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ORIGINAL ARTICLE

Which has the stronger impact on coronary artery disease, eicosapentaenoic acid or docosahexaenoic acid?

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It has been suggested that n-3 polyunsaturated fatty acids, such as eicosapentaenoic acid (EPA) and docosahexaenoic acid (DHA), protect against cardiovascular diseases, and EPA/arachidonic acid (AA) and DHA/AA ratios in serum are potential risk markers for coronary artery disease (CAD). The purpose of this study was to clarify the clinical significance of the difference in the EPA/AA ratio and the DHA/AA ratio in patients with CAD. In 369 patients with confirmed or suspected CAD who underwent diagnostic coronary angiography, we measured serum levels of EPA, DHA and AA and calculated the EPA/AA and DHA/AA ratios. The EPA/AA ratio was significantly lower in patients with acute coronary syndrome (ACS) than in patients with chronic CAD or chest pain syndrome (0.27 ± 0.19 *vs.* 0.44 ± 0.20, respectively; $P < 0.01$), whereas the DHA/AA ratio was similar in the two groups (0.78 ± 0.27 *vs.* 0.79 ± 0.37). Multiple logistic regression analyses using various biomarkers related to coronary risk discriminated ACS from other disease entities and demonstrated that the EPA/AA ratio (odds ratio: 0.0012, 95% confidence interval: 0.00–0.16, $P < 0.01$) but not the DHA/AA ratio (odds ratio: 1.05, 95% confidence interval: 0.98–1.12) was a significant independent predictive factor. Our findings suggest that the EPA/AA ratio might be more closely associated with the pathophysiology of CAD, especially with that of ACS, than the DHA/AA ratio. Our findings suggest that interventions with EPA agents or supplemental EPA intake, compared with DHA agents or supplemental DHA, may confer greater benefit for plaque stabilization to prevent the onset of ACS in patients with CAD.
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Keywords: acute coronary syndrome; coronary artery disease; DHA/AA ratio; EPA/AA ratio; risk factor

INTRODUCTION

It has long been noted that fish or fish oil consumption may prevent cardiovascular events. Since the 1980s, various epidemiological studies around the world have suggested that n-3 polyunsaturated fatty acids (PUFA), such as eicosapentaenoic acid (EPA) and docosahexaenoic acid (DHA), which are present in fish oils, protect against the occurrence of cardiovascular events compared with n-6 PUFA such as arachidonic acid (AA). Accordingly, supplemental intake of n-3 PUFA is recommended.[1–3] The JELIS (Japan Eicosapentaenoic acid Lipid Intervention Study) has demonstrated that administration of high-purity EPA agents in addition to statins reduces the incidence of cardiovascular events.[4] EPA and DHA differ in their biochemical effects and functions, and it is not clear which is more effective for preventing cardiovascular events. In addition, the EPA/AA and DHA/AA ratios, which reflect the intake of n-3 PUFA relative to n-6 PUFA, have attracted attention as possible new cardiovascular risk markers. In addition to various conventional risk markers,[5] the EPA/AA and DHA/AA ratios also may predict future cardiovascular events. The purpose of this study was to clarify the clinical significance of the EPA/AA and DHA/AA ratios in patients with coronary artery disease (CAD).

METHODS

Study design

We recruited 369 consecutive patients with confirmed or suspected CAD (285 men, 84 women, aged 66 ± 11 years) who underwent diagnostic coronary angiography. Those patients who were diagnosed with ST elevation myocardial infarction, non-ST elevation myocardial infarction and unstable angina were categorized as having acute coronary syndrome (ACS). In patients without physical coronary artery stenosis, an acetylcholine provocation test was performed. The patients in whom coronary artery spasm was provoked were diagnosed with coronary spastic angina (CSA), and those in whom it was not provoked were diagnosed with chest pain syndrome. The patients who had taken EPA or DHA as an agent or dietary supplement were excluded from the study. Among these 369 patients, we measured the serum concentrations of EPA, DHA and AA in venous blood collected before the coronary angiography and calculated the EPA/AA and DHA/AA ratios. As conventional biomarkers for coronary risk factors, the levels of serum creatinine, hemoglobin A1c, total cholesterol, low-density lipoprotein cholesterol, high-density lipoprotein

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cholesterol, triglycerides and uric acid were also measured. The estimated glomerular filtration rate was calculated using the method described in the Japanese Society of Nephrology CKD Practice Guide.[6] The study was approved by the ethics committee of Dokkyo Medical University, and informed consent was obtained from each patient.

