Original Article

Effect of Cerivastatin on Endothelial Dysfunction and Aortic CD36 Expression in Diabetic Hyperlipidemic Rats

Tetsuya NAKAMURA, Yuichiro SAITO*, Yoshio OHYAMA*, Tsuyoshi UCHIYAMA*, Hiroyuki SUMINO*, and Masahiko KURABAYASHI*

A mutation of the CD36 gene that encodes a fatty acid transporter has been reported to play a role in insulin resistance in spontaneously hypertensive rat (SHR). Statins reduce circulating cholesterol and triglyceride concentrations. The objective of this study was to determine the role of CD36 and the significance of statin therapy in insulin-resistance syndromes. We determined the isometric relaxation induced by acetylcholine or lecithinized superoxide dismutase (SOD) in aortas obtained from Otsuka Long Evans Tokushima Fatty (OLETF) rats, a model of insulin resistance and dyslipidemia, and normal control (Long Evans Tokushima Otsuka; LETO) rats with or without cerivastatin treatment. We also determined the effect of cerivastatin on aortic expression of CD36 and PPARγ. The CD36 genotype and microsatellite markers on chromosome 4 were also determined. The relaxation induced by acetylcholine and lecithinized SOD were attenuated in OLETF rats but restored by a low dose of cerivastatin without significant changes in serum cholesterol. These relaxations were also restored by a high dose of cerivastatin with significant reductions in serum cholesterol and triglyceride. Cerivastatin increased the aortic expression of CD36 and PPARγ mRNA in both LETO and OLETF rats. However, the basal level of CD36 mRNA and the increase in CD36 mRNA in response to cerivastatin were significantly lower in OLETF rats than in LETO rats. Although the abnormal CD36 genotype reported in SHR was not found in OLETF rats, the microsatellite markers of D4Rat151 and D4Rat115 differed between OLETF and LETO rats. In conclusion, insulin resistance in OLETF rats may be partially due to an altered expression of CD36. Increased aortic expression of CD36 in response to cerivastatin could explain the reduction in serum triglyceride concentrations with statin therapy and may have pronounced beneficial effects in insulin-resistance syndromes. (Hypertens Res 2004; 27: 589–598)

Key Words: endothelium, superoxide dismutase, insulin

Introduction

Insulin resistance and dyslipidemia are important cardiovascular risk factors that often coexist in patients with type II diabetes, essential hypertension, or other clinical disorders, even in the absence of obesity (1–3). However, the mechanisms responsible for this clustering of metabolic risk factors for cardiovascular disease remain poorly understood. Although primary abnormalities in insulin sensitivity can give rise to disorders of lipid metabolism, a considerable body of evidence suggests that primary defects in lipid metabolism may also cause insulin resistance and impaired glucose tolerance. In the most widely studied rat model of spontaneous hypertension, a naturally occurring mutation in the gene for CD36 that encodes a fatty acid transporter has been identified as a primary determinant of both insulin resistance and dyslipidemia (4, 5). Therefore, at least in the rat, sponta-
neous variations in a gene that regulates lipid metabolism can contribute to the inherited variation in insulin action.

The Otsuka Long Evans Tokushima Fatty (OLETF) strain of rat is a useful model of obese type II diabetes characterized by hypertension, hypertriglyceridemia, hyperinsulinemia, hyperglycemia, insulin resistance, and abundant visceral fat (6–8). Their lean littermates are Long Evans Tokushima Otsuka (LETO) rats. Genome-wide quantitative trait locus (QTL) analyses have identified 12 statistically significant QTLs that are associated with the type II diabetes, obesity, or dyslipidemia phenotypes of the OLETF rat (6–8). Using genetic dissection with two kinds of F2 intercross progeny generated by mating between the OLETF rat and non-diabetic F344 or Brown Norway (BN) rats, QTLs associated with features of diabetes have been found on chromosomes 1, 7 and 14, designated as diabetes mellitus, OLETF type Dmo1, Dmo2, and Dmo3, respectively (6–8). Significant loci on chromosomes 4 and 13 have also been identified in the [OLETF x BN] F2 cross (6–8). These results indicate that diabetes in the OLETF rat is polygenic, and that different phenotypic effects are produced by different genetic backgrounds from the same locus.

