



Association Between Serum Levels of n-3 Polyunsaturated Fatty Acids and Coronary Plaque Detected by Coronary Computed Tomography Angiography in Patients Receiving Statin Therapy

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Background: Intensive lipid-lowering therapy with statins reduces cardiovascular events, but residual cardiovascular risks remain. Intake of n-3 polyunsaturated fatty acids (PUFAs) has been associated with cardiovascular events. We examined the relationships between serum n-3 PUFAs and coronary atherosclerotic findings on computed tomography angiography (CTA) in patients undergoing statin treatment.

Methods and Results: We enrolled 172 subjects (mean age: 68.2 years; 64% men) prior to statin treatment for 6 months. Serum PUFAs, including eicosapentaenoic acid (EPA), docosahexaenoic acid (DHA), and arachidonic acid, were measured. When the patients were divided into 2 groups according to the median EPA level (61.3 $\mu\text{g/ml}$), the low-EPA group showed higher incidences of 3-vessel plaque involvement (62% vs. 43%, $P=0.015$), noncalcified plaques (NCPs) (74% vs. 52%, $P=0.0016$), extensive NCPs (≥ 2 segments) (56% vs. 34%, $P=0.0036$), and high-risk plaques (minimum CT density < 39 HU and remodeling index > 1.05) (43% vs. 22%, $P=0.0034$). Multivariate analyses revealed that low EPA levels were an independent factor for these coronary plaque findings. The DHA levels were not independently associated with these findings.

Conclusions: Low serum EPA level, but not serum DHA, is associated with the presence and extent of NCPs and high-risk plaques detected by coronary CTA in patients undergoing lipid-lowering therapy with statins. (*Circ J* 2013; **77**: 2578–2585)

Key Words: Coronary computed tomography angiography; Coronary plaques; Eicosapentaenoic acid; N-3 polyunsaturated fatty acid

Several interventional studies have reported that lipid-lowering therapy with statins reduces cardiovascular morbidity and mortality^{1,2} and induces plaque regression and stabilization.^{3,4} Despite the fact that intensive statin therapy reduces cardiovascular events, residual cardiovascular risks remain.² It has been reported that half of the patients hospitalized with coronary artery disease (CAD) have low-density lipoprotein cholesterol (LDL-C) levels < 100 mg/dl at admission.⁵

(PUFAs) is associated with cardiovascular events.^{6–8} Of the PUFAs, eicosapentaenoic acid (EPA) and docosahexaenoic acid (DHA) are independent predictors of cardiovascular health,⁹ with some differential cardiovascular benefits between EPA and DHA having been reported.^{10,11} However, it remains unclear whether these differences apply to patients with CAD.

Coronary computed tomography angiography (CTA) has enabled detection of coronary stenosis and coronary plaques. It also has potential for assessing the characteristics of coronary plaque. The presence of noncalcified or mixed plaques on coronary CTA has been found to be the strongest predictor of events.¹² It has also been reported that positive vascular remodeling, low-attenuation plaque content, and adjacent coro-

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Previous epidemiological and interventional studies have demonstrated that the intake of n-3 polyunsaturated fatty acids

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nary plaque calcium coexist in the culprit lesions of acute coronary syndrome (ACS).^{13,14}

Previous serial volumetric intravascular ultrasonographic (IVUS) analyses have shown that early aggressive lipid-lowering therapy by atorvastatin for 6 months significantly reduced the plaque volume in patients with ACS.¹⁵ In addition, we reported that CTA-detected low-density plaques (LDPs) occur less frequently in patients who have undergone intensive statin pretreatment.¹⁶ The current study aimed to investigate the residual cardiovascular risks after statin therapy by examining the relationship between serum n-3 PUFA levels and coronary plaque findings on CTA. We evaluated the presence, extent, and characteristics of coronary plaques in patients under statin treatment.

Methods

Subjects

From June 2010 to June 2012, we examined 230 consecutive subjects who underwent coronary CTA before they started a ≥ 6 -month statin therapy regimen. Exclusion criteria included poor CTA images because of irregular heart rhythm or inadequate contrast concentration (n=8), previous coronary revascularization including percutaneous coronary intervention or coronary artery bypass grafting (n=40), missing relevant information (eg, traditional coronary risk factors or laboratory data [n=3]), and already receiving EPA therapy (n=7). Ultimately, 172 patients were analyzed.

Risk Factor Assessment and Laboratory Tests

Medical histories were obtained for all patients. Hypertensive patients were defined as those whose systolic and diastolic blood pressures were $\geq 140/90$ mmHg or who had already received antihypertensive therapy. Fasting blood samples were obtained from the antecubital vein before coronary CTA. Diabetes mellitus was defined as hemoglobin A_{1c} (HbA_{1c}) $\geq 6.5\%$ and/or current use of hypoglycemic agents.¹⁷ Current smokers were defined as subjects who smoked regularly at the time of coronary CTA. The serum lipid profile (LDL-C, high-density lipoprotein cholesterol [HDL-C], and triglycerides), and the fasting blood glucose, HbA_{1c}, and serum C-reactive protein (CRP) concentrations were measured in the hospital's laboratory. Serum levels of PUFAs, including EPA, DHA, and arachidonic acid (AA), were measured by gas chromatography at an external laboratory (SRL, Tokyo, Japan).

