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Relationships of Fatty Acids, Delta-5 Desaturase Activity, and Lipid Profiles in Men with Acute Coronary Syndrome

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Aims: We evaluated the relationship between the ratios of eicosapentaenoic acid and arachidonic acid (EPA/AA), docosahexaenoic acid (DHA)/AA, and delta-5 desaturase activity (D5D) and atherogenic lipid profiles (ALP) and coronary atherosclerosis.

Methods: Polyunsaturated fatty acids (PUFA) and ALP were assessed in 436 men with the first episode of acute coronary syndrome (ACS) not take any lipid-lowering drugs. D5D was estimated as the ratio of AA to dihomo-gamma-linolenic acid (DGLA). These biomarkers were compared between the lower and higher levels of EPA/AA (0.41) or DHA/AA (0.93) according to the levels in Japanese general population. The thrombolysis in myocardial infarction flow (TIMI) grade of the culprit coronary artery was visually estimated during the initial angiography.

Results: Approximately 70% of patients had low EPA/AA or DHA/AA. Serum levels of LDL-cholesterol, apolipoprotein B (apoB), and remnant lipoprotein cholesterol (RL-C) were significantly higher in the low EPA/AA or DHA/AA groups, while those of triglycerides and malondialdehyde-modified LDL (MDA-LDL) were significantly higher in the low EPA/AA group alone. The levels of EPA, EPA/AA, DHA/AA, and HbA1c increased and those of DGLA and apoA1 decreased with increasing number of stenotic vessels. Patients with three stenotic coronary vessels or TIMI grade ≥ 1 had significantly higher EPA levels compared with the others. The levels of LDL-cholesterol, non-HDL-cholesterol, triglycerides, small dense LDL-cholesterol, RL-C, MDA-LDL, apoB, and apoE decreased progressively and those of EPA, DHA, EPA/AA and HDL-cholesterol increased as D5D increased.

Conclusions: The EPA/AA is a superior risk marker than DHA/AA in term of correlation with ALP in ACS patients.

Key words: Eicosapentaenoic acid, Docosahexaenoic acid, Arachidonic acid, Delta-5, Acute coronary syndrome

Introduction

Numerous studies have demonstrated that *n*-3 polyunsaturated fatty acids (PUFA), such as eicosapentaenoic acid (EPA) and docosahexaenoic acid (DHA), which are present in fish oils, protect against cardiovascular diseases (CVDs), and the ratios of serum levels of EPA and DHA to arachidonic acid (AA, *n*-6 PUFA) have been recognized as promising risk markers for coronary artery disease (CAD)[1, 2]. Previous case-control studies including one study from our group[3] have demonstrated that patients, especially younger ones, with both lower EPA/AA and DHA/AA had a greater probability of being acute coronary syndrome (ACS)[3, 4] and/or ischemic stroke[5]. Other cross-sectional studies have demonstrated that EPA/AA but not DHA/AA was significantly associated with ACS[6, 7]. A cohort study of CAD patients

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underwent non-emergency percutaneous coronary intervention (PCI) found that lower EPA/AA (but not lower DHA/AA) was significantly associated with the incidence of major adverse cardiac events[8]. However, between 20–80% of the patients in these studies were on statins[4-8]; therefore, the relationships between these ratios and disease may have been influenced by drug use. A previous study of patients with CAD who underwent PCI demonstrated that pitavastatin (but not pravastatin) increased AA and reduced DHA without affecting EPA levels[9]. Therefore, certain statins may influence the serum levels of *n*-3 and *n*-6 PUFA levels, and it is suggested that EPA/AA may better reflect the residual risk for CAD following statin treatment than DHA /AA.

The Hisayama study, a population-based prospective cohort study of 3,103 community-dwelling Japanese men and women, aged ≥ 40 years, reported that the EPA/AA (but not the DHA/AA) was significantly inversely associated with the incidence of CAD in individuals with serum high sensitivity CRP (hsCRP) levels ≥ 1.0mg/L. However, there was no clear association of either ratio with CAD in the overall population[10]. A recent report of the Hisayama study demonstrated that both EPA/AA and DHA/AA were significantly inversely associated with serum levels of resistin, an adipocyte-derived polypeptide associated with insulin resistance and subsequent atherosclerosis[11]. Therefore, it remains unclear if one of these PUFA ratios is better than the other in predicting risk for CAD.

PUFA levels depend on dietary intake, bioavailability, and PUFA metabolism. In the biosynthesis of long chain PUFA from precursor PUFA, the crucial enzymes include elongase and desaturase[12]. Delta-5 desaturase (D5D) and delta-6 desaturase (D6D) are two key enzymes in the synthesis of long-chain PUFA and are encoded by fatty acid desaturase 1 (FADS1) and FADS2 genes, respectively[12]. Previous studies have reported that the FADS1gene polymorphism (less function) was associated with increased CAD risk[13, 14]. D5D is involved in one step in the conversion of linoleic acid (LA, 18:2 *n*-6) and alpha-linoleneic acid (ALA, 18:3 *n*-3) to AA (20:4 *n*-6) and EPA (20:5 *n*-3), respectively, as the sole enzymatic source of endogenous AA and EPA. EPA and DHA are strongly influenced by the dietary intake of pre-formed PUFA, and, while human can readily retroconvert DHA to EPA, the elongation of ALA to EPA and DHA is minimal[15, 16]. However, contrary results have also been very recently reported[17]. The activities of D5D cannot be measured directly; generally, they are conventionally estimated from the ratio of AA to dihomo-gamma linolenic acid (DGLA, 20:3 *n*-6)[12, 18-20].

Aim

The aim of this study was to compare ratios of EPA/AA and DHA/AA with their respective associations with various atherogenic lipid biomarkers and elucidate how D5D activities may affect the association between EPA, DHA, EPA/AA, or DHA/AA and those biomarkers in patients with ACS who were not treated with any lipid-lowering agents. It is well established that both EPA/AA and DHA/AA are higher in elderly people and lower in patients with CAD compared with those in healthy subjects[3, 21, 22]; therefore, even patients with EPA/AA or DHA/AA higher than the median values in patients with CAD are recognized as those with lower EPA/AA or DHA/AA in healthy elder people. Therefore, the cutoff levels of EPA/AA and DHA/AA have been adopted from the results in the general Japanese population of the Hisayama study[10].

