Effects of Pitavastatin on Fasting and Postprandial Endothelial Function and Blood Rheology in Patients With Stable Coronary Artery Disease

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Background: Because postprandial hypertriglyceridermia and hyperglycemia may promote atherosclerosis, the present study investigated the effects of a clinical dose of pitavastatin on endothelial function and blood rheology in patients with coronary artery disease (CAD) before and after eating a test meal.

Methods and Results: The 16 patients with stable CAD and mild dyslipidemia and 6 age-matched healthy men as controls were recruited. In each group, forearm blood flow (FBF) was measured during postischemic reactive hyperemia and blood samples were taken before and 2h after the test meal. Urinary 8-hydroxy-2'-deoxyguanosine (8-OHdG) was also measured. The patients were started on pitavastatin 2mg/day. The tests were repeated after 6 months. Maximum FBF during hyperemia in the baseline fasting phase was significantly lower in CAD patients than in control subjects (P=0.040). Fasting and postprandial FBF during reactive hyperemia significantly improved after pitavastatin treatment (P<0.05 vs baseline data for each phase) associated with reduced urine 8-OHdG, increased plasma adiponectin and improved lipid profile. No significant differences in baseline rheological parameters remained unchanged. (Circ J 2009; 73: 1523–1530)

Key Words: Angina pectoris; Endothelial function; Oxidative stress; Postprandial dyslipidemia; Rheology

Acumulating evidence shows that postprandial metabolic changes play an important role in the development of atherosclerotic cardiovascular disease! Two recent cohort studies have reported that postprandial hypertriglyceridermia is associated with increased risk of coronary vascular events! In addition, postprandial hyperglycemia in patients with diabetes mellitus (DM) or impaired glucose tolerance has been identified as an independent risk factor for cardiovascular disease4 and a stronger predictor of cardiovascular mortality than fasting plasma glucose5. High-calorie cooked meals, rich in processed carbohydrates and saturated fat, can lead to both hyperglycemia and hypertriglyceridermia with elevated serum levels of triglyceride (TG)-rich remnant lipoproteins6. The resultant endothelial and hemorheological dysfunction may be closely related to transiently enhanced postprandial oxidative stress, one of the relevant therapeutic targets for statins7.8 Statins reduce cardiovascular events in dyslipidemic patients for both primary9,10 and secondary prevention11 of coronary arterial events by lowering of lipid levels, including the oxidized low-density lipoprotein-cholesterol (LDL-C) involved in atherosclerosis, and via pleiotropic effects, because mevalonate is a precursor of various nonsteroidal isoprenoid products as well as cholesterol. Effects of strong statins on postprandial endothelial function, blood rheology and adiponectin in patients with dyslipidemia or DM have been reported12–16 but effects in patients with coronary artery disease (CAD) are not fully understood17. Such information might be valuable in constructing better therapeutic strategies for patients with CAD.

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The present study investigated effects of a clinical dose of pitavastatin, a strong hydrophobic statin providing marked improvements in lipids (including increases in high-density lipoprotein-cholesterol (HDL-C)), postprandial lipids, endothelial function, blood rheology, and oxidative stress in Japanese patients with CAD.

Methods

Study Subjects

Entry criteria for CAD patients were age between 40 and 75 years and evidence of significant coronary artery stenosis (>70%) by coronary arteriography within the previous 5 years at Saitama Medical Center of Jichi Medical University. Exclusion criteria included unstable angina; previous
The protocol was approved by the Ethics Committee of Study Protocol
period of 6 months.