The spontaneously hypertensive rat (SHR) is another model of hypertension and insulin-resistance syndromes (4, 9). The QTLs for SHR defects in glucose and fatty acid metabolism, hypertriglyceridemia, and hypertension map to a single region on rat chromosome 4 (10). Aitman et al. (4) have identified a deletion in the SHR gene for CD36 that encodes a fatty acid receptor/transporter involved in the transmembrane transport of long-chain fatty acids in adipose tissue and in cardiac and skeletal muscle. Genetic analysis of SHR derived from a National Institute of Health colony led to the identification of a causative mutation in the SHR CD36 on chromosome 4 (4). The findings in human CD36 deficiency bear strong resemblance to the phenotype of increased plasma triglycerides and fatty acid concentrations and insulin resistance in rodent CD36 deficiency (11). In the face of such an atherogenic milieu, rodents with CD36 deficiency might be expected to show endothelial dysfunction and premature, accelerated atherosclerosis. In fact, transgenic rescue of defective CD36 ameliorates insulin resistance in SHR (12).

The clinical benefits of cholesterol reduction have been established in large-scale primary and secondary intervention trials using statins, demonstrating that treatment with these drugs results in decreased morbidity and mortality due to coronary heart disease (13). Statin exerts pleiotropic properties and interferes with various vascular events leading to the formation of atherosclerotic lesions by acting on smooth muscle cells, endothelial cells, and macrophages (14–16). Statins increase endothelial nitric oxide synthase expression in cultured human endothelial cells (17). Furthermore, powerful 3-hydroxy-3-methylglutaryl coenzyme A (HMG-CoA) reductase inhibitors, such as simvastatin or atorvastatin, not only reduce circulating cholesterol concentrations but also reduce triglyceride concentrations (18), in which CD36 may also have a role through its action of fatty acid transport.

In the present study, we investigated the effect of the HMG-CoA reductase inhibitor cerivastatin on the endothelial function and the regulation of expression of CD36 in diabetic hyperlipidemic rats. We also investigated the CD36 genotype in OLETF rats to elucidate the genetic mechanisms of insulin resistance in obesity-induced hypertension.

Methods

Animal Preparation

All procedures were performed in accordance with our institutional guidelines for animal research. Male OLETF and LETO rats (6 weeks old) were a gift from Otsuka Pharmaceuticals (Tokushima, Japan). Thirty-week-old male OLETF and LETO rats were fed standard laboratory chow (Oriental Koubo, Tokyo, Japan) that contained 5.5 mEq of sodium and 12.2 mEq of potassium per 100 g of chow, and given distilled water ad libitum. Groups of rats received low-dose (0.002 mg/l in the drinking water) or high-dose (0.006 mg/l in the drinking water) cerivastatin or distilled water for 4 weeks using the following protocols:

1) LETO vehicle group (n = 10): Distilled water was provided to LETO rats for 4 weeks. 2) LETO cerivastatin 0.002 mg/ml group (n = 9): Cerivastatin (0.002 mg/ml) was administered to LETO rats in the drinking water for 4 weeks. In preliminary studies, we determined that the presence of cerivastatin in the drinking water did not affect the rats’ drinking behavior. The daily intake of cerivastatin was approximately 0.2 mg/kg. 3) LETO cerivastatin 0.006 mg/ml group (n = 12): Cerivastatin (0.006 mg/ml) was administered to LETO rats in the drinking water for 4 weeks. The daily intake of cerivastatin was approximately 0.6 mg/kg. 4) OLETF vehicle group (n = 8): Distilled water was provided to OLETF rats for 4 weeks. 5) OLETF cerivastatin 0.002 mg/ml group (n = 10): Cerivastatin (0.002 mg/ml) was administered to OLETF rats in the drinking water for 4 weeks. 6) OLETF cerivastatin 0.006 mg/ml group (n = 13): Cerivastatin (0.006 mg/ml) was administered to OLETF rats in the drinking water for 4 weeks.

