Detrimental effects of high-fat diet loading on vascular endothelial function and therapeutic efficacy of ezetimibe and statins in patients with type 2 diabetes

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Abstract. Several recent reports from large clinical trials have described the role of postprandial hyperlipidemia in the onset of atherosclerosis. In this pilot study, the effects of postprandial lipid abnormalities induced by high-fat diet loading on vascular endothelial function in type 2 diabetes were investigated and the effects of ezetimibe and statins on endothelial function were compared. In 20 patients in Study 1, peripheral arterial tonometry tests were performed before and 4h after loading to measure the reactive hyperemia index (RHI). In Study 2, the same patients were randomly allocated to ezetimibe or rosuvastatin. After 1 week of treatment, loading tests were conducted in the same manner. In Study 1, the RHI decreased from 1.86 to 1.60. There were no significant correlations between changes in RHI and the area under the curve (AUC) or coefficient of variation (CV) of each metabolic marker. In Study 2, ezetimibe treatment resulted in a significant improvement in RHI. The two drugs had comparable effects on changes in AUC. There were no significant correlations between changes in RHI and changes in AUC or changes in CV. When age, sex, drug, hemoglobin A1c, and changes in each lipid were evaluated as independent variables with RHI improvement as the dependent variable, drug differences were found to exert the greatest effect on RHI improvement using a stepwise procedure. The results of this study suggest that the progression of atherosclerosis is due to abnormalities in postprandial lipid metabolism and that ezetimibe can potentially inhibit the aggravation of vascular endothelial dysfunction after high-fat diet loading.

Key words: Postprandial hyperlipidemia, Atherosclerosis, Type 2 diabetes, Reactive hyperemia index (RHI), Ezetimibe
which is a constituent of chylomicrons and chylomicron remnants, significantly correlated with the morbidity of atherosclerotic cardiovascular disease [5].

Statins are the most frequently used drugs for the treatment of lipid abnormalities affecting vascular endothelial function. The Collaborative Atorvastatin Diabetes (CARDS) study [6] provided clear evidence for the usefulness of statin therapy in preventing the onset of macrovasculopathies in type 2 diabetic patients lacking history of macrovascular disorders. However, statin therapy is associated with certain risks. For example, in the Treating to New Targets (TNT) study [7], high-dose statin therapy reduced the relative risk of major cardiovascular events by 22% in comparison with standard statin therapy, but the study also showed that the remaining 78% of cases required intervention by other agents. The reason for that finding was postulated to be that inhibition of cholesterol synthesis by statin therapy induced compensatory enhancement of cholesterol absorption, thereby elevating blood concentrations of the drug. In studies on the effects of lipid-lowering drugs on vascular endothelial function, 4-week treatment of patients with congestive heart failure (CHF) with 10 mg of rosuvastatin resulted in a significant improvement of flow-mediated vasodilatation (FMD) compared with 20 mg ezetimibe, demonstrating the usefulness of statin therapy in patients with heart disease [8]. Another study showed that 4-week administration of 10 mg ezetimibe in 10 healthy subjects achieved significant inhibition of decrease in FMD after high-fat diet loading in comparison with the control (no ezetimibe), suggesting that ezetimibe influences vascular endothelial function [9]. However, only a few studies have compared the effects of statins and ezetimibe on vascular endothelial function.

The outcome of FMD has been assessed in several studies and the results of FMD have been compared with those of invasive techniques that measure epicardial vascular function. In comparison, peripheral arterial tonometry (Endo-PAT) has low interobserver and intraobserver variability and less than ideal correlation with indices of microvascular function measured by invasive procedures [10]. The aim of the present study was to determine the effects of postprandial lipid abnormalities induced high-fat diet loading on vascular endothelial function and the effects of ezetimibe and statins on endothelial function tested by Endo-PAT, which is approved as the only non-invasive vascular endothelial function test by Food and Drug Administration (FDA) in diabetic patients with vasculopathies.

