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ORIGINAL

Administration of highly purified eicosapentaenoic acid to statin-treated diabetic patients further improves vascular function

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Abstract. We prospectively examined the additional effects of highly purified eicosapentaenoic acid (EPA) particularly on the vascular function of diabetic patients with hypercholesterolemia receiving statin therapy. We enrolled 28 patients with type 2 diabetes complicated by dyslipidemia who had been treated with statins for at least one year. The patients were randomly assigned to 2 groups: administration of statin alone (group S: n=13) and addition of EPA to the current statin therapy (group SE: n=15). The highly purified EPA was administered at a dose of 1,800 mg/day for 6 months. To evaluate vascular function, the duration of reactive hyperemia (DRH), which is the time required for forearm blood flow to return to the basal level after inducing reactive hyperemia, was measured using strain gauge plethysmography. There were no significant differences in the clinical background factors between the 2 groups. Low-density lipoprotein cholesterol (LDL-C), and non-high-density lipoprotein cholesterol levels significantly decreased after 6 months only in group SE. Compared with the baseline data, no significant change in DRH was observed after 6 months in group S. By contrast, DRH was significantly prolonged after 6 months in group SE, indicating that the addition of highly purified EPA improved vascular function. Our results showed that in patients with type 2 diabetes and receiving statin therapy whose LDL-C level was less than 100 mg/dL, the addition of highly purified EPA for 6 months significantly improved vascular function.

Key words: Eicosapentaenoic acid, Vascular function, Diabetes mellitus, Low-density lipoprotein cholesterol, Residual risk

EICOSAPENTAENOIC ACID (EPA), which is among the n-3 polyunsaturated fatty acids contained in fish oil, has an anti-arteriosclerotic effect [1-3]. In the Japan EPA Lipid Intervention Study (JELIS), which examined the preventive effects of EPA on CAD in Japanese patients with dyslipidemia, the addition of highly purified EPA to statin therapy lowered the incidence of major coronary events (MCE) by 19% in the patient group compared with a control group [4].

Moreover, the JELIS subanalysis published in 2008 [5] demonstrated the preventive effects of EPA on MCE, independently of glycemic control, in high-risk patients with dyslipidemia complicated by impaired glucose metabolism. Among the many drugs with preventive effects on CAD, statins are highly useful particularly in patients with diabetes [6]. Given this finding, the mechanism underlying the additional effects of highly purified EPA observed in the JELIS is of great interest.

Based on the hypothesis that a mechanism underlying improvement of vascular function by highly purified EPA is in itself an important factor contributing to the reduction of MCE incidence, we prospectively examined the effects of 6-month addition of highly purified EPA particularly on endothelium-dependent vasodilation of patients with type 2 diabetes with hypercholesterolemia who were receiving statin therapy by comparing these patients to a control group.

Methods and Subjects

We enrolled 28 patients (14 men and 14 women; mean age: 67 ± 6.7 years) with type 2 diabetes complicated by dyslipidemia who had been treated with statins at our outpatient department for at least 1 year. All of the enrolled patients provided written informed

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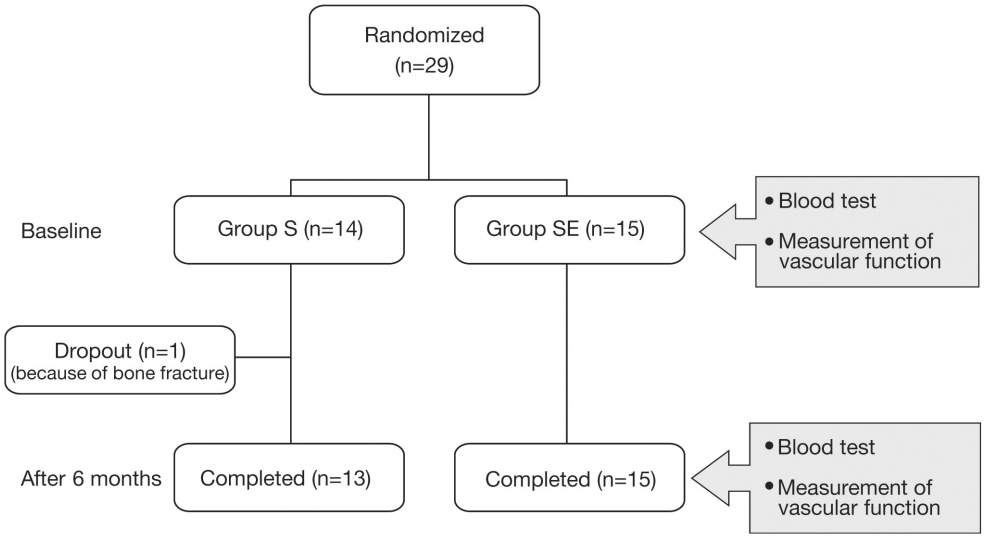