advised to maintain their usual lifestyle during the study
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lipid profile and plasma glucose concentrations. Written
examination and standard laboratory tests, including serum
medications and showed normal results on routine physical
subjects had normal blood pressure, were not taking any
percutaneous coronary intervention using stents. All control
had a history of myocardial infarction and 7 had a history of
were taking angiotensin II receptor blockers, 2 were taking
acteristics are shown in
we recruited 16 Japanese male patients with CAD and 6
them, 13 patients declined to participate in the study. Finally,
3 months; coronary revascularization by
catheter angioplasty within the previous 3 months; chronic
had a history of coronary artery bypass surgery; left ventricular
ejection fraction <50%; renal dysfunction defined as serum
creatinine >1.3 mg/dl; current smoker; cerebrovascular event
within the previous 3 months; coronary revascularization by
catheter angioplasty within the previous 3 months; chronic
heart failure; DM uncontrolled by diet with hemoglobin (Hb)
A\textsubscript{1c} \geq 7.5%; LDL-C \geq 180 mg/dl or <100 mg/dl, and taking
lipid-lowering agents, angiotensin-converting enzyme
inhibitors, nitrates or blood sugar-lowering agents includ
insulin or supplemental vitamins. A total of 29 patients
were selected as candidates according to these criteria. Of
them, 13 patients declined to participate in the study. Finally,
we recruited 16 Japanese male patients with CAD and 6
age-matched healthy male volunteers (controls); their char
acteristics are shown in Table 1. All 16 CAD patients were
taking aspirin, 8 were taking calcium-channel blockers, 7
were taking angiotensin II receptor blockers, 2 were taking
\beta-blockers, and 4 were taking ticlopidine. Four patients
had a history of myocardial infarction and 7 had a history of
percutaneous coronary intervention using stents. All control
subjects had normal blood pressure, were not taking any
medications and showed normal results on routine physical
examination and standard laboratory tests, including serum
lipid profile and plasma glucose concentrations. Written
informed consent was given by all subjects before examination.
Medications for CAD patients other than pitavastatin
were not altered during the study period. All subjects were
advised to maintain their usual lifestyle during the study
period of 6 months.

Study Protocol
The protocol was approved by the Ethics Committee of
Jichi Medical University. This was a prospective observational study in a single institution comparing pitavastatin
intervention in CAD patients with normal control subjects. Because of ethical concerns, no CAD patient without statins
was included. Subjects fasted overnight, abstained from beverages containing alcohol or caffeine and did not take
any medications for at least 12h before laboratory measure
ments. After subjects arrived at the laboratory room at
08.30h, fasting blood samples were taken for assessment of
blood rheology and the following: HbA\textsubscript{1c}; serum total cholesterol (TC); TG; LDL-C; HDL-C; remnant-like particle cholesterol (RLP-C); plasma glucose, insulin and high-sensitivity C-reactive protein (hsCRP); and plasma adiponectin. Urine samples were also obtained for measurement of 8-hydroxy-2’-deoxyguanosine (8-OHdG) levels. Forearm blood flow (FBF) following reactive hyperemia was then measured using strain-gauge plethysmography on the right
toearm to assess endothelial function. All subjects then ate
within 30min a cooked test meal (a modified standard test meal as proposed by a working group of the Japan Diabetes Society) comprising buttered and honeyed toast, boiled
egg, yogurt and vegetable soup (680kcal: carbohydrates
60g; protein 20g; fat 40g (unsaturated fatty acids, 13g))

All measurements, except HbA\textsubscript{1c} and adiponectin, were
repeated 2h after taking the test meal. After measurements
at baseline, all patients with CAD started their intake of
pitavastatin (Kowa, Tokyo, Japan) of 2 mg daily. The tests
were repeated after 6 months in each group with the excep
tion of urinary sampling for control subjects.

Blood and Urine Sampling and Assay
Serum levels of TC, TG, LDL-C, HDL-C and plasma
glucose were measured using enzymatic methods. RLP-C
was measured with 0.2-ml serum samples using an enzyme-
linked immunosorbent assay kit (JIMRO; Otsuka Pharma
ceutical, Osaka, Japan). HbA\textsubscript{1c} was measured by high-

<table>
<thead>
<tr>
<th>Table 1. Clinical Characteristics of Subjects</th>
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<tbody>
<tr>
<td>Control (n=6)</td>
</tr>
<tr>
<td>Age (years)</td>
</tr>
<tr>
<td>Hypertension (%)</td>
</tr>
<tr>
<td>Hyperlipidemia (%)</td>
</tr>
<tr>
<td>Diabetes mellitus (%)</td>
</tr>
<tr>
<td>Ex-smoker (%)</td>
</tr>
<tr>
<td>BMI</td>
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</table>