**Subjects and Methods**

**Subjects**

The research participants were 20 in patients with type 2 diabetic patients, aged between 20 and 79 years, who were not being treated for dyslipidemia, at the University of Occupational and Environmental Health, Department of Endocrinology, Metabolism and Diabetes and affiliated hospitals between April 2012 and March 2014. We excluded patients treated with insulin, and those with abnormalities in the electro-cardiogram. Although there were no restrictions on the use of oral antihyperglycemic agents at the time of admission, change of drugs was prohibited until the end of the study. The Institutional Review Board of the University of Occupational and Environmental Health approved this study. This Clinical Trial was registered with the University Hospital Medical Information Network (UMIN) (No. UMIN000018577, 000018629). The study was explained to participants in writing, and written consent was obtained. Samples were processed appropriately according to the Declaration of Helsinki.

**Study design**

Study 1 was a cross-sectional study in which the high-fat diet loading test was conducted in fasting patients in the morning of the second day of hospital stay. Blood samples were collected before high-fat diet loading as well as 1, 2, 3 and 4 h after loading, and TG, LDL-C, HDL-C, malondialdehyde modified low-density lipoprotein (MDA LDL-C), small-dense LDL-C (sd LDL-C), free-fatty acids (FFA), remnant like particles cholesterol (RLP-C), PG and apoB-48 were measured. The Endo-PAT test was performed before and 4 h after high-fat diet loading to measure the reactive hyperemia index (RHI), a marker of vascular endothelial function.

In Study 2, which was a randomized controlled study; subjects were allocated randomly by the envelope method to either of two treatment groups: ezetimibe 10 mg/day or rosuvastatin 2.5 mg/day. After 1-week oral administration of the allocated drug, the high-fat diet-loading test was conducted in the same manner as in Study 1.

The primary endpoint of Study 1 was changes in RHI before and after high-fat diet loading, and the secondary endpoint was the correlation between changes...
in each lipid profile indicator and changes in RHI. In Study 2, the primary endpoint was the difference between RHI changes following treatment with each of the two drugs, and the secondary endpoint was the difference between changes in lipid and glucose metabolism markers in the two groups.

**Biochemical analyses**

Blood samples were collected early in the morning after at least 12 h fasting, through a venous line placed in the median vein using an indwelling catheter. The PG level was measured with the glucose oxidase method. Hemoglobin A1c (HbA1c) was measured by high-performance liquid chromatography (HPLC) using Yosoh HLC-723 G8 (Tosoh Co., Kyoto, Japan). HbA1c (%) was estimated as the National Glycohemoglobin Standardization Program (NGSP) equivalent value, which was calculated as HbA1c (NGSP) (%) = HbA1c (JDS) (%) + 0.4%, considering the relationship of HbA1c (NGSP) values to HbA1c (JDS) (%) values measured by the Japanese standard and measurement method. The homeostasis model assessment for insulin resistance (HOMA-IR), which represents insulin resistance, was calculated (formula: HOMA-IR = Fasting glucose level × Fasting Insulin Level ÷ 405). Blood samples were collected during fasting and urinary C-peptide reactivity (u-CPR) levels were measured in 24 h urine samples.

Measurement of lipid profiles and other markers was outsourced to SRL Co., Ltd. Plasma lipid was measured with a Hitachi 7350 autoanalyzer (Hitachi Co., Tokyo). LDL-C was measured using the colest-est LDL (Sekisui Medical, Tokyo) by the direct method. HDL-C was measured using the colest-est NHDL (Sekisui Medical, Tokyo) by the direct method. TG was measured using the pureanto STG-N (Sekisui Medical, Tokyo) by the enzymatic method. FFA was measured using the NEFA-SS“EIKEN” (Eiken Kagaku, Tokyo) by the enzymatic method. sd LDL-C was measured using the sd LDL-EX reagent “SEIKEN” (Denka Seiken Inc., Tokyo) by the enzymatic method. MDA LDL-C was measured using the oxidative ELISA “Daichi” (Sekisui Medical, Tokyo) by a sandwich ELISA (enzyme linked immunosorbent assay) method. RLP-C was measured using the RLP-C reagent “JIRO-II” (Otsuka Inc., Tokyo) by the immunoaffinity isolation method. ApoB-48 was measured using a chemiluminescence enzyme immunoassay (CLEIA, Fuji Rebio Inc., Tokyo) [11]. All samples were stored at −80°C until measurement.