Fig. 1 Study profile

consent to participate in this study. Premenopausal women, patients with concurrent or a history of hemorrhagic lesions, and those with other serious complications (e.g., malignant tumors, hepatic cirrhosis, renal failure and heart failure) were excluded.

Study design

The patients were randomly assigned to 2 groups: administration of statin alone (group S: n=13) and addition of EPA to the current statin therapy (group SE: n=15). In principle, treatment contents were not changed except for the addition of highly purified EPA. The highly purified EPA preparation (Epadel®, Mochida Pharmaceutical Co., Ltd., Tokyo, Japan) was orally administered at a dose of 1,800 mg/day (divided into doses of 900 mg after the morning and evening meals or into 3 doses of 600 mg after each meal). The observation period was 6 months. Vascular function was evaluated before and after the observation period (Fig. 1).

This study was designed as a prospective, randomized, parallel group trial and was registered at UMIN-CTR (University Hospital Medical Information Network-Clinical Trial Registry) (No. 000005783).

Blood chemical values

We measured the levels of β-thromboglobulin (β-TG) and platelet factor 4 (PF4) as thrombocytic markers, and P-selectin as adhesion molecules. All blood samples were collected under fasting conditions. For the measurement of both β-TG and PF4 levels, blood samples were collected without a tourniquet and centrifuged under refrigeration. The plasma was then kept frozen until measurement. P-selectin, β-TG and PF4 levels were measured by enzyme immunoassays. The value for HbA1c (%) is estimated as an NGSP equivalent value (%) calculated by the formula HbA1c (%) = HbA1c (JDS) (%) + 0.4%, considering the relational expression of HbA1c (JDS) (%) measured by the previous Japanese standard substance and measurement methods and HbA1c (NGSP) [7].

Assessment of vascular function by strain gauge plethysmograph

To assess vascular function, we induced reactive hyperemia by briefly occluding the upper arm and then suddenly releasing the pressure. The duration of reactive hyperemia (DRH) was measured using a strain gauge plethysmograph (EC-5R, Hokanson Inc., USA). The measurements based on this method correlate with assessment results of increased forearm blood flow (FBF) after the injection of an endothelium-dependent vasoactive agent (e.g., acetylcholine) into the brachial artery [8], as well as correlate significantly with the number of cardiovascular risks [9]. Thus, we considered this method to be useful for obtaining endpoint data for the assessment of vascular function.