Methods

Study Subjects

This study included 436 consecutive male patients with the first episode of ACS who were not on lipid-lowering drugs and underwent successful PCI on admission at the Showa University Hospital between June 2009 and December 2018. The diagnoses of ACS were based on the clinical symptoms, electrocardiographic changes and coronary angiography (CAG) findings. The thrombolysis in myocardial infarction (TIMI) flow grade of the culprit coronary artery was visually estimated during the initial CAG (TIMI0–TIMI3)[23]. The exclusion criteria included ACS due to coronary spasm, TIMI3 without any stenosis of culprit coronary artery, previous histories of myocardial infarction (MI) and/or PCI, stable CAD, undiagnosed CAD, hemodialysis, infectious disease, malignancy, and any other serious conditions. Non-fasting serum samples were collected immediately before CAG. The institutional review board of Showa University approved this protocol (No. 2855). The investigation conformed to the principles of the Declaration of Helsinki, and the written informed consent was obtained from all subjects.

Baseline Evaluation

Serum concentrations of creatinine, total cholesterol (T-C), triglycerides, high-density lipoprotein cholesterol (HDL-C) and glycated hemoglobin (HbA1c) were measured using standard laboratory procedures. HbA1c values that were measured as HbA1c (JDS) till March 31, 2012, were estimated using the formula $1.019 \times \text{HbA1c [JDS]} + 0.3$ [24].

Serum apolipoprotein (apo) levels were determined using an immunoturbidometric assay (Daiichi Chemicals Co. Ltd. Tokyo, Japan). Malondialdehyde-modified LDL (MDA-LDL) levels were measured using an enzyme-linked immunosorbent assay[25]. Lipoprotein(a) [Lp(a)] levels were measured using a latex agglutination immunoassay. Remnant lipoprotein cholesterol (RL-C) was measured using immunoprecipitation method using immunoaffinity mixed gel containing anti-apo A-I (apoA) and anti-apo B-100 (apoB) monoclonal antibodies, known as remnant-like particle cholesterol (RLP-C) (Japan Immunoresearch Laboratories, Takasaki, Japan) till March 31, 2016 [26]. Thereafter, they were measured using another homogenous assay, known as RemL-C (Kyowa Medex, Tokyo, Japan)[27]. Although the RLP-C and RemL-C were different, they have been reported to have significant positive correlation with each other[28], and we confirmed the correlation between each assay and PUFA as well **(Supplementary Table 1)**. Serum samples were kept frozen at −80℃ until the performing the assays for LDL-C and sdLDL-C that were measured using a direct homogenous assay[29, 30]. The kits used for the LDL-C and sdLDL-C tests were both provided by Denka Seiken. Non-HDL cholesterol (non-HDL-C) level was estimated by subtracting the HDL-C concentration from the T-C concentration. Large buoyant LDL-C (lbLDL-C) levels were estimated by subtracting the sdLDL-C concentration from the LDL-C concentration. The hsCRP level was measured using the Dade Behring BN assay[31]. Plasma brain natriuretic peptide (BNP) levels were measured using chemiluminescence-enzyme immunoassay. The plasma PUFA were measured by gas-liquid chromatography at a commercially available laboratory (SRL Co., Ltd., Tokyo, Japan). The D5D was estimated as AA/DGLA[12, 18-20, 22]. D6D activity could not be estimated because the levels of ALA, LA, stearidonic acid, and gamma-linolenic acid were not measured. Serum creatinine–based estimate of glomerular filtration rate (eGFR) was calculated as follows: $eGFR = 194 \times Cr^{-1.094} \times Age^{-0.287}$ (x0.739 for women)[32].

The diagnosis of hypertension was based on a history of hypertension or blood pressure >140 mmHg systolic or >90 mmHg diastolic[33]. Diabetes mellitus (DM) was diagnosed based on fasting serum glucose value ≥ 126 mg/dL, 2-hour glucose ≥ 200 mg/dL during an oral glucose tolerance test, random serum glucose ≥ 200 mg/dL, HbA1c ≥ 6.5%, or treatment with any hypoglycemic agents[24]. Body mass index (BMI) was calculated as weight (kilograms) divided by height (meters) squared. Patients with a reported smoking habit of at least one cigarette per day at admission were classified as current smokers. The extent of coronary vessel disease (VD) was classified according to the number of stenotic coronary vessels (1VD, 2VD and 3VD) which was defined as stenosis of 50% or greater narrowing of the diameter. The left ventricular ejection fraction (LVEF) were evaluated using the Simpson's method using standard echocardiographic measurements within a few days of hospitalization.

Statistical Analysis

Statistical analyses were performed using SPSS Statistics for Macintosh, Version 23.0. (IBM Corp. Armonk, NY, USA). The patients were classified based on EPA/AA ratio and DHA/AA ratio according to the general Japanese population from the Hisayama study. The median values of the serum EPA/AA ratio and DHA/AA ratio in Hisayama study were 0.41 and 0.93, respectively.

Categorical data are expressed as numbers and percentages, and differences were analyzed using chi-square tests. Normality of distribution was tested with Shapiro-Wilk tests. Differences in continuous variables between two groups were compared using the Mann-Whitney tests because a majority of the variables did not have Gaussian distribution. Comparisons between the quartiles of D5D activities were performed using Kruskal-Wallis tests when variables showed a non-normal distribution, and by one-way analysis of variance (ANOVA) with Tukey's honest significant difference test to identify differences between the groups. The correlations were analyzed using the Spearman tests. Age-adjusted logistic regression analysis was used to assess independent associations between FA and the presence of 3VD. All the statistical analyses were two tailed. $P<0.05$ was considered statistically significant.

