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

Atsushi Shinnakasu, Kiyoki Yamamoto, Mihoko Kurano, Hiroshi Arimura, Aiko Arimura, Akira Kikuti, Hiroshi Hashiguchi, Takahisa Deguchi and Yoshihiko Nishio

Department of Diabetes and Endocrine Medicine, Kagoshima University Graduate School of Medical and Dental Sciences, Kagoshima, Japan

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

Vascular endothelial dysfunction independently predicts post treatment cardiovascular diseases. 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. FMD is reduced in patients with type 2 diabetes. Numerous factors have been reported to be related to this dysfunction. Among these, lipid abnormalities in the postprandial and fasting states are the important factors. Increased level of serum TG associated with metabolic syndrome or insulin resistant state is particularly closely related to endothelial dysfunction.

**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...
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 ( systolic blood pressure ≥ 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...

Table 1a. 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/m²)  |                   | 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* **          |

F/E group, fenofibrate and ezetimibe combination group; AST, Aspartate transaminase; ALT, Alanine transaminase; γGT, Gamma-Glutamyltransferase; 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.
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
### Table 1b. 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 |
| CM-C (mg/dL) | before 3.2 ± 3.4 | 4.1 ± 4.6 |
| VLDL-C (mg/dL) | before 36.3 ± 8.4 | 42.5 ± 14.1 |
| LDL-C (mg/dL) | before 86.8 ± 15.5 | 88.1 ± 16.2 |
| HDL-C (mg/dL) | before 53.1 ± 14.2 | 51.8 ± 9.10 |
| Total-triglyceride (mg/dL) | before 131.8 ± 54.6 | 154.6 ± 72.3 |
| CM-TG (mg/dL) | before 14.0 ± 16.1 | 17.9 ± 21.3 |
| VLDL-TG (mg/dL) | before 74.8 ± 35.7 | 92.4 ± 41.3 |
| LDL-TG (mg/dL) | before 26.5 ± 5.2 | 27.1 ± 7.4 |
| HDL-TG (mg/dL) | before 27.6 ± 5.0 | 29.9 ± 6.9** |
| RLP-C (mg/dL) | before 4.6 ± 3.0 | 5.2 ± 3.3 |
| MDA-LDL (U/l) | before 121.8 ± 39.1 | 134.4 ± 34.1 |

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 < 0.01 (vs statin group); between statin and F/E group comparison by unpaired *t* test or Mann-Whitney test.

### Table 1c. 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 |
| apoB (mg/dL) | before 91.0 ± 16.6 | 98.2 ± 18.3 |
| apoA1 (mg/dL) | before 148.2 ± 30.4 | 152.7 ± 24.1 |
| Lp(a) (mg/dL) | before 14.9 ± 13.9 | 31.1 ± 33.7 |
| LPL (ng/mL) | before 94.8 ± 37.7 | 87.1 ± 31.3 |
| sitosterol (µg/mL) | before 3.6 ± 2.5 | 2.8 ± 0.8 |
| campesterol (µg/mL) | before 7.0 ± 4.1 | 5.8 ± 1.7 |
| lathosterol (µg/mL) | before 1.2 ± 0.8 | 1.7 ± 0.7 |

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 < 0.01 (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, \( \Delta \text{Lp(a)} (\beta = -0.656, p < 0.01) \) and \( \Delta \text{very small HDL (VS-HDL) cholesterol} (\beta = 0.438, p = 0.01) \) were independent predictors for determining \( \Delta \text{FMD} \) [adjusted \( R^2 = 0.412, \text{ANOVA} p < 0.01 \)] (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 significa-
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 significantly 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
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
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. Thus, treatment with ezetimibe is reasonable for hyper-LDL cholesterolemia in patients with type 2 diabetes.

Oikawa et al. reported that the combination increase in the fraction of small dense LDL-C, known as an atherogenic LDL. 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. 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. Thus, treatment with ezetimibe is reasonable for hyper-LDL cholesterolemia in patients with type 2 diabetes.

