A New Transgenic Rat Model of Hepatic Steatosis and the Metabolic Syndrome

Nathan R. Qi, Jiaming Wang, Vaclav Zidek, Vladimir Landa, Petr Mlejnek, Ludmila Kazdová, Michal Pravenec, Theodore W. Kurtz

Abstract—Fatty liver is extremely common in insulin-resistant patients with either obesity or lipodystrophy and it has been proposed that hepatic steatosis be considered an additional feature of the metabolic syndrome. Although insulin resistance can promote fatty liver, excessive hepatic accumulation of fat can also promote insulin resistance and could contribute to the pathogenesis of the metabolic syndrome. We sought to create a new nonobese rat model with hypertension, hepatic steatosis, and the metabolic syndrome by transgenic overexpression of a sterol-regulatory element-binding protein (SREBP-1a) in the spontaneously hypertensive rat (SHR). SREBPs are transcription factors that activate the expression of multiple genes involved in the hepatic synthesis of cholesterol, triglycerides, and fatty acids. The new transgenic strain of SHR overexpressing a dominant-positive form of human SREBP-1a under control of the phosphoenolpyruvate carboxykinase (PEPCK) promoter exhibited marked hepatic steatosis with major biochemical features of the metabolic syndrome, including hyperglycemia, hyperinsulinemia, and hypertriglyceridemia. Both oxidative and nonoxidative skeletal muscle glucose metabolism were significantly impaired in the SHR transgenic strain and glucose tolerance deteriorated as the animals aged. The SHR transgenic strain also exhibited reduced body weight and reduced adipose tissue stores; however, the level of hypertension in the transgenic SHR was similar to that in the nontransgenic SHR control. The transgenic SHR overexpressing SREBP-1a represents a nonobese rat model of fatty liver, disordered glucose and lipid metabolism, and hypertension that may provide new opportunities for studying the pathogenesis and treatment of the metabolic syndrome associated with hepatic steatosis. (Hypertension. 2005; 45:1004-1011.)

Key Words: hypertension ■ insulin resistance ■ liver ■ metabolism ■ rats ■ transcription, genetic

It is widely recognized that hypertension often occurs as part of a complex metabolic syndrome characterized by the clustering of multiple risk factors for cardiovascular disease, including insulin resistance and dyslipidemia. The metabolic syndrome affects 15% to 25% of individuals in a variety of populations and is associated with significantly increased risks for cardiovascular mortality and for development of type 2 diabetes.1–3 Although the metabolic syndrome is frequently associated with obesity, the prevalence of this syndrome can also be remarkably high even in subjects that are not obese by Western standards.4–6

In addition to disturbances in blood pressure, glucose metabolism, and lipid metabolism, subjects with the metabolic syndrome often exhibit evidence of other disorders, including chronic inflammation, hypercoagulability, and liver abnormalities.7–10 The relationship between the metabolic syndrome and nonalcoholic fatty liver disease has recently begun to attract considerable attention.8,9,11 In subjects with clinical features of the metabolic syndrome, the prevalence of nonalcoholic fatty liver disease can be very high even in the absence of diabetes, obesity, or abnormal liver enzymes. For example, Donati et al found that 30% of nonobese, nondiabetic subjects with hypertension, insulin resistance, and normal liver enzymes may have fatty liver.10 Moreover, 50% of subjects with pure fatty liver and up to 90% of subjects with nonalcoholic steatohepatitis have the metabolic syndrome according to Adult Treatment Panel III (ATPIII) criteria.9 Although insulin resistance can be a determinant of fatty liver, it has also been suggested that hepatic steatosis may play a role in the pathogenesis of the metabolic syndrome and promote insulin resistance in liver and skeletal muscle.11–13 Some investigators have further proposed that nonalcoholic fatty liver disease be considered an additional feature of the metabolic syndrome.8 The availability of animal models with hepatic steatosis, as well as insulin resistance, dyslipidemia, and hypertension, could be valuable for studying the pathogenesis and treatment of the metabolic syndrome.
syndrome and its relationship to nonalcoholic fatty liver disease.

