Anti-Atherosclerotic Effect of Eicosapataenoic Acid in Patients Undergoing Percutaneous Coronary Intervention

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ABSTRACT

Background: Statins exert a range of pleiotropic effects, not only reducing serum levels of low density lipoprotein (LDL), but also inhibiting the development of coronary atherosclerosis. Even if optimal reduction is achieved in LDL levels, however, there is still no guarantee that cardiovascular events will be prevented. The Japan Eicosapentaenoic Acid (EPA) Lipid Intervention Study demonstrated that intake of purified EPA in addition to statins reduced cardiovascular mortality in patients with coronary artery disease (CAD) and identified a low serum EPA/arachidonic acid (AA) ratio as a novel cardiovascular risk factor. On the other hand, correlations have been shown between CAD and flow-mediated dilation (FMD), intima-media complex thickness (IMT), and the cardio-ankle velocity index (CAVI). The purpose of this study was to evaluate the effects of administration of EPA on FMD, IMT, and CAVI in patients with CAD undergoing percutaneous coronary intervention (PCI).

Method: A total of 30 CAD patients undergoing PCI were included in the study. The EPA/AA ratio in the serum of all patients was below 0.4. The patients were divided into a 1800 mg/day EPA administration group and a non-administered control group. The FMD, IMT and CAVI were determined at baseline and 6 and 12 months later.

Results: Flow-mediated dilation showed a time-dependent increase in the EPA group, but not in the controls. No significant change was observed in the IMT in the EPA group, whereas it tended to increase time-dependently in the controls. No significant change was observed in the CAVI in either group.

Conclusion: Administration of EPA improves vascular endothelial function, thus inhibiting progression of atherosclerosis in patients with high risk CAD such as those requiring PCI, and possibly enhancing secondary prevention.

INTRODUCTION

Statins exert a range of pleiotropic effects, not only reducing serum levels of low density lipoprotein (LDL), but also inhibiting the development of coronary atherosclerosis. Even if optimal reduction is achieved in LDL levels, however, there is still no guarantee that cardiovascular events will be prevented, indicating the need to develop novel therapeutic strategies for dyslipidemia beyond statins. The results of an earlier Danish epidemiological study comparing Caucasian and Inuit populations suggested that intake of n-3 polyunsaturated fatty acids (PUFA) such as eicosapentaenoic acid (EPA) and docosahexaenoic acid (DHA) decreases the incidence of cardiovascular events,[1] and since then many other studies have reached a similar conclusion.[2] The Japan EPA Lipid Intervention Study (JELIS) demonstrated that intake of purified EPA in addition to statins reduced cardiovascular mortality[3] to the same extent that percutaneous coronary intervention (PCI) decreased the incidence of major adverse cardiac events.[4]

Vascular endothelial dysfunction is a major factor in the progression of coronary atherosclerosis, indicating the importance of assessing such function in patients with coronary artery disease (CAD). Recently, brachial artery flow mediated dilation (FMD) has been recognized as a promising method of assessing vascular endothelial function.[5,6] The intima-media complex thickness (IMT) of the carotid artery and cardio-ankle velocity index (CAVI) have also been widely used to assess progression of atherosclerosis; moreover, their association with progression of CAD has

also been reported.[7, 8)]

Serum levels of long chain PUFA are determined almost exclusively by PUFA intake. A lower ratio of n-3 PUFA to n-6 PUFA has been associated with a higher incidence of acute coronary syndrome (ACS).[2)] In an earlier study, this group also noted that a low EPA/arachidonic acid (AA) ratio might be a prognostic predictor of ACS.[9)] The purpose of the present study was to clinically assess the effects of administration of a purified EPA agent on vascular function in patients with a low EPA/AA ratio undergoing PCI.

METHODS

Study design

A total of 30 CAD patients undergoing PCI comprising 25 men and 5 women aged 67.1 ± 9.1 years were included in the study. The EPA/AA ratio in the serum of all patients was below 0.4; no patient had a history of n-3 PUFA treatment and/or supplementation. After baseline assessment with FMD, IMT, and CAVI, the patients were divided into an EPA administration group (n=19) and a control group (n=11). Patients in the EPA group received an EPA agent at 1800 mg/day, while no agent was given in the control group. Flow-mediated dilation, IMT, and the CAVI were determined again at 6 and 12 months after the baseline assessment.