The systolic blood pressure was measured by the tail-cuff method in each group (UR-5000; Ueda Co., Tokyo, Japan). Four weeks after starting cerivastatin, the rats were anesthetized with pentobarbital (40 mg/kg i.p.). Serum concentrations of creatinine, sodium, potassium, glucose, total cholesterol and triglyceride were determined.

Effect of Cerivastatin on Endothelium-Dependent Vascular Responses in OLETF Rats

Aortic ring preparations were obtained from OLETF and LETO rats with or without cerivastatin treatment. Cylindrical segments 3.0-mm long were cut from the aorta and were
bathed in 10 ml of Krebs bicarbonate saline (120 mmol/l NaCl; 5.2 mmol/l KCl; 2.4 mmol/l CaCl₂; 1.2 mmol/l MgSO₄; 25 mmol/l NaHCO₃; 0.03 mmol/l Na₂-EDTA; and 11 mmol/l dextrose [pH, 7.4]) equilibrated with 95% O₂ and 5% CO₂, and maintained at 37 ºC.

The rings were suspended under 2 g of tension and preconstricted by adding 10⁻⁷ mol/l norepinephrine. The force of isometric contraction was measured using a force-displacement transducer (Model UR-50GR; Minebea Co., Nagano, Japan). After the contraction force had reached a plateau, acetylcholine 10⁻⁸ to 10⁻⁵ mol/l was added incrementally to the bath. The effects of NO synthesis inhibition with N⁢G⁻nitro-L-arginine methyl ester (LNAME, 10⁻⁵ mol/l) were also studied. To confirm that the vascular smooth muscle cells were intact, the vasodilator response to sodium nitroprusside (10⁻¹⁰ to 10⁻⁷ mol/l) was determined. The amount of superoxide necessary to shorten the half-life of endothelium-derived NO was also evaluated by administering lecithinized superoxide dismutase (lecithinized SOD) (0.25 to 4.0 units/l) ([19], [20]). Acetylcholine (Sigma Chemical Co., St. Louis, USA) and LNAME (Calbiochem Co., La Jolla, USA) were dissolved in Krebs bicarbonate solution.

In each experimental group, n refers to the number of animals from which the aortic rings were obtained. The maximum vasodilator response was defined as the level of vasodilation that preceded the preconstriction induced by norepinephrine. The response to each dose of drug is expressed as a percent of the maximum vasodilation as described previously ([19], [20]).

### Measurements of CD36 and Peroxisome Proliferator-Activated Receptor γ (PPARγ) mRNA Expression in the Aorta

**RNA Isolation from Thoracic Aortas**

The thoracic aorta, extending from the aortic root to the second intercostal region and up to the carotids, was weighed, flash immersed in RNA stabilization reagent (RNAlater; QIAGEN Inc., Valencia, USA) and stored at - 80 ºC. Isolation of total RNA was performed using an RNaseasy kit (QIAGEN Inc.). Total RNA was treated with deoxyribonuclease I (QIAGEN Inc.) for 20 min at room temperature to remove contaminating genomic DNA. The amount of RNA was determined by spectrophotometry, and 200 ng of RNA was loaded onto a 1.5% agarose gel to determine its quality before analysis.

**Reverse-Transcription (RT)–Polymerase Chain Reaction (PCR)-Based Quantitative Gene Expression Analysis**

Real-time detection of PCR products was performed. Using the Perkin-Elmer ABI Prism 7700 and Sequence Detection System software (Perkin-Elmer, Foster City, USA), the differential displays of mRNAs for CD36 and PPARγ were determined. Briefly, 1 µg of total RNA was used to generate cDNA using an oligo dT oligodeoxynucleotide primer (T12–18) following the protocol for Omniscript (QIAGEN Inc.). The primers for CD36 and PPARγ are shown in Table 1, and the following probes were used: CD36: 5′-GAAGA ACTCTTGTGCTATTTCC-3′; PPARγ: 5′-CCCTGGCGGAAAGCCCTTGGTGTACT-3′. The TaqMan Rodent GAPDH Control Reagents (Perkin-Elmer) were used to detect glyceraldehyde-3-phosphate dehydrogenase (GAPDH) genes. Equal amounts of cDNA were used in triplicate and amplified with the TaqMan Master Mix provided by Perkin-Elmer. Amplification efficiencies were validated and normalized against