In the current study, we sought to create a nonobese rat model with hypertension, fatty liver, and the metabolic syndrome by transgenic overexpression of a sterol-regulatory element-binding protein (SREBP) in the spontaneously hypertensive rat (SHR). SREBPs are transcription factors involved in the regulation of fatty acid and lipid metabolism and can activate the expression of multiple genes involved in the hepatic synthesis of cholesterol, triglycerides, fatty acids, and phospholipids. Transgenic overexpression of SREBPs is also known to be an effective method for causing hepatic steatosis in rodents. In addition, genetic variants in SREBPs have been reported to be associated with increased risk for obesity, type 2 diabetes, and dyslipidemia.

We now report that transgenic overexpression of human SREBP-1a in the SHR induces hepatic steatosis and multiple biochemical features of the metabolic syndrome, including hyperinsulinemia, hyperglycemia, and hypertriglyceridemia, in the absence of obesity. The transgenic SHR expressing human SREBP-1a may be a useful model for studying the relationship between hepatic steatosis and the metabolic syndrome and for investigating the pathogenesis and treatment of these very common and highly related clinical disorders.

Methods

Generation of Transgenic Rats

Transgenic rats overexpressing a dominant-positive form of human SREBP-1a were derived on the genetic background of the hypertensive SHR/Ola strain that harbors a mutant form of the CD36 fatty acid transporter. The SHR/Ola strain descends directly from the widely used SHR/NIH strain and was one of the original strains used to map the metabolic phenotypes linked to CD36 deficiency.

Transgenic SHR were produced by microinjection of zygotes with a cDNA construct encoding amino acids 1 to 460 of human SREBP-1a without proteolysis and is not subject to feedback regulation. The cDNA transgene was provided by J. Horton and J. Goldstein. The cDNA transgene generates a truncated form of SREBP-1a that can enter the nucleus and the hepatic synthesis of cholesterol, triglycerides, fatty acids, and phospholipids.

In the current study, we sought to create a nonobese rat model with hypertension, fatty liver, and the metabolic syndrome by transgenic overexpression of a sterol-regulatory element-binding protein (SREBP) in the spontaneously hypertensive rat (SHR). SREBPs are transcription factors involved in the regulation of fatty acid and lipid metabolism and can activate the expression of multiple genes involved in the hepatic synthesis of cholesterol, triglycerides, fatty acids, and phospholipids. Transgenic overexpression of SREBPs is also known to be an effective method for causing hepatic steatosis in rodents. In addition, genetic variants in SREBPs have been reported to be associated with increased risk for obesity, type 2 diabetes, and dyslipidemia.

We now report that transgenic overexpression of human SREBP-1a in the SHR induces hepatic steatosis and multiple biochemical features of the metabolic syndrome, including hyperinsulinemia, hyperglycemia, and hypertriglyceridemia, in the absence of obesity. The transgenic SHR expressing human SREBP-1a may be a useful model for studying the relationship between hepatic steatosis and the metabolic syndrome and for investigating the pathogenesis and treatment of these very common and highly related clinical disorders.

Methods

Generation of Transgenic Rats

Transgenic rats overexpressing a dominant-positive form of human SREBP-1a were derived on the genetic background of the hypertensive SHR/Ola strain that harbors a mutant form of the CD36 fatty acid transporter. The SHR/Ola strain descends directly from the widely used SHR/NIH strain and was one of the original strains used to map the metabolic phenotypes linked to CD36 deficiency.

Transgenic SHR were produced by microinjection of zygotes with a cDNA construct encoding amino acids 1 to 460 of human SREBP-1a under control of the rat PEPCk promoter, which produces high levels of gene expression in the liver. Gene expression levels were assessed in 22- to 25-week-old rats that had been fed a high-fructose diet for 2 weeks. For real-time PCR, total RNA was isolated using standard methods and cDNA was prepared and analyzed by real-time PCR testing using QuantiTect SYBR Green reagents (Qiagen, Inc., Valencia, Calif) on an Opticon continuous fluorescence detector (MJ Research, Waltham, Mass) as previously described. Gene expression levels were normalized relative to the expression of cyclophilin (peptidylprolyl isomerase A), which served as the internal control, with results being determined in triplicate using the preferred method of Muller et al. For SREBP-1a target genes, the results in the transgene-positive rats (n = 3) are displayed in comparison to the results in the transgene-negative rats (n = 3).

