Original Article

Do Eicosapentaenoic Acid Supplements Attenuate Age-Related Increases in Arterial Stiffness in Patients with Dyslipidemia ?: A Preliminary Study

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The present study was conducted to examine the effect of eicosapentaenoic acid supplements on pulse wave velocity (PWV) in patients with dyslipidemia as a prospective open-labeled study. Eicosapentaenoic acid supplements (1,800 mg/day) were prescribed to 40 patients, and diet therapy in consultation with a nutritionist was conducted in 44 patients as a control group. These interventions were continued for 12 months, and PWV and blood examinations were performed at the start and end of these interventions. PWV increased in the control group but not in the eicosapentaenoic acid group. After adjustment for age, gender, the initial PWV, and the changes in mean blood pressure during the study period, a general linear model univariate analysis post hoc comparison demonstrated that the change in PWV during the period of study was significantly larger in the control group (42±20 cm/s) than in the eicosapentaenoic acid group (−9±19 cm/s) ($p<0.05$). Thus, this preliminary study suggested that eicosapentaenoic acid supplements attenuate age-related increases in arterial stiffness in patients with dyslipidemia. A further study with a larger number of subjects is proposed to confirm this beneficial effect of eicosapentaenoic acid supplements on arterial stiffness. (*Hypertens Res* 2005; 28: 651–655)

***Key Words*: arterial stiffness, dyslipidemia, eicosapentaenoic acid**

Introduction

Several epidemiological studies have demonstrated the beneficial effects of the high dietary consumption of fish oils against atherosclerotic cardiovascular diseases (*1*, *2*). Furthermore, recent prospective studies have demonstrated that fish oil supplements prevent the progression of coronary atherosclerosis in patients with coronary artery disease (*3*) and reduce cardiovascular events in patients with myocardial infarction (*4*). However, the precise mechanisms underlying these beneficial effects of fish oils are not fully understood.

Pulse wave velocity, which reflects arterial stiffness, is a known indicator of cardiovascular risk (*5*, *6*). An elevated pulse wave velocity is a predictor of future cardiovascular events (*5*, *6*), and Geurin *et al*. suggested that pulse wave velocity could be used as a surrogate marker of arterial stiffness during anti-hypertensive treatment (*7*). However, the effect of fish oils on pulse wave velocity is not clear. If fish oils reduce pulse wave velocity, a regression in arterial stiff-

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Table 1. Clinical Characteristics of Subjects and Their Changes in the Eicosapentaenoic Acid Supplement Group or Diet Therapy Control Group

	EPA		Control	
	Start	End	Start	End
Number of subjects	40		44	
Age (years)	65±2		64±2	
Gender (male/female)	22/18		19/25	
BMI (kg/m^2)	22.8±0.5	22.2±0.6	23.0±0.7	23.2±0.6
SBP (mmHg)	136±3	132±3	134±2	136±3
DBP (mmHg)	77±2	77±2	76±2	77±2
MBP (mmHg)	96±2	95±2	94±2	96±2
PP (mmHg)	56±2	55±2	56±2	56±2
FBS (mg/dl)	110±4	112±4	110±4	113±4
TC (mg/dl)	224±5	216±6	223±5	217±6
HDL (mg/dl)	53±2	56±2	58±2	59±2
TG (mg/dl)	175±10	154±12*	154±12	142±12
baPWV (cm/s)	1,655±57	1,649±57	1,556±33	1,610±43*
Number of subjects with (percentage of total subjects)				
Smoking habits	20 (50)		19 (43)	
Hypertension	22 (55)		30 (68)	
Diabetes mellitus	8 (20)		16 (36)	
Coronary heart disease	10 (22)		10 (23)	
Stroke	1 (3)		2 (5)	

EPA: patients taking eicosapentaenoic acid supplements; Control: patients receiving diet therapy; start: at the start of the protocol; end: at the end of the protocol. BMI, body mass index; SBP, systolic blood pressure; DBP, diastolic blood pressure; MBP, mean blood pressure; PP, pulse pressure; FBS, fasting blood sugar; TC, total cholesterol; HDL, high density lipoprotein cholesterol; TG, triglycerides; baPWV, brachial-ankle pulse wave velocity. *$p<0.05$ *vs.* start.

ening may be one possible mechanism by which the beneficial effects of fish oils are brought about in patients with atherosclerotic cardiovascular diseases.