Coronary CT Scan Protocol and Image Reconstruction

Coronary CT examinations were performed using a 64-slice CT scanner (LightSpeed VCT; GE Healthcare, Waukesha, WI, USA). If the patients' resting heart rate was >60 beats/min, a β -blocker (metoprolol 20–40 mg p.o.) was administered at 60 min before the CT scan to avoid motion artifacts. All patients received 0.3 mg of nitroglycerin just before the CT scan. Prior to a contrast-enhanced scan, a noncontrast scan was performed to measure the Agatston coronary artery calcium score, which was then analyzed using SmartScore software, version 4.0 (GE Healthcare).¹⁸ Following a test bolus examination to determine the start of the contrast-enhanced scan, a retrospective ECG-gated scan using the helical mode based on our previous report¹⁹ or prospective ECG-triggered CTA (center of the imaging window corresponding to 75% of the R-R interval)²⁰ was performed during an inspiratory breath-hold. A body weight-adjusted volume (0.6 ml/kg) of iodine contrast material was injected into the antecubital vein over the course of 10 s, followed by a 25-ml saline solution chas-

er at 5.0 ml/s.

Coronary CTA image reconstruction was performed with image-analysis software (Card IQ Xpress; GE Healthcare) on a dedicated computer workstation (Advantage Workstation Version 4.4; GE Healthcare).

Evaluation of Coronary CTA

Two blinded, experienced observers assessed the coronary CTA images. Using the 18-segment Society of Cardiovascular Computed Tomography model,²¹ all coronary segments >2 mm in diameter were evaluated on curved multiplanar reformation and cross-sectional images in a direction perpendicular to the vessel's center line. A "significant" stenotic lesion in the epicardial coronary arteries was defined as cross-sectional stenosis of $>50\%$, regardless of whether it was in a calcified or noncalcified area.

Coronary plaque was defined as tissue that was >1 mm² in size, located within the vessel wall, and clearly distinguishable from the contrast-enhanced coronary lumen and surrounding pericardial tissue. Based on our previous report,²² coronary plaque was classified into 2 types: calcified (CP) and noncalcified (NCP). CP was defined as having only high CT density compared with the contrast-enhanced lumen. NCP was defined as having any distinguishable region with a low-density area >1 mm² in size. Low density was defined as CT density <130 Hounsfield units (HU), but greater than that of the surrounding pericardial tissue.

When coronary plaque was present in all 3 vessels (left anterior descending artery including the left main trunk, left circumflex artery, and right coronary artery), we defined it as 3-vessel plaque involvement. With regard to the extent of CP or NCP, we defined it as a binary variable: extensive plaque was diagnosed when present in 2 or more segments, and focal plaque was diagnosed when present in 1 or part of 1 segment.

NCP Characteristics

Based on our previous reports,^{13,19} NCP characteristics were evaluated by determining the minimum CT density and the vascular remodeling index. The minimum CT density was assessed to be the lowest CT density of at least 5 regions of interest (area: 1 mm²) in each noncalcified region. LDP was defined as a lesion with minimum CT density <39 HU. The vascular remodeling index was calculated by dividing the cross-sectional vessel lesion area by the proximal cross-sectional vessel reference area. Positive remodeling (PR) was defined as a remodeling index >1.05 . Plaques with both features (LDP+PR) were identified as high risk.^{13,16}

Statistical Analysis

Categorical variables are presented as the number of patients and percentage. Triglycerides, CRP, and the Agatston score are reported as the median (interquartile range), and other continuous data are presented as the mean \pm SD. The differences between 2 groups were analyzed using Pearson's χ^2 test for categorical variables and Student's t-test or Wilcoxon's test for continuous variables. Univariate logistic analyses were performed to assess the associations between serum levels of n-3 PUFAs (EPA, DHA, and EPA+DHA), age, sex, hypertension, diabetes mellitus, current smoking, and serum levels of LDL-C and HDL-C with the coronary plaque findings on coronary CTA, comprising 3-vessel plaque involvement, presence and extent (≥ 2 segments) of NCPs, and presence of high-risk coronary plaques (LDP+PR). Age, sex, and all variables with values of $P < 0.05$ on univariate analyses were considered in the

