markers

		Coefficient (β)	S.E	95% CI	*P*
Lipid-related					
TC	EPA/AA	0.035	8.146	-11.618 / 20.317	0.596
	DHA/AA	0.014	6.645	-11.646 / 14.455	0.833
LDL-C	EPA/AA	-0.059	6.810	-19.331 / 7.421	0.382
	DHA/AA	0.047	5.603	-7.037 / 14.974	0.479
HDL-C	EPA/AA	0.391	3.388	14.999 / 28.307	< 0.0001
	DHA/AA	-0.312	2.761	19.877 / -9.031	< 0.0001
TG*	EPA/AA	-0.492	0.040	-0.396 / -0.240	< 0.0001
	DHA/AA	0.474	0.033	0.184 / 0.322	< 0.0001
non HDL-C	EPA/AA	-0.138	7.733	-31.271 / -0.839	0.039
	DHA/AA	0.157	6.287	2.809 / 27.504	0.016
MDA-LDL	EPA/AA	-0.153	10.200	-42.200 / -2.148	0.030
	DHA/AA	0.292	8.279	11.721 / 44.277	0.001
Lp (a)*	EPA/AA	0.060	0.103	-0.188 / 0.288	0.411
	DHA/AA	-0.122	0.083	-0.309 / 0.018	0.082
RLP-C*	EPA/AA	-0.340	0.048	-0.342 / -0.154	< 0.0001
	DHA/AA	0.342	0.039	0.131 / 0.281	< 0.0001
VLDL fraction*	EPA/AA	-0.560	1.309	-14.404 / -9.312	< 0.0001
	DHA/AA	0.526	1.071	7.251 / 11.456	< 0.0001
Apolipoprotein					
apo A-1	EPA/AA	0.255	6.450	12.647 / 37.984	< 0.0001
	DHA/AA	-0.195	5.269	-26.581 / -5.584	0.002
apo A-2	EPA/AA	0.088	1.307	-0.781 / 4.353	0.172
	DHA/AA	-0.108	1.068	-3.918 / 0.276	0.089
apo B	EPA/AA	-0.074	4.613	-14.216 / 3.903	0.264
	DHA/AA	0.154	3.768	73.860 / 106.144	0.017
apo C-2	EPA/AA	-0.107	0.411	-1.482 / 0.132	0.100
	DHA/AA	0.238	0.335	0.598 / 1.915	0.0002
apo C-3	EPA/AA	-0.007	0.740	-1.526 / 1.382	0.923
	DHA/AA	0.123	0.605	-0.052 / 2.352	0.061
apo E	EPA/AA	-0.056	0.255	-0.717 / 0.285	0.397
	DHA/AA	-0.197	0.208	0.227 / 1.045	0.002
Lipid, apo ratio					
LDL-C/HDL-C	EPA/AA	-0.287	0.170	-1.059 / -0.392	< 0.0001
	DHA/AA	0.225	0.139	0.206 / 0.754	0.0006
apo B/apo A-1	EPA/AA	-0.202	0.044	-0.219 / -0.047	0.0025
	DHA/AA	0.218	0.036	0.050 / 0.190	0.0008
Inflammatory markers					
hs-CRP*	EPA/AA	-0.122	0.114	-0.432 / 0.016	0.068
	DHA/AA	0.131	0.093	0.003 / 0.368	0.046
leukocyte count	EPA/AA	-0.146	0.340	-1.421 / -0.084	0.027
	DHA/AA	0.010	0.278	-0.504 / 0.589	0.879

Abbreviations are as in Table II. SE indicates standard error; CI, confidence interval; adjusted for the age, gender, body mass index, hypertension (+/-), diabetes mellitus (+/-), smoking (+/-), lipid disorder (+/-), CKD (+/-), and statin use. The serum EPA/AA ratio was log-transformed. *tested after log-transformed.

undetected cases of asymptomatic CAD which can be diagnosed primarily by an exercise stress test or non-invasive tests[31] such as coronary artery computed tomography cannot be ruled out. Fourth, because this study was a pilot cross-sectional single-center study involving a relatively small number of subjects and there were missing test data for 51 of the 700 consecutive cases, we cannot rule out the existence of case selection bias. Furthermore, the multivariate analysis carried out to identify the correlations between the serum EPA/AA and DHA/AA ratios and atherosclerosis-related markers in the present study was nothing more than a comparison between these two parameters and the results of this analysis do not allow arrival at the conclusion that atherosclerosis-related markers are higher in populations with a higher DHA/AA ratio.

Conclusions: This cross-sectional study suggested that populations with higher serum EPA/AA ratios, but not higher serum DHA/AA ratios, had a lower prevalence of CAD as compared to populations with lower EPA/AA ratios. Furthermore, elevation of the serum EPA/AA ratio might be associated with improved HDL and TG metabolism and ameliorated inflammatory reactions.

Disclosure

The authors have no financial conflicts of interest to disclose.

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Table V. Summary of the Associations between High Serum n-3PUFAs/AA Ratios and Shifts in the Serum Levels of Atherosclerosis-related Markers

High levels of the n-3PUFAs / AA	EPA/AA	DHA/AA
TC	NS	NS
LDL-C	NS	NS
HDL-C	↑	↓
TG	↓	↑
non HDL-C	↓	↑
MDA-LDL	↓	↑
Lp (a)	NS	NS
RLP-C	↓	↑
VLDL fraction	↓	↑
apo A-1	↑	↓
apo A-2	NS	NS
apo B	NS	↑
apo C-2	NS	↑
apo C-3	NS	↑
apo E	NS	↓
LDL-C/HDL-C	↓	↑
apo B/apo A-1	↓	↑
hs-CRP	NS	↑
leukocyte count	↓	NS

Effects of serum EPA/AA and DHA/AA on atherosclerosis-related markers: increase ↑ ($\beta > 0$), decrease ↓ ($\beta < 0$) (refer to Table IV).

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