Lipid profiles and EPA/AA ratio

Serum levels of total cholesterol, triglycerides, high-density lipoprotein (HDL), and low-density lipoprotein (LDL) were determined to obtain a lipid profile in each patient. The total cholesterol and triglyceride levels were determined using enzymatic methods. The HDL level was measured using the precipitation method. The LDL level was calculated using the Friedewald formula (LDL = total cholesterol − HDL − triglyceride/5). Patients with a triglyceride level of over 400 mg/dl were excluded from calculation of the LDL

Serum fatty acids were assayed by gas chromatography (SRL, Tokyo, Japan). Briefly, total lipids in plasma were extracted according to the method of Folch, followed by hydrolysis 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 the GC-17A gas chromatograph (Shimadzu Corporation, Kyoto, Japan) with an Omegawax-250 Capillary Column (SUPELCO, Sigma-Aldrich, Tokyo, Japan). Reproducibility (i.e., the coefficient of variation) in determining serum EPA and AA levels by this method has been reported to be 4.4% and 3.8%, respectively.[7] The EPA/AA ration was calculated based on serum concentrations of EPA and AA.

Flow-mediated vasodilatation measurement

Flow-mediated dilation in the brachial artery was ultrasonically measured according to the standard protocol described in the guidelines[10], and the Japanese guidelines published by the Vascular Failure Working Group.[11] Vasoactive drugs were withheld on the day of measurement. Patients were required to fast for at least 4 hours and then rest in a supine position for at least 15 minutes in a temperature-controlled

quiet room. Flow-mediated dilation was then measured in the right or left arm taking a right brachial approach. The ultrasound system used was equipped with an edge-tracking system for 2-D imaging and a pulsed Doppler flow velocimeter for automatic measurement, the UNEXEF 38G (Unex Co. Ltd., Nagoya, Japan). Regarding the reproducibility of FMD measurements using this system, the correlation coefficient between two examinations was reported to be 0.86 with a coefficient of variance of 11.2%.[12] In brief, the diameter of the brachial artery at rest was measured in the cubital region and the cuff subsequently inflated to 50 mmHg above systolic blood pressure for 5 min and then deflated. The diameter at the same point was monitored continuously and maximum dilation of the brachial artery determined according to a plateau or no increase in that diameter using real-time monitoring for at least one minute after cuff deflation. Flow-mediated dilation was calculated as follows: FMD(%) = (maximum diameter – diameter at rest) × 100/diameter at rest. All measurements were performed by a single technician who was blinded to the study design.

Intima-media thickness measurement

Color Doppler ultrasound was performed with a probe frequency of 7.5 MHz by using an ultrasound device (NEMIO XG, Toshiba Corp., Tokyo, Japan). Examination was carried out with the patient in the supine position with their head turned contralaterally by 45°. A long axial section of the carotid artery was probed to determine the location of the common carotid artery bifurcation and internal carotid artery. The IMT was defined as the cross-sectional distance between the main surface of the intima and the interface of the tunica media and adventitia. The IMT at 1.5 cm proximal to the

carotid bifurcation was measured and the maximum value of either the right or left common carotid artery recorded.

Cardio-ankle vascular index measurement

The CAVI was determined using the VaSera VS-1000 (Fukuda Denshi Co. Ltd, Tokyo, Japan) by methods described previously.[13,14] Briefly, cuffs were applied to the four extremities, with the participants lying in a supine position. A microphone was attached to the right sternal border of the second intercostal space and leads placed on both wrists to obtain a phonocardiogram. The examination was performed after the patient had rested for 10 minutes. Pulse wave velocity (PWV) was determined by dividing vascular length by the period of time taken for the pulse to travel from the aortic valve to the ankle. Pulse transit time was calculated as the time lapsed between rise of the brachial and ankle pulse waves added to that between the sound of the closing of the aortic valve and the notch of the brachial pulse wave. Scale conversion from PWV to CAVI was performed using the following equation: $CAVI = a\{(2\rho/\Delta p) \times \ln(Ps/Pd) \times PWV2\} + b$, where Ps denotes systolic blood pressure; Pd, diastolic blood pressure; Δp, Ps-Pd; ρ, blood density; and a and b, constants. The average value of the right and left CAVI was used for the analysis.