Oikawa et al. reported that the combination...
therapy with fenofibrate and ezetimibe reduces concentrations of LDL-cholesterol and triglyceride and is safe in a long-term treatment. 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, 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, and the serum levels of small dense LDL and MDA-LDL, a form of oxidized LDL, are positively correlated. 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. 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

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

| Characteristic change                                      | Standardized partial regression coefficient | P-value | 95% confidence interval |
|-----------------------------------------------------------|---------------------------------------------|---------|-------------------------|
| ΔLipoprotein (a)                                          | -0.656                                      | <0.01   | -0.106 - 0.533          |
| ΔVery Small-HDL (19 fraction) cholesterol                | 0.438                                       | 0.01    | 0.153 - 0.253           |

R²=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.
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

References
1) Turner RC, Millns H, Neil HA, Stratton IM, Manley SE, Matthews DR, Holman RR: Risk factors for coronary artery disease in non-insulin dependent diabetes mellitus: United Kingdom Prospective Diabetes Study (UKPDS: 23). BMJ, 1998; 316: 823-828
2) Kearney PM, Blackwell L, Collins R, Keech A, Simes J, Peto R, Armitage J, Baigent C: Efficacy of cholesterol-lowering therapy in 18,686 people with diabetes in 14 randomised trials of statins: a meta-analysis. Lancet, 2008; 371: 117-125
3) Lorenzo C, Hartnett S, Hanley AJ, Rewers MJ, wagen-knecht LE, Karter AJ, Haffner SM: Impaired fasting glucose and impaired glucose tolerance have distinct lipoprotein and apolipoprotein changes: the insulin resistance atherosclerosis study. J Clin Endocrinol Metab, 2013; 98: 1622-1630
4) Tanaka A: Postprandial hyperlipidemia and atherosclerosis. J Atheroscler Thromb, 2004; 11: 322-329
5) Ai M, Tanaka A, Orita K, Sekine M, Numano F, Numano F, Reaven GM: Relationship between plasma insulin concentration and plasma remnant lipoprotein response to an oral fat load in patients with type 2 diabetes. J Am Coll Cardiol, 2001; 38: 1628-1632
6) Taskinen MR, Boren J: New insights into the pathophysiology of dyslipidemia in type 2 diabetes. Atherosclerosis, 2015; 239: 483-495
7) Ras RT, Streppel MT, Draijer R, Zock PL: Flow-mediated dilation and cardiovascular risk prediction: a systematic review with meta-analysis. Int J Cardiol, 2013; 168: 344-351
8) Mori H, Maeda A, Wakabayashi K, Sato T, Sasaki M, Tashiro K, Iso T, Ebato M, Suzuki K: The Effect of Cilostazol on Endothelial Function as Assessed by Flow-Mediated Dilation in Patients with Coronary Artery Disease. J Atheroscler Thromb, 2016; 23: 1168-1177
9) Fakhhrzadeh H, Sharifi F, Alizadeh M, Arzaghi SM, Tajalizade-Khoob Y, Tootee A, Alatab S, Mirarefin M, Badamzadeh Z, Kazami H: Relationship between insulin resistance and subclinical atherosclerosis in individuals with and without type 2 diabetes mellitus. J Diab Metab Disord, 2015; 15: 41
10) Benjamin EJ, Larson MG, Keyes MJ, Mitchell GF, Vasan RS, Keaney JF, Jr., lehman BT, Fan S, Ospyui F, Vita JA: Clinical correlates and heritability of flow-mediated dilation in the community: the Framingham Heart Study. Circulation, 2004; 109: 613-619
11) Widlansky ME, Gokce N, Keaney JF, Jr., Vita JA: The clinical implications of endothelial dysfunction. J Am Col Cardiol, 2003; 42: 1149-1160
12) Marchesi S, Lupattelli G, Schillaci G, Pirro M, Siepi D, Roscini AR, Pasqualini L, Mannarino E: Impaired flow-mediated vasoreactivity during post-prandial phase in young healthy men. Atherosclerosis, 2000; 153: 397-402
13) Watts GF, O’Brien SF, Silvester W, Millar JA: Impaired endothelium-dependent and independent dilatation of forearm resistance arteries in men with diet-treated non-insulin-dependent diabetes: role of dyslipidaemia. Clin Sci, 1996; 91: 567-573
14) Carmena R: Type 2 diabetes, dyslipidaemia, and vascular risk: rationale and evidence for correcting the lipid imbalance. Am Heart J, 2005; 150: 859-870