Measurement of basal FBF

Each patient was instructed to fast for 9 hours or lon-

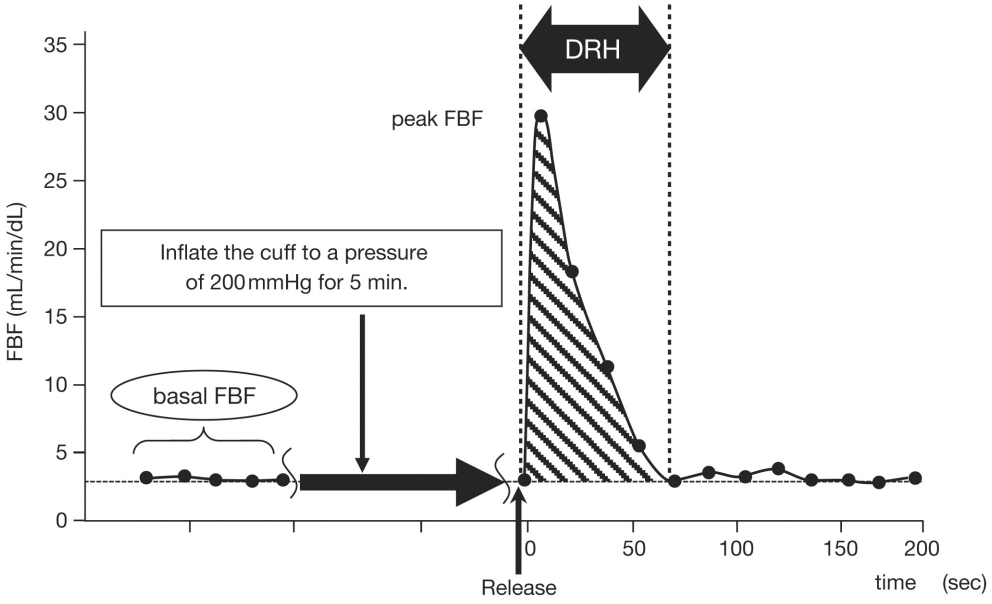

Fig. 2 Evaluation of vascular function by strain-gauge plethysmography
DRH, duration of reactive hyperemia; FBF, forearm blood flow

ger, and measurements of basal FBF were performed between 8:30 a.m. and 10:00 a.m. During the measurements, the patient was in the supine position and the forearm was placed at the level of the right atrium. The strain gauge filled with mercury was placed around the forearm and connected to the plethysmograph. From 1 minute before the start until the end of the basal FBF measurement, a pressure 50 mm Hg higher than the patient's systolic blood pressure was applied to the cuff placed around the wrist. Then, venous return from the forearm was prevented by applying a pressure of 50 mm Hg to the cuff placed around the upper arm for 7 seconds, using a rapid cuff inflator (EC-20, Hokanson Inc.; Bellevue, WA, USA). Blood flow was calculated from the rate of increase in forearm blood volume while pressure was applied to the upper arm. Measurements were performed 5 times at 15-second intervals. The average of 3 values excluding the minimum and maximum values was regarded as basal FBF.

Measurement of DRH

After basal FBF measurement, a pressure of 200 mm Hg was applied to the cuff placed around the left upper arm to completely occlude the antebrachial artery for 5 minutes. From 1 minute before releasing the occlusion until the end of measurement, a pressure 50 mm Hg higher than the patient's systolic blood pressure was applied to the cuff placed around the wrist. After occluding the upper arm for 5 minutes, the occlusion was suddenly released to repeatedly measure FBF after inducing reactive hyperemia 15 times at 15-second intervals. Based on the obtained flow rate curve, DRH, which is the time required for FBF to return to the basal level after inducing reactive hyperemia (i.e., after releasing the occlusion) was determined. We compared DRH before and after the observation period as an endpoint for the assessment of vascular function (Fig. 2).

Assessment of endothelium-independent vasodilation

To assess endothelium-independent vasodilation, sublingual nitroglycerin (0.075 mg) was administered to the patient, and a pressure 50 mm Hg higher than the patient's systolic blood pressure was applied to the cuff placed around the wrist from 1 minute before the start until the end of measurement. A pressure of 50 mm Hg was applied to the cuff placed around the left upper arm 20 times at 15-second intervals. Blood flow was calculated from the rate of increase in forearm blood volume while pressure was applied to the upper arm. The mean values were determined for endothelium-independent vasodilation.

Statistical analysis

Each item was compared between the 2 groups using an unpaired *t*-test and the χ^2 test. For the comparisons

Table 1 Baseline characteristics of the patients.