Results

Clinical Charcteristics between High and Low EPA/AA or DHA/AA

Table 1 and 2 summarize and compare the general characteristics and biomarkers between the participants with low and high EPA/AA and DHA/AA. Approximately 70% of patients with ACS were classified into the low EPA/AA and DHA/AA groups. Serum levels of LDL-C, non-HDL-C, RL-C, and apoB, and eGFR were significantly higher in the low EPA/AA and low DHA/AA groups, while serum levels of triglycerides, MDA-LDL, and hsCRP were significantly higher in only the low EPA/AA group. The serum BNP level was signicantly higher in the high EPA/AA and low DHA/AA group. The serum levels of HDL-C, sdLDL-C, apoA-1, apoE, Lp(a), and HbA1c were comparable between the two groups.

Table 1. Comparison of clinical characteristics between low and high EPA/AA or DHA/AA

	EPA/AA		DHA/AA	
	Low (n = 299)	High (n = 137)	Low (n = 302)	High (n = 134)
Age, years	62.5 ± 13.9	71.1 ± 10.5***	62.1 ± 13.8	72.1 ± 9.8***
BMI, kg/m^2	24.4 ± 4.3	23.8 ± 3.6	24.6 ± 4.4	23.2 ± 3.2**
LVEF, %	50.3 ± 10.5	51.4 ± 10.7	50.1 ± 10.8	51.9 ± 10.0
Risk factors				
Hypertension, %	55.2	62.0	56.6	59.0
Diabetes mellitus, %	23.7	22.6	24.2	21.6
Family History of CAD, %	11.0	8.8	10.9	9.0
Smoking (Current and Former), %	85.3	74.5**	83.4	78.4
Cardiovascular medication				
Calcium Channel Blocker, %	19.1	21.2	21.2	16.4
ACE-I, %	1.7	2.2	2.3	0.7
ARB, %	16.7	25.5*	18.5	21.6
Beta-blocker, %	3.0	8.0*	3.6	6.7
Antiplatelet, %	5.7	7.3	5.0	8.2

Data are presented as mean ± standard deviation or number (%). *p < 0.05; **p < 0.01; ***p < 0.001 vs. low EPA/AA (< 0.41) or low DHA/AA (< 0.93). ACE-I, angiotensin-converting enzyme inhibitor; ACS, acute coronary syndrome; ARB, angiotensin II type 1 receptor blocker; BMI, body mass index; MI, myocardial infarction; PCI, percutaneous coronary intervention.

Table 2. Comparison of various biomarkers between patients with low EPA/AA or DHA/AA and those with high EPA/AA or DHA/AA

	EPA/AA		DHA/AA	
	Low (n = 299)	High (n = 137)	Low (n = 302)	High (n = 134)
EPA, μg/dl	41.0 ± 19.3	105.6 ± 51.1***	46.5 ± 29.5	94.6 ± 53.6***
DHA, μg/dl	114.5 ± 39.2	169.0 ± 44.6***	113.4 ± 37.0	172.7 ± 45.0***
AA, μg/dl	189.4 ± 58.6	155.3 ± 39.9***	192.6 ± 56.9	147.3 ± 37.3***
DGLA, μg/dl	41.2 ± 17.9	29.6 ± 21.3***	40.6 ± 17.4	30.5 ± 22.7***
AA/DGLA	5.05 ± 1.71	6.01 ± 2.08***	5.22 ± 1.82	5.65 ± 1.99*
LDL-C, mg/dL	131.7 ± 35.8	121.7 ± 32.6**	132.6 ± 35.3	119.4 ± 33.0**
Non-HDL-C, mg/dL	156.9 ± 42.0	145.8 ± 34.8**	157.3 ± 41.3	144.7 ± 36.1**
HDL-C, mg/dL	44.2 ± 11.3	46.4 ± 12.1	44.7 ± 11.4	45.4 ± 12.3
Triglycerides, mg/dL	114.0 (71.0-175.0)	95.0 (66.5-128.5)**	112.0 (68.0-175.0)	103.0 (70.1-135.3)
sdLDL-C, mg/dL	38.9 ± 21.1	35.8 ± 16.9	39.1 ± 21.2	35.2 ± 16.2
RL-C, mg/dL	5.5 (3.4-9.0)	4.3 (3.0-6.1)**	5.3 (3.4-8.8)	4.4 (3.0-6.1)**
MDA-LDL, mg/dL	148.7 ± 67.8	131.3 ± 48.4*	146.3 ± 66.8	136.2 ± 52.3
ApoA1, mg/dL	118.6 ± 22.5	122.9 ± 25.4	119.4 ± 22.1	121.1 ± 26.4
ApoB, mg/dL	106.2 ± 27.2	99.9 ± 24.2*	106.3 ± 27.7	99.5 ± 22.7*
ApoE, mg/dL	4.4 ± 1.6	4.1 ± 1.1	4.4 ± 1.5	4.2 ± 1.4
Lp(a), mg/dL	17.3 ± 19.3	16.5 ± 19.0	17.4 ± 19.3	16.4 ± 19.0
HbA1c, %	6.3 ± 1.4	6.1 ± 1.1	6.3 ± 1.4	6.1 ± 1.0
eGFR, mL/min/1.73 m^2	74.4 ± 23.5	68.3 ± 22.8**	74.3 ± 23.2	68.4 ± 23.5**
hsCRP, mg/dL	1.10 ± 2.70	0.74 ± 2.24*	1.03 ± 2.64	0.89 ± 2.41
BNP, pg/mL	180.2 ± 334.4	186.4 ± 357.7*	184.3 ± 347.7	177.4 ± 328.5*

Data are presented as mean ± standard deviation or median (25% and 75% quartiles). Abbreviations are provided in the text. AA/DGLA is presented as estimated D5D. *p < 0.05; **p < 0.01; ***p < 0.001 vs. lower EPA/AA (< 0.41) or lower DHA/AA (< 0.93) by the Mann-Whitney tests.

