J Atheroscler Thromb, 2017; 24: 735-748.
http://doi.org/10.5551/jat.39446

Original Article

The Combination Therapy of Fenofibrate and Ezetimibe Improved Lipid Profile and Vascular Function Compared with Statins in Patients with Type 2 Diabetes

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***Aim*: Elevated level of serum triglyceride (TG) is a characteristic of type 2 diabetes. We evaluated the clinical significance of intervention for the serum TG levels in the fasting and postprandial states in patients with type 2 diabetes.**
***Methods*: Fifty patients with type 2 diabetes, treated with statins, were selected and divided into two groups. One group was treated with a combination of fenofibrate and ezetimibe (F/E group) and the other group with statins (statin group) for 12 weeks. The lipoprotein profile of both groups was compared using high-performance liquid chromatography, and the vascular function was assessed using flow-mediated dilation (FMD) at the forearm.**
***Results*: The levels of very low-density lipoprotein (VLDL) cholesterol, malondialdehyde low-density lipoprotein (MDA-LDL), total TG, chylomicron-TG, VLDL-TG, and HDL-TG decreased in the F/E group, whereas those of HDL cholesterol increased. Furthermore, the peak particle size of LDL increased, but that of HDL decreased in the F/E group. The combination treatment significantly improved the FMD. The change in the cholesterol level in a very small fraction of HDL was a significant independent predictor for determining the improvement of FMD ($p<0.01$).**
***Conclusions*: Compared with the treatment with statins, the treatment with the combination of fenofibrate and ezetimibe effectively controlled the LDL cholesterol and TG levels, increased the HDL cholesterol level, especially in its small fraction, and improved vascular function of patients with type 2 diabetes.**

Key words: Endothelial function, Triglyceride, Small dense LDL, HDL

Introduction

Patients with type 2 diabetes are at increased risk of cardiovascular events. The United Kingdom Prospective Diabetes Study showed that the level of low-density lipoprotein cholesterol (LDL-C) is the most important risk factor for cardiovascular events even in patients with type 2 diabetes[1]. The intervention for the LDL-C of patients with type 2 diabetes using statins significantly reduces cardiovascular events by 21%[2]. However, approximately 70% of cardiovascular events cannot be prevented by statin treatment[3]. Thus, the residual risk factors (other than LDL-C) may also have a crucial role. The characteristics of lipid abnormality in patients with type 2 diabetes are qualitative changes as well as quantitative abnormalities in lipoproteins such as an increase in the level of the triglyceride (TG)-containing lipoprotein and a decrease in the level of high-density lipoprotein cholesterol (HDL-C)[4]. The major qualitative abnormalities in lipoproteins found in patients with type 2 diabetes include changes in the size of lipoproteins and appearance of remnant particles. Furthermore,

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Received: January 9, 2017
Accepted for publication: March 6, 2017

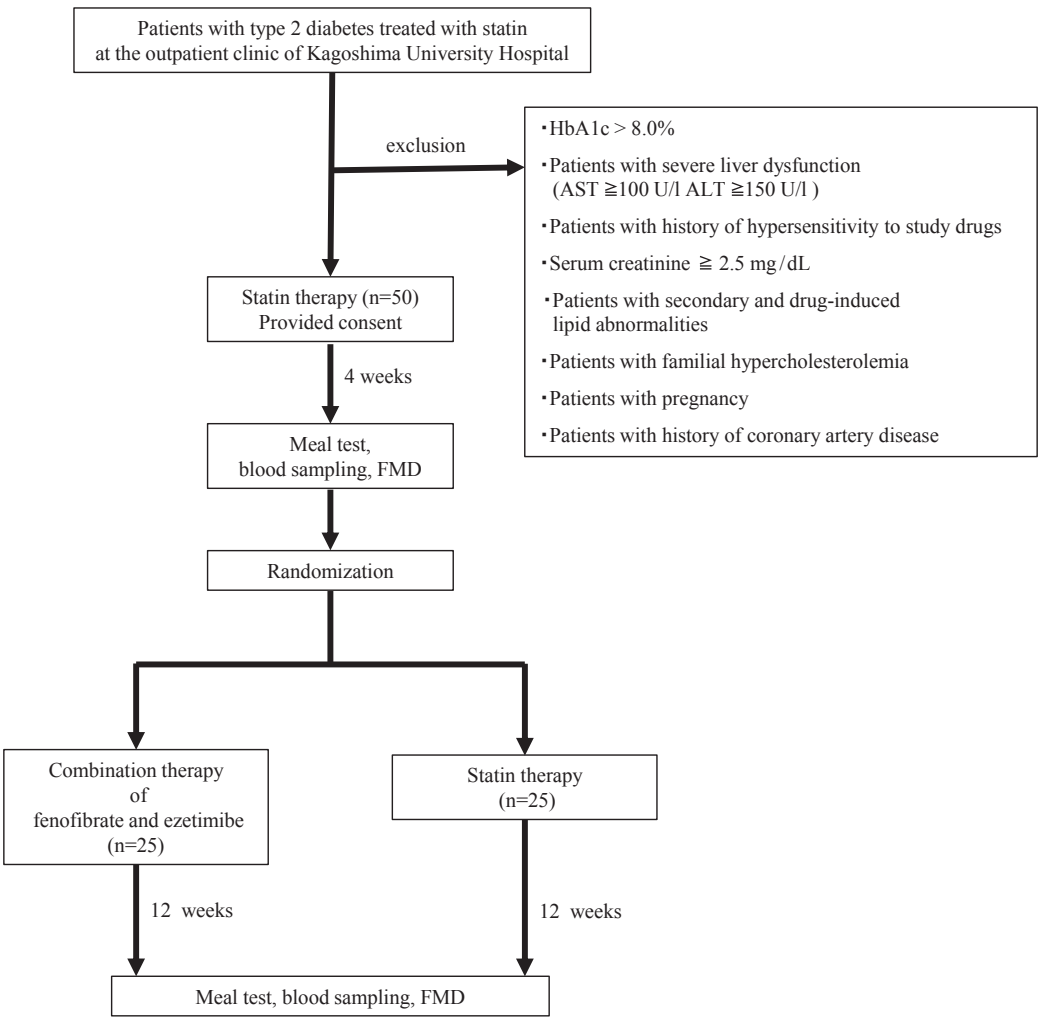

Fig. 1. Protocol of the study design

Blood samples were taken at 0, 60, and 120 min after the meal test. FMD (flow mediated dilation) was performed before and at 120 min after the meal test.

patients with type 2 diabetes often show lipid abnormalities after meals in spite of normal lipid levels at fasting[5]. These changes in the lipoproteins of patients with type 2 diabetes are closely related to the abnormality of serum TG levels and its metabolism[6].