<table>
<thead>
<tr>
<th>Genotype</th>
<th>Primer</th>
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| CD36 | Forward: TCAAGGTGTGCTCAACAGCC  
Reverse: AGGATAAAAAACAACAGTGT |
| PPARγ | Forward: ATAAAGTCTTTCGCTGACCAAGCC  
Reverse: GCGGTCCTCCAAGTGAATAATGACACG |
| D4Rat9 | Forward: TTTATGGTTAAATTTGGTCTTGT  
Reverse: CCGTGAAGGACAAACAGC |
| D4Rat151 | Forward: GGTTGAAAATCACAGGATT  
Reverse: TCAAGGGGTAGATGATGGAGTTT |
| D4Rat115 | Forward: CAGCTCACGAACACACAT  
Reverse: CCGTTTTTATGATCACTGCTTC |
| D4Rat101 | Forward: TGGATGAATCCTCTGATGTCA  
Reverse: TGGTCATGACTTCAGTGAAC |
| D4Rat24 | Forward: AGGGACAGTGATGAGCCATG  
Reverse: GATAAAATCTATGCTAAAGC |

PPARγ, peroxisome proliferator-activated receptor γ.
Table 2. Effects of Cerivastatin on Body Weight, Systolic BP, Serum Glucose, Triglyceride and Total Cholesterol in LETO and OLETF Rats

<table>
<thead>
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<th>LETO rats</th>
<th>OLETF rats</th>
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<tr>
<td></td>
<td>Distilled water (n = 10)</td>
<td>Distilled water (n = 10)</td>
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<td></td>
<td>Low dose (n = 9)</td>
<td>Low dose (n = 10)</td>
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<tr>
<td></td>
<td>High dose (n = 12)</td>
<td>High dose (n = 13)</td>
</tr>
<tr>
<td>Body weight (g)</td>
<td>504 ± 10</td>
<td>628 ± 15*</td>
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<tr>
<td></td>
<td>520 ± 10</td>
<td>615 ± 16*</td>
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<tr>
<td></td>
<td>523 ± 8</td>
<td>581 ± 12*</td>
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<tr>
<td>Systolic BP (mmHg)</td>
<td>134 ± 4</td>
<td>151 ± 3*</td>
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<td></td>
<td>140 ± 3</td>
<td>163 ± 0.4*</td>
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<td></td>
<td>144 ± 3</td>
<td>158 ± 3*</td>
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<tr>
<td>Serum glucose (mg/dl)</td>
<td>166 ± 9</td>
<td>236 ± 17*</td>
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<td></td>
<td>154 ± 6</td>
<td>225 ± 12*</td>
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<td></td>
<td>158 ± 10</td>
<td>203 ± 13*</td>
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<tr>
<td>Triglyceride (mg/dl)</td>
<td>54 ± 8</td>
<td>165 ± 17*</td>
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<td></td>
<td>69 ± 6</td>
<td>155 ± 16*</td>
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<tr>
<td></td>
<td>60 ± 5</td>
<td>112 ± 13*</td>
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<tr>
<td>Total cholesterol (mg/dl)</td>
<td>93 ± 2</td>
<td>115 ± 3*</td>
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<td></td>
<td>96 ± 2</td>
<td>109 ± 4*</td>
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<td></td>
<td>92 ± 2</td>
<td>94 ± 2*</td>
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Data are mean ± SEM. BP, blood pressure; LETO, Long Evans Tokushima Otsuka; OLETF, Otsuka Long Evans Tokushima Fatty; Low dose, cerivastatin 0.002 mg/ml in drinking water; High dose, cerivastatin 0.006 mg/ml in drinking water. * p < 0.05 vs. LETO rats, distilled water. † p < 0.05 vs. LETO rats, low dose. ‡ p < 0.05 vs. LETO rats, high dose, ‡ p < 0.05 vs. OLETF rats, distilled water.