Measurement of serum fatty acids

Serum fatty acids were assayed by gas chromatography (SRL, Tokyo, Japan). All plasma lipids were extracted according to the Folch procedure, and hydrolysis was performed to release free fatty acids. Free fatty acids were then esterified with potassium methoxide/methanol and boron trifluoride/methanol. Methylated fatty acids were analyzed using a GC-17A gas chromatograph (Shimadzu Corporation, Kyoto, Japan) with an omegawax-250 capillary column (SUPELCO, Sigma-Aldrich Japan, Tokyo, Japan). The reproducibility (that is, coefficients of variation) of serum EPA, DHA and AA determination by this method have been reported to be 4.4, 2.3 and 3.8%, respectively.[7]

Statistical analysis

The data are expressed as the mean ± s.d. Two groups were compared using Student's unpaired *t*-test for continuous variables. Multi-group comparisons were performed using one-way analysis of variance, followed by a *post hoc* Fisher's least significant difference test. Multiple logistic regression analysis was performed for the discrimination of ACS from chronic CAD, such as stable angina pectoris (SAP), old myocardial infarction, CSA and chest pain syndrome, using various biomarkers of coronary risk. Statistical significance was defined by values of $P<0.05$. All the statistical analyses were performed using statistical software (Excel To-kei 2012, SSRI, Tokyo, Japan).

Table 1 Clinical background of the patients ($n=369$)

Age, years	66 ± 11
Male gender, *n* (%)	292 (79)
Risk factor	
Hypertension, *n* (%)	273 (74)
Diabetes, *n* (%)	202 (55)
Dyslipidemia, *n* (%)	246 (67)
Current smoking, *n* (%)	104 (28)
Systolic BP, mm Hg	124 ± 11
Diastolic BP, mm Hg	72 ± 9
Total cholesterol, mg dl^{-1}	148 ± 29
LDL-cholesterol, mg dl^{-1}	86 ± 25
HDL-cholesterol, mg dl^{-1}	49 ± 14
Triglyceride, mg dl^{-1}	102 ± 59
Creatinine, mg dl^{-1}	0.86 ± 0.30
eGFR; ml min^{-1} 1.73 m^{-2}	71 ± 19
Uric acid, mg dl^{-1}	5.5 ± 7.2
Hemoglobin A1c, %	6.3 ± 1.2
EPA, mg dl^{-1}	59 ± 34
DHA, mg dl^{-1}	126 ± 46
AA, mg dl^{-1}	146 ± 40
EPA/AA ratio	0.43 ± 0.29
DHA/AA ratio	0.90 ± 0.36
Medication	
Statin; *n* (%)	302 (82)
ACEI/ARB; *n* (%)	248 (67)

Abbreviations: AA, arachidonic acid; ACEI, angiotensin-converting enzyme inhibitor; ARB, angiotensin receptor blocker; BP, blood pressure; DHA, docosahechisaenoic acid; eGFR, estimated glomerular filtration rate; EPA, eicosapentaenoic acid; HDL, high-density lipoprotein; LDL, low-density lipoprotein.

RESULTS

The clinical backgrounds of the patients are shown in Table 1. In all the subjects, lipid metabolism appeared to be well controlled on statin treatment. Serum creatinine, estimated glomerular filtration rate and hemoglobin A1c levels were 0.86 ± 0.30 mg dl^{-1}, 71 ± 19 ml min^{-1} per 1.73 m^2 and 6.3 ± 1.2%, respectively.

