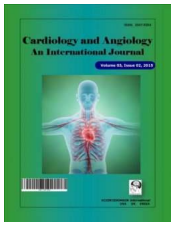

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Serum Polyunsaturated Fatty Acid Profile and Carotid Intima-media Thickness in Japanese Atherosclerotic Patients Hospitalized for Endovascular Therapy

Hisataka Nakamura[1], Keita Odashiro[1], Masahiko Fujihara[1], Mitsuhiro Fukata[1], Takeshi Arita[1], Taku Yokoyama[1], Shioto Yasuda[1], Toru Maruyama[1*] and Koichi Akashi[1]

[1]Department of Medicine and Biosystemic Science, Kyushu University Graduate School of Medical Sciences, Fukuoka 812-8582, Japan.

Authors' contributions

This work was performed in collaboration with all authors. Author HN performed practical data extraction from medical records. Author KO made the ultrasound measurements in vascular laboratory and contributed to writing the manuscript. Authors MF and TA performed the ultrasound data acquisition, input and assessment. Authors TY and MF made the statistical analyses and manuscript writing. Author TM had the initial research concept and revised the manuscript. Author KA is the team leader and supervised the team collaboration. All authors approved the final version of the manuscript to be sent for publication.

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ABSTRACT

Aims: Serum eicosapentaenoic acid (EPA) to arachidonic acid (AA) ratio (EPA/AA) in Japanese is rapidly changing according to the senescence and food westernization, and sonographic carotid artery intima-media thickness (IMT) is increasingly used as a surrogate of systemic atherosclerosis. However, the relationship between IMT and EPA/AA in Japanese remains unclear.

**Corresponding author: E-mail: maruyama@artsci.kyushu-u.ac.jp;*

Place of the Study: Vascular Laboratory of Heart Center, Kyushu University Hospital, Fukuoka, Japan.
Methodology: Seventy consecutive Japanese atherosclerotic patients (69.7±7.8 years) hospitalized for elective endovascular therapy without purified EPA agent administration were enrolled in this study. IMT was estimated by high-resolution duplex ultrasonography in vascular laboratory. Conventional vascular risk factors were under the intensive treatment. Demographic variables were extracted from medical records after obtaining informed consent.
Results: IMT was strongly age-dependent (P = .003) and age-adjusted multiple correlation analyses revealed no significant correlations among vascular risk factors, although positive partial correlation between serum EPA/AA and IMT (r = .277, P = .056) and negative partial correlation between EPA/AA and HbA1c (r = -.281, P = .053) were marginal. Stepwise multiple regression analyses demonstrated age and serum EPA/AA as positive contributors to the IMT (P < .001). EPA/AA was a seemingly paradoxical positive contributor to IMT due to age-dependent complicated profile of EPA/AA. Multiple correlation analyses were performed by multiple adjustments, which yielded significant negative correlation between EPA/AA and IMT (r = -.262, P = .049).
Conclusions: This study demonstrated that EPA/AA is a determinant of IMT among other vascular risk factors at least in Japanese atherosclerotic patients hospitalized for endovascular therapy.

Keywords: Atherosclerosis; carotid artery; eicosapentaenoic acid; intima-media thickness.

1. INTRODUCTION

Polyunsaturated fatty acids (PUFA) are involved in inflammatory process including gene expression in response to physiological stress. Eicosapentaenoic acid (EPA), as a representative ω-3 PUFA, has favorable cardiovascular protective effects including vasodilatory, anti-platelet and anti-inflammatory actions, improving dyslipidemia, blood rheology, arterial stiffness, and stabilizing atherosclerotic plaque and autonomic nervous activity. On the other hand, eicosanoid series derived from ω-6 PUFA such as arachidonic acid (AA) are prostaglandin I_2/E_2, thromboxane A_2, and leukotriene B_4. These eicosanoids show pro-inflammatory, platelet aggregating, and vasoconstrictive actions [1]. Therefore, serum EPA/AA ratio is reported to reflect the risk of cardiovascular diseases in community-dwelling Japanese residents [2].