**Assessment of endothelial function with Endo-PAT**

We assessed vascular function in all 20 patients by Endo-PAT 2000. The method used for digital assessment of vascular function using PAT has been described in detail previously [12]. Briefly, after 30 min acclimatization period in a room controlled for temperature and light in the fasting state, the baseline pulse amplitude was recorded during a period of 5 min before the induction of ischemia, which was induced by placing a blood pressure cuff on the upper arm, while the opposite arm served as a control. The PAT probes were placed on one finger of each hand. After 5 min, the blood pressure cuff was inflated to 60 mmHg above the systolic pressure or 200 mmHg for 5 min and then deflated to induce reactive hyperemia. As a measure of reactive hyperemia, RHI was calculated as the ratio of the average amplitude of the PAT signal over 1 min beginning 1.5 min after cuff deflation (control arm, A; tested arm, C) divided by the average amplitude of the PAT signal over the 2.5 min time period before cuff inflation (baseline) (control arm, B; tested arm, D). Thus, RHI = (C/D) / (A/B) × baseline correction. Because RHI has a heteroscedastic error structure, we used a natural logarithm transformation in all analyses.

**Oral fat loading test (OFLT)**

The high-fat diet-loading test was conducted in the morning after 12 h overnight fasting. The high-fat diet contained a total of 928 kcal, 58.6 g lipid (57% of the total calories), 68.3 g carbohydrates (30% of the total calories), and 31.1 g protein (13% of the total calories). The ingredients were similar to those of an American fast-food meal (Big Mac® with French fries, Orange-juice®). Patients were asked to eat this high-fat and high-glucose meal (cake sále) within 20 min. Blood samples were collected during the fasting state and 1, 2, 3 and 4 h after the load. In all patients, the OFLT was performed under stable conditions.

**Statistical analysis**

Data were expressed as mean ± standard deviation (SD). Normality was determined by the Shapiro-Wilk test. Values of TG, FFA, RLP-C, sd LDL-C, PG, apoB-48 showed skewed distribution. For one-sample comparison, the paired t-test was used for parameters with normal distribution, whereas the Wilcoxon test was used for parameters with skewed distribution. The
two sample t-test was used for normally distributed data and Mann-Whitney U test was used for data with skewed distribution. Regarding time-course changes in each metabolic marker in Study 1, preprandial and postprandial values were compared by the Bonferroni t-test. With regard to univariate analysis, we used Pearson correlation for normally distributed data and Spearman rank correlation for variables with skewed distribution. Multivariate stepwise regression analysis was conducted using RHI as the dependent variable and several parameters found to be significantly related to RHI on univariate analysis. We calculated AUC by using trapezoidal method. The level of significance was set as P<0.05. All Statistical analyses were conducted using The Statistical Package for Social association version 21.0 (SPSS Inc., Chicago, IL).

Results

Clinical characteristics

The demographic details are shown in Table 1. Of the 20 participants, 10 were males and 10 were females. The mean age of participants was 54.4±11.3 years. Participants were mildly obese, with mean BMI of 24.8±5.2 kg/m². Blood glucose was generally poorly controlled on admission, with mean HbA1c levels at 10.3±1.4%. In addition, participants were insulin resistant on average, with a mean HOMA-IR of 2.4±1.7 and u-CPR level of 72.0±47.0 μg/day. The LDL-C level was 114.4±38.9 mg/dL, the HDL-C level was 44.0±12.7 mg/dL, and the TG level was 166.4±97.8 mg/dL, showing hypertriglyceridemia. TG level was ≥150 mg/dL in 10 (50%) of the 20 subjects. Table 2 shows the demographic details of patients of the ezetimibe and rosuvastatin groups in Study 2. There was no significant difference in all parameters between the two groups.