Statistical analysis

All statistical analyses were performed using Excel To-kei (SSRI, Tokyo, Japan). Values were expressed as the mean ± standard deviation. Intra-group comparisons were performed using an unpaired t-test for continuous variables and the

chi-square test for categorical variables. Serial changes in data were analyzed with a repeated measures analysis of variance (ANOVA) and a post hoc Bonferroni test for inter- and intra-group comparisons. Receiver operating characteristics (ROC) analysis was performed to evaluate accuracy of EPA/AA ratio for prediction of FMD, and area under the curve (AUC) was calculated. A P value of < 0.05 was considered to indicate statistical significance.

RESULTS

No significant differences were observed in patient characteristics between the EPA administration and control groups (Table 1). Table 2 shows serial changes in the lipid profiles and EPA/AA ratio during the 12-month follow-up period. Total cholesterol, triglycerides, and LDL levels showed no significant change during the follow-up period in either group, with these levels at baseline, 6 months, and 12 months remaining comparable. The level of HDL showed a time-dependent increase in the EPA group ($P < 0.05$), but not in the control group. However, the HDL levels at baseline, 6 months, and 12 months were comparable between the two groups. The EPA/AA ratio also showed a time-dependent increase in the EPA group ($P < 0.05$), but not in the control group. Although the EPA/AA ratio at baseline was comparable between the two groups, that at 6 months ($P < 0.05$), and that at 12 months was higher in the EPA group.

Flow-mediated dilation was comparable between the EPA and control groups at baseline, at 4.5% ± 1.6% and 5.3% ± 3.6%, respectively. After that, it showed a

time-dependent increase in the EPA group to 5.5% ± 2.1% and 5.9% ± 2.0% at 6 months and 12 months, respectively ($P < 0.01$), however. The increase in FMD from 6 to 12 months was even greater in the EPA group ($P < 0.05$). No significant change was observed in the control group, however, with a change to 6.0% ± 2.7% and to 5.6% ± 2.0% at 6 and 12 months, respectively (Fig. 1). In the EPA group, the ROC analysis showed that the cut-off value of EPA/AA to achieve an over 1.0% increase in FMD at 6 months was 1.03 (AUC = 0.65).

At baseline, the IMT tended to be lower in the EPA group (1.8 ± 0.8 mm vs. 2.3 ± 1.1 mm, $P = 0.06$). No significant change was observed at 6 or 12 months in the EPA group, with values of 1.6 ± 0.6 mm and 1.7 ± 0.5 mm, respectively. It tended to increase time-dependently, however, in the control group, showing values of 2.5 ± 1.4 mm ($P = 0.05$) and 2.6 ± 1.2 mm ($P < 0.05$) at 6 and 12 months, respectively (Fig. 2).

The CAVI was significantly lower in the EPA group at baseline (8.6 ± 1.6 vs. 9.4 ± 1.8, $P < 0.05$). No significant serial change was observed in either group, with the EPA group showing values of 8.1 ± 1.8 and 9.1 ± 1.8 and the control group 9.3 ± 1.1 and 9.6 ± 0.9 at 6 and 12 months, respectively. In the EPA group, however, it significantly increased at 12 months compared with at 6 months ($P < 0.01$). The CAVI at 6 months was significantly lower in the EPA group than in the control group ($P < 0.01$)(Fig. 3).

DISCUSSION

The purpose of the present study was to assess serial change in the FMD, IMT, and CAVI in CAD patients with a low EPA/AA ratio undergoing PCI. The patients were divided into two groups, one with and one without administration of 1800 mg/day of an EPA agent. The major finding of our study is that FMD showed a time-dependent increase at 6 and 12 months in the EPA administration group, but not in the control group. No significant change was observed in the IMT in the EPA group, whereas it tended to increase time-dependently in the control group. These results suggest that EPA agents improve vascular endothelial function, thereby preventing progression of atherosclerosis in high risk CAD patients such as those requiring PCI.