Both the EPA/AA ratio and the DHA/AA ratio were lower in younger patients (<60 years) than in older patients (⩾60 years) (0.36 ± 0.20 *vs.* 0.47 ± 0.30, respectively, $P<0.0001$; and 0.72 ± 0.28 *vs.* 0.96 ± 0.33, $P<0.001$). The EPA/AA ratio was lower in females than in males (0.36 ± 0.25 *vs.* 0.44 ± 0.29, $P<0.05$), but a gender difference was not observed in the DHA/AA ratio (0.87 ± 0.55 *vs.* 0.91 ± 0.37).

In the disease entities of ACS, SAP, old myocardial infarction, CSA and chest pain syndrome, the EPA/AA ratios were 0.35 ± 0.13, 0.49 ± 0.20, 0.47 ± 0.20, 0.47 ± 0.23, 0.42 ± 0.19, respectively, and the DHA/AA ratios were 0.78 ± 0.23, 0.95 ± 0.37, 0.86 ± 0.36, 0.95 ± 0.32, 0.95 ± 0.36, respectively. The EPA/AA ratio was significantly lower in the ACS group than in each of the SAP ($P<0.05$), CSA ($P<0.01$) and chest pain syndrome ($P<0.05$) groups. The DHA/AA ratio in the ACS group was significantly lower than in the SAP group ($P<0.05$, Figure 1).

Table 2 compares various biomarkers for coronary risk, including the EPA/AA and DHA/AA ratios between ACS patients and patients with other disease entities, that is, chronic CAD such as SAP, old myocardial infarction and CSA, and chest pain syndrome. In patients with ACS, the EPA/AA ratio was significantly lower, and the DHA/AA ratio also tended to be lower than in other patients. Total cholesterol and high-density lipoprotein cholesterol levels were significantly lower, and hemoglobin A1c levels tended to be higher in the ACS patients compared with the others.

Multiple logistic regression analysis for the discrimination of ACS from other disease entities revealed that only the EPA/AA ratio (odds ratio: 0.0012, 95% confidence interval: 0.00–0.16, $P<0.01$) but not the DHA/AA ratio (odds ratio: 1.05, 95% confidence interval: 0.98–1.12) was a significant independent predictor (Table 3).

DISCUSSION

In this study, we measured the EPA/AA and DHA/AA ratios in patients with definite or suspected CAD. We determined that both the EPA/AA ratio and the DHA/AA ratio were lower in young patients than in older patients. The EPA/AA ratio was also lower in females than in males, but a similar difference was not observed in the DHA/AA ratio. In addition, the EPA/AA ratio was significantly lower in ACS patients compared with chronic CAD or chest pain syndrome patients, whereas the difference in the DHA/AA ratio was less significant. In multivariate analysis, the EPA/AA ratio but not the DHA/AA ratio was an independent predictor for discriminating ACS from chronic CAD or chest pain syndrome.

It has been demonstrated previously that the EPA/AA ratio is low in young people.[8] In the present study, the DHA/AA ratio was also lower in younger people than in older people. This may be a consequence of young Japanese people adopting a Western diet, which provides low n-3 PUFA and high n-6 PUFA. PUFAs are essential fatty acids, and their consumption directly affects their plasma concentration. It has also been suggested that estrogens affect PUFA metabolism in women, although details are not available.[9] We have also previously reported gender differences in the EPA/AA ratio.[8] The incidence of hypercholesterolemia is higher in men than women under 40 years old, but this trend reverses as women enter menopause and estrogen production decreases, because estrogen has a known mechanism that lowers low-density lipoprotein cholesterol and raises high-density