Vascular medicine shows recent progress associated with sophisticated high-resolution vascular imaging techniques. Sonographic intima-media thickness of common carotid artery (IMT) has been recognized as an alternative of systemic atherosclerosis [3] and a predictor of future cerebral and cardiovascular events [4]. Routine evaluation of IMT in ultrasound laboratory is essential considering the close link of carotid atherosclerosis to cerebrovascular and cardiovascular diseases. However, the association of IMT with the serum PUFA profile in Japanese is still unclear. Therefore, we investigated the relationship between the sonographic IMT and the serum EPA/AA in comparison with other conventional vascular risk factors in Japanese atherosclerotic patients.

2. METHODOLOGY

2.1 Subjects

This study was performed in accordance with the updated Declaration of Helsinki (2008). Seventy consecutive Japanese atherosclerotic patients (53 males and 17 females, mean age of 69.7±7.8 years) were enrolled in this study in the period ranging from April 2011 to October 2015. They were hospitalized in Heart Center of Kyushu University Hospital (Fukuoka, Japan) for elective endovascular therapy. Inclusion criteria were patients admitted for evaluation and intervention of stable coronary or peripheral artery diseases. Exclusion criteria were acute coronary syndrome (ACS), type 1 diabetes mellitus, advanced heart failure, malignancy in any organs, dementia, and medication of the highly purified EPA agents.

Demographic variables of the enrolled patients were extracted from medical records after obtaining signed informed consent on admission. Interviews concerning lifestyle including food intake were performed by nurses. Hypertension was defined as casual BP ≥ 140/90 mmHg or treatment with antihypertensive drugs. Type 2 diabetes mellitus was defined as fasting serum glucose ≥ 126 mg/dl, casual serum glucose ≥ 200 mg/dl, HbA1c (NGSP) ≥ 6.5% or current

antidiabetic medication. Dyslipidemia was defined as serum LDL cholesterol ≥ 140 mg/dl, serum HDL cholesterol < 40 mg/dl or prescription of lipid-lowering agents including statin [5]. Chronic kidney disease (CKD) covers the stage of impaired renal function of estimated glomerular filtration rate (eGFR) ≤ 60 ml/min/1.73 m^2 [6].

2.2 Measurements of IMT

Carotid intima-media thickness (IMT) was estimated routinely by two kinds of high-resolution duplex ultrasonography in our vascular laboratory (Xario, Toshiba Medical System, Otawara, Japan; iE33, Philips Electronics Japan, Tokyo, Japan) in patients hospitalized to our Center. Patients were examined at supine position after the bed rest with at least five minutes. The scanning area included bilateral common carotid arteries, carotid bifurcations, and internal and external carotid arteries on both sides. Longitudinal B-mode and Doppler images of these areas at the end-diastolic phase were stored to observe vessel morphology and atherosclerotic plaque. Analyses included whether the carotid intima was smooth, and the location, amount and property of the plaque. For IMT estimation, scanning area was segmented on each side into common carotid arteries proximal to the bifurcation (1.0 cm in length), the carotid bulb, and the proximal portion of the internal carotid arteries (1.0 cm in length). IMT was defined as the perpendicular distance between the main surface of the intima and the interface of the tunica media and adventitia. The average of one side of IMT was calculated by taking the mean of the three segments, and bilateral IMT were averaged. An average IMT ≥ 1.0 mm was considered as carotid arterial thickening, and an IMT ≥ 1.2 mm was labeled as atherosclerotic plaque. IMT was estimated by three experienced sonographers in a blind manner. By using preliminary off-line analysis, observer variations of IMT measurements were estimated. Intra-observer and inter-observer variations were 5.4±1.9% and 8.2±2.6%, respectively.

2.3 Statistical Analyses

All data were expressed as means±standard deviation (SD). Data sets were examined by Kolmogorov-Smirnov test and Shapiro-Wilks test for normality. Linear regression was fitted by the standard least squares method. Intergroup comparisons of normally distributed continuous variables were performed by Student's *t*-test. After the Pearson's multiple correlation analyses, significant contributors to IMT were determined by stepwise multiple regression analysis. Significance of the decision coefficient was estimated by F test. Multi-co-linearity was monitored by variance inflation factors. None of the variables with missing data qualified, and dropout case was not anticipated in this retrospective study. These analyses were performed using Predictive Analytics Software (PASW) version 18.0 for Windows (Statistical Package for Social Science (SPSS), Inc., IBM, Chicago, IL, USA). Differences with two-sided $P < .05$ were considered significant.