Table 1. Baseline Characteristics According to Serum Levels of EPA, DHA, and AA

Variable	Low-EPA group (≤61.3 μg/ml) (n=86)	High-EPA group (>61.3 μg/ml) (n=86)	P value	Low-DHA group (≤145.5 μg/ml) (n=86)	High-DHA group (>145.5 μg/ml) (n=86)	P value	Low-AA group (≤173.8 μg/ml) (n=86)	High-AA group (>173.8 μg/ml) (n=86)	P value
Age (years)	67.8±9.0	68.5±6.4	0.96	68.8±8.8	67.5±6.7	0.12	70.9±6.9	66.4±8.3	0.0047
Male, n (%)	58 (67)	52 (61)	0.34	57 (66)	53 (62)	0.53	58 (67)	52 (60)	0.34
BMI (kg/m ²)	24.9±3.5	24.4±3.3	0.29	24.3±3.1	25.1±3.6	0.28	24.3±3.3	25.0±3.4	0.26
Hypertension, n (%)	69 (80)	66 (77)	0.58	67 (78)	68 (79)	0.85	70 (81)	65 (76)	0.35
Diabetes mellitus, n (%)	40 (47)	37 (43)	0.65	39 (45)	38 (44)	0.88	38 (44)	39 (45)	0.88
Current smoking, n (%)	22 (26)	12 (14)	0.060	20 (23)	14 (16)	0.25	15 (17)	19 (22)	0.44
Total cholesterol (mg/dl)	174.0±28.4	183.7±29.6	0.055	168.1±25.1	189.6±29.4	<0.0001	166.3±26.2	191.4±26.9	<0.0001
LDL-C (mg/dl)	100.3±23.5	99.4±27.5	0.44	94.2±22.3	105.5±27.4	0.0047	91.6±24.4	108.0±24.1	<0.0001
HDL-C (mg/dl)	56.5±15.3	65.4±18.2	0.00070	59.5±15.8	62.4±18.7	0.39	59.9±18.2	62.0±16.5	0.27
Triglycerides (mg/dl) [25–75% quartiles]	114.0 [82.0–165.3]	127.5 [81.0–172.0]	0.48	97.5 [73.8–132.8]	146.5 [108.0–210.5]	<0.0001	106.0 [75.0–136.0]	142.5 [103.3–210.8]	<0.0001
Hemoglobin A _{1c} (%)	6.23±1.05	6.14±0.94	0.60	6.17±0.93	6.19±1.06	0.74	6.12±0.94	6.25±1.05	0.48
CRP (mg/dl) [25–75% quartiles]	0.06 [0.03–0.12]	0.06 [0.03–0.10]	0.90	0.05 [0.03–0.11]	0.06 [0.03–0.11]	0.46	0.06 [0.03–0.12]	0.05 [0.02–0.10]	0.46
Intensive statin*, n (%)	70 (81)	61 (71)	0.11	69 (80)	62 (72)	0.21	63 (73)	68 (79)	0.37
ACEI or ARB, n (%)	46 (54)	41 (48)	0.45	49 (57)	38 (44)	0.09	49 (57)	38 (44)	0.09
Aspirin, n (%)	18 (21)	14 (16)	0.43	16 (19)	16 (19)	1.00	13 (15)	19 (22)	0.24
Agatston CACS [25–75% quartiles]	137 [10–444]	101 [9–430]	0.63	162 [10–503]	97 [10–374]	0.31	144 [10–481]	97 [9–352]	0.40

*Intensive statin=atorvastatin, rosuvastatin and pitavastatin. Data expressed as number (percent), mean±SD or median (interquartile range). ACEI, angiotensin-converting enzyme inhibitor; ARB, angiotensin II receptor blocker; BMI, body mass index; CACS, coronary artery calcium score; CRP, C-reactive protein; DHA, docosahexaenoic acid; EPA, eicosapentaenoic acid; HDL-C, high-density lipoprotein cholesterol; LDL-C, low-density lipoprotein cholesterol.

multivariate analyses of each coronary plaque finding on coronary CTA. Spearman correlation analyses were used to estimate the correlation between EPA or AA and the EPA/AA ratio. Receiver-operating characteristic (ROC) curves were constructed from regression models. ROC analyses were used to compare the prediction accuracies for the presence of NCPs by the serum EPA levels and the EPA/AA ratio. Incremental improvements were compared after adding either the serum EPA level or the EPA/AA ratio to the HDL-C level. Values of $P<0.05$ were considered statistically significant in all instances. All statistical analyses were performed using JMP 10 software (SAS Institute, Tokyo, Japan).

Results

Baseline Characteristics

The mean age of the 172 study subjects was 68.2 ± 7.8 years, and 64% of the subjects were men. The prevalence of coronary risk factors was as follows: hypertension (79%), diabetes mellitus (45%) and current smoking (20%). The mean body mass index was 24.7 ± 3.4 kg/m². The serum levels of LDL-C and HDL-C were 99.8 ± 25.5 and 61.0 ± 17.3 mg/dl, respectively. The levels of AA, EPA, DHA, and EPA+DHA were 182.1 ± 47.6 , 74.1 ± 41.0 , 151.6 ± 54.0 , and 225.8 ± 86.8 μg/ml, respectively.

The clinical characteristics of the 2 groups were compared according to the median serum level of EPA (61.3 μg/ml), DHA (145.5 μg/ml) or AA (173.8 μg/ml) (Table 1). Patients in the low-DHA group had significantly lower levels of serum total cholesterol, LDL-C, and triglycerides than those in the high-DHA group. Likewise, patients in the low-AA group had significantly lower levels of serum total cholesterol, LDL-C, and triglycerides than those in the high-AA group. In contrast, patients in the low-EPA group had significantly lower levels

of HDL-C than those in the high-EPA group. The subjects in the low-AA group were significantly older than those in the high-AA group. Medication use or Agatston coronary calcium scores did not differ significantly between groups based on the EPA, DHA, and AA levels.