<table>
<thead>
<tr>
<th>Fasting blood test</th>
<th>Control</th>
<th>CAD</th>
<th>P value</th>
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</thead>
<tbody>
<tr>
<td>TC (mg/dl)</td>
<td>176.8±18.8</td>
<td>218.2±34.3</td>
<td>0.012</td>
</tr>
<tr>
<td>HDL-C (mg/dl)</td>
<td>97.7±21.2</td>
<td>139.9±26.3</td>
<td>&lt;0.001</td>
</tr>
<tr>
<td>LDL-C (mg/dl)</td>
<td>55.0±6.5</td>
<td>40.6±7.5</td>
<td>&lt;0.001</td>
</tr>
<tr>
<td>Triglycerides (mg/dl)</td>
<td>1.82±0.53</td>
<td>3.53±0.81</td>
<td>&lt;0.001</td>
</tr>
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<td>RLP-C (mg/dl)</td>
<td>81.7±38.9</td>
<td>160.6±45.9</td>
<td>0.001</td>
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<td>FFA (mmol/L)</td>
<td>3.1±0.76</td>
<td>5.7±2.30</td>
<td>0.013</td>
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<td>Adiponectin (ug/ml)</td>
<td>0.46±0.11</td>
<td>0.47±0.13</td>
<td>0.880</td>
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<td>IRI (\mu U/ml)</td>
<td>10.5±1.65</td>
<td>6.19±2.76</td>
<td>&lt;0.001</td>
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<td>hsCRP (mg/L)</td>
<td>1.00±0.80</td>
<td>1.80±1.70</td>
<td>0.286</td>
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<td>HbA\textsubscript{1c} (%)</td>
<td>5.0±0.10</td>
<td>6.3±0.12</td>
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<td>Plasma glucose (mg/dl)</td>
<td>102.2±8.2</td>
<td>120.4±26.7</td>
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<tr>
<td>IRI (\mu U/ml)</td>
<td>6.6±2.6</td>
<td>8.6±3.9</td>
<td>0.253</td>
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<td>Hematocrit</td>
<td>41.5±2.3</td>
<td>44.1±4.3</td>
<td>0.180</td>
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<td>Blood passage time (s)</td>
<td>44.7±8.0</td>
<td>46.8±7.9</td>
<td>0.595</td>
</tr>
<tr>
<td>Basal FBF</td>
<td>5.1±0.45</td>
<td>6.0±1.4</td>
<td>0.141</td>
</tr>
<tr>
<td>Max. FBF during RH</td>
<td>36.7±4.6</td>
<td>28.8±8.2</td>
<td>0.040</td>
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<tr>
<td>FBF after NTG s.l.</td>
<td>7.7±1.5</td>
<td>6.8±1.8</td>
<td>0.096</td>
</tr>
</tbody>
</table>

Values are mean±SD or the percentage of participants in that category.

CAD, coronary artery disease; BMI, body mass index; TC, total cholesterol; LDL-C, low-density lipoprotein-cholesterol; HDL-C, high-density lipoprotein-cholesterol; RLP-C, remnant-like particle cholesterol; hsCRP, high-sensitivity C-reactive protein; Hb, hemoglobin; IRI, insulin; FBF, forearm blood flow; Max., maximum; RH, reactive hyperemia; NTG, nitroglycerine; s.l., sublingual.

history of coronary artery bypass surgery; left ventricular
ejection fraction <50%; renal dysfunction defined as serum
creatinine >1.3 mg/dl; current smoker; cerebrovascular event
within the previous 3 months; coronary revascularization by
catheter angioplasty within the previous 3 months; chronic
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were not altered during the study period. All subjects were
advised to maintain their usual lifestyle during the study
period of 6 months.
performance liquid chromatography, serum insulin levels by enzyme immunoassay, and serum hsCRP by a monoclonal antibody-based latex agglutination test that allows measurement of samples with low (0.01 mg/dl) to high (42 mg/dl) concentrations (Nanopia CRP; Daichi Pure Chemical, Tokyo, Japan). Plasma adiponectin levels were measured using an enzyme-linked immunosorbent assay kit (Otsuka Pharmaceutical, Osaka, Japan). Oxidative damage to DNA and its precursors were measured by determining 8-OHdG levels in urine. Urine samples were stored in polypropylene tubes at –80°C. Urinary 8-OHdG levels were measured using a competitive enzyme-linked immunosorbent assay kit (New 8-OHdG Check; Japan Institute for the Control of Aging, Nikken SEIL, Fukuroi, Japan). Levels of urinary 8-OHdG were expressed as a ratio corrected by urinary creatinine levels.