For the expression level of the human transgene encoding SREBP-1a, the results are displayed compared with the expression level of the endogenous rat gene encoding SREBP-1a. Expression of the transgenic encoding human SREBP-1a was quantified using the following primers: upstream, 5′-tgc tga cca tac gaa; downstream, 5′-agg tgg aca gag ggt g. Expression of endogenous gene encoding rat SREBP-1a was quantified using the following primers: upstream, 5′-tgc tga cca tac gaa; downstream, 5′-agg tgg aca gag ggt g. Expression of endogenous gene encoding rat SREBP-1a was quantified using the following primers: upstream, 5′-tgc tga cca tac gaa; downstream, 5′-agg tgg aca gag ggt g.
glucose into glycogen and CO₂ as previously described.²¹,²⁵ The soleus muscles were attached to a stainless steel frame in situ at in vivo length by special clips and separated from other muscles and tendons and immediately incubated for 2 hours in Krebs-Ringer bicarbonate buffer, pH 7.4, that contained 5.5 mmol/L unlabeled glucose, 0.5 μCi/mL of ¹⁴C-U glucose, and 3 mg/mL bovine serum albumin (Armour, Fraction V) with or without 250 μU/mL insulin. After 2-hour incubation, 0.3 mL of 1 mol/L hyamine hydroxide was injected into the central compartment of the incubation vessel and 0.5 mL of 1 mol/L H₂SO₄ into the main compartment to liberate CO₂. The vessels were incubated for another 30 minutes, and the hyamine hydroxide was then quantitatively transferred into the scintillation vial containing 10 mL of toluene-based scintillation fluid for counting of radioactivity. Measurement of insulin-stimulated incorporation of glucose into glycogen was determined after extraction of glycogen as previously described.²¹,²⁵

Tissue Triglyceride Measurements
For determination of triglycerides in liver and soleus muscle, tissues were powdered under liquid N₂ and extracted for 16 hours in chloroform:methanol, after which 2% KH₂PO₄ was added, and the solution was centrifuged. The organic phase was removed and evaporated under N₂. The resulting pellet was dissolved in isopropyl alcohol, and triglyceride content was determined by enzymatic assay (Pliva-Lachema, Brno, Czech Republic).

Hemodynamic Studies
Arterial blood pressures were measured continuously in unanestheitized, unrestrained rats using radiotelemetry. All rats were allowed to recover for at least 10 to 14 days after surgical implantation of radiotelemetry transducers before the start of blood pressure recordings. Pulsatile pressures were recorded in 5-second bursts every 5 minutes throughout the day and 24-hour averages for systolic arterial blood pressure were calculated for each rat on each day of the study. The results from each rat in the same group were then averaged to obtain the group means. Blood pressure salt-sensitivity was also assessed by measuring arterial pressures during a 1-week period in which 1% NaCl solution was provided in place of drinking water.

Biochemical Analyses
Blood glucose levels were measured by the glucose oxidase assay (Pliva-Lachema) using tail vein blood drawn into 5% trichloroacetic acid and promptly centrifuged. Serum nonesterified fatty acid levels were determined using an acyl-coenzyme A oxidase-based colorimetric kit (Roche Diagnostics GmbH, Mannheim, Germany). Serum triglyceride concentrations were measured by standard enzymatic methods (Pliva-Lachema). Serum insulin concentrations were determined using a rat insulin radioimmunoassay kit (Amersham Pharmacia Biotech UK Ltd). Serum levels of leptin and adiponectin were determined using radioimmunoassay kits from Linco Research (St. Charles, Mo).

Statistical Analysis
All data are expressed as means±SEM. Arterial pressures were analyzed by repeated measures analysis of variance (ANOVA). Individual group means for metabolic measurements were compared by t test. Statistical significance was defined as P<0.05.

Results
Expression of the Human Transgene Gene Encoding SREBP-1a
Transgene-positive SHR and transgene-negative controls were identified by genotyping offspring derived from crosses of a founder male with transgene-negative SHR females. Real-time PCR analysis confirmed abundant hepatic expression of the human transgene encoding SREBP-1a compared with expression of the endogenous SREBP-1a gene (Figure 1). Lower levels of transgene expression could also be...
Body weight, g 253
state.
obtained in the fasting state; other measurements obtained in the nonfasting
Serum glucose and glucose tolerance testing area under the curve (AUC)
/H11006
0.1 1.5
Liver weight, g 11.1
/H11005
12) and SHR controls (open symbols, n = 9) fed a high-
/H11005
8) fed a high-fructose diet for 2 weeks before study. NS indicates no significant difference between the 2 groups in the area under the curve of the glucose tolerance tests.