A number of epidemiological studies have found that intake of EPA prevented onset of cardiovascular events. In the JELIS study, the benefits of EPA were greater in patients with a prior history of CAD undergoing PCI,[4] a result similar to that observed in the present study. This suggests that administration of EPA would be particularly beneficial in secondary prevention in high risk CAD patients. Interestingly, although such benefits were obtained without LDL levels being affected, they were more pronounced in populations consuming low amounts of n-3 fatty acids, namely those with a low EPA/AA ratio. [4] However, the mechanism by which EPA suppresses atherogenesis, if indeed it does so, is not well understood. Anti-inflammatory,[15,16] antithrombotic,[17,18] and lipid-lowering[19] effects have all been proposed as potential underlying mechanisms. Improvement in vascular endothelial function is also a major candidate.[20-22] The vascular endothelial functional status of the individual may reflect their propensity to develop atherosclerotic disease. Therefore, endothelial dysfunction may serve as an indicator of the initial stage of atherosclerosis. Strong evidence for

endothelial dysfunction as an independent predictor of cardiovascular events stems from several studies investigating the presence and prognosis of endothelial dysfunction in systemic circulations.[23,24] Flow-mediated dilation is widely used as a noninvasive method of assessing vascular endothelial function, and is an established predictive surrogate marker of cardiovascular events.[25,26] Recently, Kubo et al.[27] reported that the incidence of cardiovascular disease was greater in a low (median FMD < 4.2 %) than a high FMD group in patients with CAD undergoing PCI (median FMD ≥ 4.2 %). In addition, their multivariate analysis using various prognostic parameters demonstrated that only low FMD was an independent predictor of cardiovascular events. Taken together with the present results, this strongly suggests that administration of EPA induces an increase in FMD, which may help prevent future cardiovascular events. In addition, the results of the present ROC analysis indicated that the cut-off value of EPA/AA to achieve an over 1.0% increase in FMD at 6 months was 1.03. Only patients in whom the EPA/AA ratio was below 0.4 were included in the present study. However, the results suggest that administration of an EPA agent would still be desirable, even in CAD patients with an EPA/AA ratio of over 0.4 in targeting a level of over 1.0.

Determining the carotid artery IMT is also an established noninvasive method of assessing risk of atherosclerosis. The Rotterdam study, which was based on a short follow-up period of 2.7 years, provided evidence that an increase in common carotid IMT was associated with future cerebrovascular and cardiovascular events.[28] In the present study, increase in IMT was suppressed in the EPA group, whereas it showed an increase in the control group. However, here we only measured the IMT at 1.5 cm proximal to the carotid bifurcation and assessed the maximum value of either the right

or left common carotid artery. Further study should employ individual IMT assessment at the bifurcation, and proximal common carotid and internal carotid arteries in both the right and left side vessels for greater accuracy.

The CAVI is widely used to evaluate arterial stiffness in a clinical setting. In the present study, the CAVI showed no serial change between 6 and 12 months in the EPA group, but significantly increased at 12 compared with 6 months. This suggests that administration of EPA does not affect arterial stiffness. It is possible that the effect of EPA was different here as CAVI reflects the vessel stiffness of not only the large arteries, but also the aorta, and the mechanism underlying progression of atherosclerosis may differ between these sites.

Conclusion

The present results suggest that administration of EPA improves vascular endothelial function, thereby inhibiting progression of atherosclerosis in patients with high risk CAD such as those requiring PCI, and possibly enhancing secondary prevention.

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Figure legend

Figure 1 Comparison of serial change in flow mediated dilation (FMD) between eicosapentaenoic acid (EPA) administration and control groups. FMD was comparable between both groups at baseline. It increased time-dependently in EPA group. It increased still more at 12 compared with 6 months in control group, but did not change significantly.

Figure 2 Comparison of serial change in intima-media complex thickness (IMT) between EPA administration and control groups. At baseline, IMT tended to be lower in EPA group. Nos significant change was observed at 6 or 12 months in EPA group. However, it tended to increase time-dependently in control group, increasing significantly at 12 compared with 6 months.