Table 1 Baseline characteristics of all patients, ezetimibe group and rosuvastatin group

<table>
<thead>
<tr>
<th></th>
<th>All patients</th>
<th>Ezetimibe group</th>
<th>Rosuvastatin group</th>
<th>p value</th>
</tr>
</thead>
<tbody>
<tr>
<td>Sex (male: female)</td>
<td>(10:10)</td>
<td>(5:5)</td>
<td>(5:5)</td>
<td>&gt;0.999</td>
</tr>
<tr>
<td>Age, years</td>
<td>54.4±11.1</td>
<td>51.2±10.9</td>
<td>57.5±11.4</td>
<td>0.272</td>
</tr>
<tr>
<td>Body weight, kg</td>
<td>64.8±14.2</td>
<td>69.8±14.1</td>
<td>59.9±13.3</td>
<td>0.096</td>
</tr>
<tr>
<td>Body mass index, kg/m²</td>
<td>24.8±5.2</td>
<td>26.6±6.6</td>
<td>23.1±2.7</td>
<td>0.162</td>
</tr>
<tr>
<td>Duration of diabetes, years</td>
<td>6.8±7.9 (1-23)</td>
<td>6.7±8.8</td>
<td>6.8±7.3</td>
<td>0.639</td>
</tr>
<tr>
<td>Diabetic neuropathy, n (%)</td>
<td>15 (75%)</td>
<td>6 (60%)</td>
<td>9 (90%)</td>
<td>0.121</td>
</tr>
<tr>
<td>Diabetic retinopathy, n (%)</td>
<td>6 (30%)</td>
<td>2 (20%)</td>
<td>4 (40%)</td>
<td>0.329</td>
</tr>
<tr>
<td>Diabetic nephropathy, n (%)</td>
<td>5 (25%)</td>
<td>2 (20%)</td>
<td>3 (30%)</td>
<td>0.606</td>
</tr>
<tr>
<td>HbA1c, %</td>
<td>10.3±1.4 (8.4-12.8)</td>
<td>10.4±1.6</td>
<td>10.1±1.1</td>
<td>0.791</td>
</tr>
<tr>
<td>Fasting plasma glucose, mg/dL</td>
<td>171±47.9 (117-269)</td>
<td>186±53.9</td>
<td>156±38.2</td>
<td>0.150</td>
</tr>
<tr>
<td>Insulin, μU/mL</td>
<td>6.4±3.9 (2.3-16.9)</td>
<td>5.9±3.6</td>
<td>4.8±2.4</td>
<td>0.496</td>
</tr>
<tr>
<td>HOMA-IR</td>
<td>2.4±1.7 (0.5-6.8)</td>
<td>2.9±2.1</td>
<td>1.9±1.2</td>
<td>0.364</td>
</tr>
<tr>
<td>u-C peptide, μg/day</td>
<td>72.0±47.0 (11.5-214.0)</td>
<td>61.7±27.2</td>
<td>83.5±62.2</td>
<td>0.567</td>
</tr>
<tr>
<td>TG, mg/dL</td>
<td>166±98 (67.0-423.0)</td>
<td>182±134</td>
<td>151±39.7</td>
<td>0.650</td>
</tr>
<tr>
<td>LDL-C, mg/dL</td>
<td>114±38.9 (61.0-218.0)</td>
<td>116±52</td>
<td>113±21.9</td>
<td>0.650</td>
</tr>
<tr>
<td>HDL-C, mg/dL</td>
<td>44.0±12.7 (21.0-76.0)</td>
<td>44.9±15.2</td>
<td>43.1±10.4</td>
<td>0.623</td>
</tr>
<tr>
<td>RLP-C, mg/dL</td>
<td>5.9±4.1</td>
<td>7.3±5.3</td>
<td>4.6±1.5</td>
<td>0.677</td>
</tr>
<tr>
<td>apoB-48, μg/mL</td>
<td>2.73±2.72</td>
<td>1.64±1.90</td>
<td>3.82±3.06</td>
<td>0.140</td>
</tr>
</tbody>
</table>

Data are mean±SD (range: minimum-maximum) or n (%). P values indicate the difference between the ezetimibe and rosuvastatin groups, by two-sample t-test for normally distributed data and by Mann-Whitney U test for data with skewed distribution. Diabetic neuropathy, retinopathy and nephropathy were valued by chi-square test. HbA1c, hemoglobin A1c; HOMA-IR, homeostasis model assessment insulin resistance; TG, triglyceride; LDL, low-density lipoprotein; HDL, high-density lipoprotein; RLP-C, Remnant Like Particles Cholesterol; apoB, apolipoprotein B.