Coronary CTA

Of the 2576 segments in the 172 subjects, significant (>50%) stenotic lesions were detected in 201 segments of 80 patients (47%). Among all subjects, any type of coronary plaque was found in 832 segments (33%) of 146 subjects (85%). CPs were visualized in 629 segments of 134 (78%) subjects and NCPs in 280 segments of 108 (63%) subjects. LDPs with PR were observed in 73 segments of 56 (33%) subjects.

Coronary CTA Findings: Low vs. High EPA, DHA, and AA Groups

There were no significant differences with regard to the prevalence of significant stenosis, any plaque, and CPs between the 2 groups stratified by EPA levels. The frequency of 3-vessel plaque involvement (62% vs. 43%, $P=0.015$), presence of NCPs (74% vs. 52%, $P=0.0016$), and extent of NCPs (≥2 segments) (56% vs. 34%, $P=0.0036$) were significantly higher in the low-EPA group than in the high-EPA group. LDPs with PR were also observed more often in the low-EPA group than in the high-EPA group (43% vs. 22%, $P=0.0034$) (Figure 1A). As shown in Figure 1B, the frequency of significant stenosis (56% vs. 37%, $P=0.014$) and presence of NCPs (71% vs. 55%, $P=0.027$) were significantly higher in the low-DHA group than in the high-DHA group. There were no significant differences in other coronary plaque findings. There were no significant differences in the coronary CTA findings between the low-AA and high-AA groups (Figure 1C).

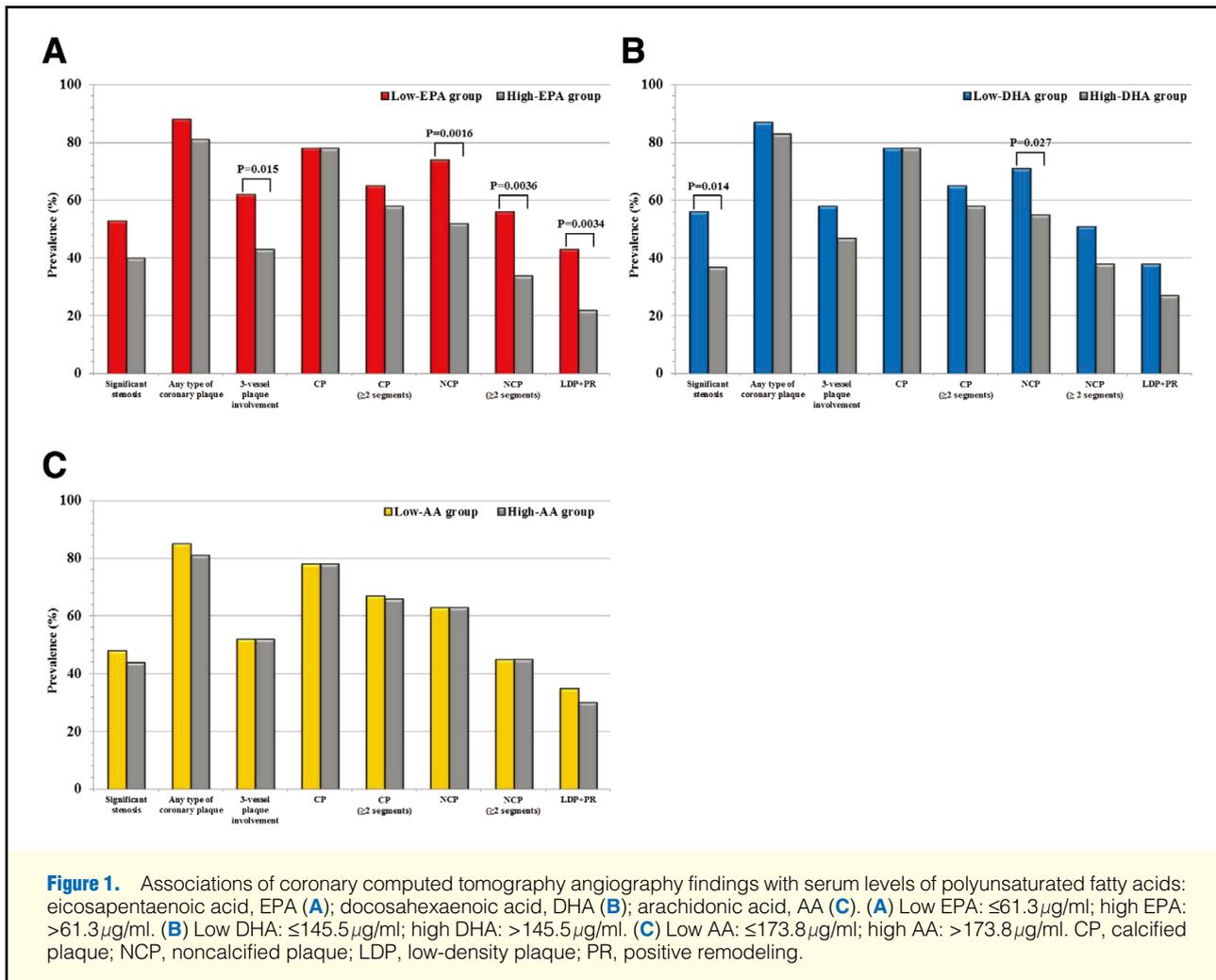


Table 2. Univariate Analyses of Coronary Plaque Findings By Computed Tomography Angiography