**Blood Kinetics in Narrow Microchannels Ex Vivo**
Fasting blood samples were carefully taken from the left cubitus vein into a 5-ml syringe containing heparin sodium (50 units/ml blood). The passage time for 0.1 ml of blood through microchannels (8700-parallel, 7-μm equivalent diameter, 20-μm-long channels; Kowa) under a constant suction pressure of –20 cmH₂O was determined using a Microchannel Flow Analyzer (Kowa) as a whole blood rheological parameter. Saline passage time was determined before each blood measurement for calibration.

**Noninvasive Assessment of Endothelial Function**
Endothelial function was assessed by FBF in response to reactive hyperemia using strain-gauge plethysmography as described elsewhere. In brief, patients remained seated in a quiet, air-conditioned room (constant temperature, 23–24°C) throughout the procedure. FBF measurements were deliberately made contralateral to the arm from which blood had been drawn. A strain-gauge was attached to upper part of the forearm and connected to a plethysmography device (EC-5R; D.E. Hokanson Issaquah, WA, USA), and supported at the height of the right atrium. A wrist cuff was inflated to 50 mmHg above systolic blood pressure 1 min before first measurement of basal flow and throughout the measurement of FBF to exclude hand circulation from the measurement. The upper arm cuff was inflated to 40 mmHg for 7 s in each 15-s cycle to occlude venous outflow from the arm using a rapid cuff inflator (EC-20; D.E. Hokanson).

The blood flow output signal was transmitted to a recorder for further processing (U-228; Advance, Nagoya, Japan). FBF was expressed as milliliters per minute per 100 ml of forearm tissue volume.

**Statistical Analysis**
Data were statistically analyzed using JMP 7.01 J software (SAS Institute, Cary, NC, USA). Continuous variables are described as mean±standard deviation (SD), and where appropriate, Student’s t-test or 2-way analysis of variance followed by the Tukey-Kramer post-hoc test were used. Categorical variables are described as frequencies and were compared using Fisher’s exact test. Probability values of P<0.05 were considered to indicate statistical significance.

**Results**
All subjects completed the study protocol without any cardiovascular events. Pitavastatin was continued until the end of the study in all subjects and no adverse effects occurred in the CAD patients. In 1 patient, β-blocker administration was stopped because of sinus bradycardia. In all other patients, medications were unaltered during the study period. At baseline, serum levels of TC, LDL-C, LDL-C/HDLC ratio, and HDL-C were normal. Plasma glucose levels were 120.4±26.7 mg/dl, total cholesterol 218.2±24.1 mg/dl, triglyceride 104.6±23.1 mg/dl, and hemoglobin A1c 5.5±0.7% in all patients. On the other hand, levels of HDL-C and HDL-C/TC were lower in patients with CAD than in controls (HDL-C: 40.6±9.4 vs 50.1±6.7, P<0.01, HDL-C/TC: 0.18±0.01 vs 0.21±0.01, P<0.05).

In the postprandial phase, plasma glucose levels increased to 132.6±48.8 mg/dl, and the HDL-C/TC ratio decreased to 0.16±0.01. The baseline FBF was 4.1±1.3 ml/min·100 ml tissue⁻¹, with the peak FBF after reactive hyperemia measured for 3 min after cuff release. Only in the postprandial phase was the FBF at baseline significantly lower than the preprandial phase (4.1±1.3 vs 6.0±1.2 ml/min·100 ml tissue⁻¹, P<0.05). The peak FBF after reactive hyperemia was 22.9±9.5 ml/min·100 ml tissue⁻¹, significantly higher than the baseline FBF (P<0.01). The hyperemic response was maintained over 20 min (4.1±1.3 vs 6.0±1.4 ml/min·100 ml tissue⁻¹).