3. RESULTS

Clinical background of the enrolled subjects (n = 70) are detailed in Table 1. All the listed continuous variables showed normality. Average BMI was close to the lower limit of mild obesity in Japanese criteria. Mean HbA1c value (NGSP) was already within the range of suspected diabetes. Approximately half of the enrolled patients had such vascular risk factors as hypertension, diabetes, dyslipidemia or current smoking. Consequently, average IMT was already in the range of atherosclerotic plaque. Aspirin and statins were prescribed to more than 80% of the patients, and renin-angiotensin-system (RAS) inhibitors such as angiotensin receptor blockers (ARB) or angiotensin converting enzyme inhibitors (ACEI) were administered to more than half of the enrolled patients (Table 1). Although Japanese National Institute of Public Health (NIPH) promotes nutritional education, all the enrolled patients had an average diet which gradually tends to be westernized [7].

As in literature [8], strong association between IMT and age was observed in our simple regression analysis ($r = .420$, $P = .003$). Atherosclerotic risk factors of continuous parameters were considered as independent variables and age was set as a control variable. Table 2 indicates the partial correlation between age-adjusted IMT and risk factors. Although positive partial correlation between serum EPA/AA and IMT ($r = .277$, $P = .056$) and negative partial correlation between EPA/AA and HbA1c ($r = -.281$, $P = .053$) were found to be marginal, no significant partial correlation coefficients were obtained after the adjustment for age (Table 2).

Table 1. Baseline characteristics of enrolled subjects (n = 70)

Parameter	Mean±SD	Range	Parameter	n	%
age (y.o.)	69.7±7.8	50–88	gender (M/F)	53/17	—
IMT (mm)	1.93±0.85	0.6–4.9	CAD	43	61.4
ABI	0.98±0.21	0.45–1.27	PAD	38	54.3
BMI (kg/m^2)	24.1±3.6	15.5–35.7	hypertension	65	92.9
serum EPA/AA	0.41±0.19	0.15–0.94	diabetes	41	58.6
HbA1c (NGSP) (%)	6.4±1.2	3.3–9.8	dyslipidemia	57	81.4
UA (mg/dl)	6.0±1.5	2.3–10.1	BMI ≥ 25	26	37.1
T.chol (mg/dl)	175.7±35.9	94–270	current smoking	53	75.7
LDL-chol (mg/dl)	99.4±30.2	35–167	Aspirin	59	84.3
HDL-chol (mg/dl)	47.3±10.6	25–75	Clopidogrel	29	41.4
TG (mg/dl)	140.4±65.4	45–308	Statins	58	82.8
CRP (mg/dl)	0.38±0.74	0.01–3.80	RAS-inhibitors	45	64.3
BNP (pg/ml)	82.3±128.0	4–928	Ca-blockers	35	50.0
Cr (mg/dl)	1.30±1.37	0.50–8.16	β-blockers	21	30.0
eGFR (ml/min/1.73m^2)	59.7±21.0	6–110	anticoagulants	4	5.7

AA, Arachidonic acid; ABI, ankle-brachial index; BMI, body mass index; CAD, coronary artery disease; EPA, eicosapentaenoic acid; IMT, carotid intima-media thickness; PAD, peripheral artery disease

Table 2. Age-adjusted partial correlation coefficients and probability (n = 70)

	IMT	BMI	EPA/AA	HbA1c	LDL	CRP	eGFR
IMT	1.000	-.165	.277	-.165	.053	-.165	-.019
		.262	.056	.262	.718	.262	.899
BMI	-.165	1.000	.050	.076	.015	.281	.077
	.262		.734	.610	.919	.053	.603
EPA/AA	.277	.050	1.000	-.281	-.042	-.037	.050
	.056	.734		.053	.778	.802	.734
HbA1c	-.165	.076	-.281	1.000	-.021	-.070	.147
	.262	.610	.053		.889	.638	.320
LDL	.053	.015	-.042	-.021	1.000	.042	-.073
	.718	.919	.778	.889		.777	.624
CRP	-.165	.281	-.037	-.070	.042	1.000	.076
	.262	.053	.802	.638	.777		.609
eGFR	-.019	.077	.050	.147	-.073	.076	1.000
	.899	.603	.734	.320	.624	.609	