Correlation between PUFA and Various Biomarkers

Spearman correlation coefficients between PUFA and biomarkers were computed (**Supplementary Table 2**). Not EPA, but either DHA, AA or DGLA was significantly directly associated with LDL-C, triglycerides, RL-C, and MDA-LDL, whereas PUFA

Table 3. Comparison of biomarkers between TIMI flow grade 0 and ≥ 1 on the initial coronary angiography

	TIMI ≥ 1 (n = 193)	TIMI0 (n = 243)
Age	67.0 ± 14.0	63.8 ± 13.0**
EPA, μg/dl	64.7 ± 41.6	58.3 ± 46.4*
DHA, μg/dl	135.1 ± 48.8	128.7 ± 47.5
AA, μg/dl	178.7 ± 58.5	178.7 ± 53.5
DGLA, μg/dl	35.5 ± 15.2	39.2 ± 22.6
EPA/AA	0.40 ± 0.30	0.35 ± 0.33
DHA/AA	0.81 ± 0.35	0.77 ± 0.33
AA/DGLA	5.54 ± 1.95	5.20 ± 1.81
LDL-C, mg/dL	126.3 ± 37.2	130.4 ± 33.4
Non-HDL-C, mg/dL	150.7 ± 42.5	155.6 ± 38.2
HDL-C, mg/dL	46.2 ± 12.6	43.9 ± 10.6
Triglycerides, mg/dL	109.0 (68.0-151.5)	108.0 (70.0-171.0)
sdLDL-C, mg/dL	36.2 ± 19.8	39.3 ± 19.9
RL-C, mg/dL	4.5 (3.2-7.4)	5.3 (3.5-8.7)
MDA-LDL, mg/dL	141.6 ± 65.4	144.5 ± 60.8
ApoA1, mg/dL	122.4 ± 25.8	118.0 ± 21.3
ApoB, mg/dL	103.1 ± 27.8	105.1 ± 25.3
ApoE, mg/dL	4.2 ± 1.5	4.4 ± 1.5
Lp(a), mg/dL	17.5 ± 20.0	16.8 ± 18.6
HbA1c, %	6.3 ± 1.3	6.3 ± 1.3
eGFR, mL/min/1.73 m^2	74.1 ± 23.0	71.1 ± 23.8
hsCRP, mg/dL	1.03 ± 2.69	0.95 ± 2.47
BNP, pg/mL	210.3 ± 369.6	159.8 ± 316.3*

Data are presented as mean ± standard deviation or median (25% and 75% quartiles). Abbreviations are presented in the main text. AA/DGLA is presented as estimated D5D. *$p < 0.05$; **$p < 0.01$ using the Mann-Whitney tests.

were significantly directly associated with non-HDL-C, sdLDL-C, apoB, and apoE. In contrast, (EPA + DHA) /AA was significantly and inversely associated with LDL-C, non-HDL-C, triglycerides, RL-C, and apoB.

Comparisons between the Severities of Coronary Lesions

The biomarkers were compared between the patients with and without TIMI0 **(Table 3)**. Of the lipid biomarkers, only EPA was significantly lower in the TIMI0. The biomarkers were compared between three groups based on the number of stenotic coronary vessels **(Table 4)**. The prevalence of TIMI0 gradually decreased with increasing number of stenotic vessels. Age and serum levels of EPA, EPA/AA, DHA/AA, HbA1c and BNP gradually increased, while the prevalences of low EPA/AA and low DHA/AA, DGLA and apoA1 gradually decreased from 1VD to 3VD. Approximately, 55% and 60% of patients in the 3VD group were classified into the low EPA/AA and low DHA/AA groups, respectively. The Japanese dietary practices have markedly changed since the 1960s, and fish consumption is decreasing in the younger generation[34]. The present patients aged ≥ 60 years might have consumed more fish during their lives. In elder patients (age ≥ 60 years), only EPA/AA gradually increased with increasing involvement of the number of vessels **(Supplementary Table 3)**. According to the age-adjusted logistic regression analysis, independent association of EPA/AA and DHA/AA with the presence of 3VD could not be observed **(Supplementary Table 4)**.

Comparisons between Quartiles According to the Estimated D5D Activities

Table 5 summarizes the levels of biomarkers between the four groups based on quartiles of D5D. The levels of EPA, DHA, EPA/AA, HDL-C and BNP increased progressively, while those of LDL-C, non-HDL-C, triglyceride, sdLDL-C, RL-C, MDA-LDL, apoB, and apoE decreased progressively from the lowest to the highest D5D quartile. In contrast, the prevalence of DM, HbA1c, DHA/AA, lbLDL-C, and hsCRP did not differ between the quartiles of D5D. Multiple studies have demonstrated that decreased D5D is associated with insulin resistance, obesity, and incident of DM[19, 20, 35-39]. D5D activity, DGLA, and

Table 4. Comparisons of various biomarkers between the three groups based on the number of stenotic coronary arteries