**Table 2. Serial Changes in Laboratory Data in Patients With CAD (n=16)**

<table>
<thead>
<tr>
<th>Parameter</th>
<th>Baseline</th>
<th>Preprandial</th>
<th>Postprandial</th>
<th>Baseline</th>
<th>Preprandial</th>
<th>Postprandial</th>
</tr>
</thead>
<tbody>
<tr>
<td>BMI (kg/m²)</td>
<td>23.5±3.3</td>
<td>23.4±3.4</td>
<td>23.6±3.5</td>
<td>23.2±3.1</td>
<td>23.1±3.2</td>
<td>23.0±3.3</td>
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<tr>
<td>TC (mg/dl)</td>
<td>218.2±34.3</td>
<td>209.4±33.0</td>
<td>209.3±32.4</td>
<td>215.5±35.7</td>
<td>214.5±35.5</td>
<td>213.5±35.3</td>
</tr>
<tr>
<td>HDL-C (mg/dl)</td>
<td>40.6±7.5</td>
<td>38.5±6.8</td>
<td>38.4±6.5</td>
<td>39.8±7.9</td>
<td>39.6±7.8</td>
<td>39.5±7.6</td>
</tr>
<tr>
<td>LDL-C (mg/dl)</td>
<td>139.2±263</td>
<td>132.9±24.4</td>
<td>132.6±24.1</td>
<td>140.2±18.7</td>
<td>140.1±18.5</td>
<td>139.9±18.4</td>
</tr>
<tr>
<td>LDL-C/HDL-C</td>
<td>3.54±10.8</td>
<td>3.54±10.8</td>
<td>3.54±10.8</td>
<td>3.54±10.8</td>
<td>3.54±10.8</td>
<td>3.54±10.8</td>
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<tr>
<td>TG (mg/dl)</td>
<td>160.6±45.9</td>
<td>231.3±59.9</td>
<td>231.2±59.9</td>
<td>113.8±48.4</td>
<td>170.5±61.3</td>
<td>170.5±61.3</td>
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<tr>
<td>RLP-C (mg/dl)</td>
<td>5.8±3.2</td>
<td>9.5±3.2</td>
<td>9.5±3.2</td>
<td>3.9±1.7</td>
<td>3.9±1.7</td>
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<tr>
<td>FFA (nmol/L)</td>
<td>0.47±0.13</td>
<td>0.29±0.11</td>
<td>0.29±0.11</td>
<td>0.43±0.20</td>
<td>0.31±0.11</td>
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<tr>
<td>Adiponectin (μg/ml)</td>
<td>6.19±2.76</td>
<td>7.45±3.33</td>
<td>7.45±3.33</td>
<td>12.4±3.1</td>
<td>18.6±4.2</td>
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<tr>
<td>Plasma glucose (mg/dl)</td>
<td>120.4±26.7</td>
<td>191.9±61.6</td>
<td>191.9±61.6</td>
<td>124.6±31.1</td>
<td>186.6±70.0</td>
<td>186.6±70.0</td>
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<tr>
<td>hsCRP (mg/L)</td>
<td>1.80±1.70</td>
<td>1.70±1.60</td>
<td>1.70±1.60</td>
<td>1.50±1.90</td>
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<tr>
<td>HbA1c (%)</td>
<td>6.3±1.2</td>
<td>6.3±1.2</td>
<td>6.3±1.2</td>
<td>6.5±1.2</td>
<td>6.5±1.2</td>
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<tr>
<td>Hematocrit (%)</td>
<td>44.1±4.3</td>
<td>42.8±3.6</td>
<td>42.5±3.6</td>
<td>42.5±3.6</td>
<td>41.8±3.2</td>
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<td>Blood passage time (s)</td>
<td>46.8±7.9</td>
<td>46.9±10.7</td>
<td>46.9±10.7</td>
<td>44.9±8.1</td>
<td>42.5±9.1</td>
<td>42.5±9.1</td>
</tr>
<tr>
<td>Basal FBF</td>
<td>4.1±1.3</td>
<td>5.1±2.2</td>
<td>5.1±2.2</td>
<td>4.6±2.6</td>
<td>6.8±3.2</td>
<td>6.8±3.2</td>
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<tr>
<td>FBF after NTG (ml·min⁻¹·100 ml tissue⁻¹)</td>
<td>6.8±1.8</td>
<td>7.2±3.4</td>
<td>7.2±3.4</td>
<td>6.8±1.8</td>
<td>7.2±3.4</td>
<td>7.2±3.4</td>
</tr>
</tbody>
</table>

*P<0.05 vs preprandial baseline data, **P<0.01 vs preprandial baseline data, †P<0.05 vs postprandial baseline data, ‡P<0.01 vs postprandial baseline data, §P<0.05 vs preprandial data at 6 months.