Table 3. Effects of Cerivastatin on Serum Creatinine, BUN, Electrolytes, AST and ALT in LETO and OLETF Rats

<table>
<thead>
<tr>
<th></th>
<th>LETO rats</th>
<th>OLETF rats</th>
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<tbody>
<tr>
<td></td>
<td>Distilled water (n = 10)</td>
<td>Distilled water (n = 10)</td>
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<tr>
<td></td>
<td>Low dose (n = 9)</td>
<td>Low dose (n = 10)</td>
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<tr>
<td></td>
<td>High dose (n = 12)</td>
<td>High dose (n = 13)</td>
</tr>
<tr>
<td>Serum creatinine (mg/dl)</td>
<td>0.43 ± 0.01</td>
<td>0.32 ± 0.01</td>
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<td>0.52 ± 0.04</td>
<td>0.36 ± 0.01</td>
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<td>0.46 ± 0.02</td>
<td>0.35 ± 0.01</td>
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<tr>
<td>Serum BUN (mg/dl)</td>
<td>24.4 ± 0.8</td>
<td>22.2 ± 1.0</td>
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<td>23.6 ± 0.5</td>
<td>21.8 ± 0.3</td>
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<td></td>
<td>23.1 ± 0.5</td>
<td>21.3 ± 0.5</td>
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<tr>
<td>Serum sodium (mEq/l)</td>
<td>144.3 ± 0.7</td>
<td>142.3 ± 0.6</td>
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<td></td>
<td>140.9 ± 0.5</td>
<td>141.0 ± 0.4</td>
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<td></td>
<td>142.0 ± 0.5</td>
<td>141.9 ± 0.6</td>
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<tr>
<td>Serum potassium (mEq/l)</td>
<td>5.1 ± 0.1</td>
<td>5.0 ± 0.1</td>
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<td></td>
<td>5.2 ± 0.3</td>
<td>5.0 ± 0.2</td>
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<td></td>
<td>4.7 ± 0.1</td>
<td>4.8 ± 0.2</td>
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<tr>
<td>AST (IU/l)</td>
<td>131 ± 7</td>
<td>120 ± 5</td>
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<tr>
<td></td>
<td>159 ± 11</td>
<td>108 ± 8</td>
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<td></td>
<td>149 ± 7</td>
<td>129 ± 10</td>
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<tr>
<td>ALT (IU/l)</td>
<td>50 ± 2</td>
<td>57 ± 5</td>
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<td>43 ± 1</td>
<td>46 ± 2</td>
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<td></td>
<td>44 ± 2</td>
<td>54 ± 6</td>
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Data are mean ± SEM. Low dose, cerivastatin 0.002 mg/ml in drinking water; High dose, cerivastatin 0.006 mg/ml in drinking water; BUN, blood urea nitrogen; AST, aspartate aminotransferase; ALT, alanine aminotransferase; LETO, Long Evans Tokushima Otsuka; OLETF, Otsuka Long Evans Tokushima Fatty.

GAPDH, and nanograms of product were calculated using the standard curve method for quantitation against cDNA that was reverse-transcribed from isolated aortas of OLETF and LETO rats. Total RNA that was not reverse-transcribed was also analyzed to determine genomic DNA contamination.

CD36 Genotyping

We analyzed the CD36 genotypes in both OLETF (n = 80) and LETO rats (n = 80) using PCR and a high throughput capillary sequencer (ABI PRISM 3100 Genetic Analyzer; Perkin-Elmer). PCR amplification of exon 6 of CD36 between 486 and 633 bp (Genbank accession no. L19658) was performed using the same primers as for the real-time RT-PCR (Table 1). PCR products were purified with a PCR purification kit (Perkin Elmer) and directly sequenced using a Big Dye sequencing kit (Perkin Elmer) on an ABI Prism 3100 Genetic Analyzer. This sequencing detects both the normally transcribed wild-type copy of CD36 and the SHR deletion variant in CD36 described previously by Altman et al. (4).