Vascular endothelial dysfunction independently predicts post treatment cardiovascular diseases[7]. Flow-mediated dilation (FMD) of the forearm artery, often serve as a marker of vascular endothelial function, is used as an indicator of evaluation in various therapeutic interventions[8]. FMD is reduced in patients with type 2 diabetes[9]. Numerous factors have been reported to be related to this dysfunction[9-11]. Among these, lipid abnormalities in the postprandial and fasting states are the important factors[12]. Increased level of serum TG associated with metabolic syndrome or insulin resistant state is particularly closely related to endothelial dysfunction[13].

Aims

In this study, to evaluate the role of elevation of serum TG levels in the fasting and post prandial states in patients with type 2 diabetes treated with statins, we reduced the serum TG levels using a combination of ezetimibe and fenofibrate and assessed the endothelial function and quality of lipoproteins.

Methods

Subjects and Study Design

Fig. 1 shows the protocol of this study design. Patients with type 2 diabetes who had normal LDL-C levels and were treated with statins were recruited

Table1a. Patient clinical characteristics, diabetic control, endothelial function and treatment types before and 12 weeks after the drug intervention

	Drug intervention	statin group ($n=25$)	F/E group ($n=25$)
Age (yr)		63.3 ± 10.0	60.2 ± 11.2
Sex (M/F)		16/9	15/10
Body mass index (kg/m^2)		24.9 ± 4.5	27.4 ± 5.1
AST (U/l)	before	22.6 ± 6.3	27.1 ± 14.2
	after	22.7 ± 8.1	44.6 ± 57.7* ††
ALT (U/l)	before	24.7 ± 9.0	27.2 ± 27.7
	after	24.6 ± 12.1	47.4 ± 79.0*
γGT (U/l)	before	41.0 ± 40.1	29.2 ± 15.6
	after	37.0 ± 31.2	51.1 ± 64.6
sCr (mg/dL)	before	0.8 ± 0.3	0.9 ± 0.3
	after	0.9 ± 0.3	1.1 ± 0.4** †
CPK (U/l)	before	94.4 ± 24.2	132.6 ± 75.1
	after	118.6 ± 46.9**	136.1 ± 80.1
hsCRP (ng/mL)	before	1240 ± 2715	968 ± 1060
	after	1290 ± 2602	824 ± 818
FBG (mg/dL)	before	121.7 ± 19.9	137.3 ± 38.2
	after	118.4 ± 18.2	117.1 ± 31.3**
HbA1C (%)	before	6.9 ± 0.5	7.0 ± 0.4
	after	6.8 ± 0.6	6.8 ± 0.4**
FMD (%)	before	5.2 ± 2.6	5.5 ± 2.4
	after	4.8 ± 2.4	6.5 ± 2.2* †
medication (*n*)			
Hypoglycemic agent		23	21
Insulin		9	5
Antihypertensive drug		15	10

F/E group, fenofibrate and ezetimibe combination group; AST, Aspartate transaminase; ALT, Alanine transaminase; γGT, Gamma-Glutamyltranspeptidase; sCr, serum Creatinine; CPK, Creatine Phosphokinase; hsCRP, High-sensitivity C-reactive protein; FBG, fasting blood glucose; HbA1c, Hemoglobin A1c; FMD, Flow-mediated dilation.
Values are given as mean ± SD unless otherwise stated., *$P<0.05$, **$P<0.01$ (vs before the drug intervention); between before and after the drug intervention comparison by paired *t* test or Wilcoxon test, †$P<0.05$, ††$P<0.01$ (vs statin group); between statin and F/E group comparison by unpaired *t* test or Mann-Whitney test

from the outpatient clinic of the authors' institution between October 2014 and November 2015. Fifty patients (31 men and 19 women) were included in the study. (Please refer to **Supplemental Table 1** for detail information on statins used in this study.) Patients were excluded if they met one of the following criteria: age <20 years, uncontrolled hypertension (≧ 180/100 mmHg), HbA1c (National Glycohemoglobin Standardization Program: NGSP) ≧8.0%, severe liver dysfunction, serum creatinine (sCr) ≧2.5 mg/dL, secondary or drug-induced lipid abnormalities, familial hypercholesterolemia, pregnancy, history of cardiovascular diseases, and use of lipid-lowering medications, except statins. All subjects were randomly assigned to an open-label treatment with either statin (statin group) or fenofibrate (160 mg/day) and ezetimibe (10 mg/day) (F/E group). There was no significant difference in the ratio of strong statins used between the two groups (see **Supplemental Table 2**). Background characteristics of both groups are shown in **Table 1a**. Before and after the 12-week intervention, we compared the metabolic parameters and FMD of the forearm to evaluate the endothelium-dependent vascular function between the two groups. We also performed a meal test to assess postprandial dyslipidemia. The meal test was performed after overnight fasting. The meal consisted of 75 g of carbohydrate (flour starch and maltose), 28.5 g of fat (butter), and 8 g of protein, providing a total of 592 kcal (meal test C; SARAYA Corp., Osaka, JPN). The subjects were instructed to ingest the meal with water or black tea within 20 min. Time measurement was started when they began to ingest the meal. Venous blood samples were drawn, and the FMD was assessed in the

fasting state and at 120 min after the meal test. During the study, the subjects were requested to continue the diet and exercise therapy as before the intervention and to make no alterations in the medications. We did not set a wash-out period in this study. Because we treated the patients with the combination therapy of fenofibrate and ezetimibe for 12 weeks after changing from statin, the effects of statin have already disappeared at the end of the study.

All of the studies were approved by the Ethics Committee of Kagoshima University Graduate School of Medicine and Dentistry Sciences (approval number 26-34), and written informed consent was obtained from all subjects before the procedure. This study was registered with UMIN (UMIN000016676, March 2, 2015).

Laboratory Methods

Before and after the 12-week intervention, blood samples were obtained during the overnight fasting and at 120 min after the meal test. Biochemical variables were determined immediately except for cholesterol and TG contents of the lipoprotein subclasses. Serum levels of aspartate transaminase (AST), alanine transaminase, gamma-glutamyltranspeptidase, sCr, creatine phosphokinase, and HbA1c were determined by routine biochemical assays in the authors' institution. High-sensitivity C-reactive protein (hsCRP), fasting blood glucose, apolipoprotein A1 (apoA1), apolipoprotein B (apoB), apolipoprotein B48 (apoB48), remnant-like lipoprotein cholesterol, malondialdehyde low-density lipoprotein (MDA-LDL), and lipoprotein (a) [Lp(a)] were measured with SRL (Hachioji, JPN). Sitosterol and campesterol were measured as cholesterol absorption markers, and lathosterol was measured as a cholesterol synthesis marker by SRL. Lipoprotein lipase (LPL) was measured with Skylight Biotech (Akita, JPN). Serum samples were separated into 20 different lipoprotein subclasses using high-performance liquid chromatography by Skylight Biotech. Cholesterol and TG concentrations of the major lipoproteins and their subclasses were calculated with a computer software program designed to process complex chromatograms with a modified Gaussian curve-fitting function (LipoSERCH; Skylight Biotech).