Figure 3 Comparison of serial change in cardio-ankle velocity index (CAVI) between EPA administration and control groups. CAVI was significantly lower in EPA group at baseline. No significant serial change was observed at 6 or 12 months in either group. In EPA group, however, significantly increase was observed at 12 compared with 6 months. CAVI at 6 months was significantly lower in EPA than control group.

Table 1 Comparison of baseline characteristics between ~~two groups of~~ EPA administration and control groups

	EPA group (n = 19)	Control (n = 11)	*P* value
Age (yr~~e~~)	67.3 ± 8.6	66.6 ± 10.6	0.84
Male ~~gender~~ n (%)	9 (84)	16 (82)	0.87
Diagnosis			0.95
ACS	4 (21)	2 (18)	
SAP	4 (21)	2 (18)	
OMI	11 (58)	7 (64)	
Risk factors			
Diabetes	7 (37)	3 (27)	0.59
Hypertension	12 (63)	9 (82)	0.28
Dyslipidemia	10 (53)	7 (64)	0.56
Obesity	3 (36)	4 (16)	0.19
Family history	6 (32)	3 (27)	0.80
Current smoking	12 (63)	5 (46)	0.35
Baseline medication~~s~~			
Beta blockade	9 (47)	3 (27)	0.27
RAS inhibitors	19 (100)	11 (87)	0.054
Statins	19 (100)	11 (100)	1.00
PCI procedures			0.41
BMS stenting	15 (78.9)	8 (72.7)	
DES stenting	4 (21.1)	2 (18.2)	
Balloon angioplasty	0 (0)	1 (9.1)	
Quantitative coronary angiography			
Lesion length (mm)	14.5 ± 7.3	13.5 ± 4.0	0.7
MLD (mm)	0.71 ± 1.12	0.49 ± 0.53	0.58
% Diameter stenosis	81 ± 22	78 ± 23	0.71
% Area stenosis	92 ± 9	90 ± 13	0.65
Acute gain (mm)	2.34 ± 0.96	2.21 ± 0.90	0.72
Late loss (mm)	0.96 ± 0.77	0.90 ± 0.68	0.85

ACS; acute coronary syndrome, SAP; stable angina pectoris, OMI; old myocardial infarction, RAS; renin-angiotensin system, BMS; bare metal stent, DES; drug-eluting stent, MLD; minimal lumen diameter. Values are expressed as number, (percentage) and mean ± SD

Table 2 Comparison of changes in lipid profiles and EPA/AA ratio between ~~two groups of~~ EPA administration and control groups

	EPA group (n=19)	Control (n=11)	*P* value
Total cholesterol (mg/dL)			
Baseline	132 ± 23	158 ± 37	0.13
6 ~~-month~~ later	143 ± 18	161 ± 36	0.11
12 mo ~~-month~~ later	147 ± 22	160 ± 27	0.15
Triglycerides (mg/dL)			
Baseline	90 ± 37	123 ± 31	0.90
6 mo ~~-month~~ later	89 ± 27	126 ± 41	0.22
12 mo ~~-month~~ later	88 ± 31	141 ± 68	0.03
HDL ~~-cholesterol~~ (mg/dL)			
Baseline	45 ± 11	49 ± 13	0.94
6 mo ~~-month~~ later	53 ± 13 ‡	48 ± 10	0.16
12 mo ~~-month~~ later	56 ± 14	52 ± 11	0.56
LDL ~~-cholesterol~~ (mg/dL)			
Baseline	73 ± 16	86 ± 28	0.13
6 mo ~~-month~~ later	72 ± 13	88 ± 30	0.14
12 mo ~~-month~~ later	79 ± 21	84 ± 20	0.75
EPA/AA ratio			
Baseline	0.30 ± 0.11	0.34 ± 0.19	0.38
6 mo ~~-month~~ later	1.09 ± 0.42 ‡	0.47 ± 0.16	0.048
12 mo ~~-month~~ later	1.10 ± 0.35	0.39 ± 0.08	0.01

‡; ~~-~~ *P* < 0.05 among baseline, 6 mo ~~-month,~~ and 12 mo ~~-month~~. Values are expressed as mean ± SD

HDL; high~~-~~density lipoprotein LDL; low~~-~~density lipoprotein, EPA; eicosapentaenoic acid