	3-vessel plaque involvement		NCP		Extensive NCP (≥ 2 segments)		LDP with PR	
	OR (95% CI)	P value	OR (95% CI)	P value	OR (95% CI)	P value	OR (95% CI)	P value
Serum EPA, \leq median (61.3 $\mu\text{g/ml}$)	2.13 (1.16–3.94)	0.015	2.78 (1.47–5.34)	0.0018	2.48 (1.35–4.64)	0.0039	2.66 (1.38–5.25)	0.0039
Serum DHA, \leq median (145.5 $\mu\text{g/ml}$)	1.60 (0.88–2.93)	0.13	2.02 (1.08–3.83)	0.028	1.68 (0.92–3.10)	0.093	1.71 (0.90–3.28)	0.11
Serum EPA+DHA, \leq median (198.9 $\mu\text{g/ml}$)	1.71 (0.90–3.28)	0.051	3.65 (1.86–7.47)	0.0002	2.13 (1.15–3.97)	0.016	2.83 (1.48–5.52)	0.0019
Age, per 10 years	1.29 (0.88–1.92)	0.20	1.03 (0.69–1.53)	0.89	1.10 (0.75–1.63)	0.62	1.00 (0.66–1.51)	0.99
Sex, men	1.74 (0.93–3.28)	0.083	3.62 (1.89–7.06)	< 0.0001	2.26 (1.19–4.39)	0.0013	1.64 (0.84–3.33)	0.15
Hypertension	1.39 (0.67–2.90)	0.38	0.89 (0.41–1.88)	0.77	0.94 (0.45–1.97)	0.87	1.66 (0.75–4.00)	0.22
Diabetes mellitus	1.89 (1.03–3.51)	0.039	1.31 (0.70–2.46)	0.40	1.27 (0.69–2.33)	0.44	1.52 (0.80–2.90)	0.20
Current smoking	1.62 (0.76–3.56)	0.22	3.38 (1.40–9.52)	0.0059	1.51 (0.71–3.23)	0.29	2.18 (1.01–4.71)	0.047
LDL-C, per 10 mg/dl	0.95 (0.84–1.06)	0.35	0.93 (0.82–1.04)	0.21	1.01 (0.90–1.14)	0.81	0.99 (0.87–1.13)	0.91
HDL-C, per 10 mg/dl	0.89 (0.74–1.06)	0.19	0.77 (0.63–0.92)	0.0043	0.76 (0.62–0.92)	0.0039	0.86 (0.70–1.04)	0.13
Triglycerides, per 10 mg/dl	1.02 (0.99–1.07)	0.17	1.04 (0.99–1.09)	0.067	1.03 (0.99–1.08)	0.060	0.99 (0.96–1.03)	0.77

CI, confidence interval; LDP, low-density plaque; NCP, noncalcified plaque; OR, odds ratio; PR, positive remodeling. Other abbreviations as in Table 1.

Table 3. Multivariate Analysis Between Serum Polyunsaturated Fatty Acids and Coronary Plaque Findings By Computed Tomography Angiography

	Serum EPA ≤median (61.3 μg/ml)		Serum DHA ≤median (145.5 μg/ml)		Serum EPA+DHA ≤median (198.9 μg/ml)	
	OR (95% CI)	P value	OR (95% CI)	P value	OR (95% CI)	P value
3-vessel plaque involvement*	2.12 (1.14–4.03)	0.018	1.51 (0.81–2.82)	0.19	1.83 (0.97–3.48)	0.061
NCP†	2.36 (1.18–4.83)	0.016	1.84 (0.94–3.68)	0.077	3.51 (1.70–7.59)	0.0006
Extensive NCP ≥2 segments‡	2.15 (1.13–4.15)	0.020	1.56 (0.83–2.95)	0.16	1.93 (1.02–3.67)	0.044
LDP with PR§	2.47 (1.27–4.92)	0.0077	1.59 (0.82–3.10)	0.17	2.68 (1.38–5.29)	0.0034

Multivariate: age, sex and all variables with a P value <0.05 on univariate analyses were considered in each coronary plaque finding on CTA. *3-vessel plaque involvement adjusted for age, sex and diabetes mellitus. †NCP adjusted for age, sex, current smoking and serum levels of HDL-C. ‡Extensive NCP adjusted for age, sex and serum levels of HDL-C. §LDP with PR adjusted for age, sex and current smoking. CTA, computed tomography angiography. Other abbreviations as in Tables 1,2.