Ultrasonographic Measurement of Endothelial Function

The FMD of the right brachial artery was evaluated using A- and B-mode ultrasonography (UNEX Corp, Nagoya, JPN). The subjects were instructed to lie down for 15 min. The baseline diameter of the brachial artery was defined as its mean diameter 5 cm proximal to the elbow joint during 10 consecutive diastoles on an electrocardiogram before hyperemia. After the baseline diameter was determined, forearm hyperemia was produced using a sphygmomanometric cuff inflation 50 mmHg greater than the systolic blood pressure, which was applied for 5 min. The maximum diameter of the brachial artery after hyperemia was measured for 120 s after the cuff was deflated. The rate of change in diameter (%) determined using the maximum diameter at baseline and after hyperemia was defined as the FMD.

Statistical Analysis

All statistical analyses were performed with the SPSS version 22.0 (SPSS, Inc., Chicago, USA). Results were presented as mean ± standard deviation (SD). Differences in the continuous variables between the two groups were compared. To compare normally distributed variables, an unpaired *t*-test was used; otherwise, the Mann–Whitney *U* test was performed. Differences in the continuous variables within each group were compared using the two-sided paired *t*-test or the two-sided Wilcoxon's signed rank test. The former test was performed when the variables showed a normal distribution; otherwise, the latter test was used. The association between the variables, including lipid-related variables or covariates [age, sex, and body mass index (BMI)], was examined using multiple regression models. Adjustment for the differences in the baseline covariates and changes in the variables of the study were performed with analysis of variance (ANOVA) using general linear models. Significance was defined at the 5% level using a two-tailed test ($p < 0.05$).

Results

Characteristics of the Subjects

Tables 1a, **1b**, and **1c** show the characteristics of the statin and F/E groups before and after the intervention. Age, sex, BMI, medication, and glycemic parameters were not significantly different between the two groups. AST and sCr were also not significantly different between the two groups before the intervention; however, AST and sCr were higher in the F/E group compared with those in the statin group after the intervention. AST, ALT, and sCr were higher after the drug intervention compared with those before the drug intervention in the F/E group. FBG and HbA1c were lower after the drug intervention compared with those before the drug intervention in the F/E group (**Table 1a**). Lipid profiles were not significantly different between the two groups before the drug intervention. In the F/E group, the levels of

Table1b. Lipid levels before and 12 weeks after the drug intervention

	Drug intervention	statin group ($n=25$)	F/E group ($n=25$)
Total cholesterol (mg/dL)	before	179.4 ± 27.6	186.6 ± 28.1
	after	179.7 ± 24.5	178.3 ± 27.1
CM-C (mg/dL)	before	3.2 ± 3.4	4.1 ± 4.6
	after	1.3 ± 1.5**	0.6 ± 1.0**
VLDL-C (mg/dL)	before	36.3 ± 8.4	42.5 ± 14.1
	after	34.7 ± 10.3	27.5 ± 10.1** ††
LDL-C (mg/dL)	before	86.8 ± 15.5	88.1 ± 16.2
	after	91.6 ± 15.4	90.7 ± 21.6
HDL-C (mg/dL)	before	53.1 ± 14.2	51.8 ± 9.10
	after	52.1 ± 12.0	59.4 ± 10.2** †
Total-triglyceride (mg/dL)	before	131.8 ± 54.6	154.6 ± 72.3
	after	145.4 ± 64.0	100.2 ± 49.0** ††
CM-TG (mg/dL)	before	14.0 ± 16.1	17.9 ± 21.3
	after	7.4 ± 8.9**	3.0 ± 5.5** †
VLDL-TG (mg/dL)	before	74.8 ± 35.7	92.4 ± 41.3
	after	93.6 ± 51.9**	54.5 ± 36.5** ††
LDL-TG (mg/dL)	before	26.5 ± 5.2	27.1 ± 7.4
	after	27.6 ± 5.0	29.9 ± 6.9**
HDL-TG (mg/dL)	before	16.4 ± 5.1	17.2 ± 7.8
	after	16.9 ± 5.9	12.9 ± 4.8** †
RLP-C (mg/dL)	before	4.6 ± 3.0	5.2 ± 3.3
	after	4.6 ± 3.0	4.5 ± 2.0
MDA-LDL (U/l)	before	121.8 ± 39.1	134.4 ± 34.1
	after	123.8 ± 31.1	101.8 ± 22.8** †

F/E group, fenofibrate and ezetimibe combination group; CM-C, Chylomicron Cholesterol; VLDL-C, Very Low-Density Lipoprotein Cholesterol; LDL-C, Low-Density Lipoprotein Cholesterol; HDL-C, High-Density Lipoprotein Cholesterol; CM-TG, Chylomicron Triglyceride; VLDL-TG, Very Low-Density Lipoprotein Triglyceride; LDL-TG, Low-Density Lipoprotein Triglyceride; HDL-TG, High-Density Lipoprotein Triglyceride; RLP-C, Remnant Like particles Cholesterol; MDA-LDL, Malondialdehyde Low-Density Lipoprotein.
Values are given as mean ± SD. *$P<0.05$, **$P<0.01$ (vs before the drug intervention); between before and after the drug intervention comparison by paired t test or Wilcoxon test, †$P<0.05$, ††$P<0.01$ (vs statin group); between statin and F/E group comparison by unpaired t test or Mann-Whitney test

Table1c. Apolipoprotein, lipoprotein, and lipase levels and synthesis and resorption markers before and 12 weeks after the drug intervention

	Drug intervention	statin group ($n=25$)	F/E group ($n=25$)
apoB48 (μg/mL)	before	5.5 ± 3.7	6.1 ± 4.7
	after	5.7 ± 2.9	3.4 ± 2.8** ††
apoB (mg/dL)	before	91.0 ± 16.6	98.2 ± 18.3
	after	88.9 ± 14.5	84.9 ± 18.2**
apoA1(mg/dL)	before	148.2 ± 30.4	152.7 ± 24.1
	after	150.2 ± 28.8	164.4 ± 21.5*
Lp(a) (mg/dL)	before	14.9 ± 13.9	31.1 ± 33.7
	after	16.4 ± 13.9	24.5 ± 23.2*
LPL (ng/mL)	before	94.8 ± 37.7	87.1 ± 31.3
	after	118.6 ± 154.6	74.9 ± 26.0*
sitosterol (μg/mL)	before	3.6 ± 2.5	2.8 ± 0.8
	after	3.9 ± 3.0	0.8 ± 0.4** ††
campesterol (μg/mL)	before	7.0 ± 4.1	5.8 ± 1.7
	after	7.1 ± 4.7	2.1 ± 0.5** ††
lathosterol (μg/mL)	before	1.2 ± 0.8	1.7 ± 0.7
	after	1.2 ± 0.8	3.2 ± 1.9** ††

F/E group, fenofibrate and ezetimibe combination group; Apo, apolipoprotein; Lp(a), lipoprotein (a); LPL, Lipoprotein lipase.
Values are given as mean ± SD. *$P<0.05$, **$P<0.01$ (vs before the drug intervention); between before and after the drug intervention comparison by paired t test or Wilcoxon test, †$P<0.05$, ††$P<0.01$ (vs statin group); between statin and F/E group comparison by unpaired t test or Mann-Whitney test

chylomicron (CM) cholesterol, very low-density lipoprotein (VLDL) cholesterol, total TG, CM-TG, VLDL-TG, and HDL-TG decreased after the drug intervention compared with those before the drug intervention. The levels of HDL-C and LDL-TG increased in the F/E group after the drug intervention compared with those before the drug intervention.