High fat load test

Study 1

As shown in Fig. 1, high-fat diet loading resulted in increased serum levels of TG, RLP-C, FFA, PG,
Table 2  Area under the curve (AUC) of various parameters of lipid and glucose metabolism, change in AUC for ezetimibe and rosuvastatin groups

<table>
<thead>
<tr>
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<th>Ezetimibe group</th>
<th>Rosuvastatin group</th>
<th>p value</th>
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<tbody>
<tr>
<td></td>
<td>Before</td>
<td>After</td>
<td>Change in AUC</td>
</tr>
<tr>
<td>TG AUC (mg/dL·h)</td>
<td>1011 ± 670</td>
<td>497 ± 273</td>
<td>-514 ± 464</td>
</tr>
<tr>
<td>LDL-C AUC (mg/dL·h)</td>
<td>443 ± 194</td>
<td>334 ± 108</td>
<td>-109 ± 109</td>
</tr>
<tr>
<td>HDL-C AUC (mg/dL·h)</td>
<td>166 ± 56</td>
<td>155 ± 36</td>
<td>-11.7 ± 26.6</td>
</tr>
<tr>
<td>MDA LDL-C AUC (mg/dL·h)</td>
<td>508 ± 151</td>
<td>395 ± 165</td>
<td>-113 ± 98</td>
</tr>
<tr>
<td>sd LDL-C AUC (mg/dL·h)</td>
<td>150 ± 76</td>
<td>80.3 ± 28.5</td>
<td>-69.5 ± 61.1</td>
</tr>
<tr>
<td>FFA AUC (μEq/L·h)</td>
<td>4665 ± 4265</td>
<td>3325 ± 2404</td>
<td>-1340 ± 3018</td>
</tr>
<tr>
<td>RLP-C AUC (mg/dL·h)</td>
<td>45.7 ± 28.5</td>
<td>18.2 ± 7.7</td>
<td>-27.5 ± 25.7</td>
</tr>
<tr>
<td>apoB-48 AUC (μg/mL·h)</td>
<td>18.9 ± 16.9</td>
<td>11.1 ± 10.6</td>
<td>-7.8 ± 16.3</td>
</tr>
<tr>
<td>PG AUC (mg/dL·h)</td>
<td>1012 ± 263</td>
<td>649 ± 110</td>
<td>-363 ± 242</td>
</tr>
</tbody>
</table>

Data are mean±SD. P values represent the difference between the ezetimibe and rosuvastatin groups, by two-sample t-test for normally distributed data and by Mann-Whitney U test for data with skewed distribution. AUC was calculated by the trapezoidal method. Abbreviations as in Table 1, MDA LDL-C, malondialdehyde modified low-density lipoprotein; sd LDL-C, small dense low-density lipoprotein; FFA, free fatty acid; PG, plasma glucose.

Fig. 1  Serum levels of various metabolic parameters measured before high-fat load and 4 h after the load. (A) TG, PG, (B) LDL-C, HDL-C, MDA LDL-C, sd LDL-C, (C) FFA, RLP-C, apoB-48. P values indicate the difference between fasting stage and each time point, by Bonferroni t-test. * p<0.05, ** p<0.01, *** p<0.001, vs. 0 h.
and apoB-48 and reduced the levels of LDL-C, HDL-C, sdLDL-C, and MDA LDL-C. Fig. 2 shows the effects of high-fat diet loading on changes in vascular endothelial function. The RHI value was 1.86 ± 0.40 before high-fat diet loading, but decreased significantly to 1.62 ± 0.29 4 h after loading (p=0.028). The AUC of each metabolic marker and the coefficient of variation (CV) were examined in relation to changes in RHI. Assessment of apoB-48, AUC of each metabolic marker, and the CV value showed that fasting apoB-48 correlated positively with TG AUC (r=0.468, p=0.037) and TG CV (r=0.446, p=0.049). Moreover, apoB-48 AUC correlated with TG AUC (r=0.575, p=0.008), TG CV (r=0.482, p=0.031), and RLP-C CV (r=0.482, p=0.031), but not with other parameters (data not shown).