15) Lally S, Tan CY, Owens D, Tomkin GH: Messenger RNA levels of genes involved in dysregulation of postprandial lipoproteins in type 2 diabetes: the role of Niemann-Pick C1-like 1, ATP-binding cassette, transporters G5 and G8, and of microsomal triglyceride transfer protein. Diabetologia, 2006; 49: 1008-1016
16) Phan BA, Dayspring TD, Toth PP: Ezetimibe therapy: mechanism of action and clinical update. Vasc Health Risk Manag, 2012; 8: 415-427
17) Oikawa S, Yamashita S, Nakaya N, Sasaki J, Kono S for the Effect of Fenofibrate and Ezetimibe Combination Treatment on Lipid (EFECTL) Study Investigators: Efficacy and Safety of Long-term Coadministration of Fenofibrate and Ezetimibe in Patients with Combined Hyperlipidemia: Results of the EFECTL Study. J Atheroscler Thromb, 2017; 24: 77-94
18) Lamarache B, Tcherno H, Moorjani S, Cantin B, Dagenais GR, Lupien PJ, Despres JP: Small, dense low-density lipoprotein particles as a predictor of the risk of ischemic heart disease in men. Prospective results from the Quebec Cardiovascular Study. Circulation, 1997; 95: 69-75
19) Takahashi R, Imamura A, Yoshikane M, Suzuki M, Cheng XW, Numaguchi Y, Ikeda N, Murohara T, Okumura K: Circulating malondialdehyde-modified low-density lipoprotein is strongly associated with very small low-density lipoprotein cholesterol concentrations in healthy men. Clin Chim Acta, 2009; 399: 74-78
20) Tanaka K, Bujo H, Inoue M, Mikami K, Kotani K, Takahashi K, Kanno T, Saito Y: Increased circulating malondialdehyde-modified LDL levels in patients with coronary artery diseases and their association with peak sizes of LDL particles. Arterioscler Thromb Vasc Biol, 2002; 22: 662-666
21) Kontush A: HDL particle number and size as predictors of cardiovascular disease. Front Pharmacol, 2015; 6: 218
22) Camont L, Lhomme M, Rached F, Le Goff W, Negre-Salvayre A, Salvare R, Calzada C, Lagarde M, Chapman MJ, Kontush A: Small, dense high-density lipoprotein-3 particles are enriched in negatively charged phospholipids: relevance to cellular cholesterol efflux, antioxidative, anti-thrombotic, anti-inflammatory, and antiapoptotic functionalities. Arterioscler Thromb Vasc Biol, 2013; 33: 2715-2723
23) Mineo C, Deguchi H, Griffin JH, Shaul PW: Endothelial and anti-thrombotic actions of HDL. Circ Res, 2006; 98: 1352-1364
24) Kratzer A, Giral H, Landmesser U: High-density lipoproteins as modulators of endothelial cell functions: alterations in patients with coronary artery disease. Cardiovasc Res, 2014; 103: 350-361
25) Schofield I, Malik R, Izzard A, Austin C, Heagerty A: Vascular structural and functional changes in type 2 diabetes mellitus: evidence for the roles of abnormal myogenic responsiveness and dyslipidemia. Circulation, 2002; 106: 3037-3043
26) Jones GT, van Rij AM, Cole J, Williams MJ, Bateman EH, Marcovina SM, Deng M, McCormick SP: Plasma lipoprotein(a) indicates risk for 4 distinct forms of vascular disease. Clin Chem, 2007; 53: 679-685
27) Hiramitsu S, Miyagishima K, Ishi J, Matsui S, Naruse H, Shino K, Kitagawa F, Ozaki Y: Effect of ezetimibe on lipid and glucose metabolism after a fat and glucose load. J Cardiol, 2012; 60: 395-400
28) Masuda D, Nakagawa-Toyama Y, Nakatanai K, Inagaki M, Tsubaki-Yamamoto K, Sandovall JC, Ohama T, Nishida M, Ishigami M, Yamashita S: Ezetimibe improves postprandial hyperlipidaemia in patients with type IIb hyperlipidaemia. Eur J Clin Invest, 2009; 39: 689-698
29) Yunoki K, Nakamura K, Miyoshi T, Enko K, Kohno K, Morita H, Kusano KF, Ito H: Ezetimibe improves postprandial hyperlipemia and its induced endothelial dys...
function. Atherosclerosis, 2011; 217: 486-491
30) Moriyama K, Takahashi E: Non-HDL Cholesterol is a More Superior Predictor of Small-Dense LDL Cholesterol than LDL Cholesterol in Japanese Subjects with TG Levels < 400 mg/dL. J Atheroscler Thromb, 2016; 23: 1126-1137
31) Kuwabara K, Harada S, Sugiyama D, kurihara A, Kubota Y, Higashiyama A, Hirata T, Nishida Y, Kawasaki M, Takebayashi T, Okamura T: Relationship between Non-

High-Density Lipoprotein Cholesterol and Low-Density Lipoprotein Cholesterol in the General Population. J Atheroscler Thromb, 2016; 23: 477-490
32) Furuta M, Ueyama M, Morita S, Yamana A, Sanke T: Combined examination of glyceryl trinitrate-mediated vascular dilation with flow-mediated vascular dilation is essential for assessment of vascular function in type 2 diabetes. J Diabetes Investig, 2013; 4: 304-309

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 |
| rosvastatin | 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, rosvastatin