The LDL-C level did not change; however, the MDA-LDL level decreased in the F/E group after the drug intervention compared with that before the drug intervention (**Table 1b**). The levels of apolipoprotein, Lp(a), and LPL were not significantly different between the two groups before the drug intervention. In the F/E group, the levels of apolipoprotein, Lp(a), and LPL were significantly different between before and after the drug intervention. However, only ApoB48 level decreased in the F/E group compared with that in the statin group after the drug intervention (**Table 1c**). The levels of sitosterol, campesterol, and lathosterol were not significantly different between the two groups before the drug intervention. In the F/E group, the levels of sitosterol and campesterol, which are markers of cholesterol absorption, significantly decreased, and the levels of lathosterol, which is a marker of cholesterol synthesis, significantly increased after the drug intervention compared with those before the drug intervention. In the statin group, the levels of sitosterol, campesterol, and lathosterol were not significantly different after the drug intervention compared with those before the drug intervention (**Table 1c**).

Lipid and Glycemic Profiles after the Meal Test

Table 2 shows the results of the meal test after the drug intervention. The levels of total cholesterol, VLDL-TG, HDL-TG, and LDL were not significantly different between 0 and 120 min. The levels of CM cholesterol, total TG, CM-TG, VLDL-TG, HDL-TG, MDA-LDL, and apoB48 in the F/E group were lower than those in the statin group at 120 min of the meal test. The levels of HDL-C and apoA1 in the F/E group were higher than those in the statin group at 120 min of the meal test. In particular, when the results of the meal test were compared between 0 and 120 min, the CM cholesterol level in the F/E group was significantly lower only at 120 min of the meal test, while the significance of the low VLDL cholesterol level in the F/E group disappeared at 120 min of the meal test.

Cholesterol and TG Contents in the Lipoprotein Subclasses

Cholesterol and TG contents in 20 lipoprotein subclasses are shown in **Fig. 2**. In the statin group, the fasting cholesterol levels in CM, large VLDL, and very large HDL were slightly lower, whereas those in small VLDL and large LDL were slightly higher after the drug intervention than before the drug intervention. In the F/E group, the fasting cholesterol levels in CM, large VLDL, middle VLDL, small LDL, very small LDL, very large HDL, and large HDL were lower, whereas those in large LDL, middle HDL, small HDL, and very small HDL were higher after the drug intervention than before the drug intervention. The same tendency was observed before and after the meal test in the statin group. However, in the F/E group, the significant decrease in the cholesterol fraction in VLDL caused by the change in the treatment disappeared after the meal test (**Fig. 2a**).

In the F/E group, either at fasting or after the meal test, the TG levels in CM, large VLDL, middle VLDL, very small LDL, very large HDL, large HDL, and middle HDL decreased after the drug intervention compared with those before the drug intervention (**Fig. 2b**).

The peak particle sizes of LDL and HDL were compared between the two groups. The peak particle-size diameter of LDL increased, but that of HDL decreased in the F/E group compared with the statin group (**Fig. 3**).

Vascular Function

The results of FMD at fasting and 120 min after the meal test are shown in **Fig. 4**. Before the intervention, no significant difference in FMD was observed between the two groups. After the intervention, FMD significantly improved in the F/E group compared with that in the statin group either at fasting or after the meal test (**Fig. 4**).

Association between FMD and Lipid Profile

To elucidate the factors associated with the improvement of FMD in this study, a stepwise multiple linear regression analysis was performed. In the F/E group, ΔLp(a) ($\beta = -0.656$, $p < 0.01$) and Δvery small HDL (VS-HDL) cholesterol ($\beta = 0.438$, $p = 0.01$) were independent predictors for determining ΔFMD [adjusted $R^2 = 0.412$, ANOVA $p < 0.01$] (**Table 3**). The results of the comparison of Lp(a) and VS-HDL cholesterol are shown in **Fig. 5**. In the statin group, significant differences were not found in the results before and after the intervention. In the F/E group, both the levels of Lp(a) and VS-HDL cholesterol were significantly different before and after the intervention. However, the levels of Lp(a) were not different between the statin group and the F/E group before and after the drug intervention.

Table 2. Lipid and glycemic profiles and endothelial function changes before and after meal test, 12 weeks after the drug intervention

	time (min.)	statin group	F/E group
hsCRP (ng/mL)	0	1290 ± 2602	824 ± 818
	120	1144 ± 2295**	702 ± 656**
FBG (mg/dL)	0	118.4 ± 18.2	117.1 ± 31.3
	120	239.1 ± 61.9**	220.8 ± 49.3**
FMD (%)	0	4.8 ± 2.4	6.5 ± 2.2†
	120	3.8 ± 2.1**	5.0 ± 1.8** †
Total cholesterol (mg/dL)	0	179.7 ± 24.5	178.3 ± 27.1
	120	157.0 ± 21.5**	156.9 ± 22.9
CM-C (mg/dL)	0	1.3 ± 1.5	0.6 ± 1
	120	1.5 ± 1.2*	0.9 ± 0.8** †
VLDL-C (mg/dL)	0	34.7 ± 10.3	27.5 ± 10.1††
	120	28 ± 8.7**	24.6 ± 8.8**
LDL-C (mg/dL)	0	91.6 ± 15.4	90.7 ± 21.6
	120	81.9 ± 14.1**	79.3 ± 18.8**
HDL-C (mg/dL)	0	52.1 ± 12	59.4 ± 10.2†
	120	45.5 ± 9.8**	52.1 ± 8.6** †
Total-Triglyceride (mg/dL)	0	145.4 ± 64	100.2 ± 49††
	120	158.7 ± 67.2**	119 ± 54.8** †
CM-TG (mg/dL)	0	7.4 ± 8.9	3.0 ± 5.5†
	120	13.8 ± 11**	7.3 ± 6.3** †
VLDL-TG (mg/dL)	0	93.6 ± 51.9	54.5 ± 36.5††
	120	105.3 ± 53**	73.4 ± 42.7†
LDL-TG (mg/dL)	0	27.6 ± 5	29.9 ± 6.9
	120	23.8 ± 4.6**	25.6 ± 6**
HDL-TG (mg/dL)	0	16.9 ± 5.9	12.9 ± 4.8††
	120	15.8 ± 5.3**	12.7 ± 4.2†
RLP-C (mg/dL)	0	4.6 ± 3	4.5 ± 2.0
	120	6.5 ± 3**	5.6 ± 1.9**
MDA-LDL (U/l)	0	123.8 ± 31.1	102.7 ± 22.8††
	120	106.9 ± 38.7**	82.2 ± 20.2** ††
apoB48 (μg/mL)	0	5.7 ± 2.9	3.4 ± 2.8††
	120	9.2 ± 3.7**	6.3 ± 2.8** ††
apoB (mg/dL)	0	88.9 ± 14.5	84.9 ± 18.2
	120	78.4 ± 14.9**	74.5 ± 15.5**
apoA1(mg/dL)	0	150.2 ± 28.7	164.4 ± 21.5
	120	132.8 ± 22.8**	145 ± 17.3** †
LPL (ng/mL)	0	118.6 ± 154.6	74.9 ± 26.0
	120	81.4 ± 28.4*	71.2 ± 24.9