Abbreviations as in Table 1.
Figure 1. Maximum forearm blood flow (FBF) following postischemic reactive hyperemia in control subjects (a, n=6) and patients with stable angina pectoris (b, n=16) before (white bar) and 2 h after intake of the test meal (black bar). Pre- and postprandial FBF following reactive hyperemia were significantly increased after 6-month pitavastatin treatment in patients with angina pectoris (b). In control subjects, FBF following reactive hyperemia remained unchanged after the period of 6 months. *P<0.05 vs baseline preprandial data; #P<0.05 vs baseline postprandial data. CAD, coronary artery disease.

Figure 2. Changes in urine 8-hydroxy-2'-deoxyguanosine (8-OHdG) as a parameter of oxidative stress in control subjects (a, n=6) at baseline and in patients with stable angina pectoris (b, n=16) before (white bar) and 2 h after intake of the test meal (black bar). In patients with angina pectoris, urinary 8-OHdG levels were significantly increased at postprandial phase compared with fasting data (P<0.01), although those levels remained unchanged in the control subjects. Note that postprandial urine 8-OHdG level was significantly decreased compared with baseline postprandial data in patients with stable angina pectoris (P<0.001).
HDL-C, TG, RLP-C and HbA1c were significantly higher and levels of HDL-C and adiponectin significantly lower in the CAD patients than in healthy controls (Table 1). Serum levels of free fatty acids were not different. Maximum FBF during hyperemia in the baseline fasting phase was significantly lower in CAD patients (28.8±8.2 ml·min⁻¹·100 ml tissue⁻¹) than in control subjects (36.7±4.6 ml·min⁻¹·100 ml tissue⁻¹; P=0.040). No differences were seen at baseline in blood passage time through the microchannels.

At 2 h after taking the test meal, serum levels of TG, insulin and RLP-C were significantly increased from fasting levels in both groups, particularly in the CAD patients, and plasma glucose levels were increased only in CAD patients. Serum levels of free fatty acids remained unchanged at 2 h after the test meal. Basal FBF was slightly but significantly increased after the test meal in the CAD group (Table 2). Maximum FBF during hyperemia (Figure 1) remained unchanged in each group when compared with the fasting data. FBF after NTG administration tended to be decreased in the CAD patients as compared with healthy controls (Table 1). In the CAD patients, urinary 8-OHdG levels were significantly increased in the postprandial phase over fasting levels (P<0.01).

After 6-month pitavastatin treatment, serum levels of TC, LDL-C, LDL-C/HDL-C, TG and RLP-C, both fasting and postprandial, were significantly decreased while HDL-C and adiponectin levels were significantly increased compared with baseline data in the CAD patients (Table 2). The number of CAD patients with LDL-C >100 mg/dl decreased from 16 (100%) to 2 (13%, P<0.001 vs baseline), with TG >150 mg/dl decreased from 8 (100%) to 2 (13%, P<0.05 vs baseline), with HDL-C <40 mg/dl decreased from 1 (63%) to 0 (0%, P<0.001), and with LDL-C/HDL-C >2.0 decreased from 10 (63%) to 2 (13%, P<0.05 vs baseline).

Both fasting and postprandial maximum FBF during hyperemia were significantly increased after 6-month pitavastatin treatment (fasting, from 28.9±8.2 to 36.3±11.5 ml·min⁻¹·100 ml tissue⁻¹; postprandial, from 31.5±9.1 to 39.3±11.5 ml·min⁻¹·100 ml tissue⁻¹; P<0.05 vs baseline each phase data) (Figure 1). Fasting and postprandial maximum FBF during hyperemia were unchanged from baseline at 6 months in the control subjects. FBF after sublingual NTG administration was unchanged in both groups. Postprandial urinary 8-OHdG levels in CAD patients decreased significantly from 16.9±7.6 to 6.7±3.8 ng·ml⁻¹·min⁻¹·100 ml tissue⁻¹ (P<0.001) after 6-month pitavastatin treatment (Figure 2). No significant correlation was seen between changes in reactive hyperemia and those in lipid profile or 8-OHdG in each prandial phase according to single logistic regression analysis (data not shown). Fasting and postprandial blood passage time through the microchannels in both groups showed no significant change after the 6-month period (Tables 2, 3).