	1VD ($n=206$)	2VD ($n=135$)	3VD ($n=95$)	P
Age	62.5 ± 13.7	66.4 ± 12.9*	69.4 ± 12.8***	< 0.001
TIMI 0, %	62.1	54.8	43.2	0.008
Low EPA/AA, %	75.2	68.1	54.7	0.002
Low DHA/AA, %	76.7	64.4	60.0	0.005
EPA, μg/dl	55.5 ± 35.6	64.2 ± 55.4	69.1 ± 42.7**	0.024
DHA, μg/dl	126.3 ± 43.7	136.4 ± 56.5	135.9 ± 43.5	0.098
AA, μg/dl	182.5 ± 54.4	178.2 ± 56.8	171.4 ± 56.9	0.203
DGLA, μg/dl	38.3 ± 15.6	37.8 ± 19.2	35.5 ± 27.3**	0.011
EPA/AA	0.33 ± 0.25	0.39 ± 0.41	0.44 ± 0.28**	0.004
DHA/AA	0.74 ± 0.30	0.81 ± 0.37*	0.86 ± 0.34**	0.005
AA/DGLA	5.23 ± 1.83	5.30 ± 1.86	5.68 ± 2.01	0.096
LDL-C, mg/dL	128.6 ± 34.4	130.3 ± 39.1	125.9 ± 30.5	0.793
Non-HDL-C, mg/dL	153.4 ± 38.8	156.0 ± 42.7	150.0 ± 39.7	0.494
HDL-C, mg/dL	45.9 ± 11.6	44.3 ± 11.8	43.8 ± 11.3	0.216
Triglycerides, mg/dL	108.5 (70.0-171.0)	109.0 (70.0-157.0)	108.0 (64.0-159.0)	0.586
sdLDL-C, mg/dL	37.6 ± 19.1	40.2 ± 20.2	35.3 ± 21.0	0.099
RL-C, mg/dL	5.1 (3.3-8.9)	5.1 (3.5-7.8)	4.3 (3.0-7.7)	0.298
MDA-LDL, mg/dL	142.2 ± 65.0	148.0 ± 61.4	138.7 ± 59.9	0.398
ApoA1, mg/dL	122.4 ± 21.5	118.4 ± 26.4*	116.8 ± 22.9*	0.032
ApoB, mg/dL	104.1 ± 24.9	105.4 ± 28.3	102.6 ± 27.0	0.762
ApoE, mg/dL	4.3 ± 1.4	4.4 ± 1.5	4.2 ± 1.7	0.446
Lp(a), mg/dL	17.3 ± 20.2	17.8 ± 18.3	15.7 ± 18.2	0.324
HbA1c, %	6.1 ± 1.1	6.4 ± 1.5	6.5 ± 1.4**	0.016
eGFR, mL/min/1.73 m^2	74.6 ± 23.3	71.4 ± 22.8	69.2 ± 24.4	0.156
hsCRP, mg/dL	0.86 ± 2.45	1.03 ± 2.41	1.19 ± 3.03	0.456
BNP, pg/mL	146.8 ± 309.5	189.2 ± 283.7**	248.6 ± 456.7**	0.003

Data are expressed as mean ± standard deviation or median (25% and 75% quartiles). AA/DGLA is presented as estimated D5D. # Comparisons between the three groups were performed using Kruskal-Wallis tests. Abbreviations are provided in the main text. *$p<0.05$, **$p<0.01$, ***$p<0.001$ vs. 1VD, using the Mann-Whitney tests.

other biomarkers were compared between obese and non-obese patients **(Supplementary Table 5)**. Compared with non-obese patients, obese patients had significantly higher levels of AA, DGLA, LDL-C, non-HDL-C, triglyceride, sdLDL-C, RL-C, MDA-LDL, apoB, apoE, and HbA1c; significantly lower levels of DHA/AA, estimated D5D, HDL-C, and Lp(a); similar EPA/AA and lbLDL-C.

Discussion

There are four novel findings in this report. First, according to the cutoff levels in the general Japanese population, levels of LDL-C, non-HDL-C, RL-C, and apoB were significantly higher in the low EPA/AA or low DHA/AA groups, while levels of triglycerides, MDA-LDL and hsCRP were significantly higher in the low EPA/AA group alone. Second, total thrombotic occlusion of coronary artery determined by TIMI0 was significantly associated with low EPA alone. Third, levels of EPA, EPA/AA, and DHA/AA were unexpectedly the highest in 3VD group, while more than half the patients were classified into low EPA/AA and DHA/AA groups. However, this result was not observed after adjusting for age. Fourth, levels of LDL-C, non-HDL-C, triglycerides, sdLDL-C, RL-C, MDA-LDL and apoB decreased progressively from the lowest to the highest D5D quartile. However, D5D activity was not associated with coronary atherosclerosis.

These results are in good agreement with those of previous reports that both EPA/AA and DHA/AA were positively associated with age at onset of ACS[4, 40]. In those studies, more than 15% of patients were on statins. A previous cross-sectional study with 507 patients (384 men) with acute ST elevation MI (STEMI) reported that levels of EPA, DHA, EPA/AA and DHA/AA were significantly lower in patients with 3VD and/or left main trunk disease (LMTD) than 2VD or 1VD. However, in that study, the blood samples were collected within 8 days of admission, and the mean LDL-C in 3VD/LMTD was 96.6 ± 31.6

Table 5. Comparisons of various biomarkers between the quartiles of D5D estimated by AA/DGLA