F/E group, fenofibrate and ezetimibe combination group; hsCRP, High-sensitivity C-reactive Protein; FBG, Fasting Blood Glucose; FMD, Flow-mediated Dilation; CM-C, Chylomicron Cholesterol; VLDL-C, Very Low-Density Lipoprotein Cholesterol; LDL-C, Low-Density Lipoprotein Cholesterol; HDL-C, High-Density Lipoprotein Cholesterol; CM-TG, Chylomicron Triglyceride; VLDL-TG, Very Low-Density Lipoprotein Triglyceride; LDL-TG, Low-Density Lipoprotein Triglyceride; HDL-TG, High-Density Lipoprotein Triglyceride; RLP-C, Remnant Like particles Cholesterol; MDA-LDL, Malondialdehyde Low-Density Lipoprotein; Apo, apolipoprotein; LPL, Lipoprotein lipase.
Values are given as mean ± SD. *$P<0.05$, **$P<0.01$ (vs 0min after test meal); between 0min and 120min after test meal comparison by paired *t* test or Wilcoxon test, †$P<0.05$, ††$P<0.01$ (vs statin group); between statin and F/E group comparison by unpaired *t* test or Mann-Whitney test.

Discussion

In this study, treatment with statins was compared with that with the combination of fenofibrate and ezetimibe in patients with type 2 diabetes. The patients treated with the combination showed signifi-

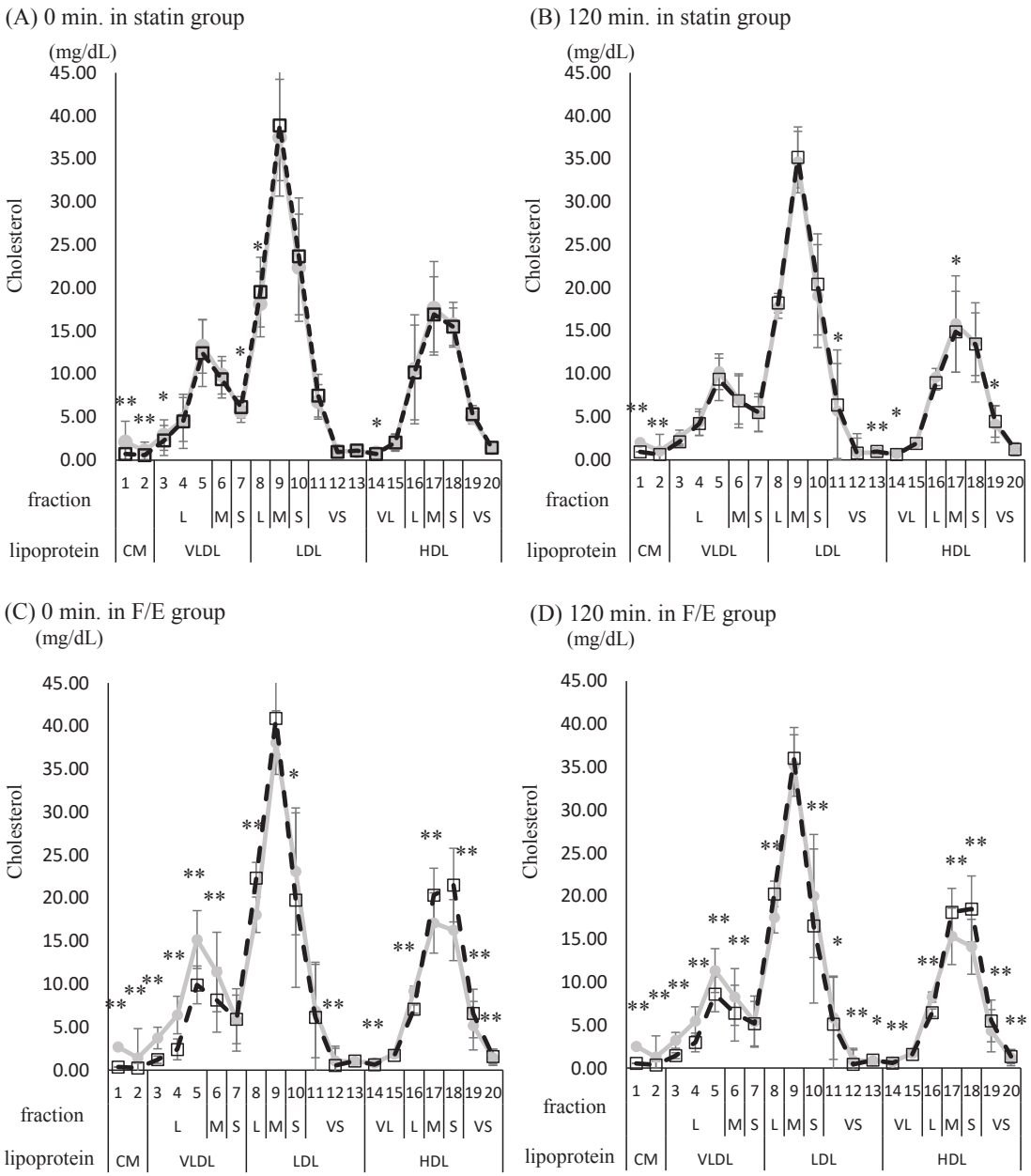

Fig. 2a. Twenty fractionated cholesterol, comparison of before and after the drug intervention at 0 and 120 min of the meal test