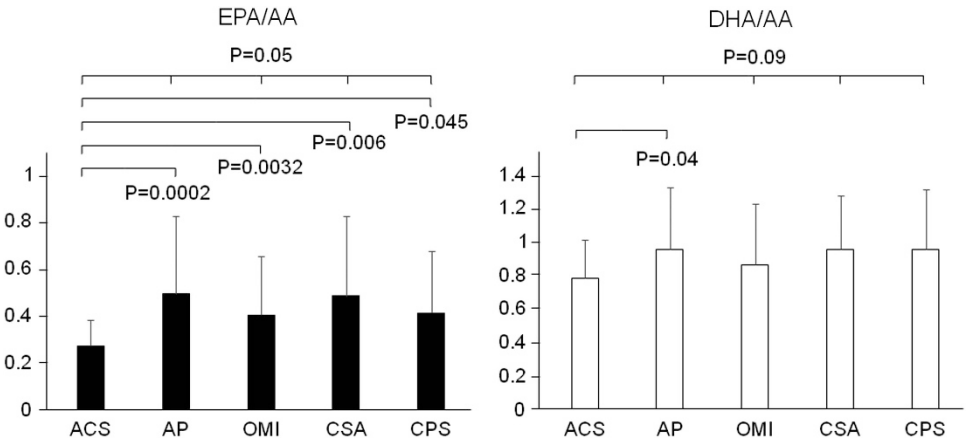

Figure 1 Comparisons of the EPA/AA and DHA/AA ratios among acute coronary syndrome (ACS), stable angina pectoris (SAP), old myocardial infarction (OMI), coronary spastic angina (CSA) and chest pain syndrome (CPS). The EPA/AA ratio was significantly lower in the ACS group compared with each of the SAP ($P<0.05$), CSA ($P<0.01$) and CPS ($P<0.05$) groups. The DHA/AA ratio in the ACS group was significantly lower than in the SAP group. AA, arachidonic acid; DHA, docosahechisaenoic acid; EPA, eicosapentaenoic acid.

Table 2 Comparisons of biomarker for coronary risk between ACS patients and patients with other disease entities such as chronic CAD and chest pain syndrome

	ACS (n = 24)	*Others (n = 345)*	P-*value*
Total cholesterol, mg dl^{-1}	133 ± 35	148 ± 29	0.029
LDL-cholesterol, mg dl^{-1}	81 ± 29	86 ± 25	0.38
HDL-cholesterol, mg dl^{-1}	42 ± 12	49 ± 13	0.016
Triglyceride, mg dl^{-1}	95 ± 49	102 ± 59	0.58
Creatinine, mg dl^{-1}	0.96 ± 0.65	0.85 ± 0.26	0.10
eGFR, ml min^{-1} 1.73 m^{-2}	73 ± 24	71 ± 19	0.94
Uric acid, mg mg dl^{-1}	5.5 ± 1.4	5.6 ± 1.4	0.81
Hemoglobin A1c, %	6.4 ± 1.4	6.2 ± 1.2	0.051
EPA/AA ratio	0.27 ± 0.19	0.44 ± 0.20	0.0063
DHA/AA ratio	0.78 ± 0.27	0.79 ± 0.37	0.093

Abbreviations: AA, arachidonic acid; ACS, acute coronary syndrome; CAD, coronary artery disease; DHA, docosahechisaenoic acid; eGFR, estimated glomerular filtration rate; EPA, eicosapentaenoic acid; HDL, high-density lipoprotein; LDL, low-density lipoprotein.

Table 3 Multiple logistic regression analysis for discrimination of ACS from other disease entities, using various biomarkers for coronary risk

	Coefficient	*Wald* χ^2	*Odds ratio (95% CI)*	P-*value*
Total cholesterol	−0.043	1.881	0.96 (0.90–1.02)	0.17
LDL-cholesterol	0.047	2.185	1.05 (0.98–1.12)	0.14
HDL-cholesterol	−0.046	1.331	0.96 (0.88–1.03)	0.25
Triglyceride	−0.005	0.568	1.00 (0.98–1.01)	0.45
eGFR	0.001	0.010	1.00 (0.97–1.03)	0.92
Uric acid	−0.137	0.552	0.87 (0.61–1.25)	0.46
Hemoglobin A1c	0.075	0.208	1.08 (0.78–1.48)	0.65
EPA/AA ratio	−6.721	7.232	0.0012 (0.00–0.16)	0.007
DHA/AA ratio	0.757	0.383	2.13 (0.19–23.5)	0.53

Abbreviations: AA, arachidonic acid; ACS, acute coronary syndrome; CI, confidence interval; DHA, docosahechisaenoic acid; eGFR, estimated glomerular filtration rate; EPA, eicosapentaenoic acid; HDL, high-density lipoprotein; LDL, low-density lipoprotein.