AA; arachido~~i~~nic acid

Figure 1

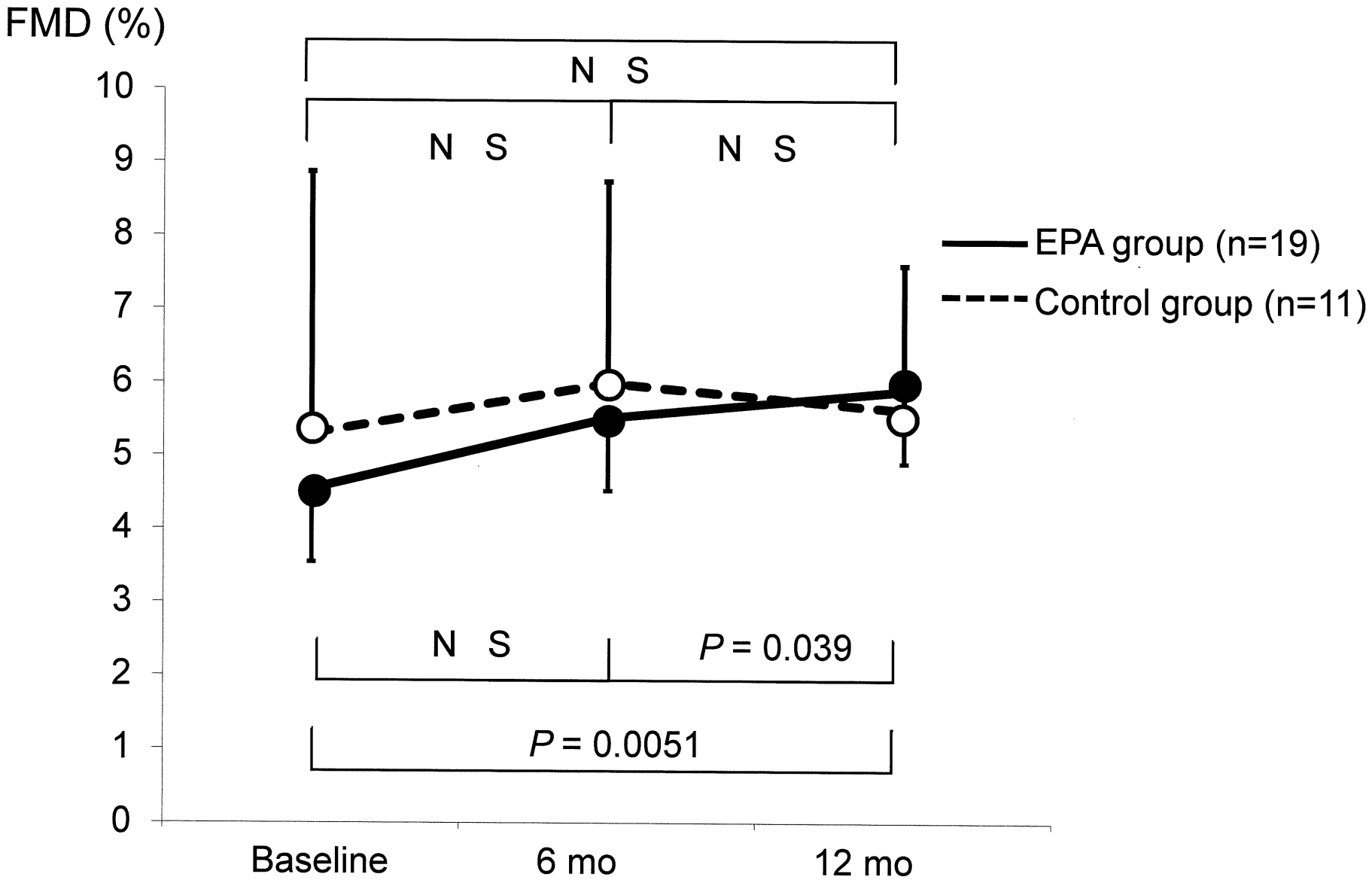

FMD (%)
10
9
8
7
6
5
4
3
2
1
0
N S
N S
N S
N S
N S
P = 0.039
P = 0.0051
EPA group (n=19)
Control group (n=11)
Baseline
6 mo
12 mo