Gray line and circles correspond to before the drug intervention, while dashed line and blank squares correspond to after the drug intervention. (A) 0 min of the meal test in the statin group. (B) 120 min of the meal test in the statin group. (C) 0 min of the meal test in the fenofibrate and ezetimibe combination group (F/E group). (D) 120 min of the meal test in the F/E group. Lipoprotein fraction: CM, Chylomicron; VLDL, Very Low-Density Lipoprotein; LDL, Low-Density Lipoprotein; HDL, High-Density Lipoprotein. Fraction size: VL; very large, L; large, M; middle, S; small, VS; very small. Values are given as mean ± SD. $^{*}p<0.05$, $^{**}p<0.01$; between-group comparison by paired *t*-test or Wilcoxon signed rank test

cantly lower levels of serum TG without any differences in LDL-C levels as compared with those treated with statins. The reduction of the serum TG levels was associated with an increase in the small HDL-C fraction and a decrease in the small LDL-C fraction. In addition to the improvement of the lipid profile, the group treated with the combination of fenofibrate and ezetimibe showed recovery of vascular function assessed using the forearm FMD. Multiple regression analysis revealed that the improvement of FMD was

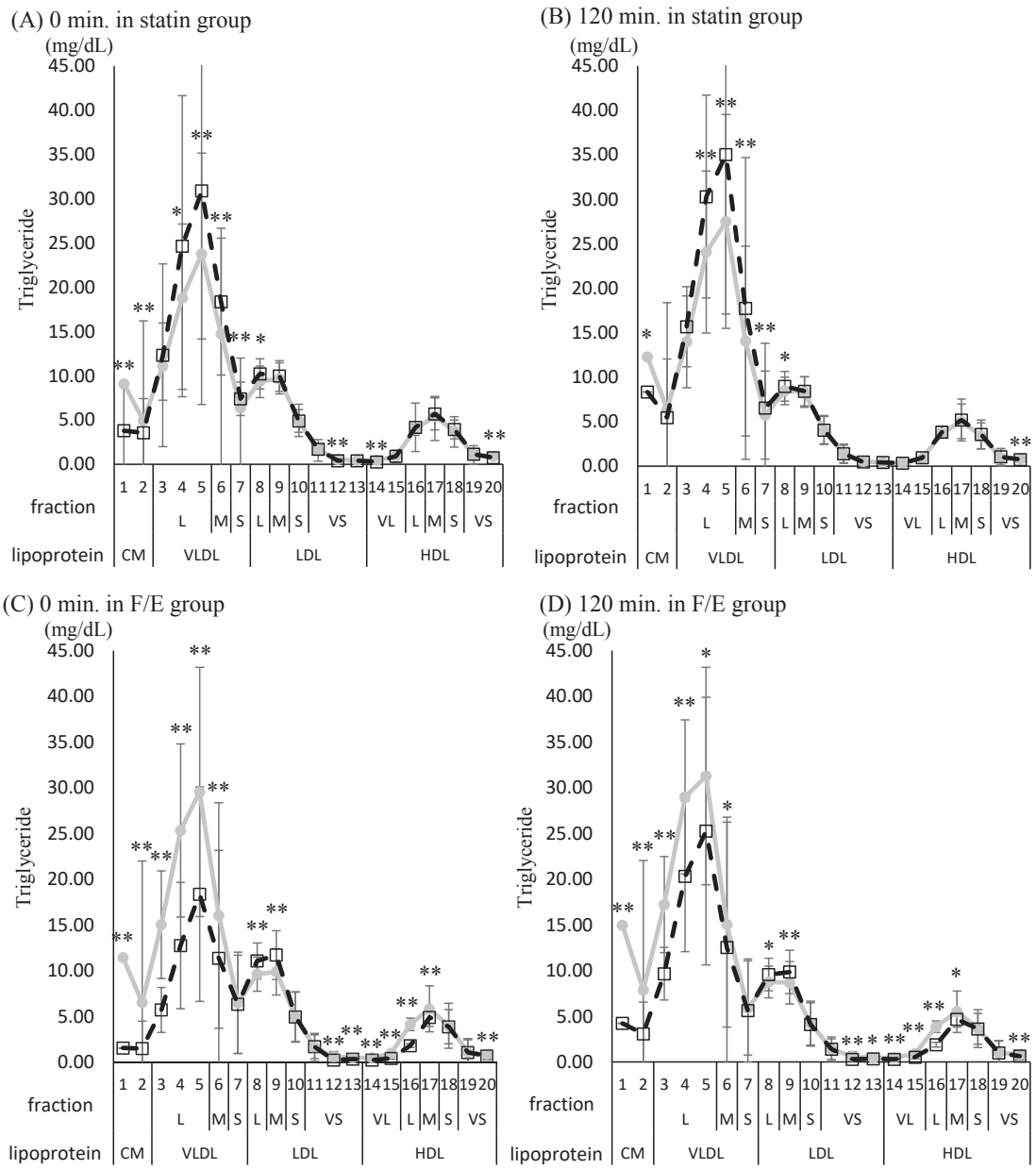

Fig. 2b. Twenty fractionated triglyceride, comparison of before and after the drug intervention at 0 and 120 min of the meal test

Gray line and circles correspond to before the drug intervention, while dashed line and blank squares correspond to after the drug intervention. (A) 0 min of the meal test in the statin group. (B) 120 min of the meal test in the statin group. (C) 0 min of the meal test in the fenofibrate and ezetimibe combination group (F/E group). (D) 120 min of the meal test in the F/E group. Lipoprotein fraction: CM, Chylomicron; VLDL, Very Low-Density Lipoprotein; LDL, Low-Density Lipoprotein; HDL, High-Density Lipoprotein. Fraction size: VL; very large, L; large, M; middle, S; small, VS; very small. Values are given as mean ± SD. $^{*}p < 0.05$, $^{**}p < 0.01$; between-group comparison by paired *t*-test or Wilcoxon signed rank test

associated with the decrease in the levels of Lp(a) and the increase in the very small HDL-C fraction. To the best of our knowledge, this study is the first to report that the intervention for the serum TG with the combination of fenofibrate and ezetimibe in patients with type 2 diabetes treated with statins not only improved the lipoprotein profile but also the vascular function.