lipoprotein cholesterol levels. The gender difference in the mortality rate from CAD lessens at menopause and disappears when people enter their 80 s. These results suggest that essential fatty acid metabolism in women may also be affected at the time that their sex hormone balances change. Oxidative metabolism of estrogen is predominantly catalyzed by hepatic cytochrome P450 (CYP450), and dietary fish oil has been shown to increase the concentration of CYP450 and some CYP450 subtypes in rats.[10] Moreover, n-3 PUFA supplementation may decrease estradiol metabolism.[11] In contrast, alpha-linolenic acid is converted to a subtype of long-chain n-3 PUFAs such as EPA and DHA by hepatic desaturase and elongase enzymes. The concentrations of long-chain n-3 PUFAs may be positively associated with the circulating concentration of estrogen and progesterone but negatively associated with testosterone. These associations suggest that sex hormones may act to modify plasma and tissue n-3 PUFA content by possibly altering the expression of desaturase and elongase enzymes. Therefore, the findings in the present study that the EPA/AA ratio was lower in female patients than in male patients and the observation of no gender difference in the DHA/AA ratio might be a consequence of an effect of sex hormones on PUFA metabolism.

Although many epidemiological studies have found that the consumption of fish or fish oils prevents the early onset of cardiovascular events,[12] the mechanism by which fish oil might suppress atherogenesis is not understood. Arita *et al.*[13] have demonstrated that the strong anti-inflammatory and anti-oxidative actions of both EPA and DHA metabolites, such as resolvins and protectins, can suppress atherogenesis. However, it is not known which EPA or DHA metabolites are most effective for atherosclerosis prevention. Conversely, the PUFA with 20 carbon chains, EPA and AA, which are present in cell membranes, are released by phospholipase A and are transported into the metabolic cascade. They are then transformed into eicosanoids, such as prostaglandins or thromboxanes, by cyclooxygenase or into leukotrienes by lipoxygenase, which mediate their biological activities.[14–16] AA-derived eicosanoids promote platelet aggregation and inflammatory cell migration, whereas EPA-derived eicosanoids do not and are thought to be anti-atherosclerotic. However, DHA, which is a 22-carbon chain PUFA, cannot be involved in such a metabolic cascade. Therefore, EPA and DHA potentially have different biological properties. This difference between EPA and DHA may be able to explain this multifactorial etiology. The Japanese JELIS trial has demonstrated that the administration of a high-purity EPA

agent inhibits the onset of CAD. In particular, an inhibitory effect of the EPA agent on the onset of ACS, demonstrated by sub-analysis of the JELIS data, merits attention.[17] ACS is pathologically assumed to be characterized by plaque destabilization, plaque rupture and plaque erosion, in which inflammation and oxidative stress have a key role.[18] Therefore, the anti-inflammatory and anti-oxidative properties of EPA might somewhat contribute to its effect on inhibiting ACS onset. However, there is no evidence of inhibitory effects of DHA on ACS onset. Domei *et al.*[19] have reported that the risk of developing cardiovascular events after percutaneous coronary intervention is increased in association with a low EPA/AA ratio but is independent of the DHA/AA ratio. Lee *et al.*[20] have reported that a low EPA/AA ratio, but not a low DHA/AA ratio, is predictive of total cardiovascular death in ACS patients. It has also been reported that the concentration of EPA, but not DHA, in erythrocytes predicts the incidence of in-hospital deaths after ACS onset.[21] In our present study, the EPA/AA ratio, but not the DHA/AA ratio, was strongly associated with ACS in CAD patients, which supports previous data. From our results, we posit that interventions with EPA agents or supplemental EPA are likely to confer a greater benefit for plaque stabilization to prevent ACS in CAD patients compared with DHA agents or DHA supplements.

CONCLUSION

The EPA/AA ratio might be more closely associated with CAD pathophysiology, especially that of ACS, compared with the DHA/AA ratio.

CONFLICT OF INTEREST

The authors declare no conflict of interest.

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