Figure 2. Oral glucose tolerance tests (OGTT) in young and old SHR transgenic rats and SHR controls. Left panel, OGTT in 10-week-old SHR transgenic rats (solid circles, n = 9) and SHR controls (open symbols, n = 8) fed a high-fructose diet for 2 weeks before study. Right panel, OGTT in 16-month-old SHR transgenic rats (solid circles, n = 12) and SHR controls (open symbols, n = 9) fed a high-fructose diet for 2 weeks before study. NS indicates no significant difference between the 2 groups in the area under the curve of the glucose tolerance tests. *P < 0.01, significant difference between the 2 groups in the area under the curve of the glucose tolerance tests.

Table 1. Metabolic Features of 10-Week-Old Transgenic SHR Expressing Human SREBP-1a

<table>
<thead>
<tr>
<th>Parameter</th>
<th>SHR Controls (n=8)</th>
<th>SHR Transgenics (n=9)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Serum glucose, mmol/L</td>
<td>5.2±0.1</td>
<td>5.7±0.1†</td>
</tr>
<tr>
<td>Serum insulin, nmol/L</td>
<td>1.1±0.1</td>
<td>1.8±0.1†</td>
</tr>
<tr>
<td>Serum triglycerides, mmol/L</td>
<td>1.9±0.3</td>
<td>6.5±0.5‡</td>
</tr>
<tr>
<td>Serum fatty acids, mmol/L</td>
<td>0.4±0.02</td>
<td>0.7±0.04‡</td>
</tr>
<tr>
<td>Serum cholesterol, mmol/L</td>
<td>1.8±0.1</td>
<td>2.4±0.1‡</td>
</tr>
<tr>
<td>Glucose tolerance AUC, mmol/L per 2 hours</td>
<td>813±13</td>
<td>797±7</td>
</tr>
<tr>
<td>Liver triglycerides, μmol/g</td>
<td>13.1±0.6</td>
<td>32.6±3.5‡</td>
</tr>
<tr>
<td>Liver cholesterol, μmol/g</td>
<td>6.9±0.2</td>
<td>10.8±0.6‡</td>
</tr>
<tr>
<td>Spleen triglycerides, μmol/g</td>
<td>4.9±0.5</td>
<td>9.2±1.2†</td>
</tr>
<tr>
<td>Body weight, g</td>
<td>253±3</td>
<td>235±8*</td>
</tr>
<tr>
<td>Liver weight, g</td>
<td>11.1±0.2</td>
<td>13.3±0.4‡</td>
</tr>
<tr>
<td>Epididymal fat pad weight, g</td>
<td>2.1±0.1</td>
<td>1.5±0.1†</td>
</tr>
<tr>
<td>Perirenal fat pad weight, g</td>
<td>1.4±0.2</td>
<td>0.6±0.1†</td>
</tr>
</tbody>
</table>

Measurements obtained after feeding rats a high-fructose diet for 2 weeks. Serum glucose and glucose tolerance testing area under the curve (AUC) obtained in the fasting state; other measurements obtained in the nonfasting state.

Metabolic Effects of Transgenic Expression of SREBP-1a in Young Transgenic Rats

In 10-week-old transgenic SHR fed a high-fructose diet for 2 weeks, serum concentrations of glucose, insulin, triglycerides, and fatty acids were all significantly increased compared with the SHR transgene-negative controls (Table 1). However, there was no significant difference in the area under the curve of the oral glucose tolerance test between the 10-week-old SHR transgenics and the age-matched SHR controls (Table 1 and Figure 2).

The transgenic SHR weighed ≈10% less than the transgene-negative controls (Table 1). Epididymal and perirenal fat pad weights were also significantly reduced (P<0.01) in the transgenic SHR compared with the transgene-negative controls (Table 1). However, liver weight was significantly increased in the transgenic strain (Table 1). In addition, the transgenic SHR exhibited increased levels of triglycerides in liver and in soleus muscle compared with the SHR transgene-negative controls (Table 1). Hepatic levels of cholesterol were also significantly increased in the transgenic SHR compared with controls (Table 1). The changes in serum and tissue lipid levels induced by transgenic expression of human SREBP-1a in the SHR model are similar to those reported to be induced by expression of the same human SREBP-1a transgene in low-density lipoprotein receptor knockout mice.28

In soleus muscle isolated from 10-week-old transgenic SHR expressing human SREBP-1a, glucose oxidation and glucose incorporation into glycogen were significantly decreased compared with SHR transgene-negative controls either in the presence or in the absence of insulin (Table 2). Thus, basal and insulin-stimulated oxidative and nonoxidative glucose metabolism were impaired in skeletal muscle of transgenic rats compared with controls.