**Discussion**

The present study provides the first evidence that in patients with stable CAD, 6-month pitavastatin treatment improved postprandial endothelium-dependent vasodilatation in forearm resistance vessels to the same level as in healthy subjects, and was associated with decreased postprandial oxidative stress, TG, RLP-C and LDL-C levels, and increased adiponectin and HDL-C.

**Fasting and Postprandial Endothelial Function in CAD**

Baseline FBF data in response to reactive hyperemia, suggesting impairment of endothelial cells in the peripheral arteries of the patients with CAD, were consistent with previous reports. In the CAD patient group, 56% had type 2 DM, which might have contributed to the impaired vasodilatory response to reactive hyperemia. In general, insulin upregulates endothelial nitric oxide synthase (eNOS) via the phosphatidylinositol 3-kinase/Akt signaling pathway but this cascade is impaired in patients with DM, which might have contributed to the impaired vasodilatory response to reactive hyperemia. In an animal model of insulin resistance, eNOS activity is reduced because of decreased levels of tetrahydrobiopterin, a coenzyme of eNOS, and both eNOS and NAD(P)H oxidase induce excessive production of reactive oxygen species, leading to endothelial dysfunction.

Despite the postprandial metabolic changes, maximum FBF in response to reactive hyperemia was not significantly changed in either group after intake of the test meal.
especially in the CAD group exposed to oxidative stress. The present data are in line with some previous reports[7,18,25] but not all[5,8,13,14] Discrepancies regarding results for postprandial endothelial function may be explained as follows. First, our test meal represented carbohydrate loading (60 g) plus moderate fat intake and was associated with a high serum level of insulin at 2 h after the meal, leading to eNOS upregulation via the phosphatidylinositol 3-kinase/Akt signaling pathway. The increase in endothelium-derived NO production by eNOS upregulation could thus reach a balance with the increase in oxidative stress in our CAD patients. Second, the increase in basal FBF after meal (from 4.1±1.3 to 5.1±2.2 ml·min⁻¹·100 ml tissue⁻¹) might have somewhat confounded the pure postprandial vasodilatory response to reactive hyperemia.

Effects of Pitavastatin on Lipid Profile, Adiponectin and Oxidative Stress

In the present study, a therapeutic dose of pitavastatin (2 mg/day) decreased serum levels of LDL-C, TG, RLP-C and LDL-C/HDL-C in the fasting phase after 6 months, similar to previous reports[5,15,16] and also decreased postprandial levels of TG and RLP-C in the patients with CAD. RLP-C has been reported as closely associated with impaired endothelium-dependent vasomotor function in human coronary arteries[26,27] and predicts future coronary events in patients with CAD and type 2 DM or insulin resistance[31] Increased plasma adiponectin levels in CAD patients following pitavastatin administration are in line with previous results from patients with dyslipidemia[16] According to our knowledge, the present data may represent the first evidence that pitavastatin increases plasma adiponectin concentrations in patients with CAD. Adiponectin stimulates NO production in vascular endothelial cells[32] and suppresses the attachment of monocytes to endothelial cells[31] Increased adiponectin levels might have contributed to the improved endothelial function observed in the present study. Pravastatin has been reported to increase plasma adiponectin in association with improved glucose metabolism in patients with impaired glucose tolerance and CAD[34] However, effects of atorvastatin on plasma adiponectin in patients with type 2 DM seem to be controversial[35–37] A more recent report by Ando et al showed that both pravastatin and atorvastatin significantly increased plasma adiponectin in obese patients with dyslipidemia, but not in non-obese patients[36] According to recent observational studies, including our own, high adiponectin levels have been observed in several selected populations with heart failure[38–40] or renal dysfunction[41] and in an aged group[39] In those selected populations, accumulation of adiponectin in patients with heart failure or chronic kidney disease, unlike the general population, may reflect the malnutrition that characterizes these disease states and is thus a marker of poor prognosis[39,41] Because we excluded patients with heart failure, serum creatinine >1.3 mg/dl or age >76 years, increased adiponectin levels after pitavastatin treatment may act as a cardioprotective factor, including improvement of endothelial function.