Dyslipidemia in patients with type 2 diabetes is characterized by an increase in serum TG level and a decrease in HDL-C level, which is associated with an

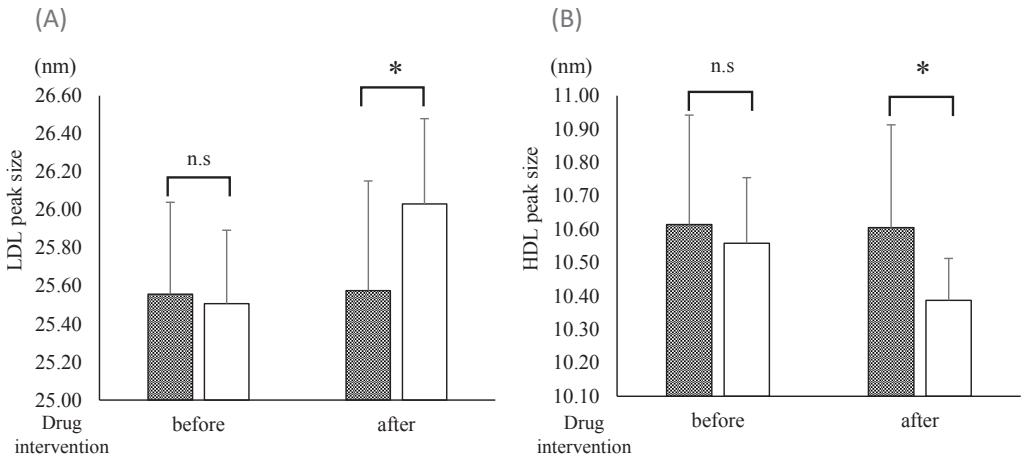

Fig. 3. Peak sizes of LDL and HDL before and after the drug intervention

Gray column: statin group. Open column: fenofibrate and ezetimibe combination group (F/E group). (A) Peak size of LDL. (B) Peak size of HDL. $^{*}p<0.01$; between-group comparison by unpaired *t*-test or Mann–Whitney *U* test.

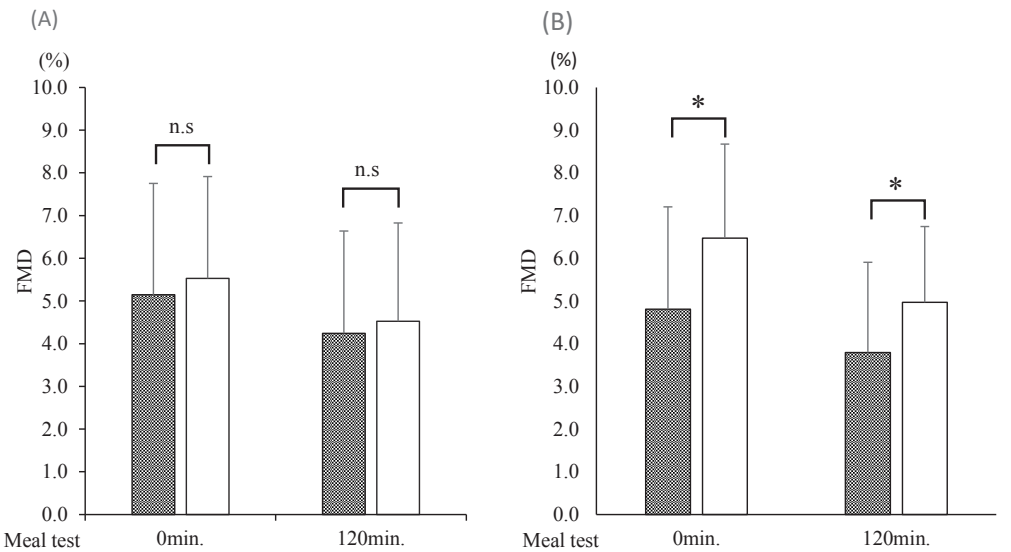

Fig. 4. Comparison of flow-mediated dilation (FMD) before and after the meal test through the drug intervention

Gray column: statin group. Open column: fenofibrate and ezetimibe combination group (F/E group). (A) Before the drug intervention. (B) After the drug intervention. $^{*}p<0.05$; between-group comparison by unpaired *t*-test or Mann–Whitney *U* test.

increase in the fraction of small dense LDL-C, known as an atherogenic LDL[14]. Treatment with statins reduces the serum LDL-C level effectively, but it does not improve the characteristics of dyslipidemia found in patients with type 2 diabetes. Therefore, we used a fibrate in the present study. The treatment with fenofibrate successfully reduced the serum TG level and increased the HDL-C level. In addition, patients with type 2 diabetes were reported to have increased intestinal cholesterol absorption mediated by the Niemann-Pick C1-like 1 (NPC1L1) protein[15]. Ezetimibe inhibits cholesterol absorption by selectively blocking the NPC1L1 protein in the jejunal brush border and depletes hepatic pools of cholesterol. This increases the expression of the LDL receptor on the hepatocyte surface, which leads to reductions in the serum levels of LDL-C[16]. Thus, treatment with ezetimibe is reasonable for hyper-LDL cholesterolemia in patients with type 2 diabetes.

Oikawa *et al.* reported that the combination

Table 3. Multiple regression analysis of the FMD changes (⊿FMD) before and after the drug intervention in the F/E group

Characteristic change	Standardized partial regression coefficient	*P*-value	95%confidence interval	
			lower	upper
⊿Lipoprotein (a)	−0.656	<0.01	−0.106	−0.533
⊿Very Small-HDL (19 fraction) cholesterol	0.438	0.01	0.153	0.253

R^2=0.412, ANOVA $p<0.01$

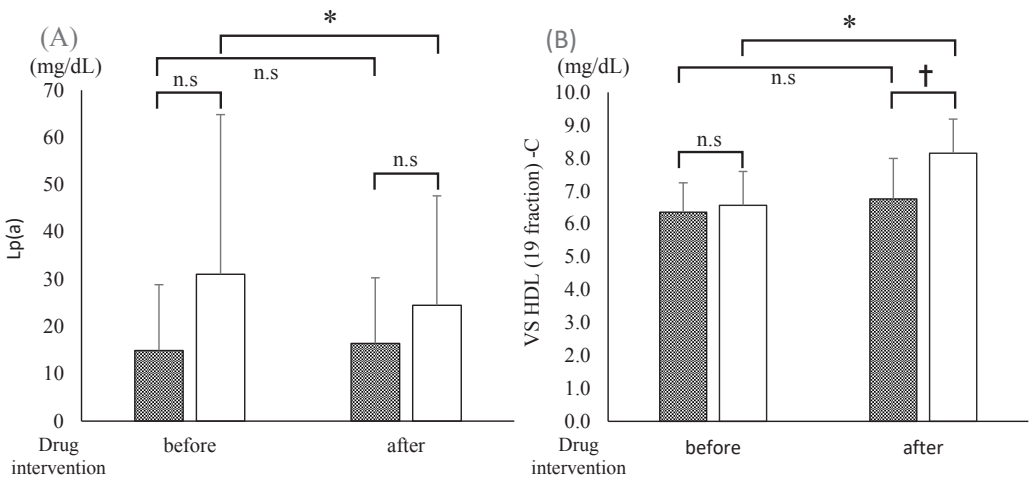

Fig. 5. Changes in the independent variable related to flow-mediated dilation (FMD) before and after the intervention