Metabolic Effects of Transgenic Expression of SREBP-1a in Old Transgenic Rats

To determine if glucose tolerance deteriorated with age, we performed oral glucose tolerance testing in 16-month-old transgenic SHR and in age-matched SHR controls after feeding a high-fructose diet for 2 weeks. In contrast to the results in the young rats, glucose tolerance was significantly impaired in the old transgenic SHR compared with age-

detected in other tissues, most notably in the kidney, with less in fat and heart (Figure 1). The greater transgene expression in liver relative to other tissues is consistent with the known expression pattern of PEPCK, which was used as the promoter to drive expression of the SREBP-1a transgene.

Effects on Expression of SREBP-1a Target Genes

The expression levels of 3 target genes of SREBP-1 that regulate hepatic lipid metabolism were tested by real-time PCR analysis. In the SHR transgenic strain, the hepatic expression levels of SCD-1 and FAS were substantially increased compared with SHR transgene-negative controls. In contrast to the results in the young rats, glucose tolerance was significantly impaired in the old transgenic SHR compared with age-

Real-time PCR analysis also showed increased expression of SCD-1 but not FAS or ACC in adipose tissue of the SHR transgenic strain (data not shown).
matched controls (Figure 2 and Table 3). Serum glucose, insulin, and lipid levels were also significantly increased in the 16-month-old transgenic SHR compared with the SHR transgene-negative controls (Table 3).

Effects on Blood Pressure

Despite significantly lower body weights throughout the course of the studies (Figure 3; Table 1), the transgenic SHR exhibited frank hypertension with blood pressures similar to those in transgene negative SHR controls (Figure 3). The blood pressure response to salt loading in the transgenic SHR compared with SHR controls (Table 3).

Effects on Liver and Fat Pad Morphology and Adipocytokine Levels

Autopsy of 24-week-old rats maintained on normal chow confirmed the presence of grossly enlarged fatty livers and reduced adipose tissue stores in the transgenic strain compared with the transgene negative control (Figure 4). The mean liver weight of the 24-week-old transgenic rats, 18.2±0.9 grams, was significantly greater than that of controls, 12.8±0.3 grams (P<0.001). In the transgenic strain, histological analysis confirmed the presence of fatty liver as expected; however, there was no apparent increase in the amount of hepatic inflammation compared with the SHR control strain. However, distinct features of steatohepatitis were evident on histological analysis of livers from 16-month-old transgenic rats (data not shown).

Epididymal and perirenal fat weights in the transgenic strain, 0.9±0.1 grams and 0.6±0.04 grams, respectively, were significantly lower than in the transgene-negative control, 3.1±0.3 grams and 2.8±0.2 grams, respectively (both P<0.0001). Although adipose tissue mass was decreased in the transgenic strain, the average volume of adipocytes in the epididymal fat pads was significantly increased in the transgenic rats compared with controls, 405±18 picoliters versus 327±28 picoliters, respectively (P<0.05). The transgenic rats showed an increase in the percentage of large adipocytes with diameters >120 μm compared with SHR controls: 10% of adipocytes in the transgenics were >120 μm, whereas only 1% of the adipocytes were >120 μm in the SHR controls. Serum levels of leptin and adiponectin in the SHR transgenic strain, 1.9±0.3 and 2.2±0.3 ng/mL, respectively, were significantly lower than in the SHR control strain, 4.2±0.4 and 5.4±0.5 ng/mL, respectively (both P<0.005).

Discussion

In the current study in the most widely used rat model of essential hypertension, we have found that transgenic over-expression of a dominant-positive form of human SREBP-1a under control of the PEPCK promoter induces marked hepatic steatosis and biochemical features of the metabolic syndrome. Transgenic SHR exhibited fatty liver and hyperglycemia, hyperinsulinemia, and hypertriglyceridemia compared with nontransgenic SHR. Oral glucose tolerance was similar in 10-week-old transgenic SHR versus SHR controls but became frankly abnormal in older transgenic rats. In addition to glucose intolerance developing, histological fea-