AA/DGLA	Q1 ($n=109$)	Q2 ($n=109$)	Q3 ($n=109$)	Q4 ($n=109$)	*P*
AA/DGLA	3.29 ± 0.56	4.55 ± 0.30	5.63 ± 0.37	7.92 ± 1.43	-----
Age, years	60.8 ± 14.1	65.6 ± 12.9	66.3 ± 13.3	68.2 ± 12.9	0.001
BMI, kg/m^2	25.7 ± 4.9	24.0 ± 3.3	24.1 ± 4.2	23.0 ± 3.5	< 0.001
LVEF, %	51.5 ± 8.8	50.3 ± 9.6	50.4 ± 11.8	50.1 ± 11.8	0.748
Prevalence of DM, %	22.0	29.4	22.9	19.3	0.353
HbA1c, %	6.3 ± 1.2	6.2 ± 1.0	6.3 ± 1.3	6.3 ± 1.6	0.597
EPA, μg/dl	44.5 ± 22.9	57.8 ± 37.5*	61.8 ± 38.1*	80.5 ± 62.2*§#	< 0.001
DHA, μg/dl	119.0 ± 44.5	130.3 ± 49.9	130.5 ± 41.6	146.2 ± 52.4*	0.001
EPA/AA	0.27 ± 0.18	0.36 ± 0.28*	0.37 ± 0.26*	0.48 ± 0.45*	< 0.001
DHA/AA	0.72 ± 0.30	0.80 ± 0.33	0.78 ± 0.30	0.85 ± 0.39	0.094
LDL-C, mg/dL	138.9 ± 32.3	127.3 ± 34.0	124.2 ± 37.6*	123.8 ± 34.8*	0.005
lbLDL-C, mg/dL	90.9 ± 26.9	88.4 ± 27.4	88.9 ± 30.2	91.2 ± 28.8	0.757
sdLDL-C, mg/dL	46.6 ± 23.0	38.7 ± 18.5*	34.3 ± 18.9*	32.3 ± 15.8*§	< 0.001
Non-HDL-C, mg/dL	168.6 ± 41.3	152.3 ± 37.5*	147.9 ± 41.5*	144.9 ± 36.7*	< 0.001
HDL-C, mg/dL	41.7 ± 9.0	43.9 ± 10.2	45.9 ± 13.9*	48.2 ± 11.9*§	0.001
Triglycerides, mg/dL	148.0 (93.3-213.8)	112.0 (66.5-170.0)*	101.0 (65.0-153.0)*	87.0 (60.1-119.5)*§#	< 0.001
RL-C, mg/dL	7.0 (4.1-11.0)	5.4 (3.3-8.2)	4.8 (3.2-7.9)*	4.1 (3.0-5.5)*§#	< 0.001
MDA-LDL, mg/dL	167.5 ± 75.3	143.4 ± 54.6*	132.2 ± 53.6*	130.1 ± 59.4*	< 0.001
ApoA1, mg/dL	118.3 ± 18.9	118.3 ± 22.0	120.0 ± 25.8	123.2 ± 26.5	0.478
ApoB, mg/dL	114.3 ± 25.0	101.9 ± 26.0*	100.9 ± 27.7*	99.8 ± 24.6*	< 0.001
ApoE, mg/dL	4.9 ± 2.1	4.2 ± 1.3*	4.1 ± 1.2*	4.0 ± 0.9*	0.001
Lp(a), mg/dL	17.8 ± 18.8	16.6 ± 18.3	17.1 ± 21.0	16.9 ± 18.8	0.856
eGFR, mL/min/1.73 m^2	75.1 ± 21.4	73.9 ± 22.4	72.4 ± 25.6	68.3 ± 24.0	0.233
hsCRP, mg/dL	0.59 ± 1.37	1.00 ± 2.92	1.02 ± 2.57	1.33 ± 3.06	0.932
BNP, pg/mL	94.2 ± 165.9	131.1 ± 185.5	197.4 ± 356.0*	306.3 ± 504.1*§	< 0.001

Data are expressed as means ± standard deviation, median (25% and 75% quartiles) or number (%). Abbreviations are presented in the main text. Kruskal-Wallis tests and analysis of variance (ANOVA) with Tukey's honest significant difference test was used to identify the differences between the groups. Q1: AA/ DGLA < 4.03, Q2: 4.04 ≦ AA/ DGLA < 5.07, Q3: 5.08 ≦ AA/ DGLA < 6.29, Q4: AA/ DGLA ≧ 6.29. *$p<0.05$ vs Q1, §$p<0.05$ vs Q2, #$p<0.05$ vs Q3 using Tukey-Kramer post-hoc test.

mg/dL[40]. These findings suggest that a majority of those patients were on statins. In contrast, LDL-C level in the patients with 3VD in this study was 125.9 ± 30.5 mg/dL and none of the patients were on any lipid-lowering agents. Therefore, these differences might have caused discrepancies in the results. Additionally, positive association of EPA, EPA/AA and DHA/AA with the presence of 3VD could not be observed after adjusting for age. Therefore, higher levels of EPA, EPA/AA and DHA/AA may solely reflect the higher number of elder patients with 3VD.

According to intravascular imaging studies such as optical coherence tomography (OCT) and intravascular ultrasound (IVUS), the coronary thrombus is mostly occlusive and sustained in STEMI, whereas it is usually incomplete and dynamic, or absent in unstable angina and non–STEMI[41-43]. The former occlusive thrombus is associated with plaque rupture that is associated with thin-cap fibroatheromas, whereas the latter is associated with plaque erosion. However, these studies included few patients with ACS with TIMI0. Silent plaque ruptures and erosions with subsequent plaque healing plays an important role in the plaque growth and the development of coronary stenosis[44]. A previous study demonstrated that low serum EPA was associated with lipid-rich plaque as evaluated using integrated backscatter IVUS in patients with CAD[45]. A randomized controlled trial to compare open-label addition of 1.86g EPA and 1.5g DHA for 30 months in 218 patients with stable CAD treated with statins reported that higher plasma omega-3 PUFA index—calculated as (EPA + DHA) / total fatty acids (≥ 4%)—prevented the progression of coronary plaque volume in non-diabetic patients compared with lower omega-3 index (< 3.4%)[46]. A recent retrospective analysis in 60 patients with CAD reported that EPA therapy was associated with coronary plaque stability, which is defined as lower lipid index, lower macrophage grade, or higher minimum fibrous cap thickness determined by optical frequency domain imaging[47]. Another previous study suggested that the incorporation of EPA into platelet membranes might reduce

platelet aggregation through the formation of prostaglandin H_3, which is then converted into prostaglandin I_3 and thromboxane A_3 [48]. In patients with DM, hyperlipidemia, and high levels of angiopoietin-2, a 6-month treatment with 1.8g EPA significantly decreased platelet-derived microparticles and sE-selectin[49]. We speculated that high EPA might limit the size of the overlying thrombus[50] and protect totally occlusive thrombus formation in coronary arteries, which will result in silent progression of coronary atherosclerosis till 3VD and advanced age. Future studies are required to evaluate the association between PUFA and coronary morphology such as ruptured or erosive plaque and healing lesion in patients with ACS.