	Group S (n=13)	Group SE (n=15)	*p*-value
Age (years)	69.2 ± 7.7	65.5 ± 5.4	0.15
Male (%)	46	54	0.72
Duration of diabetes (years)	16 ± 8	16 ± 8	0.99
BMI (kg/m^2)	24.6 ± 2.8	24.7 ± 2.7	0.91
Current smoker (%)	31	13	0.26
Clinical history of CVD (%)	31	27	0.81
Systolic BP (mmHg)	127 ± 14	129 ± 11	0.56
Diastolic BP (mmHg)	70 ± 9	67 ± 10	0.46
FPG (mg/dL)	130 ± 27	132 ± 40	0.91
HbA1c (%)	6.7 ± 0.6	6.7 ± 0.9	0.91
T-C (mg/dL)	182 ± 23	183 ± 34	0.96
LDL-C (mg/dL)	96 ± 20	96 ± 24	0.95
HDL-C (mg/dL)	60 ± 13	56 ± 14	0.44
Triglyceride (mg/dL)	123 ± 44	156 ± 120	0.36
non-HDL-C (mg/dL)	122 ± 19	127 ± 30	0.62
Medications			
Sulfonylurea (%)	31	20	0.51
Metformin (%)	46	47	0.98
Insulin (%)	31	27	0.98
ACE inhibitor or ARB (%)	30	40	0.61
Calcium channel blocker (%)	15	20	0.75
Aspirin (%)	38	60	0.26

Data are expressed as percentage or mean ± standard deviation, unless otherwise indicated.
Abbreviation : BMI, body-mass index; CVD, cardiovascular disease; BP, blood pressure; FPG, fasting plasma glucose; HbA1c, hemoglobin A1c; T-C, total cholesterol; LDL-C, low-density lipoprotein cholesterol; HDL-C, high-density lipoprotein cholesterol; ACE, angiotensin-converting-enzyme; ARB, angiotensin receptor blocker.

within each group (i.e., between values obtained before and after the start of this study), the Wilcoxon rank test was performed using SPSS for Windows. The results were expressed as mean± standard deviation. $P < 0.05$ was considered to indicate a statistically significant difference for all statistical tests.

Results

Baseline profiles of the patients in both groups are shown in Table 1. Analysis was performed in 28 patients (group S, n=13; group SE, n=15). There were no significant differences in clinical background factors, such as mean age, male-to-female ratio, body mass index (BMI), blood pressure, diabetes medications, smoking rate, glycemic control, or serum lipids between the 2 groups.

Also, the basal FBF both at baseline and after 6 months of observation showed no significant difference between the 2 groups. There was no significant change in DRH following induction of reactive hyperemia after 6 months in group S compared with baseline, whereas DRH was significantly prolonged after 6 months in group SE, indicating that the addition of highly purified EPA to statin therapy for 6 months improved vascular function. On the other hand, endothelium-independent vasodilation induced by nitroglycerin administration showed no significant change in either group before versus after the 6-month observation period (Table 2, Fig. 1).

In both group S and group SE, BMI slightly but significantly increased at 6 months. The changes observed only in group SE after 6 months were a significant decrease in total cholesterol, low-density lipoprotein cholesterol (LDL-C), and non-high-density lipoprotein cholesterol (non-HDL-C). There were no significant changes in the systolic or diastolic blood pressure as well as in the β-TG, PF4, or P-selectin levels in either group during the observation period (Table 3).

Table 2 Forearm blood flow measured by strain-gauge plethysmography

	Group S (n=13)			Group SE (n=15)		
	Baseline	6 months	*p*-value	Baseline	6 months	*p*-value
basal FBF (mL/min/dL)	3.4 ± 1.02	3.9 ± 0.7	0.05	4.4 ± 1.3	3.9 ± 1.3	0.19
max FBF (mL/min/dL)	823 ± 309	744 ± 337	0.31	685 ± 326	781 ± 280	0.10
DRH (sec)	99 ± 35	94 ± 45	0.75	82 ± 36	120 ± 41	0.01
EIV (mL/min/dL)	3.6 ± 1.1	3.6 ± 0.8	0.81	4.0 ± 1.1	3.9 ± 1.3	0.53

Data are expressed as mean ± standard deviation.
Abbreviation : FBF, forearm blood flow; DRH, duration of reactive hyperemia; EIV, endothelium-independent vasodilation. EIV was assessed by administering nitroglycerin (0.075 mg) sublingually. Data are presented as the mean ± standard deviation.

Table 3 Physical, laboratory data in the 2 groups at baseline and at 6-month follow up.