Decreased postprandial urinary 8-OHdG levels after 6-month pitavastatin treatment in the CAD patients is consistent with previous reports[42,43] Membrane translocation of rac1 GTPase, which is required for the activation of NAD(P)H oxidase, is inhibited by statins[44] In addition, statins reduces NAD(P)H subunit p22phox[45] Consequently, pitavastatin might decrease the urinary 8-OHdG levels in CAD patients.

Effects of Pitavastatin Treatment on Fasting and Postprandial Endothelial Function

Fasting and postprandial endothelial function was improved after 6 months of pitavastatin to the same extent as in healthy subjects Matsumoto et al reported that 3-day treatment with pravastatin significantly improved endothelial function as evaluated by acetylcholine-induced increases in FBF measured by plethysmography in patients with CAD[46] However, van Eten et al have reported that intensive lipid lowering with atorvastatin (80 mg/day for 4 weeks) had no effect on NO availability in forearm resistance arteries as evaluated by serotonin-induced increases in FBF measured by plethysmography in patients with type 2 DM[47] which might be associated with smooth muscle dysfunction or refractory endothelial dysfunction in these patients. Sakabe et al showed rapid improvements in fasting endothelial function with pitavastatin in patients with hyperlipidemia compared with atorvastatin, despite similar effects on lipid profiles and fibrinolytic parameters[43]

Factors contributing to improvement in endothelial function following pitavastatin administration could include pleiotropic effects[46] such as anti-oxidation[42,43,45,48] as well as the LDL-C-lowering effect. Because no significant correlation was seen between changes in reactive hyperemia and those in LDL-C or 8-OHdG level in each prandial phase, we could not conclude which factor provides the greater contribution to the improvement of postprandial endothelial function by pitavastatin. Tamai et al have clearly shown that a reduction of total LDL-C and/or oxidized LDL-C by LDL-C apheresis, an alternative LDL-C-lowering treatment, improves endothelium-dependent vasodilatory response in patients with dyslipidemia[29] The inhibition of RhoA/Rho kinase activity[40] and the enhancement of phosphatidylinositol 3-kinase/Akt signaling pathway[51] by statins upregulate endothelial eNOS. The anti-oxidant effect of pitavastatin could improve NO bioavailability[42,43] thus contributing to improvement in endothelial function. Increases in plasma adiponectin[17] and blocking leukocyte function antigen 1[52] may also partly contribute to the improvement in endothelial function by pitavastatin.

Blood Rheology

The present data showed no difference in blood passage time between control subjects and CAD patients at baseline. Blood passage time also showed no significant changes after test meal intake in both groups. Pitavastatin treatment thus did not affect blood passage time in patients with CAD, despite several reports showing beneficial effects of statins on blood rheology[53–55] Two explanations are possible for the absence of abnormal blood rheology in the present CAD patients. First, all CAD patients took aspirin before entry and continued its administration throughout the study period. As the antiplatelet and anti-inflammatory effects of aspirin reportedly improve prognosis for patients with CAD, we did not feel that withdrawal of aspirin in CAD patients was ethical. Second, the present study did not include patients with poorly controlled DM or severe dyslipidemia (LDL-C ≥180 mg/dl), for whom hemorheological parameters are reportedly worse and who thus might be better candidates for improvement with pitavastatin[53–55]

Study Limitations

First, a randomized controlled trial in patients with CAD was not performed because of ethical concerns. Second, standard deviations for measurements were somewhat large,
because the sample size was relatively small. Third, discontinuation of aspirin would have been preferable for exploring the effects of pitavastatin on blood rheology, but as administration of aspirin has gained worldwide consensus for patients with CAD in terms of plaque stability, secondary prevention of cardiac events and improvements in prognosis, use of aspirin was continued in all patients with CAD.

In conclusion, administration of a clinical dose of pitavastatin appears effective for both fasting and postprandial endothelial dysfunction, and is associated with improved lipid profile, reduction in oxidative stress and increased adiponectin in patients with stable CAD.

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Disclosure
None.

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