Original Article

Effects of Docosahexaenoic Acid on the Endothelial Function in Patients with Coronary Artery Disease

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Aim: The consumption of n-3 polyunsaturated fatty acids (PUFA), including docosahexaenoic acid (DHA), reduces the incidence of cardiovascular events, and reduced serum levels of n-3 PUFA may be associated with an increased risk of cardiovascular events. However, controversy remains regarding which components of PUFA are associated with the endothelial function in patients with coronary artery disease (CAD). We therefore examined the associations between the n-3 and n-6 PUFA levels and CAD.

Methods: We retrospectively reviewed 160 consecutive Japanese patients with CAD whose endothelial function was measured according to the percent change in flow-mediated dilation (FMD) and the serum levels of n-3 PUFA, including eicosapentaenoic acid (EPA) and DHA, and n-6 PUFA, including arachidonic acid (AA) and dihomo-gamma-linolenic acid (DHLA).

Results: A single regression analysis showed no relationships between the FMD and the serum levels of PUFA, including EPA, DHA, AA and DHLA. In contrast, a multiple regression analysis showed that the DHA level was a positive ($P < 0.01$) and age was a negative ($P < 0.001$) contributor to an increased FMD; however, sex, body mass index, systolic and diastolic blood pressure, current/past smoking and the levels of HbA1c, triglycerides, low-density lipoprotein cholesterol, high-density lipoprotein cholesterol, EPA, AA and DHLA did not significantly affect the outcome.

Conclusions: The serum level of DHA is associated with the endothelial function evaluated according to the FMD in patients with CAD, thus suggesting that a low serum level of DHA may be a predictive biomarker for endothelial dysfunction.

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Key words: Flow-mediated dilation, n-3 polyunsaturated fatty acids, Docosahexaenoic acid

Introduction

Despite advances in reducing the low-density

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lipoprotein cholesterol (LDL-C) levels to below target ranges with lipid-lowering agents, including HMG-CoA reductase inhibitors, the incidence of cardiovascular events remains high. Therefore, other therapies to reduce cardiovascular events should be identified¹.

Vascular endothelial dysfunction is recognized to be a critical factor in the development of coronary artery disease (CAD)², and evaluating the endothelial function is therefore important for determining the risk of CAD. Recently, the flow-mediated dilation

(FMD) has become widely used as a noninvasive method to assess the brachial arterial endothelial function by measuring the diameter on ultrasound before and after a period of induced ischemia of the forearm. The degree of dilation is mediated by endothelial nitric oxide (NO) release in response to increased shear stress^{3, 4}. As the FMD is thought to reflect the ability of the endothelium to release NO, which regulates vascular tone, this parameter may be a useful surrogate marker for cardiovascular events^{5, 6}.

It has been reported that reduced serum levels of n-3 polyunsaturated fatty acids (PUFA), including eicosapentaenoic acid (EPA) and docosahexaenoic acid (DHA), are associated with an increased incidence of cardiovascular events and mortality^{7, 8}, while supplementation of n-3 PUFA improves the endothelial function^{9, 10}. Therefore, the cardiovascular event risk-lowering effects of n-3 PUFA may be mediated partly via endothelial-dependent mechanisms, and reduced serum levels of n-3 PUFA may be associated with an increased risk of cardiovascular events in patients with CAD. In addition, n-6 PUFA, including arachidonic acid (AA) and dihomo-gamma-linolenic acid (DHLA), are known to be proinflammatory fatty acids, and their effects on the cardiovascular system remain controversial¹¹.

Hence, to elucidate which components of PUFA are associated with the endothelial function in patients with CAD, we evaluated the association between each PUFA component, including EPA, DHA, AA and DHLA, and the endothelial function in patients with CAD.

Methods

We retrospectively reviewed 160 consecutive Japanese patients with CAD who had undergone measurements of the FMD and serum levels of PUFA. The patients were recruited from the Department of Cardiology at Tokushima University Hospital between October 2012 and May 2013.

Patients with CAD were defined as having a history of myocardial infarction, angiographic evidence of $\geq 50\%$ stenosis in ≥ 1 coronary artery, evidence of exercise-induced ischemia or a history of coronary revascularization. The exclusion criteria were as follows: the use of fish oil supplements or n-3 fatty acid-containing drugs or a history of myocardial infarction within the past month. In addition, patients with symptomatic active malignant diseases, renal failure (serum creatinine level >4.0 mg/dL) or liver dysfunction (aspartate aminotransferase level >100 IU/L, alanine aminotransferase level >100 IU/L) were excluded.