Upper column is partial correlation coefficient (r), whereas lower column is probability for r (P). Abbreviations are as in Table 1

Stepwise multiple regression analyses were applied to investigate the significant contributors to IMT. EPA/AA was incorporated into the regression model because of the marginal significance. Age, HbA1c, LDL, BMI and smoking were included in this model as known atherosclerotic risk factors. CRP was also included as an inflammatory marker. Table 3 shows that both age (*P* = .001 or less) and serum EPA/AA (*P* = .011 to .024) are significant contributors to the IMT. Partial regression coefficient of age was positive (0.483), and this coefficient of EPA/AA ratio was also unexpectedly positive (0.237).

Before age-adjustment, simple correlation analysis demonstrated age-dependent increase in IMT (r = .420, *P* = .003), but no correlation between IMT and EPA/AA at all (r = .224, *P* = .122). Therefore, in more detail, IMT and EPA/AA were plotted as a function of the generation of the enrolled patients (Fig. 1). IMT gradually increased according to advance of senescence, whereas EPA/AA ratio tended to be reduced by aging until 80 years old (0.39±0.18 in those < 80 years) and then increased steeply at ≥ 80 years old (0.54±0.27 in those ≥ 80 years, *P* = .055). Such a complicated correlation between EPA/AA and age was considered to be the main cause of seemingly paradoxical correlation between EPA/AA and IMT. Within the same generation of enrolled patients aged < 80 years old, mean IMT tended to be greater in patients showing lower EPA/AA ratio, although

significance was not obtained due to small sample size after segmentation of patients by generation spanning 10 years. IMT in patients prescribed with statins (1.93±0.88, n = 58) did not differ from that in patients without prescription of statin (1.91±0.75, n = 12). Likewise, EPA/AA in patients with statin treatment (0.41±0.20) was not different from that in patients without it (0.40±0.17), suggesting that PUFA profile is independent of cholesterol metabolism.

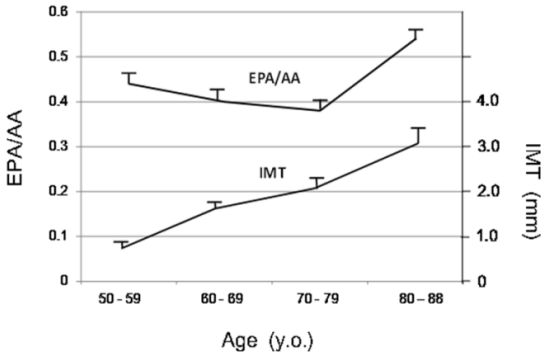

Fig. 1. Levels of serum EPA/AA and IMT in atherosclerotic patients hospitalized for vascular intervention in different generation spanning 10 years

Values are expressed as means + standard deviation (SD). Carotid intima-media thickness (IMT) increased gradually according to aging, whereas relationship between serum eicosapentaenoic acid to arachidonic acid ratio (EPA/AA) and age was complicated

As a matter of fact, representative carotid ultrasound imaging of two Japanese male patients was shown in Fig. 2. These patients are smokers. Fig. 2A is a 71-year-old hypertensive male patient with coronary artery disease. Percutaneous coronary intervention (PCI) including stent implantation was performed for left anterior descending stenotic lesion. Mean IMT was 1.29 mm, and the ratio of serum EPA/AA was 0.61 in this patient. Fig. 2B is a 74-year-old diabetic male patient with both coronary artery disease and peripheral artery disease (PAD). PCI was conducted and followed by endovascular therapy for PAD. Mean IMT was 1.78 mm and serum EPA/AA was 0.36 in this case. Considering EPA/AA as a 'residual' risk factor, partial correlation analyses were performed with conventional risk factors (age, BMI and HbA1c) set in control variables in a stepwise manner, and this analysis with multiple adjustments demonstrated significant negative correlation between EPA/AA and IMT (r = -.262, *P* = .049).