Gray column: statin group. Open column: fenofibrate and ezetimibe combination group (F/E group). (A) Lipoprotein (a) (Lp(a)). (B) Very small High-density lipoprotein (19 fraction) cholesterol (VS HDL-C). *$p<$ 0.05; between-group comparison by Mann–Whitney *U* test. †$p<0.05$; between-group comparison by paired *t*-test or Wilcoxon signed rank test.

therapy with fenofibrate and ezetimibe reduces concentrations of LDL-cholesterol and triglyceride and is safe in a long-term treatment[17]. We found that the treatment with the combination significantly reduced the cholesterol levels in small and very small fractions of LDL and increased the cholesterol levels in large fractions of LDL. Since the LDL with small diameter is known as a cardiovascular event risk[18], the change observed in the treatment with the combination may suggest an advantage of the treatment. The LDL in the small fraction can be oxidized easily[19], and the serum levels of small dense LDL and MDA-LDL, a form of oxidized LDL, are positively correlated[20]. Thus, the decrease in the MDA-LDL in patients treated with the combination may be explained by the reduction of the cholesterol level in the small LDL fraction. In contrast to the change in the diameter of LDL, the treatment with the combination of fenofibrate and ezetimibe increases the cholesterol level of small HDL fractions. Since the reduction of TG-rich lipoproteins after the treatment with the combination should have similar effects on the size of HDL as those on the size of LDL, the decreased size of HDL in patients treated with the combination suggests a direct effect of fenofibrate on the HDL synthesis due to the increase in the production of apoA1 and apoA2 in the liver. Interestingly, the association of HDL function with the size was reported[21, 22]. The small fraction of HDL is enriched with negatively charged phospholipids, which are associated with cellular cholesterol efflux, anti-oxidation, anti-thrombosis, anti-inflammation, and anti-apoptosis effects. Thus, the change in the size of HDL is another benefit of the treatment with the combination of fenofibrate and ezetimibe.

The treatment with the combination of fenofibrate and ezetimibe ameliorated the vascular function assessed with FMD. Multiple regression analysis identified that the amount of increase in the cholesterol of the very small HDL fraction and the amount of decrease in the Lp(a) were significantly correlated with the improvement of FMD. HDL, particularly the small fraction of HDL, has direct effects on the endothelium, including the promotion of nitric oxide production and prevention of nitric oxide degradation by

an anti-oxidant effect[22, 23]. HDL has been reported to increase nitric oxide production by inducing the phosphorylation of endothelial nitric oxide synthesis (eNOS) and the expression of eNOS[24]. Importantly, impairment in these activities on the endothelial cells of the HDL from the patients with type 2 diabetes was reported[25]. Recent studies have unequivocally established Lp(a) as a causal and independent risk factor for atherosclerotic cardiovascular diseases, even under maximal intensity statin treatment[26]. However, we did not find any significant differences in the level of Lp(a) between the patients treated with the combination of fenofibrate and ezetimibe and those treated with statins; thus, further investigation is necessary to determine whether the treatment with the combination reduces the level of Lp(a) in patients with type 2 diabetes.

Several investigations have reported the improvement of postprandial dyslipidemia after treatment with ezetimibe[27, 28]. Ito *et al.* found that the amelioration of postprandial dyslipidemia by ezetimibe is due to the improvement in the endothelial function[29]. In the present study, we observed that the patients did not show significant hyperlipidemia in the postprandial state. Treatment with the combination of fenofibrate and ezetimibe ameliorated serum TG and HDL-C levels at fasting and postprandial time, as well as improved vascular function. These results may indicate that the amelioration of postprandial hyperlipidemia alone cannot explain all the improvement in the vascular function found in the present study. We measured the postprandial lipid profiles and assessed the vascular function 2 h after the meal, which may not be optimal to assess the maximum effects of the meal.

Non-HDL-C has shown similar or stronger association with coronary artery disease (CAD) than LDL-C[30]. Also, it has been reported that non-HDL-C is a more superior predictor of small-dense LDL-C than LDL-C[31]. However, in this study, we did not evaluate non-HDL-C in detail.

FMD is widely accepted for evaluating the endothelial dysfunction on the premise that normality of smooth muscle function is preserved. However, it is reported that 51% of type 2 diabetes patients had low nitroglycerin-mediated dilation, which reflects vascular smooth muscle function[32]. Thus, in type 2 diabetes patients, evaluating endothelial function with FMD alone is difficult. Therefore, we consider that FMD reflects vascular function including endothelial and smooth muscle functions in this study.

Conclusions

Treatment with a combination of fenofibrate and ezetimibe effectively controlled the LDL-C and TG levels and improved vascular function in patients with type 2 diabetes. Compared with the treatment with statins, the treatment with the combination increased the HDL-C level, especially in its small fraction, and decreased the TG and small LDL-C levels. The amelioration of vascular function through treatment with the combination was significantly associated with the elevation of the very small fraction of HDL-C.

Acknowledgments

The authors thank the patients and their families and appreciate the assistance of the medical technologists in the authors' institution.

Notice of Financial Support

This study was financially supported by Bayer Yakuhin, Ltd. The authors have received research grants from the Immuno-Biological Laboratory. However, these companies were not involved in the design, execution, and analysis and interpretation of data of this study.

COI

Honoraria (lecture fee):

Astellas Pharma Inc., Ono Pharmaceutical Co., MSD KK, Mitsubishi Tanabe Pharma, Kowa Pharmaceutical Co., Bayer Yakuhin, Ltd., Takeda Pharmaceutical CO. Ltd., Sanofi KK, AstraZeneca KK, Sumitomo Dainippon Pharma., Eisai Co., and Shionogi & Co.

Grant/Research Support:

Astellas Pharma Inc., Ono Pharmaceutical Co., MSD KK, Mitsubishi Tanabe Pharma, and Bayer Yakuhin, Ltd.

Consignment/Joint Study:

Immuno-Biological Laboratory

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Supplemental Table 1. The statins used before the present study

	dosage	statin group ($n=25$)	F/E group ($n=25$)
simvastatin	5 mg	1	1
pravastatin	5 mg	8	3
	10 mg	2	2
fluvastatin	20 mg	1	0
pitavastatin	0.5 mg	1	0
	1.0 mg	4	8
	2.0 mg	1	3
atorvastatin	5 mg	3	4
	10 mg	2	2
rosuvastatin	2.5 mg	1	2
	5.0 mg	1	0

Supplemental Table 2. The number of strong and standard statins used in the statin group and fenofibrate and ezetimibe combination group

Groups	statin group	F/E group	total
Standard statin	12	6	18
Strong statin	13	19	32
total	25	25	50

$\chi^2(1)=3.125$, $P=0.070$

Standard statins; simvastatin, pravastatin, fluvastatin. Strong statins; pitavastatin, atorvastatin, rosuvastatin