Blood pressure was measured in the sitting position according to previously described guidelines¹². Hypertensive patients were defined as those with a systolic blood pressure of ≥ 140 mm Hg and/or diastolic blood pressure of ≥ 90 mm Hg and/or currently receiving antihypertensive medications. Dyslipidemic patients were defined as those with an LDL-C level of ≥ 140 mg/dL, triglyceride level of ≥ 150 mg/dL, high-density lipoprotein cholesterol (HDL-C) level of <40 mg/dL or currently receiving lipid-lowering medications. Diabetic patients were defined as individuals receiving insulin or oral hypoglycemic agents or those with an HbA1c level of $\geq 6.5\%$, fasting plasma glucose level of ≥ 126 mg/dL or casual plasma glucose level of ≥ 200 mg/dL. The smoking status (current or past smoker) was evaluated using a questionnaire. The body mass index was calculated as an index of obesity.

The FMD of the brachial artery was measured to assess endothelium-dependent vascular reactivity using a high-resolution ultrasound system coupled to a computer-assisted analysis software program (UNEX-EF18G, UNEX Co, Nagoya, Japan). The measurements were obtained by trained vascular ultrasonographers according to the recommendations of the International Brachial Artery Reactivity Task Force Guidelines for the ultrasound assessment of endothelial-dependent FMD vasodilatation of the brachial artery, as described elsewhere^{13, 14}.

The levels of four bioactive serum fatty acids, including EPA, DHA, AA and DHLA, were measured using a gas - liquid chromatograph (GC-2010; Shimadzu, Kyoto, Japan) equipped with a capillary column (TC-70; GL Sciences, Tokyo, Japan) at a commercially available laboratory (SRL, Tokyo, Japan). Because AA, an n-6 PUFA, is a proatherosclerotic fatty acid, a reduced EPA/AA ratio is widely used as a biomarker for the development of CAD¹⁵. The EPA/AA and DHA/AA ratios were therefore calculated.

In addition, other biochemical parameters, including the levels of LDL-C, triglycerides, HDL-C, random plasma glucose, HbA1c and serum creatinine, were measured.

Written informed consent was not required due to the retrospective nature of the investigation. This study protocol was approved by the Tokushima University Hospital Ethics Committee in accordance with the Declaration of Helsinki.

Statistical Analysis

Continuous variables were averaged and expressed as the mean $\pm$ standard deviation (SD) or as a percentage for categorical parameters. A single

Table 1. Clinical characteristics of the patients

Variable	
Number of patients	160
Male	123 (76.9%)
Age, (y)	70.3 ± 9.4
Body mass index, (kg/m ²)	23.1 ± 4.7
Systolic blood pressure, (mmHg)	131.7 ± 18.4
Diastolic blood pressure, (mmHg)	74.5 ± 10.1
Low-density lipoprotein cholesterol, (mg/dL)	98.9 ± 24.5
Triglycerides, (mg/dL)	131.7 ± 59.1
High-density lipoprotein cholesterol, (mg/dL)	54.1 ± 16.5
HbA1c, (%)	6.5 ± 0.90
Random plasma blood glucose, (mg/dL)	122.1 ± 40.6
Serum creatinine, (mg/dL)	0.87 ± 0.30
Fatty acid concentrations	
DHA, (μg/mL)	128.5 ± 43.2
EPA, (μg/mL)	57.5 ± 29.7
AA, (μg/mL)	167.7 ± 44.3
DHLA, (μg/mL)	34.7 ± 12.0
EPA/AA	0.37 ± 0.22
DHA/AA	0.81 ± 0.29
FMD, (%)	4.1 ± 2.2
Comorbidities	
Dyslipidemia	130 (81.3%)
Hypertension	132 (82.5%)
Diabetes mellitus	73 (45.6%)
Current/past smoker	102 (63.8%)
Drugs taken	
HMG-CoA reductase inhibitor	134 (83.8%)
Ezetimibe	14 (8.8%)
Fibrate	1 (0.6%)
ACE-I/ARB	112 (70.0%)
β-blocker	58 (36.3%)
Calcium channel blocker	60 (37.5%)