We examined the effect of eicosapentaenoic acid supplements, which contain one of the most prevalent fatty acids found in fish oils, on pulse wave velocity in patients with dyslipidemia as a prospective open-labeled study.

Methods

Study Population

The study subjects were selected from among patients under follow-up care at the investigators' (physicians) out-patient clinics for the management of atherosclerotic risk factors, coronary heart disease, or stroke. The sites included in the study were Tokyo Medical University Hospital and five other affiliated institutes from June 2001 to February 2002. The investigators first determined whether the patients being followed-up at their outpatient clinics met either the inclusion or exclusion criteria. The following criteria were used for entry into this study: patients whose total cholesterol level was >220 mg/dl and/or triglycerides were >150 mg/dl, even after treatment with statins and/or diet therapy for more than 6 months. Subjects who met the following criteria were excluded from the study: an ankle/brachial systolic blood pressure index of less than 0.95, indicating a possible diagnosis of arteriosclerosis obliterans; atrial fibrillation; valvular heart disease; heart failure (over HYHA class II); aortic aneurysm or aortic dissection; postoperative state of arteriosclerosis obliterans, aortic aneurysm, or aortic dissection; or a serum creatinine concentration of ≥176.8 μmol/l. Then, the investigators asked patients who met the inclusion criteria to enroll in this study after each of these patients had received an explanation of the protocol and the study aims. The steering committee, which consisted of all of the investigators involved in the present study, decided to enroll at least 5 study subjects for each investigator. After the enrollment of 5 subjects, the investigators were instructed not to enroll subjects in a consecutive manner, and the enrollment of all additional subjects was left to the discretion of each individual investigator.

Study Design

The present study consisted of a prospective open-labeled study. The study was performed between June 2001 and March 2003. Eicosapentaenoic acid (1,800 mg/day) supplements were prescribed to patients who had been born during an odd-numbered month (eicosapentaenoic acid group).

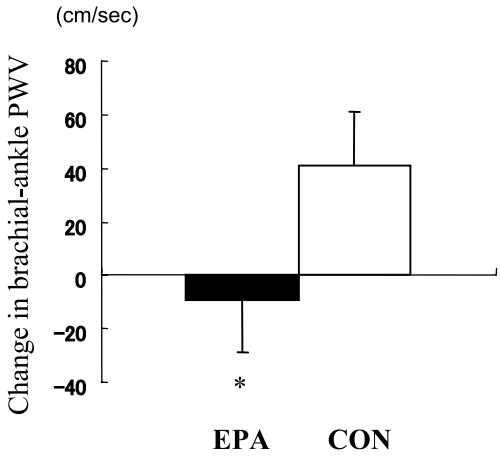

Fig. 1. *Difference in the changes in brachial-ankle pulse wave velocity during the eicosapentaenoic acid supplement or diet therapy interventions. PWV, pulse wave velocity; EPA, patients taking eicosapentaenoic acid supplements; CON, patients receiving diet therapy.* *p < *0.01* vs. *CON.*

Patients who had been born during an even-numbered month (control group) underwent diet therapy in consultation with a nutritionist; the diet was determined in accord with the guidelines of the Japan Atherosclerotic Society (total calories: adjusted total caloric intake to the desired body weight × [25–30] kcal; diet components: carbohydrate 60%, protein 15–20%, total fat 20–25%, cholesterol <300 mg per day, dietary fibers >25 g per day, and alcohol <25 g per day) (*8*). These treatments were continued for 12 months, and brachial-ankle pulse wave velocity measurements and blood examinations were performed at the start and end of these interventions. During this study period, the investigators attempted to maintain each patient on the same medication that had been administered at the onset of the study. If any problems developed that necessitated changing a patient's medication such as the elevation of blood pressure, poor glycemic control, cardiac or cerebral ischemic events, *etc*., the medication was changed at the investigator's discretion. Informed consent was obtained from all participants. The study was approved by the Ethical Guidelines Committee of Tokyo Medical University.

Pulse Wave Velocity Measurements

Brachial-ankle pulse wave velocity was measured using a volume-plethysmographic apparatus (Form/ABI, Colin Co. Ltd., Komaki, Japan) according to a previously described methodology (*9*, *10*). Each subject was examined while resting in a supine position. The brachial-ankle pulse wave velocity was then measured after the subject had rested for at least 5 min. This method was validated in a previous report; the interobserver coefficient of variation was 8.4%, and the intraobserver coefficient of variation was 10.0% (*9*).