4. DISCUSSION

The main findings of the present study are 1) age-dependent increase in IMT, 2) EPA/AA as a seemingly paradoxical positive contributor to IMT due to age-dependent complicated profile of EPA/AA, and 3) partial negative correlation between EPA/AA and IMT after multiple adjustments. In the last century, a large body of evidence has accumulated concerning health benefits of marine-derived PUFA. Fish oil consumption shows protective effects on a variety of cardiovascular and atherosclerotic diseases [1]. It is believed that such favorable effects are due to ω-3-PUFA family.

Japanese are characterized by unique serum ω-3-PUFA profile due to one of the highest mean dietary fish oil consumption in the world [9]. However, westernization of food in Japan is rapid and profound. This trend is evident especially in younger generation, which causes obvious decline of EPA/AA ratio in young people [10]. Kitagawa et al. [11] reported an age-dependent EPA/AA ratio in the TREAT-CAD study. They enrolled patients who admitted for diagnostic cardiac catheterization, and reported complicating profile of EPA/AA ratio as a function of age, i.e., age-dependent irregular increase of EPA/AA ratio in males, and gradual increase associated with clear dip of this ratio in females in their 50s. Although the baseline characteristics of our patients differ from those in their patients, similar trend was found in our study, i.e., clear dip in EPA/AA was found in the septuagenarians (Fig. 1). These indicate that increased IMT in octogenarians reflects age-related factor, whereas multiple factors including PUFA profile involve in IMT increment in the other generations. Dietary lifestyle seems to be westernized except for octogenarians.

Sonographic evaluation of IMT is known as an established noninvasive atherosclerotic imaging and as a good predictor of systemic vascular events [3,4]. There has been a variety of investigations reporting the link between IMT and conventional risk factors such as hypertension [12,13], insulin resistance leading to type 2 diabetes [14,15], dyslipidemia [16,17], obesity [18,19] and smoking [20,21]. Vascular risk factors affects synergistically on the development of carotid atherosclerotic disease [22]. Contrarily to large body of such evidence, there is small evidence showing correlation between intrinsic serum PUFA profile and IMT [23]. Moreover, literature investigating association of EPA/AA

with conventional risk factors focuses relatively young subjects joining health screening program. The present study enrolling severe atherosclerotic patients demonstrated both EPA/AA and age as strong predictors of carotid atherosclerosis among other risk factors (Table 3). Patients enrolled in this study are candidates of elective endovascular therapy. They are relatively old, and conventional risk factors were under the intensive treatment. Therefore, contribution to IMT of EPA/AA ratio as a 'residual' risk factor may have been intensified. Actually, statin exerting pleiotropic effects on carotid atherosclerosis [24] was prescribed in more than 80% of enrolled patients in our study. However, it showed no remarkable effects on IMT. This finding is compatible with our speculation concerning EPA/AA as a 'residual' risk.

The outcome of our study may keep expectation of intervention with EPA supplementation to carotid atherosclerotic regression. Colussi et al. [25] reported that one-year nutritional counseling promoting fish intake resulted in changes of erythrocyte membrane fatty acid compositions and reduction of IMT in a prospective study. Baldassarre et al. [26] also demonstrated that possible preventive effects of 2-years intake of ω-3 PUFA agents (6 g/day) on IMT progression in a double-blind pilot study, which did not reach statistical significance. Beneficial effect

Table 3. Multivariate regression analysis predicting contributors to IMT (n = 70)

Model		R^2	F	*P*
1	age (0.001), EPA/AA (0.024), smoking (0.047), LDL (0.109), CRP (0.269), HbA1c (0.439), BMI (0.732)	.387	3.873	.002
2	age (< 0.001), smoking (0.020), EPA/AA (0.024), LDL (0.096), CRP (0.189), HbA1c (0.396)	.385	4.591	.001
3	age (< 0.001), EPA/AA (0.012), smoking (0.025), LDL (0.097), CRP (0.212)	.375	5.394	.001
4	age (< 0.001), EPA/AA (0.011), smoking (0.031), LDL (0.122)	.352	6.260	< .001
5	age (0.001), EPA/AA (0.016), smoking (0.078)	.318	7.290	< .001

eGFR was excluded due to multi-co-linearity. Figures in parentheses indicate P values. Abbreviations are as in Table 1