D5D plays an important role in the production of both EPA and AA. Alterations in D5D activity have been reported to be associated with the risk of CVD[12]. In a Swedish population-based prospective cohort study of 2,009 50-year old men, D5D was reported to have an inverse correlation with CVD mortality over a follow-up of 30 years[51]. Their group reported that a diet rich in saturated fats decreased D5D[52]. An euglycemic insulin clamp study in 264 American adolescents (average age: 15 years) demonstrated that D5D was inversely related to serum levels of triglycerides and fasting insulin whereas it had no association with BMI and serum levels of glucose, LDL-C and HDL-C[53]. Another study reported that lower D5D was associated with obesity[20]. In the present study, D5D was significantly lower in obese patients, while the incidence of DM and HbA1c levels were comparable between the D5D quartiles. We previously reported that impaired glucose tolerance and DM were far more common than normal glucose regulation in patients with ACS, even though their HbA1c levels remain within the normal range[54]. In contrast to the general population, an association between D5D and incidence of DM may not be detected in patients with ACS. The present study is the first to demonstrate the markedly inverse association of serum LDL-C, especially sdLDL-C, non-HDL-C, triglycerides, MDA-LDL, RL-C, and apoB, and levels of the D5D. These findings suggest that decreased biosynthesis of long-chain PUFA were clearly associated with impaired metabolism of triglyceride-rich apoB-containing lipoproteins that are known as atherogenic lipid profiles (ALP). D5D were significantly associated with serum levels of EPA, DHA, and AA. According to a meta-analysis of prospective cohort studies investigating plasma PUFA and CAD outcome, the relative risk and 95% of confidence interval (CI) for CAD for EPA, DHA and AA were 0.78 (CI 0.65-0.94), 0.79 (CI 0.67-0.93), and 0.83 (CI 0.74-0.92), respectively[55]. Therefore, it is conceivable that lower D5D is associated with CAD. However, the present results fail to demonstrate any association between D5D and coronary atherosclerosis. EPA and AA competed for D5D; however, the mechanisms underlying the positive association between EPA/AA and D5D remain to be determined. Additionally, it remains unclear why only EPA is associated with TIMI0. Further studies are needed to clarify the association between metabolism of PUFA or ALP, and culprit coronary lesions.

The present study has several limitations. A major limitation is the single center cross-sectional analysis of only patients with ACS. Like with all cross-sectional studies, causal relationships cannot be established based on this design. Second, we used plasma concentrations of PUFA and not their compositions in non-fasting samples, and did not investigate the intake of PUFA from the last meal. Third, we could not measure PUFA in erythrocyte membranes and/or lipoprotein fractions. Fourth, D6D and stearoyl-CoA desaturase-1, another crucial enzyme of PUFA metabolism, were not investigated. Fifth, genetic variations in FADS1 were not measured[12]. These factors should be investigated in future studies. Multiple studies have demonstrated that EPA and DHA have different effects on cardiometabolic risk factors[56, 57]. The present results demonstrated that EPA/AA is a superior risk marker in assessing ALP than DHA/AA.

Conflict of Interest

The authors have no conflicts to declare.

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Author Contributions

KA composed the article and conducted data analysis, data interpretation, and critical revision of the article. SK composed the article, conducted data analysis, contributed to the study design, data interpretation, and critical revision of the article. YY, FT, and MS contributed to acquisition of data and data analysis. HT, SK and ST contributed to acquisition of data, TS contributed to data interpretation and critical revision of the article.

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Supplementary Table 1. The correlation between RLP-C assessed by Immnoprecipitation assay and RemL-C by homogenous assay and various biomarkers

	Immnoprecipitation assay ($n=311$)	Homogenous assay ($n=125$)
BMI	0.321**	0.224*
LDL-C, mg/dL	0.382**	0.409**
Non-HDL-C, mg/dL	0.613**	0.606**
Triglyceride, mg/dL	0.819**	0.910**
sdLDL-C, mg/dL	0.530**	0.666**
Apo B, mg/dL	0.547**	0.569**
Apo E, mg/dL	0.598**	0.567**
EPA, μg/dl	0.010	0.182*
DHA, μg/dl	0.207**	0.297**
AA, μg/dl	0.502**	0.379**
DGLA, μg/dl	0.563**	0.599**

Data are expressed as Spearman's Rho between RL-C measured by different assays and various biomarkers. *$p<0.05$, **$p<0.01$

Supplementary Table 2. The correlation between PUFA and various biomarkers

	EPA, μg/dl	DHA, μg/dl	AA, μg/dl	DGLA, μg/dl	(EPA + DHA)/AA
EPA, μg/dl	----------	0.823**	0.081	-0.155**	0.782**
DHA, μg/dl	0.823**	----------	0.206**	0.001	0.719**
AA, μg/dl	0.081	0.206*	----------	0.604**	-0.453**
DGLA, μg/dl	-0.155**	0.001	0.604**	----------	-0.442**
(EPA + DHA) /AA	0.782**	0.719**	-0.453**	-0.442**	----------
LDL-C, mg/dL	0.067	0.137**	0.451**	0.446**	-0.169**
Non-HDL-C, mg/dL	0.127**	0.265**	0.571**	0.554**	-0.151**
HDL-C, mg/dL	0.133**	0.065	0.091	-0.092	0.041
Triglycerides, mg/dL	0.030	0.230**	0.394**	0.538**	-0.115*
sdLDL-C, mg/dL	0.187**	0.319**	0.470**	0.556**	-0.053
RL-C, mg/dL	0.056	0.225**	0.464**	0.562**	-0.143**
MDA-LDL, mg/dL	0.061	0.219**	0.384**	0.486**	-0.093
ApoA1, mg/dL	0.199**	0.188**	0.287**	0.162**	0.001
ApoB, mg/dL	0.139**	0.295**	0.539**	0.527**	-0.117*
ApoE, mg/dL	0.156**	0.348**	0.495**	0.474**	-0.060
Lp(a), mg/dL	-0.002	0.012	0.014	-0.034	-0.013
HbA1c, %	-0.007	0.039	0.062	0.101*	-0.016
BMI, kg/m^2	0.016	-0.012	0.210**	0.325**	-0.130**

Data are expressed as Spearman's Rho between PUFA and various biomarkers. *$p<0.05$, **$p<0.01$