Laboratory Measurements

Serum creatinine, fasting plasma glucose, total cholesterol, high-density lipoprotein cholesterol, and triglyceride levels were measured using enzymatic methods. All blood samples were obtained in the morning after an overnight fast.

Statistical Analysis

Data were expressed as the mean±SEM. The changes in brachial-ankle pulse wave velocity and other variables during the study period (comparison between before and at the end of the interventions in each group) were assessed using Wilcoxon's paired test. The distributions of categorical variables were assessed using a χ^2 test. A general linear model univariate analysis with post hoc comparisons was used to assess the differences in changes in the brachial-ankle pulse wave velocity between the eicosapentaenoic acid and control groups, which were adjusted for age, gender, brachial-ankle pulse wave velocity before the intervention, and the changes in mean blood pressure during the study period. Linear regression analysis was applied to examine the relationship between the changes in brachial-ankle pulse wave velocity and the changes in either total cholesterol or triglycerides before and after the treatment with eicosapentaenoic acid. All analyses were conducted using SPSS software for Windows, version 11.0J (SPSS, Chicago, USA). p values of <0.05 were considered statistically significant.

Results

In total, 99 patients agreed to enroll in the study (control group: $n=52$, eicosapentaenoic acid group: $n=47$), and 95 patients successfully completed the protocol without a change in medication regimen (control group: $n=51$, eicosapentaenoic acid group: $n=44$). However, because blood pressure is a major determinant of brachial-ankle pulse wave velocity, 11 patients were additionally excluded from the analysis due to a wide-range alteration in the mean blood pressure during the study period (*i.e.*, >20 mmHg). Finally, the data from 84 subjects were included in the analysis. Because the number of cases used in the present analysis was reduced from 99 to 84, we re-examined the sample size of this experiment (*11*). The crude value of the mean±SD of the changes in brachial-ankle pulse wave velocity during the study period in the control group ($n=44$) was 46±78 cm/s, and the corresponding value was −14±122 cm/s in the eicosapentaenoic acid group ($n=40$). Therefore, the effect size was calculated as 46 + 14 = 60, and the standard effect size was calculated as 60/78 = 0.77. According to a previous report (*11*), in the case with an α value of 0.05 and a power of 80%, the minimum sample size for each group was 23, and thus the sample size in the present study was adequate for analysis of the data.

Table 1 shows the clinical characteristics of the patients, the changes in lipid profiles, and other demographic variables

during the study period in both groups. In both groups, the variables related to atherosclerosis were similar. While blood pressure and the plasma levels of total cholesterol and high-density lipoprotein cholesterol did not change, the triglyceride plasma levels decreased significantly in the eicosapentaenoic acid group, but not in the control group. On the other hand, brachial-ankle pulse wave velocity significantly increased in the control group, but not in the eicosapentaenoic acid group. Figure 1 shows the changes in the brachial-ankle pulse wave velocity, which are represented by a bar graph with an error bar in both groups. The change in brachial-ankle pulse wave velocity during the study period was significantly larger in the control group than in the eicosapentaenoic acid group. Linear regression analysis showed that, in the eicosapentaenoic acid group, changes in brachial-ankle pulse wave velocity was not correlated with changes in either total cholesterol or triglycerides.

Discussion

Although Nestel *et al*. demonstrated the beneficial effect of eicosapentaenoic acid plus docosahexaenoic acid supplements on arterial compliance in dyslipidemic subjects (*12*), in a review article, von Schacky noted that eicosapentaenoic acid supplements plus docosahexaenoic acid taken for a 2-year period did not have any significant influence on pulse wave velocity in patients with coronary heart disease (*13*). Racial differences and patient profiles might have contributed to these conflicting results. In the present study, during the 12-month intervention period, the change in brachial-ankle pulse wave velocity in the eicosapentaenoic acid group was smaller than that in the control group. Therefore, eicosapentaenoic acid supplements appear to have a beneficial effect on arterial stiffness in Japanese dyslipidemic subjects.