Microsatellite Genotyping

The size of the differential segment was estimated by genotyping of microsatellite markers on chromosome 4 in both OLETF (n = 20) and LETO rats (n = 20) (21). Primer sets for microsatellite markers of chromosome 4 (D4Rat9, D4Rat151, D4Rat115, D4Rat101, and D4Rat24) were obtained from commercial sources (MapPairs; ResGen Inc., Invitrogen, Boston, USA) and are shown in Table 1. The forward and reverse primers were fluorescent-labeled with FAM. PCR was performed according to the manufacturer’s protocol. PCR products were visualized on an ABI Prism 3100 Genetic Analyzer.

Statistical Analyses.

All data are expressed as the mean ± SEM. Aortic vasodilation, biochemical markers, blood pressure, body weights and aortic expressions of CD36 and PPARγ mRNA during the course of the study were analyzed by ANOVA. Statistical significance was defined as p < 0.05.
**Results**

The systolic blood pressure and body weight were significantly higher in OLETF rats under the treatments with distilled water, low-dose cerivastatin, or high-dose cerivastatin for 4 weeks than in LETO rats under the corresponding treatments, respectively (Table 2). Serum concentrations of glucose and triglycerides were significantly higher in OLETF rats compared with LETO rats. The treatment with high doses of cerivastatin (0.006 mg/ml in the drinking water) decreased serum concentrations of cholesterol and triglycerides significantly only in OLETF rats. The treatment with low-dose cerivastatin (0.002 mg/ml in the drinking water) did not affect concentrations of cholesterol or triglycerides significantly in either OLETF or LETO rats (Table 2). There were no significant differences in the serum creatinine, urea nitrogen, sodium, or potassium concentrations or the aspartate aminotransferase or alanine aminotransferase activities among the groups (Table 3).

The relaxation induced by acetylcholine and lecithinized SOD was attenuated in OLETF compared with LETO rats.

**Fig. 1.** Vasodilation induced by acetylcholine, lecithinized superoxide dismutase (SOD) and sodium nitroprusside in LETO or OLETF rats treated with or without cerivastatin. Open and closed circles represent rats receiving no drug. Open and closed squares represent rats treated with cerivastatin 0.002 mg/ml. Open and closed triangles represent rats treated with cerivastatin 0.006 mg/ml. Open circles, squares, and triangles represent experiments without N⁶-nitro-L-arginine methyl ester (LNAME) 10⁻⁵ mol/l in the tissue bath. Closed circles, squares, and triangles represent experiments with LNAME in the tissue bath. The vasodilator response to each dose of drug is expressed as a percent of the level that preceded the preconstriction induced by norepinephrine. * p<0.05 compared with rats given distilled water without LNAME.
and was abolished by the NO synthase inhibitor, LNAME (Fig. 1). Treatment with low-dose cerivastatin restored the relaxation induced by acetylcholine and lecithinized SOD in OLETF rats without significantly affecting the serum cholesterol or triglyceride concentrations. Treatment with high-dose cerivastatin significantly reduced the serum cholesterol and triglyceride concentrations (Table 2) and also restored the relaxation by acetylcholine and lecithinized SOD in OLETF (Fig. 1).

The levels of CD36 mRNA expression in the aorta of OLETF rats were significantly lower than in LETO rats (Figs. 2 and 3). Treatment with low- and high-dose cerivastatin significantly increased the expression of CD36 mRNA in both LETO and OLETF rats. Although treatment with low-dose cerivastatin did not change PPARγ mRNA significantly in either LETO or OLETF rats, treatment with high-
Dose cerivastatin increased the expression of PPARγ mRNA in both LETO and OLETF rats (Figs. 2 and 3). The genotypes of CD36, determined by direct sequencing, were identical between OLETF and LETO rats (n = 80/group) (Fig. 4).