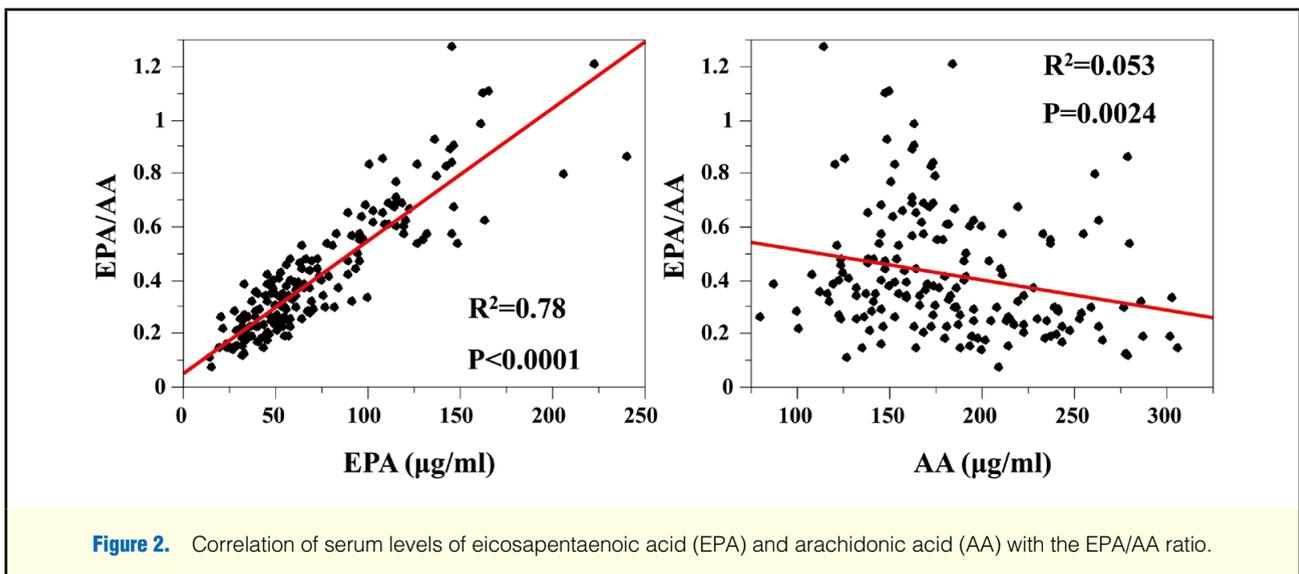


Figure 2. Correlation of serum levels of eicosapentaenoic acid (EPA) and arachidonic acid (AA) with the EPA/AA ratio.

CTA Associations Between Serum PUFAs and Coronary Plaque Findings

Univariate analyses of the associations among age, sex, traditional coronary risk factors, serum levels of LDL-C, HDL-C, and triglycerides, and the coronary plaque findings are shown in **Table 2**. Low EPA ($\leq 61.3 \mu\text{g/ml}$) and diabetes mellitus were significantly associated with 3-vessel plaque involvement. The presence of NCPs was significantly associated with low EPA, low DHA ($\leq 145.5 \mu\text{g/ml}$), low EPA+DHA ($\leq 198.9 \mu\text{g/ml}$), male sex, current smoking, and decreased serum levels of HDL-C. Low EPA, low EPA+DHA, male sex, and decreased serum levels of HDL-C were associated with extensive NCPs. Low EPA, low EPA+DHA, and current smoking were significantly associated with the presence of LDPs with PR. Multivariate analyses showed that low EPA ($\leq 61.3 \mu\text{g/ml}$) was independently associated with 3-vessel plaque involvement (odds ratio [OR]=2.12, 95% confidence interval [95% CI]=1.14–4.03; $P=0.018$), presence of NCPs (OR=2.36, 95% CI=1.18–4.83; $P=0.016$), extent of NCPs (≥ 2 segments) (OR=2.15, 95% CI=1.13–4.15; $P=0.020$), and LDPs with PR (OR=2.47, 95% CI=1.27–4.92; $P=0.0077$). In contrast, low serum DHA was not independently associated with any type of plaque finding on CTA. When stratified by the serum EPA+DHA level, low serum EPA+DHA (median: $198.9 \mu\text{g/ml}$) was an independent factor for the presence and extent of NCPs (presence of