Some prospective studies have confirmed the beneficial effects of fish oil supplements on atherosclerotic cardiovascular disease (*3*, *4*); moreover, improvement in both hemostatic and fibrinolytic balance, as well as endothelial function with the intake of such supplements has been reported; these supplements thus appear to contribute to such beneficial effects (*14–16*). The stiffening of the central arteries, reflected by the aortic pulse wave velocity, exerts a large impact on atherosclerotic cardiovascular risk because of the resulting increases in cardiac overload and cardiac oxygen demands, the impaired coronary blood supply, and the direct atherogenic actions of stiffening artery (*5*, *17*). Although brachial-ankle pulse wave velocity measurements include both central and peripheral artery segments (*9*, *10*), brachial-ankle pulse wave velocity values are closely correlated with aortic pulse wave velocity (*9*). Therefore, the findings of the present study suggest that eicosapentaenoic acid supplements may counteract age-related increases in the atherosclerotic cardiovascular risk associated with arterial stiffening.

Blood pressure is known to be a major determinant of arterial stiffness. In a previous study, we demonstrated that the changes in brachial-ankle pulse wave velocity (cm/s) were 10 to 15 times larger than changes in blood pressure (mmHg) (*18*). The fluctuation in blood pressure levels should be taken into account in order to assess the changes in arterial stiffness in a longitudinal study. Fundamentally, this study protocol recommended the maintenance of the same medication during the entire study period in order to avoid the direct influence of medications for either hypertension or diabetes mellitus on arterial stiffness (*19*, *20*). Approximately 12% of the patients enrolled in this study demonstrated a wide fluctuation in blood pressure (>20 mmHg in the mean blood pressure), and the mean value of the changes in brachial-ankle pulse wave velocity in these subjects during this study period was 332±124 cm/s. In these subjects, fluctuations in blood pressure, rather than effects of any particular interventions, might have influenced the changes in brachial-ankle pulse wave velocity. Therefore, we excluded such subjects from our analysis in order to avoid any such blood pressure-related fluctuation artifacts. These results confirmed that the influence of fluctuations in blood pressure is an important factor in the assessment of changes in pulse wave velocity in a longitudinal study.

Alterations in the arachidonic acids system and lipid profiles (*21*), as well as reductions in the production of atherogenic cytokines, are thought to underlie the beneficial effects of eicosapentaenoic acid supplements (*21*, *22*). Eicosapentaenoic acid was found to decrease the plasma level of triglycerides, without affecting the plasma levels of total cholesterol and high-density lipoprotein cholesterol. However, triglycerides are not a major determinant of arterial stiffness (*10*), and changes in brachial-ankle pulse wave velocity did not correlate with changes in triglycerides in this study. Beneficial alterations in the arachidonic acids system have been shown to induce vasodilatation and improve endothelial function (*13*, *14*), and these changes are thought to reduce arterial stiffness. In addition, such alterations and a reduction in the production of atherogenic cytokines may directly inhibit vascular wall fibrosis. These actions are thought to be involved in the attenuation of arterial stiffening, and this attenuation appears to be enhanced by the intake of eicosapentaenoic acid supplements.

This study had some limitations. First, while the period in which subjects were enrolled in this study was limited, the protocol of this study did not require the investigators to enroll patients who met the study criteria in a consecutive manner. Therefore, this study did not fully satisfy the criteria of a randomized study. In addition, when planning this protocol, we could not have estimated the incidence of such a wide fluctuation in blood pressure. Therefore, as noted above, approximately 12% of the study subjects had to be excluded from the analysis who exhibited a large blood pressure fluctuation during the study period. Finally, we were able to analyze the data obtained from 84 of 99 subjects who had originally entered the study (85%). Accordingly, we concluded that this study was preliminary; to confirm our results,

a study including a larger number of subjects will still be necessary. Second, whereas the beneficial effect of diet therapy is well recognized, the lipid profiles and body weights did not change among those subjects in this study who were administered diet therapy (the control group). Therefore, the subjects who underwent diet therapy appear to have incompletely followed the instructions of the nutritionist. This non-compliance with diet therapy might possibly contribute to increased arterial stiffening.

In conclusion, the results of this preliminary study suggested that eicosapentaenoic acid supplements attenuate age-related increases in arterial stiffness in patients with dyslipidemia. This attenuation may at least in part contribute to the beneficial effects of eicosapentaenoic acid in the prevention of atherosclerotic cardiovascular events. A further study involving a large number of subjects is proposed in order to confirm this beneficial effect of eicosapentaenoic acid supplements on arterial stiffness.

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