	Group S (n=13)			Group SE (n=15)		
	Baseline	6 months	*p*-value	Baseline	6 months	*p*-value
BMI (kg/m^2)	24.6 ± 2.8	24.8±2.8	0.03	24.7 ± 2.8	25.2 ± 2.2	0.01
Systolic BP (mmHg)	127 ± 14	128 ± 15	0.83	129 ± 11	130 ± 15	0.78
Diastolic BP (mmHg)	69 ± 9	74 ± 10	0.05	67 ± 10	69 ± 12	0.51
FPG (mg/Dl)	130 ± 27	140 ± 26	0.36	132 ± 40	142 ± 34	0.04
HbA1c (%)	6.7 ± 0.6	7.0 ± 1.0	0.17	6.7 ± 0.9	6.8 ± 1.0	0.85
T-C (mg/dL)	182 ± 23	185 ± 26	0.92	182 ± 34	171 ± 36	0.03
LDL-C (mg/dL)	96 ± 20	101 ± 20	0.55	96 ± 24	84 ± 29	0.01
HDL-C (mg/dL)	60 ± 12.9	58 ± 13	0.31	56 ± 14	55 ± 16	0.53
Triglyceride (mg/dL)	123 ± 44	114 ± 46	0.28	156 ± 120	163 ± 104	0.70
non-HDL-C (mg/dL)	122 ± 19	127 ± 24	0.62	127 ± 30	116± 27	0.03
P-selectin (ng/mL)	115 ± 40	131 ± 51	0.26	95 ± 42	111 ± 68	0.28
PF-4 (ng/mL)	27 ± 28	21 ± 26	0.42	28 ± 24	22 ± 38	0.07
β-TG (ng/mL)	70 ± 60	52.1 ± 49	0.33	75 ± 61	56 ± 75	0.09

Data are expressed as mean ± standard deviation.
Abbreviation : BMI, body-mass index; BP, blood pressure; FPG, fasting plasma glucose; HbA1c, hemoglobin A1c; T-C, total cholesterol; LDL-C, low-density lipoprotein cholesterol; HDL-C, high-density lipoprotein cholesterol; PF-4, platelet factor-4; β-TG, β-thromboglobulin.

Discussion

The main finding of present study demonstrates that the addition of highly purified EPA to statin therapy for 6 months clearly improved vascular function of patients with type 2 diabetes. Regarding the further improvement of vascular function in addition to the various actions of statins, we examined whether highly purified EPA increases statin efficacy as well as improves vascular function *via* a mechanism different from that of statins.

One possible mechanism underlying improved vascular function is the additive effect of stain which increases the action of nitric oxide (NO), the major contributor to endothelium-dependent vasodilation. Because the reduced biological activity [10] or increased inactivation [11] of NO due to increased oxidative stress suppresses endothelium-dependent vasodilatory responses in patients with diabetes, the eNOS increasing [12] and antioxidant effects [13, 14] of statins may markedly improve vascular function particularly in these patients. On the other hand, the administration of EPA alone (or intake of dietary fish oil) inhibits “the decrease in NO production” in human umbilical vein endothelial cells under hyperglycemic conditions [15]. Clinical studies have also revealed

that EPA increases NO actions, leading to coronary endothelium-dependent vasodilation [16] and endothelium-dependent vasodilation of the brachial artery [17] in patients with diabetes. Thus, it is likely that the combination of a statin and highly purified EPA exerted additive effects to increase NO production, resulting in improved vascular function.

The effects of the methods used to assess vascular function should also be considered. When changes in the diameter of the antebrachial artery are measured by ultrasound, the endothelial function of the conduit vessel is assessed. With this method, the major modulator of vascular function is NO. On the other hand, the employed strain gauge plethysmography mainly assesses the endothelial function of resistance vessels [8]. In this case, the major modulators of vascular function are non-NO vasoactive agents represented by endothelium-derived hyperpolarizing factor (EDHF). The associations of statins with non-NO modulators are not yet fully established. On the other hand, EPA improves endothelial function by increasing the production of EDHF and simultaneously decreasing the production of endothelium-derived contracting factors, such as endothelin-1 and prostanoids derived from arachidonic acid [18]. The difference in the effects on these non-NO factors may explain our finding that highly purified EPA exerts an additive effect on the actions of statins.