The microsatellite markers of D4Rat9, D4Rat101, and D4Rat24 in OLETF were identical with those in LETO rats. However, the microsatellite markers D4Rat151 and D4RAT115 differed between OLETF and LETO rats (n = 20/group) (Fig. 5).

**Discussion**

We tested for the presence of hyperglycemia, dyslipidemia, high blood pressure, and endothelial dysfunction in 34-week-old male OLETF and LETO rats. We have shown that endothelial dysfunction, metabolic disturbances, and hypertension are present in OLETF rats. In previous linkage studies in the OLETF rat, putative QTLs for increased blood pressure and abnormal carbohydrate and lipid metabolism were mapped to relatively broad regions of chromosome 4. Kanemoto et al. (6) targeted their genetic dissection studies on male OLETF progenies of [female OLETF \( \times \) male control rats] F2 crosses, and the population included 164 male [OLETF \( \times \) BN] F2 rats. They detected QTLs on chromosome 4 in the [OLETF \( \times \) BN] F2 cross, which showed linkage to serum glucose concentrations at 60 min (lod = 3.02) and at 90 min (lod = 3.00) of an oral glucose tolerance test to markers D4Wox10 and D4Mgh7, respectively. The locus of CD36 is located at approximately 30 cM away from D4Wox10 (Fig. 6). Gauguier and colleagues (22) also mapped a QTL linked to postprandial insulin secretion in the region of chromosome 4 of Goto-Kakizaki type 2 diabetic rats, which is close to the CD36 locus.

Aitman et al. (4) showed that Southern analysis of SHR genomic DNA detected missing bands caused by deletion of...
the normally transcribed copy of CD36. To investigate whether CD36 is responsible for the phenotypic effects linked to D4Wox10, we sequenced the region that distinguishes the deletion variant of CD36 described by Aitman et al. (4), but found no differences between OLETF and LETO rats. Though we cannot exclude the possibility of alterations in a distant enhancer region, this result would imply the participation of another gene in this region of rat chromosome 4. In fact, we detected differences in the microsatellite markers of D4Rat151 and D4Rat115 in chromosome 4 between OLETF and LETO rats (Figs. 5 and 6). We know of no other candidate genes mapped to this region, mainly because far fewer genes have been mapped on the rat genome in contrast to the human and mouse.

The findings by Aitman et al. (4) raise fundamental questions about the role of CD36 in metabolic and cardiovascular disease and about the relationship between CD36 and the nuclear receptor PPARγ, the target of the insulin-sensitizing thiazolidinione class of drugs (23). Because PPARγ transcriptionally induces the CD36 gene, the effects are hard to predict (24, 25). Some of the reported effects of PPARγ are pro-atherogenic, for example, induction of foam-cell formation from macrophages (24, 26), whereas others, including repression of inflammatory cytokines and induction of cholesterol efflux from macrophages, are potentially anti-atherogenic (25, 27). Miyaoka and colleagues (11) found that their study group of 26 people with CD36 deficiency had higher plasma triglycerides and glucose concentrations, lower plasma high-density lipoprotein (HDL)-cholesterol concentrations, and much higher blood pressure than did controls. The data showed a striking similarity between the phenotype of these CD36-deficient subjects and the range of metabolic and cardiovascular abnormalities found in the common syndromes of insulin resistance. The findings in human CD36 deficiency also bear a strong resemblance to the phenotypes of increased plasma triglyceride and fatty acid concentrations and insulin resistance in SHR with CD36 deficiency. In the face of such an atherogenic milieu, SHR with CD36 deficiency might be expected to show premature, accelerated atherosclerosis. However, mice with CD36 deficiency have a six-fold reduction in the amount of atherosclerotic plaques compared with control animals (28). The assumption is that the mice with CD36 deficiency are protected from the development of arterial disease because the absence of CD36 results in a failure of uptake of oxidized low-density lipoprotein (LDL) cholesterol by macrophages, reduced foam-cell formation, and a reduced severity of atherosclerotic lesions (28). Whether this presumed mechanism is correct and whether it occurs in human beings must await the results of further studies. However, in the present study, the increase in CD36 expression in response to cerivastatin was accompanied by the restoration of endothelial function in OLETF rats. In the present study, the oral administration of cerivastatin improved aortic endothelial function in OLETF rats; however, blood pressure was not significantly altered by the treatment with cerivastatin. This result implies that microvascular endothelial function, which may have a more major role in blood pressure regulation (29), was not improved by this 4-week treatment with cerivastatin enough to lower blood pressure in OLETF rats.