---

**TABLE 2. Glucose Metabolism in Isolated Soleus Muscle from 10-Week-Old Transgenic SHR**

<table>
<thead>
<tr>
<th>Parameter</th>
<th>SHR Controls (n=8)</th>
<th>SHR Transgenics (n=9)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Soleus muscle</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Glucose oxidation, nmol glucose/g per 2 hours</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Without insulin</td>
<td>167±13</td>
<td>93±8†</td>
</tr>
<tr>
<td>With insulin</td>
<td>268±37</td>
<td>129±14†</td>
</tr>
<tr>
<td>Glycogenesis, nmol glucose/g per 2 hours</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Without insulin</td>
<td>925±75</td>
<td>388±82‡</td>
</tr>
<tr>
<td>With insulin</td>
<td>1915±269</td>
<td>765±162†</td>
</tr>
</tbody>
</table>

Measurements obtained after feeding rats a high-fructose diet for 2 weeks. *P<0.05; †P<0.01; ‡P<0.001 compared with control.

---

**TABLE 3. Metabolic Features of 16 Month Old Transgenic SHR Expressing Human SREBP-1a**

<table>
<thead>
<tr>
<th>Parameter</th>
<th>SHR Controls (n=9)</th>
<th>SHR Transgenics (n=12)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Serum glucose (mmol/L)</td>
<td>5.3±0.2</td>
<td>6.1±0.2†</td>
</tr>
<tr>
<td>Serum insulin (nmol/L)</td>
<td>0.2±0.03</td>
<td>0.9±0.1†</td>
</tr>
<tr>
<td>Serum triglycerides (mmol/L)</td>
<td>0.5±0.03</td>
<td>5.6±1.0‡</td>
</tr>
<tr>
<td>Serum fatty acids (mmol/L)</td>
<td>0.5±0.01</td>
<td>0.8±0.04‡</td>
</tr>
<tr>
<td>Serum cholesterol (mmol/L)</td>
<td>1.9±0.1</td>
<td>2.5±0.2†</td>
</tr>
<tr>
<td>Glucose tolerance AUC (mmol/L/2 hours)</td>
<td>792±16</td>
<td>944±35†</td>
</tr>
</tbody>
</table>

Measurements obtained after feeding rats a high fructose diet for two weeks. Serum glucose and glucose tolerance testing AUC (area under the curve) obtained in the fasting state; other measurements obtained in the non-fasting state. * P<0.05; † P<0.01; ‡ P<0.001 compared to control.
tures of steatohepatitis developed in the older transgenic SHR. Progressive impairment in glucose metabolism is known to occur in humans with the metabolic syndrome and some studies suggest that insulin resistance further deteriorates as patients progress from fatty liver to nonalcoholic steatohepatitis.29 The transgenic SHR were also characterized by impaired oxidative and nonoxidative glucose metabolism in skeletal muscle. However, the level of hypertension in the transgenic SHR was similar to that in the nontransgenic SHR despite lower body weights, reduced amounts of adipose tissue, and multiple disturbances in glucose and lipid metabolism. These findings suggest that the SHR transgenic strain represents a lipodystrophic form of the metabolic syndrome.

In both lean and obese individuals, ectopic deposition of fat in liver and muscle is widely suspected to play a role in the pathogenesis of the metabolic syndrome and diabetes by impairing hepatic and skeletal muscle glucose metabolism.30–32 Ectopic accumulation of fat has also been proposed to explain why patients with peripheral lipodystrophy are at increased risk for insulin resistance and diabetes despite having decreased total body fat.30,33,34 In the current study, the transgenic SHR overexpressing SREBP-1a exhibited hyperglycemia and hyperinsulinemia associated with increases in skeletal muscle and liver fat, decreases in adipose tissue stores, and decreases in body weight. These findings, together with evidence of impaired oxidative and nonoxidative glucose metabolism in isolated soleus muscle of the transgenic SHR, suggest that the metabolic disturbances in this strain are at least partly related to disordered peripheral glucose metabolism secondary to the increased deposition of fat in skeletal muscle.