The values are presented as the mean ± SD or *n* (%) as appropriate. HbA1c, hemoglobin A1c; DHA, docosahexaenoic acid; EPA, eicosapentaenoic acid; AA, arachidonic acid; DHLA, dihomogamma-linolenic acid; FMD, flow-mediated dilation; ACE-I, angiotensin-converting enzyme inhibitor; ARB, angiotensin II receptor blocker

regression analysis was used to assess the correlations between the FMD and the CAD risk factors. A multiple regression analysis was used to assess the degree of association between the FMD and the CAD risk factors. The Kolmogorov-Smirnov test was performed to evaluate whether the parameters were normally distributed. Non-normally distributed parameters, including the FMD, systolic blood pressure, triglycerides, HDL-C, HbA1c, EPA, EPA/AA, DHA and DHA/AA, were natural log transformed and analyzed. All statistical analyses were performed using the JMP 10 software program. Statistical significance was

Fig. 1. Distribution of the n-3 and n-6 polyunsaturated fatty acid concentrations stratified by sex and age

The data are presented as the mean ± SD. EPA, eicosapentaenoic acid; DHA, docosahexaenoic acid; AA, arachidonic acid; DHLA, dihomogamma-linolenic acid

defined as a *P* value of <0.05.

Results

Clinical Characteristics of the Subjects

The patient characteristics are shown in **Table 1**. Of note, 83.8% of the subjects were taking HMG-CoA reductase inhibitors. The distribution of the n-3 and n-6 PUFA concentrations stratified by sex and age is shown in **Fig. 1**. Although there were no significant differences, the DHA levels were higher in women than in men and the DHA levels were higher in

Table 2. Multiple regression analysis for determinants of the percent change in the flow-mediated dilation

Model 1			
Variable	Coefficient	95% Confidence Interval	P-value
Age	-0.08	-0.13 to -0.04	<0.001
Male sex	0.15	-0.36 to 0.67	0.56
Body mass index	-0.02	-0.11 to 0.08	0.72
Systolic blood pressure	-0.01	-0.03 to 0.01	0.47
Diastolic blood pressure	-0.02	-0.06 to 0.02	0.41
Triglycerides	-0.01	-0.01 to 0.001	0.06
Low-density lipoprotein cholesterol	-0.01	-0.02 to 0.01	0.61
High-density lipoprotein cholesterol	-0.02	-0.05 to 0.01	0.08
HbA1c	-0.06	-0.46 to 0.34	0.77
Current/past smoker	-0.13	-0.55 to 0.29	0.53
DHA	0.02	0.01 to 0.03	<0.01
EPA	-0.02	-0.03 to 0.001	0.06
AA	0.003	-0.01 to 0.01	0.63
DHLA	-0.02	-0.06 to 0.02	0.31
HbA1c, hemoglobin A1c; DHA, docosahexaenoic acid; EPA, eicosapentaenoic acid; AA, arachidonic acid; DHLA, dihomogamma-linolenic acid			
Model 2			
Variable	Coefficient	95% Confidence Interval	P-value
Age	-0.08	-0.13 to -0.03	<0.001
Male sex	0.08	-0.42 to 0.59	0.74
Body mass index	-0.01	-0.11 to 0.08	0.80
Systolic blood pressure	-0.01	-0.03 to 0.01	0.49
Diastolic blood pressure	-0.01	-0.06 to 0.03	0.45
Triglycerides	-0.01	-0.01 to 0.001	0.09
Low-density lipoprotein cholesterol	-0.01	-0.02 to 0.01	0.62
High-density lipoprotein cholesterol	-0.01	-0.03 to 0.01	0.31
HbA1c	-0.08	-0.49 to 0.33	0.71
Current/past smoker	-0.11	-0.53 to 0.30	0.59
DHA/AA	1.70	-0.10 to 3.51	0.06
EPA/AA	-1.39	-3.67 to 0.88	0.23
HbA1c, hemoglobin A1c; DHA, docosahexaenoic acid; AA, arachidonic acid; EPA, eicosapentaenoic acid			

patients ≥ 70 years age than in those <70 years of age.