**Study 2**

As shown in Fig. 3, RHI tended to decrease in both the ezetimibe and rosuvastatin groups before drug administration, similar to Study 1 (ezetimibe group, 1.89 to 1.62, p=0.117, rosuvastatin group, 1.83 to 1.58, p=0.157, respectively) (Fig. 3A, B). However, after 1 week of treatment, RHI still showed a decreasing trend in the rosuvastatin group (1.87 to 1.54, p=0.050) (Fig. 3B), whereas it increased in the ezetimibe group (1.70 to 2.03, p=0.073) (Fig. 3A). With regard to changes in RHI based on the administered drug, ezetimibe resulted in a significant improvement in RHI compared with rosuvastatin (p=0.014) (Fig. 3C). On the other hand, there were no significant correlations between changes in RHI and changes in AUC (a marker of AUC improvement), and between changes in RHI and

![Fig. 2](image1.png)

**Fig. 2** Differences in changes in RHI between before high-fat load and 4 h after high-fat load. *P* values indicate the difference between before load and 4 h after load, by the paired *t*-test.

![Fig. 3](image2.png)

**Fig. 3** Differences in changes in RHI between before high-fat load and 4 h after high-fat load. (A) the ezetimibe group (B) the rosuvastatin group. *P* values indicate the difference between before load and 4 h after load, by paired *t*-test. (C) Comparison of changes in ΔRHI in the ezetimibe group and rosuvastatin group. *P* values indicate the difference between the two groups, by the paired *t*-test.
changes in CV (a marker of CV improvement). Table 2 shows changes in AUC according to the administered drug. The effects of the two drugs on changes in AUC were similar irrespective of the parameters examined. When age, sex, drug, hemoglobin A1c, and changes in each lipid parameter were examined as independent variables with improvement in RHI as the dependent variable, drug difference was found to have the largest influence on improvement in RHI in all subjects, using the stepwise procedure (adjusted multiple $R^2=0.431$, standardized coefficient $\beta=-0.542, p=0.006$).

### Discussion

In Study 1, high-fat diet loading reduced vascular endothelial function in patients with type 2 diabetes, suggesting that the existence of postprandial hyperlipidemia may facilitate the progression of endothelial dysfunction. Several clinical studies have previously investigated the lipid profile and the effects of dietary load on vascular endothelial function. In healthy adult volunteers, a cookie load (75 g carbohydrate, 28.5 g fat and 8 g protein, total 592 kcal (SARAYA Corp, Osaka, Japan) decreased FMD in a manner that correlated significantly with variations in TG and apolipoprotein B-48 (apoB-48) [9]. On the other hand, we showed in patients with type 2 diabetes that the diet loading test (using Test meal A: total 450 kcal; carbohydrate 51.4%, fat 33.3%, protein 15.3%, a recipe proposed by a working group of the Japan Diabetes Society) resulted in a significant decrease in vascular endothelial function, which neither correlated with plasma glucose (PG) AUC ($r=-0.475, p=0.074$) nor with immunoreactive insulin (IRI) AUC ($r=0.093, p=0.742$), but with TG AUC ($r=-0.780, p=0.001$) [13]. Although the direct relation between vascular endothelial function and abnormalities of lipid metabolism was not investigated in this study, the results demonstrated that increased remnants due to fat loading correlated with decreased vascular endothelial function. Other factors (in addition to remnants) that aggravate vascular endothelial dysfunction, such oxysterols (oxidized derivatives of cholesterol) and oxidative stress, were not investigated in this study, and could be involved. Because the increase of remnants in postprandial hyperlipidemia causes inflammatory reactions through oxysterol and oxidative stress [14, 15], these reactions may also have a synergistic effect on aggravating vascular endothelial reaction. In previous studies in healthy subjects, measurements up to 8 h after cookie loading (a total of 592 kcal with 28.5 g fat constituting 43.3% of the total calories) showed that FMD was lowest at 4 h, and changes in this parameter had a significant negative correlation with the maximum values of TG and apoB-48 (both TG and apoB-48 reached maximum values at 4 h) [9]. In the present study, the proportion of lipid was very high at 57%, and measurement was carried out only up to 4 h after loading. Therefore, the correlation between other metabolic markers and the observed vascular endothelial dysfunction could have been observed had the loading test been prolonged to determine the peak TG at a time point longer than 4 h after loading.