Figure 2

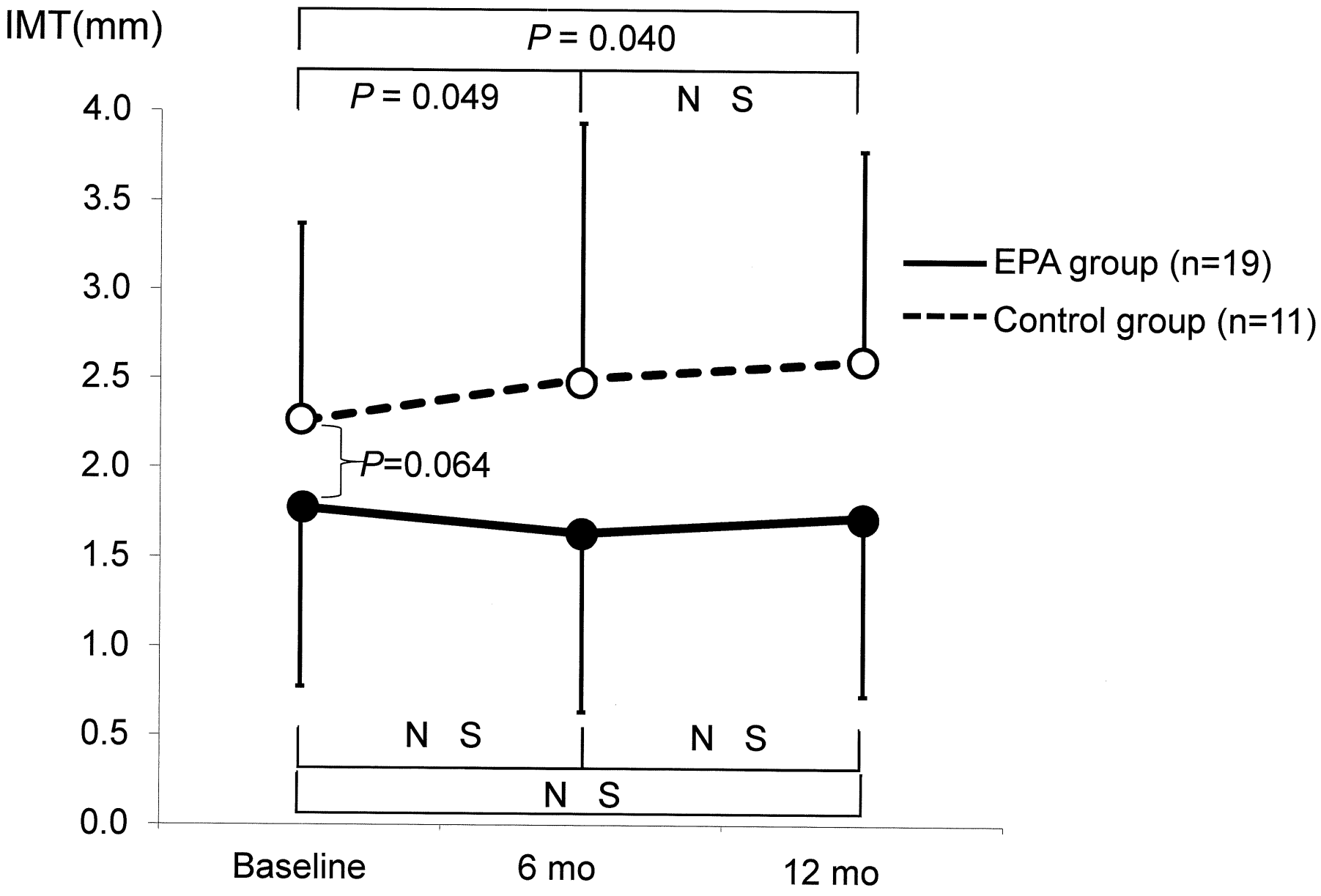
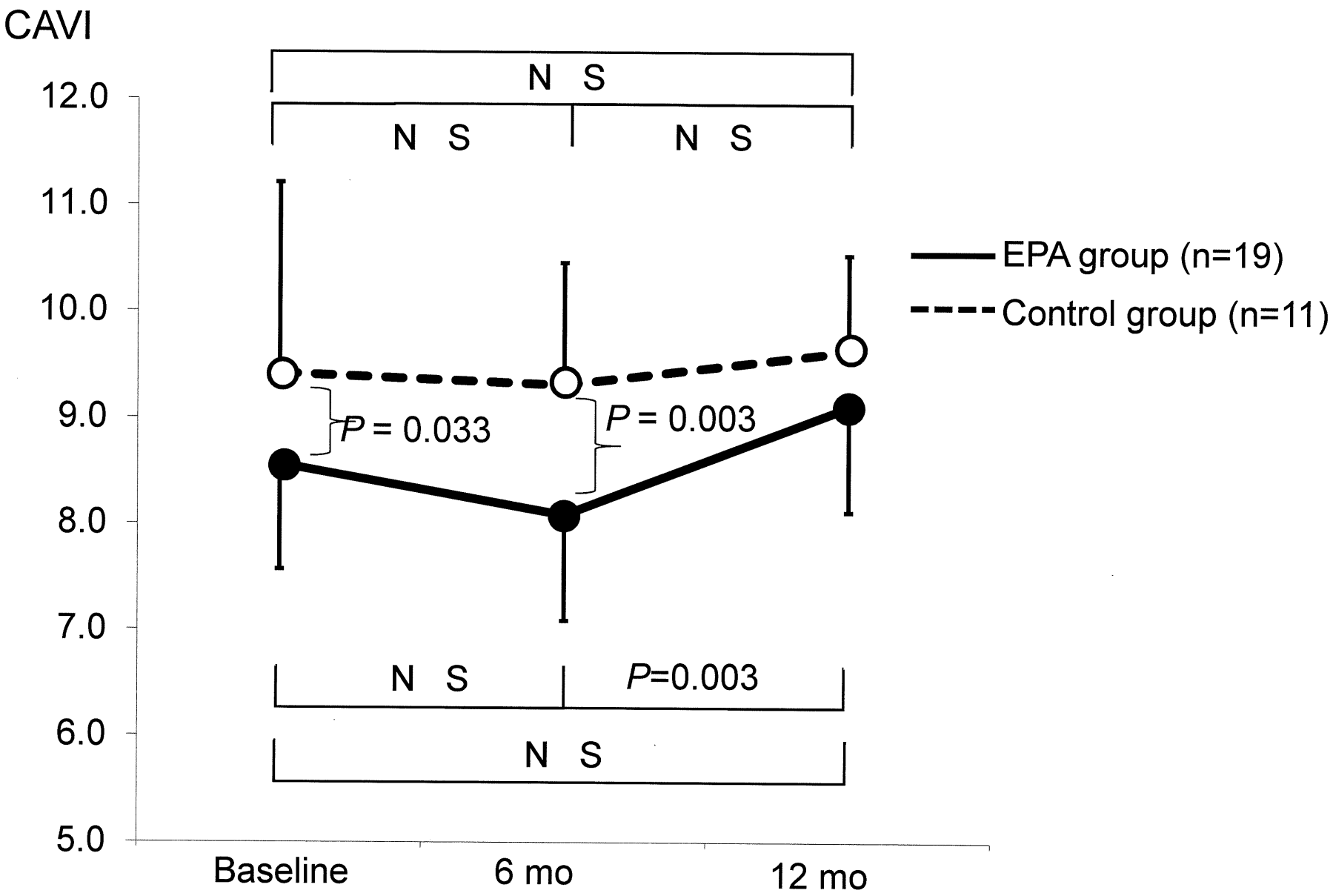

IMT(mm)
P = 0.040
P = 0.049
N S
4.0
3.5
3.0
2.5
2.0
1.5
1.0
0.5
0.0
P=0.064
EPA group (n=19)
Control group (n=11)
N S
N S
N S
Baseline
6 mo
12 mo

Figure 3


CAVI
12.0
11.0
10.0
9.0
8.0
7.0
6.0
5.0
N S
N S
N S
P = 0.033
P = 0.003
N S
P=0.003
N S
Baseline
6 mo
12 mo
EPA group (n=19)
Control group (n=11)