Correlation between n-3 PUFA and FMD

The single regression analysis showed no relationship between the FMD and the serum levels of fatty acids, including EPA, DHA, AA and DHLA (data not shown). A multiple regression analysis was performed to identify determinants of FMD. Consequently, age ($P<0.001$) was found to be a negative contributor and the DHA level ($P<0.01$) was found to be a positive contributor to the FMD. In contrast, sex, body mass index, systolic and diastolic blood pres-

sure, current/past smoking and the levels of HbA1c, triglycerides, LDL-C, HDL-C, EPA, AA and DHLA did not show any statistically significant correlations (Model 1 in **Table 2**). Neither the EPA/AA nor DHA/AA ratio were determinants of the FMD in the CAD patients (Model 2 in **Table 2**). In addition, the subanalyses of men and patients taking HMG-CoA reductase inhibitors demonstrated age to be a negative contributor and the DHA and DHA/AA levels to be positive contributors to the FMD (**Supplemental Tables 1 and 2**). Due to the small number of patients, subanalyses of women and patients not taking HMG-

CoA reductase inhibitors and were not performed.

Discussion

The present study demonstrated that the serum levels of DHA contribute significantly to increased FMD values in patients with CAD. In particular, the present subanalysis of patients taking HMG-CoA reductase inhibitors showed that the DHA and DHA/AA levels are positive contributors to an increased FMD. These results indicate that a low DHA level is a residual risk factor for endothelial dysfunction in CAD patients taking HMG-CoA reductase inhibitors.

In addition to its triglyceride-lowering effects, n-3 PUFA has several positive effects on the cardiovascular system, including effects on endothelial nitric oxide synthase (eNOS) enhancement¹⁶⁾ and anti-inflammatory actions. It has also been reported that n-3 PUFA increases the expression and activation of eNOS, leading to vasodilation¹⁷⁾. Furthermore, the EPA-induced translocation of eNOS from caveolin in the cell membrane to the cytosol results in the enhancement and activation of the eNOS system and may be a cause of EPA-induced vasodilation¹⁸⁾. Meanwhile, DHA has been reported to decrease the expression of proinflammatory cytokines (e.g. tumor necrosis factor- α , interleukin 1 β and interleukin-6) and cell adhesion molecules (e.g. vascular cell adhesion molecule 1, E-selectin and intercellular adhesion molecule 1), which may have protective effects on the endothelium^{19, 20)}.

Many clinical studies have demonstrated the effects of fish oil or n-3 PUFA on the incidence of cardiovascular events; however, the differential effects of DHA and EPA are less well established²¹⁾. It has been reported that a low level of DHA is associated with all-cause death, although this relationship is not observed in patients with a low level of EPA after acute myocardial infarction⁸⁾, and that higher plasma levels of DHA, but not EPA, are associated with a reduced progression of coronary atherosclerosis in women with CAD²²⁾. These findings suggest that DHA exerts a protective effect on CAD and indicates the possibility that the administration of DHA may reduce the onset of cardiovascular events.

Several previous studies have shown the effects of DHA on the endothelial function. For example, a double-blind placebo-controlled trial showed that DHA, but not EPA, enhances vasodilation in response to acetylcholine, which induces endogenous NO release^{23, 24)}. Another study showed that DHA supplementation increases the FMD values in hyperlipidemic children²⁵⁾. Hence, the positive effects of n-3

PUFA on cardiovascular events may occur via DHA-induced improvements in the endothelial function.

In addition, DHA achieves better tissue distribution than EPA, which exhibits very little dispersion into tissue^{26, 27, 28)}. The properties of being a bioavailable fatty acid may explain the preferable effects of DHA.

A large clinical trial recently provided evidence of the effects of EPA or fish oil on reducing cardiovascular events²⁹⁾; however, there have been no large clinical trials showing the positive effects of DHA on cardiovascular events. Therefore, large clinical cohort studies are needed to clarify the differential effects of the components of n-3 PUFA on the endothelial function and incidence of cardiovascular events.

This study is associated with several limitations. First, this study was a retrospective study with a small sample size at a single center. Second, the cross-sectional nature of the study design does not allow for evaluations of the direct effects of DHA on the endothelial function.

Conclusion

The serum levels of DHA, but not EPA, are associated with the endothelial function evaluated according to the FMD in patients with CAD, thus suggesting that a low serum DHA level may be a predictive biomarker for endothelial dysfunction.

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Disclosure

The authors declare that they have no conflicts of interest to disclose.