In this study, the target of measurement was apoB-48, a major constituent apoprotein of intestine-derived, exogenous lipoproteins (chylomicrons and chylomicron remnants). Because only one molecule of apoB-48 is present in each lipoprotein particle, it can be a quantitative marker that represents chylomicron and chylomicron remnants. Its blood level in the fasting state is useful for screening postprandial hyperlipidemia [16]. According to the report by Tanimura et al. [17], high fasting apoB-48 levels are present in diabetic patients with carotid artery plaques; TG incremental AUC and fasting apoB-48 correlated significantly after high-fat diet-loading in 10 healthy men. The present study investigated the correlation between apoB-48 level and vascular endothelial function, but no direct correlation was found between changes in RHI (which reflect vascular endothelial function) and apoB-48 AUC. However, fasting apoB-48 levels correlated with TG AUC and TG CV and apoB-48 AUC correlated with TG AUC, TG CV, and RLP-C CV. Thus, apoB-48 levels correlated with apoB-48 AUC under the condition of postprandial hyperlipidemia. These findings are consistent with those of previous studies [16, 17], suggesting the usefulness of apoB-48 as an index of postprandial hyperlipidemia.

The results of Study 2 suggested that ezetimibe can improve RHI after high-fat loading to a significantly greater extent than rosvastatin. However, analysis of parameters that could affect the improvement of RHI in the ezetimibe group showed no significant correlation with improvement in blood glucose levels, AUC of lipid metabolic markers or CV values. In a long-term study that compared the effects of statins and ezetimibe on vascular endothelial function in CHF patients, 10 mg/day simvastatin and 10 mg/day ezetimibe had similar effects on LDL-C, but the improvement in FDD
The results showed that the combination therapy of ezetimibe and statin on cardiovascular events examined the inhibitory effects of the combination therapy of its lipid-improving action. Improved vascular endothelial function, independent of RHI, suggesting that ezetimibe directly inhibit aggravation of vascular endothelial function in diabetic patients with lipid abnormalities. The present study has several limitations. First, the study was an open-label study with a small sample size; therefore, selection bias might have been involved. Further investigation in a large number of patients will be required. Second, the study did not examine the direct effects of ezetimibe on vascular endothelial function, including inflammatory cytokines, adhesion factors, and oxidative stress mediators. Interrelated factors for improvement of postprandial vascular endothelial function in this study were not identified, and any direct effect of ezetimibe on vascular endothelial function that was not investigated in this study cannot be ruled out. Ezetimibe is reported to inhibit disorders of vascular endothelial function by suppressing oxidative stress and inflammation. The results of this pilot study in a small number of type 2 diabetic patients suggest that 1-week treatment with ezetimibe improves vascular endothelial function-based RHI. Further long-term studies of larger sample size are necessary to determine the cellular and molecular mechanisms of such improvement, especially the effects of longer treatment with ezetimibe on inflammatory cytokines, adhesion factors, and mediators of oxidative stress.

In summary, study 1 showed that high-fat diet-loading decreased vascular endothelial function in patients with type 2 diabetes, suggesting that it is due to the progression of atherosclerosis, at least in part, derived from abnormalities in postprandial lipid metabolism. Also study 2 showed that ezetimibe can potentially inhibit aggravation of vascular endothelial function after high-fat diet loading.

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