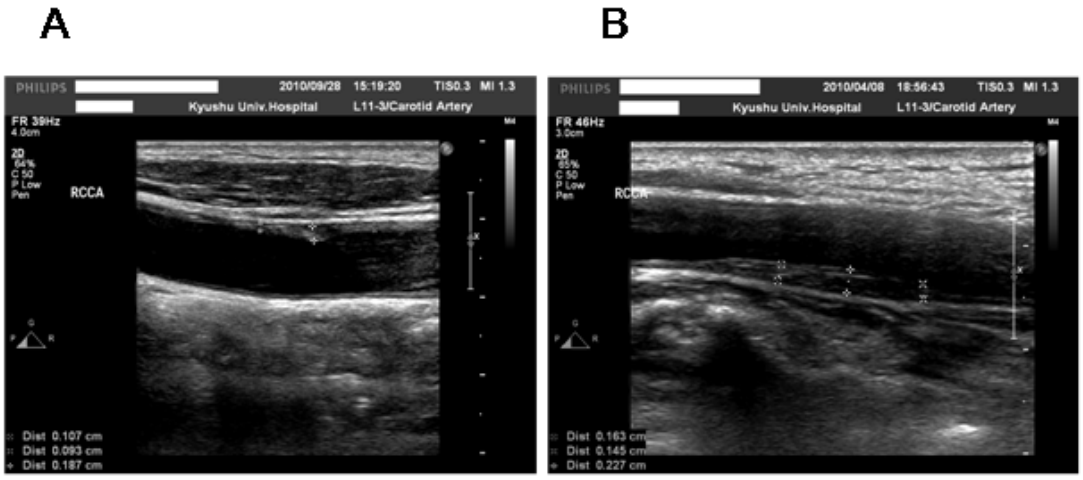

Fig. 2. Two representative carotid ultrasound imaging

Carotid intima-media thickness (IMT) was estimated at three segments of common carotid arteries proximal to the bifurcation, the carotid bulb, and the proximal portion of the internal carotid arteries. The average was calculated by taking the mean of the three segments.

***A**: Mean IMT was 1.29 mm in 71-year-old male patient with coronary artery disease for which percutaneous coronary intervention (PCI) including stent implantation was performed. Serum eicosapentaenoic acid to arachidonic acid ratio (EPA/AA) was 0.61. **B**: Mean IMT was 1.78 mm in 74-year-old male patient with both coronary and peripheral artery diseases. PCI was conducted and followed by endovascular therapy for peripheral artery diseases. EPA/AA was 0.36. These Japanese patients were smokers*

of ω-3 PUFA is not limited to adulthood, i.e., ω-3 PUFA exerts positive effects on bronchial asthma, neuropsychiatric disorder and brain cognitive function in childhood [27]. Although dietary supplementation of commercial PUFA agents is promising for anti-atherosclerotic intervention, it warrants future large-scale cohort. Because meta-analysis of carotenoids (i.e., α- and β-carotene, lycopene, lutein) rich in fruits and vegetables demonstrates conflicting results showing both anti-oxidant and pro-oxidant effects in cardiovascular care [28]. With respect to atherosclerotic quantification, there is no global standard or detailed guideline for measuring carotid IMT. IMT measurement technique should be standardized internationally to increase statistical power to predict future vascular events per unit IMT increment.

5. CONCLUSION

Since PUFA is not intrinsically synthesized in human body, serum EPA/AA ratio depends solely on dietary lifestyle. This small-sample, single-center study demonstrated that EPA/AA is a determinant of IMT among other vascular risk factors at least in Japanese carotid atherosclerotic patients hospitalized for endovascular therapy against coronary or peripheral artery diseases. The age-dependent complicated profile of EPA/AA in the severe atherosclerotic patients underlay the EPA/AA ratio as a seemingly paradoxical positive contributor to IMT. Although multiple adjustments yielded significant negative correlation between EPA/AA and IMT, it is premature to extrapolate these findings to the general population in health screening program promoting EPA supplementation for atherosclerotic regression.

CONSENT

Medical information extraction was informed to and signed informed consent was obtained from all the enrolled patients on admission.

ETHICAL APPROVAL

All procedures performed in this study involving patients were in accordance with the ethical standards of our institutional and/or national research committee and with the updated Declaration of Helsinki (2008).

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COMPETING INTERESTS

Authors have declared that no competing interests exist.

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