It is also possible that highly purified EPA improved vascular function by alleviating dyslipidemia in patients with diabetes. In this aspect, the first factor to consider is quantitative improvement of LDL-C. Steinberg *et al.* reported a negative linear relationship between serum LDL-C levels and endothelial function (endothelium-dependent vasodilation), which is maintained even if serum LDL-C levels are within the normal range [19]. From this report, it is not unreasonable to speculate that the significantly reduced LDL-C levels with the 6-month addition of highly purified EPA to statin therapy (-12 mg/dL) in our study contributed to the improvement of vascular function. However, because all participants had continuously received statins for at least 1 year before enrollment in the study, the mean basal LDL-C levels were less than 100 mg/dL in both groups. Thus, this quantitative reduction of -12 mg/dL is unlikely to have substantially contributed to the improvement of vascular function.

Moreover, it is necessary to consider whether the qualitative improvement of lipid profiles due to the administration of highly purified EPA may have improved vascular function. In particular, the administration of highly purified EPA reduced non-high-density lipoprotein-cholesterol (HDL-C) by approximately 11 mg/dL. However, non-HDL-C comprises most atherogenic lipoproteins including not only LDL, intermediate density lipoprotein, very low density lipoprotein, and remnants, but also small dense LDL particles [20], and that non-HDL-C is superior to LDL-C as a risk marker for coronary disease [21]. The meta-analysis of 28 studies by Robinson *et al.* [22] showed an approximately 1:1 relationship between percent non-HDL-C lowering and CAD reduction. Vakkilainen *et al.* showed using plethysmography that endothelial function was more highly correlated with LDL size than lipoprotein concentration in both healthy volunteers [23] and diabetic patients [24]. Thus, it would be appropriate to consider the "qualitative improvement" of lipid metabolism to be more associated with the improvement of vascular function than the "quantitative improvement" of lipid metabolism.

There were no significant changes in the hemoglobin A1c (HbA1c) levels in any of our patient groups during the observation period. However, based only on this observation, we cannot rule out an association between the improvement of vascular function by EPA and glucose metabolism. This is because vascular function is very closely associated with insulin resistance [25, 26]. If there are changes in underlying insulin resistance even without a significant improvement in HbA1c levels, changes in vascular function may also occur.

Recently, several interesting studies on EPA have been reported. Wang *et al.* [27] showed that EPA exerts an endothelial protective effect by acting as an agonist of peroxisome proliferator-activated receptor gamma, the key molecule for insulin resistance in vascular endothelial cells. Oh *et al.* [28] identified G protein-coupled receptor 120 (GPR120) as a functional receptor/sensor for omega-3 fatty acids including EPA. GPR120 signals exert potent anti-inflammatory effects on macrophages and enhance insulin sensitivity. Thus, EPA may both indirectly and directly alleviate insulin resistance to exert an endothelial protective effect, which may also be a factor underlying our present results.

Our study showed that in patients with type 2 diabetes and receiving statin therapy for hypercholesterolemia and who had achieved the therapeutic goal of reducing LDL-C levels to less than 100 mg/dL, the

addition of highly purified EPA for 6 months to the anti-CAD treatment regimen produced quantitative and qualitative improvement of blood lipid levels independently of glycemic control, as well as significant improvement of vascular function.

It is very interesting that the addition of highly purified EPA in this study further improved the vascular function-enhancing effect of statins. Our results also suggest that highly purified EPA may be effective for reducing so-called "residual risk," a phenomenon whereby cardiovascular events occur at certain rates even after starting statin therapy.

Limitations

This study involved a small number of patients and was conducted for a relatively short period. The universal applicability of our results might be enhanced by designing a larger scale and longer duration study. Moreover, there were no significant changes in the measured levels of thrombocytic markers before versus after EPA administration. However, there are many other thrombocytic markers associated with endothelial function, including the aforementioned PDMP. Because we did not measure the levels of all of these markers, we cannot rule out the possibility that highly purified EPA improved vascular function *via* antiplatelet effects.

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