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Supplemental Table 1. Multiple regression analysis for determinants of the percent change in the flow-mediated dilation in the patients taking HMG-CoA reductase inhibitors

Model 1			
Variable	Coefficient	95% Confidence Interval	P-value
Age	-0.03	-0.04 to -0.01	<0.001
Male sex	0.01	-0.16 to 0.18	0.90
Body mass index	-0.01	-0.04 to 0.01	0.57
Systolic blood pressure	-0.52	-1.52 to 0.49	0.31
Diastolic blood pressure	-0.01	-0.02 to 0.01	0.32
Triglycerides	-0.29	-0.60 to 0.03	0.07
Low-density lipoprotein cholesterol	-0.01	-0.01 to 0.01	0.60
High-density lipoprotein cholesterol	-0.23	-0.71 to 0.26	0.36
HbA1c	-0.30	-1.14 to 0.53	0.48
Current/past smoker	-0.03	-0.17 to 0.11	0.66
DHA	0.64	0.13 to 1.14	0.01
EPA	-0.27	-0.56 to 0.02	0.07
AA	0.0003	-0.003 to 0.004	0.85
DHLA	-0.01	-0.02 to 0.01	0.27
HbA1c, hemoglobin A1c; DHA, docosahexaenoic acid; EPA, eicosapentaenoic acid; AA, arachidonic acid; DHLA, dihomo-gamma-linolenic acid			
Model 2			
Variable	Coefficient	95% Confidence Interval	P-value
Age	-0.02	-0.04 to -0.01	<0.001
Male sex	-0.01	-0.16 to 0.16	0.99
Body mass index	-0.01	-0.04 to 0.02	0.69
Systolic blood pressure	-0.53	-1.5 to 0.45	0.28
Diastolic blood pressure	-0.01	-0.02 to 0.01	0.36
Triglycerides	-0.29	-0.56 to -0.03	0.03
Low-density lipoprotein cholesterol	-0.01	-0.01 to 0.01	0.46
High-density lipoprotein cholesterol	-0.14	-0.59 to 0.31	0.53
HbA1c	-0.31	-1.15 to 0.53	0.46
Current/past smoker	-0.04	-0.18 to 0.10	0.57
DHA/AA	0.49	0.03 to 0.95	0.03
EPA/AA	-0.21	-0.49 to 0.07	0.14
HbA1c, hemoglobin A1c; DHA, docosahexaenoic acid; AA, arachidonic acid; EPA, eicosapentaenoic aci			

Supplemental Table 2. Multiple regression analysis for determinants of the percent change in the flow-mediated dilation in men

Model 1			
Variable	Coefficient	95% Confidence Interval	P-value
Age	−0.03	−0.05 to −0.02	<0.001
Body mass index	−0.01	−0.03 to 0.03	0.81
Systolic blood pressure	0.10	−0.94 to 1.14	0.85
Diastolic blood pressure	−0.01	−0.03 to 0.01	0.06
Triglycerides	−0.23	−0.55 to 0.08	0.14
Low-density lipoprotein cholesterol	0.01	−0.01 to 0.01	0.41
High-density lipoprotein cholesterol	−0.32	−0.81 to 0.17	0.20
HbA1c	−0.50	−1.34 to 0.31	0.22
Current/past smoker	−0.03	−0.16 to 0.11	0.69
DHA	0.72	0.19 to 1.25	<0.01
EPA	−0.12	−0.43 to 0.19	0.43
AA	−0.01	−0.01 to 0.01	0.37
DHLA	−0.01	−0.02 to 0.01	0.45
HbA1c, hemoglobin A1c; DHA, docosahexaenoic acid; EPA, eicosapentaenoic acid; AA, arachidonic acid; DHLA, dihomogamma-linolenic acid			
Model 2			
Variable	Coefficient	95% Confidence Interval	P-value
Age	−0.03	−0.05 to −0.02	<0.001
Body mass index	−0.01	−0.03 to 0.03	0.88
Systolic blood pressure	−0.01	−1.00 to 0.98	0.99
Diastolic blood pressure	−0.01	−0.02 to 0.01	0.08
Triglycerides	−0.22	−0.48 to 0.04	0.10
Low-density lipoprotein cholesterol	0.01	−0.01 to 0.01	0.59
High-density lipoprotein cholesterol	−0.21	−0.64 to 0.22	0.33
HbA1c	−0.50	−1.31 to 0.32	0.23
Current/past smoker	−0.03	−0.16 to 0.10	0.68
DHA/AA	0.62	0.14 to 1.10	0.01
EPA/AA	−0.08	−0.37 to 0.22	0.60
HbA1c, hemoglobin A1c; DHA, docosahexaenoic acid; AA, arachidonic acid; EPA, eicosapentaenoic acid			