Supplementary Table 3. Comparisons of various biomarkers between the three groups based on the number of stenotic coronary arteries in elderly patients (≥ 60 years)

	1VD (n = 121)	2VD (n = 92)	3VD (n = 77)	P
Age	72.1 ± 8.1	73.3 ± 8.7	74.1 ± 8.4	0.199
EPA, μg/dl	59.4 ± 37.2	67.8 ± 41.8	69.9 ± 39.2	0.094
DHA, μg/dl	131.3 ± 44.7	143.4 ± 56.1	136.1 ± 42.1	0.288
AA, μg/dl	163.0 ± 47.0	158.9 ± 39.9	157.0 ± 43.8	0.790
DGLA, μg/dl	33.2 ± 12.5	31.3 ± 12.9	32.9 ± 27.8	0.156
EPA/AA	0.38 ± 0.26	0.43 ± 0.28	0.47 ± 0.28*	0.039
DHA/AA	0.84 ± 0.31	0.92 ± 0.33	0.92 ± 0.33	0.084
AA/DGLA	5.38 ± 1.96	5.57 ± 1.80	5.66 ± 1.96	0.226
LDL-C, mg/dL	120.1 ± 31.3	119.8 ± 33.0	120.4 ± 29.1	0.889
Non-HDL-C, mg/dL	142.1 ± 34.6	143.2 ± 33.6	140.7 ± 33.4	0.830
HDL-C, mg/dL	47.0 ± 12.0	45.7 ± 12.7	44.3 ± 11.9	0.246
Triglycerides, mg/dL	93.0 (62.0-135.0)	97.0 (66.0-125.8)	95.0 (63.0-139.5)	0.944
sdLDL-C, mg/dL	32.9 ± 15.9	35.8 ± 16.2	31.5 ± 17.3	0.129
RL-C, mg/dL	4.2 (3.1-6.6)	4.3 (3.1-6.8)	4.1 (2.9-7.0)	0.548
MDA-LDL, mg/dL	135.1 ± 65.4	132.8 ± 52.4	130.0 ± 55.4	0.859
ApoA1, mg/dL	122.1 ± 22.8	120.0 ± 27.9	116.4 ± 24.5	0.153
ApoB, mg/dL	97.4 ± 22.0	97.1 ± 23.5	96.9 ± 24.4	0.952
ApoE, mg/dL	4.1 ± 1.1	4.0 ± 1.1	3.9 ± 0.9	0.776
Lp(a), mg/dL	20.0 ± 21.0	17.0 ± 18.4	16.3 ± 18.0	0.584
HbA1c, %	5.9 ± 0.6	6.4 ± 1.4*	6.2 ± 1.0*	0.035
eGFR, mL/min/1.73 m^2	67.7 ± 22.7	66.4 ± 22.4	65.1 ± 22.1	0.760
hsCRP, mg/dL	1.13 ± 3.01	0.83 ± 2.12	1.06 ± 2.74	0.805
BNP, pg/mL	206.7 ± 376.5	208.8 ± 287.6	286.9 ± 494.7	0.375

Data are expressed as mean ± standard deviation or median (25% and 75% quartiles). AA/DGLA is presented as estimated D5D. Comparisons between the three groups were performed using Kruskal-Wallis tests. Abbreviations are presented in the text. *$p < 0.05$ vs. 1VD using the Mann-Whitney tests.

Supplementary Table 4. Logistic regression analysis for the determination of the presence of 3VD

	Univariate		Age-adjusted	
	OR	p	OR	p
Age	1.031	0.001	----------	----------
EPA	1.005	0.06	1.004	0.096
EPA/AA	2.100	0.037	1.692	0.125
DHA/AA	2.085	0.026	1.434	0.317

OR, odds ratio

Supplementary Table 5. Comparisons of biomarkers between obese (BMI ≥ 25 kg/m^2) and non-obese (BMI < 25 kg/m^2) patients

	BMI < 25 (n = 277)	BMI ≧ 25 (n = 159)
Age	68.4 ± 12.4	59.4 ± 13.5***
BMI, kg/m^2	21.9 ± 2.1	28.2 ± 3.7***
EPA, μg/dl	62.8 ± 48.3	58.9 ± 36.6
DHA, μg/dl	132.5 ± 47.7	130.3 ± 49.1
AA, μg/dl	170.4 ± 48.6	194.1 ± 63.5***
DGLA, μg/dl	33.8 ± 15.6	44.2 ± 24.2***
EPA/AA	0.39 ± 0.34	0.33 ± 0.25
DHA/AA	0.82 ± 0.34	0.72 ± 0.32**
AA/DGLA	5.62 ± 1.97	4.89 ± 1.63***
LDL-C, mg/dL	126.0 ± 34.8	133.5 ± 35.0*
Non-HDL-C, mg/dL	148.5 ± 37.8	162.8 ± 42.3**
HDL-C, mg/dL	46.6 ± 11.9	42.0 ± 10.6***
Triglycerides, mg/dL	95.0 (63.3-141.0)	132.5 (92.3-213.3)***
lbLDL-C, mg/dL	90.5 ± 28.9	89.1 ± 27.3
sdLDL-C, mg/dL	35.4 ± 17.7	42.5 ± 22.6**
RL-C, mg/dL	4.4 (3.1-6.9)	6.5 (4.1-11.6)***
MDA-LDL, mg/dL	139.5 ± 64.9	150.5 ± 58.3*
ApoA1, mg/dL	121.5 ± 24.3	117.3 ± 21.9
ApoB, mg/dL	101.1 ± 25.4	109.9 ± 27.0**
ApoE, mg/dL	4.1 ± 1.2	4.7 ± 1.9***
Lp(a), mg/dL	18.8 ± 20.0	14.2 ± 17.5**
HbA1c, %	6.1 ± 1.2	6.5 ± 1.5*

Data are expressed as mean ± standard deviation or median (25% and 75% quartiles). AA/DGLA is presented as estimated D5D. *p < 0.05, **p < 0.01, ***p < 0.001 using the Mann-Whitney tests.