Fajas et al. (30) have shown that PPARγ protein expression is induced in cells treated with HMG-CoA reductase inhibitors and is not affected by fatty acids. Treatment with HMG-CoA reductase inhibitors, which block the enzyme responsible for the rate-limiting step of cholesterol synthesis, modifies cellular cholesterol levels (30). Cholesterol depletion induced by statins triggers the cleavage of cholesterol-sensitive transcription factors, called SREBPs. Upon treatment with compounds such as mevastatin or simvastatin, cells become cholesterol-depleted and the production of the active forms of ADD-1/SREBP-1 increases (30). Upregulation of ADD-1/SREBP-1 leads to the production of endogenous ligands for PPARγ (30, 31). Treatment of cells with simvastatin for 6h results in robust and fast induction of PPARγ protein levels, which may be sustained 12h after addition, further supporting the notion that cellular cholesterol concentrations influence the expression of PPARγ(30, 31).

The regulation of the expression of PPARγ, a nuclear receptor that is activated by fatty acid metabolites, by the cholesterol-regulated transcription factors of the ADD-1/SREBP-1 family, links transcriptional control by these two
important classes of lipids (30). Changes in intracellular cholesterol levels will, via modulation of ADD-1/SREBP-1 and/or SREBP-2 activity, profoundly affect fatty acid and triglyceride metabolism, which is controlled by PPARγ activity. One interesting example of such an interrelationship between cholesterol and fatty acid metabolism, is the observation that powerful HMG-CoA reductase inhibitors, such as simvastatin or atorvastatin, not only reduce circulating cholesterol but also triglyceride concentrations (18). The triglyceride-lowering effects of cerivastatin were also observed in the present study. If statins cause an induction of PPARγ expression, as shown by Fajas et al. (30) and also by the present study, the increased PPARγ transcriptional activity would induce the expression of several genes involved in triglyceride clearance, including the CD36 gene. This new data could provide a basis for both the pronounced beneficial effects of the statins in patients with diabetic hyperlipidemia and for the development of agents which have a broader or more specific ability to regulate different aspects of lipid metabolism.

PPARγ has been shown to stimulate transcription of the CD36 gene (24, 25). Li et al. (25) have shown that the PPARγ-specific agonists rosiglitazone and GW845 increase expression of the CD36 scavenger receptor in the arterial wall and strongly inhibit the development of atherosclerosis in LDL-cholesterol receptor-deficient male mice. Qi et al. (32) reported that CD36 is a key determinant of the metabolic effects of pioglitazone. They showed that administration of pioglitazone was associated with significantly lower circulating concentrations of fatty acid, triglyceride, and insulin in congenic and transgenic SHR than in SHR harboring mutant CD36 (32). Praveneč et al. (12) reported that transgenic rescue of defective CD36 ameliorates insulin resistance in SHR. These findings confirm that the increase in CD36 expression by cerivastatin in the present study should also ameliorate insulin resistance.

In conclusion, the present study demonstrated that the mechanisms of insulin resistance of OLETF may be partially due to an altered expression of CD36. Although the abnormal CD36 genotype reported in SHR was not found in OLETF rats, a causative gene may still exist on chromosome 4 because of a reported QTL and differences in the microsatellite markers D4Rat151 and D4Rat115 in the present study. Increased expression of CD36 by cerivastatin could be the mechanism responsible for the reduction in serum triglycerides by HMG-CoA reductase inhibitors. Restoration of endothelial function and increased CD36 expression by statin therapy may have pronounced beneficial effects in insulin-resistance syndromes.

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References