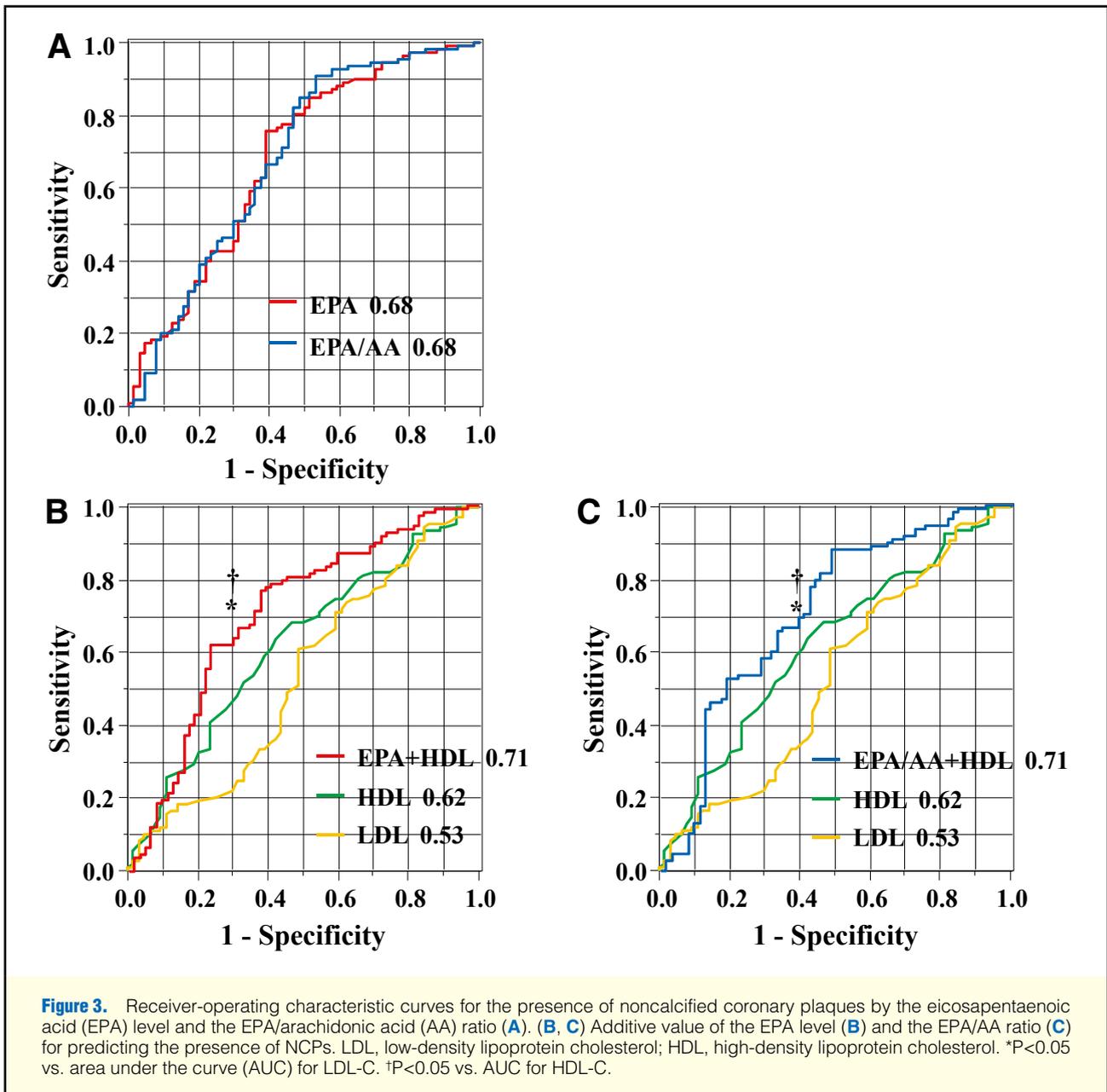
NCPs: OR=3.51, 95% CI=1.70–7.59; $P=0.0006$; extent of NCPs: OR=1.93, 95% CI=1.02–3.67; $P=0.044$). For the same stratification, the values for the presence of LDPs with PR were: OR=2.68, 95% CI=1.38–5.29; $P=0.0034$ (**Table 3**).

Value of Serum LDL-C, HDL-C, and EPA Levels and EPA/AA Ratio for Predicting the Presence of NCPs

Figure 2 shows the highly positive correlation between the serum EPA level and the EPA/AA ratio ($R^2=0.78$), but not between the serum AA level and the EPA/AA ratio ($R^2=0.053$). In the ROC analyses, the area under the curve (AUC) values for the serum EPA level and the EPA/AA ratio for predicting the presence of NCPs were equivalent (0.68 vs. 0.68). Moreover, the AUC of HDL-C (0.62) was significantly improved after adding the serum EPA level (0.71) ($P=0.029$) and the serum EPA/AA ratio (HDL-C+EPA/AA ratio) (0.71) ($P=0.023$) (**Figure 3**).

Discussion

In the present study, a low serum EPA level was associated with the extent of NCPs as detected by coronary CTA in proven and suspected CAD patients who had undergone at least 6 months of treatment with a statin. High-risk plaque features of LDPs with PR were also associated with low serum



EPA. These results suggest that even after at least 6 months of statin therapy, there remains a residual risk for coronary atherosclerotic progression that leads to future cardiovascular events, including ACS.

Association of Serum EPA and Coronary Plaque

Epidemiological and interventional studies have reported that the intake and administration of n-3 PUFAs are associated with cardiovascular events.⁶⁻⁸ In fact, the Japanese EPA Lipid Intervention Study (JELIS) reported that EPA administration had the potential to prevent major coronary events in Japanese hypercholesterolemic patients.⁸ Although the relationship between serum n-3 PUFAs and coronary plaque in patients on statin therapy is unclear, possible mechanisms have been demonstrated; EPA was reported to have protective effects against atherosclerosis by reducing platelet aggregation,^{23,24} counterbalancing inflammatory eicosanoids derived from AA,²⁵ and in-

hibiting monocyte adhesion to endothelial cells.²⁶ In addition, metabolic products derived from EPA, such as resolvin E1, have been reported to have anti-inflammatory and tissue-protective effects.^{27,28}

In the present study, we demonstrated that a low serum EPA level was associated with extensive coronary plaque (ie, 3-vessel plaque involvement) as well as the presence and extent of NCPs when adjusted for traditional coronary risk factors and serum LDL-C and HDL-C levels. It has been established that statin treatment is useful for both primary²⁹ and secondary^{1,2} prevention of cardiovascular disease. In the present study, the average LDL-C level was 99.8 mg/dl after more than 6 months of statin treatment. Ueeda et al reported that serum EPA levels were significantly associated with the extent of coronary plaques in patients with acute myocardial infarction.³⁰

The presence of high-risk coronary plaques (ie, LDP+PR) was associated with low serum EPA levels in the present

study. We previously demonstrated that coronary CTA enabled identification and characterization of plaque components more efficiently than IVUS in patients with ACS.^{13,19} Recent studies reported that low serum EPA levels were significantly associated with lipid-rich coronary plaques detected by integrated backscatter IVUS³¹ and vulnerable coronary plaques assessed by coronary angiography.³² Those reports support our finding that LDPs with PR were frequently observed in the low-EPA group.

Taking all the findings into consideration, even when statin treatment reduced the serum LDL-C level to <100 mg/dl, there was still an underlying residual risk of developing ACS in patients with low serum EPA levels. It is possible that administration of EPA may reduce the residual risk of future cardiovascular events in patients who have received statin therapy and have a mean LDL-C <100 mg/dl.

Difference in Effects of Serum EPA and DHA

We have shown in this study that low serum EPA and EPA+DHA levels, but not the DHA level alone, were independently associated with coronary CTA findings, including the extent of coronary plaques and their characteristics. Several interventional studies have demonstrated that treatment with EPA+DHA or EPA alone reduced the number of cardiac events.^{7,8} Administration of DHA alone has not been reported to do so. The mechanisms by which EPA and DHA prevent cardiac events are thought to be as follows. Advanced atherosclerotic plaques appeared to readily incorporate EPA, and a high content of EPA in carotid plaques has been associated with inhibition of inflammation and increased plaque stability.¹⁰ Serum LDL-C levels increased by 8% in patients treated with DHA.³³ It is also reported that a low plasma level of EPA, but not DHA, was an independent predictor for all-cause mortality in patients with acute myocardial infarction.³⁴ The results of those reports are consistent with our CTA results that a low EPA level, but not low DHA, was associated with coronary plaque findings, especially the presence, extent, and characteristics of NCPs.

Diagnostic Value of EPA and the EPA/AA Ratio for Detecting NCPs

The serum level of EPA and the EPA/AA ratio were equally more useful for predicting the presence of NCPs than the serum LDL-C level in patients treated with statins. Currently, statins are commonly prescribed, and reduction of serum LDL-C to <100 mg/dl is the goal recommended for Japanese CAD patients.³⁵ Several studies have reported that the EPA/AA ratio is associated with cardiac events^{36,37} because of the competition between EPA and AA for enzymatic metabolism.²⁵ Our present study showed that the accuracy of the serum EPA level for predicting NCPs was almost equal to that of the EPA/AA ratio. The reason for this equivalency is that the serum level of EPA used for determining the EPA/AA ratio is similar to the serum EPA level alone, but not to the AA level. In the present study, the serum EPA level and the EPA/AA ratio had incremental predictive value for detecting NCPs as well as the serum HDL-C level. Thus, the serum EPA level and the EPA/AA ratio may be useful for identifying high-risk CAD patients who are undergoing statin treatment.

Study Limitations

First, this study had a cross-sectional design with a small number of suspected or proven CAD subjects. Coronary CTA detected coronary plaques in 85% of the subjects and significant CAD in 47%. Thus, our results might not reflect those for

a population with a lower probability of CAD. Furthermore, the usefulness of our results might be limited to the elderly, as our study population consisted of subjects with a mean age of 68.2±7.8 years. Second, the serum levels of PUFAs might be affected by statin therapy because it has been reported that statins directly and indirectly stimulate the $\Delta 5$ -desaturase, $\Delta 6$ -desaturase, and elongase enzyme activities associated with the metabolism of n-3 or n-6 PUFAs.³⁸ Third, the duration of statin treatment was different for each subject. Therefore, the n-3 PUFA levels and the duration of statin treatment might influence the coronary CTA findings. Fourth, no consensus has yet been reached regarding high-risk plaques identified by coronary CTA with regard to vulnerable plaques causing ACS. Finally, it has not been sufficiently established that decreased serum EPA levels are associated with coronary plaque formation in patients treated with statins.

Taking all of the limitations into consideration, we believe that a prospective, interventional study is needed to investigate whether the addition of n-3 PUFAs (especially EPA) to statin treatment can inhibit the appearance of coronary plaques currently being detected by coronary CTA in patients over a broad age range.

Conclusions

A low serum EPA level is associated with the presence and extent of NCPs and the high-risk feature of LDPs with PR, detected by coronary CTA, in patients with suspected or proven CAD who are undergoing statin treatment. These results suggest that there is a residual risk of cardiovascular events in patients receiving statin treatment. Administration of supplemental EPA may prevent plaque progression and alter plaque characteristics.

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Disclosures

Conflict of interest: none.

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