Although we did not assess hepatic glucose metabolism, it is likely that the marked hepatic steatosis is also impairing glucose metabolism in the liver. Studies across multiple animal models with hepatic steatosis have shown an inverse relationship between hepatic triglyceride content and insulin-mediated inhibition of hepatic glucose production.11 Moreover, Samuel et al have found that in rats fed a high-fat diet, hepatic steatosis and hepatic resistance to insulin-mediated suppression of endogenous glucose production precede the peripheral accumulation of fat in skeletal muscle and the development of muscle insulin resistance.13 The decreased leptin and adiponectin levels observed in the transgenic SHR could also be contributing to their metabolic disturbances, because deficiencies in these adipocytokines are known to promote impaired glucose metabolism in liver and skeletal muscle.35–38 Thus, in future studies in the transgenic SHR, it will be of interest to simultaneously determine the time course for development of fat accumulation and insulin resistance in liver and skeletal muscle and to determine whether supplemental administration of leptin or adiponectin can ameliorate the hepatic steatosis and metabolic status of the transgenic SHR.

In contrast to the results in transgenic SHR expressing SREBP-1a, transgenic mice that express the same SREBP-1a construct used in this study do not display typical biochemical features of the metabolic syndrome despite marked hepatic steatosis and significantly reduced amounts of epididymal fat.20,39 However, severe hypertriglyceridemia and hypercholesterolemia develop when SREBP-1a is transgenically expressed on the genetic background of LDL low-density lipoprotein receptor knockout mice.28 Thus, when coupled with variants in other genes that influence lipid metabolism, changes in SREBP activity can have profound effects on circulating lipid levels. In the current study, we transgenically expressed SREBP-1a on the genetic background of the SHR that is known to harbor a spontaneous genetic defect in the CD36 fatty acid transporter.22,40,41 In previous studies, it has been shown that defective CD36 promotes increased susceptibility to insulin resistance and dyslipidemia in the SHR.22,40,41 These observations raise the possibility that the metabolic abnormalities in transgenic SHR expressing SREBP-1a may depend in part on the interaction between impaired fatty acid transport caused by mutant CD36 and disordered hepatic lipid metabolism caused by unregulated production of SREBP-1a. In future studies, it
should be possible to test the potential role of defective CD36 in modulating the metabolic effects of transgenic SREBP-1a by expressing the SREBP-1a transgene on the genetic background of SHR with wild-type CD36.41 Studies in additional transgenic lines will also be helpful to insure that the metabolic disturbances observed in our model are secondary to overexpression of SREBP-1a and not simply caused by an insertional mutagenesis event caused by the transgene.

Increased blood pressure is an important cardiovascular risk factor that frequently occurs as part of the metabolic syndrome. In the current study, the severity of hypertension in transgenic SHR was similar to that in SHR controls despite lower body weights and reduced adipose tissue stores in the transgenic strain. Given that reductions in body weight often lead to reductions in blood pressure, this observation raises the possibility that the metabolic abnormalities (eg, reduced adiponectin levels) and/or hepatic steatosis might be serving to maintain some portion of the hypertension in the transgenic strain. However, it is also possible that the hypertension in this model of the metabolic syndrome is entirely accounted for by the increased blood pressure characteristic of the SHR progenitor strain. In future studies, it should be possible to test the hypertensinogenic effects of overexpressing SREBP-1a by transferring the SREBP-1a transgene onto the genetic background of a normotensive strain.

Paterson et al have reported that selective overexpression of 11β-hydroxysteroid dehydrogenase type 1 (11β-HSD1) in liver of normotensive mice can produce hepatic steatosis, insulin resistance, dyslipidemia, and hypertension in the absence of obesity.42 In the SHR transgenic rats overexpressing SREBP-1a, the hepatic expression of 11β-HSD1 mRNA was similar to that in SHR controls (unpublished observations). Thus, it does not appear that the cardiovascular or metabolic effects of transgenic SREBP-1a are likely related to changes in expression of the gene encoding 11β-HSD1. Nevertheless, the findings of Paterson et al support the possibility that primary alterations in liver function may have the capacity to elicit hemodynamic as well as biochemical disturbances characteristic of the metabolic syndrome.

**Perspectives**

Transgenic SHR overexpressing SREBP-1a could provide valuable opportunities for investigating pathogenetic mechanisms that may relate fatty liver disease to the metabolic syndrome. In addition, because this model has been created on the background of a strain with increased blood pressure and an inherited defect in fatty acid transport, it could aid in studying the interaction between hepatic steatosis and genetic risk factors for insulin resistance, dyslipidemia, and hypertension. Finally, the availability of a rat model with hepatic steatosis and disordered glucose and lipid metabolism may also be helpful for investigating new therapies for fatty liver disease and the metabolic syndrome.

**Acknowledgments**

The authors acknowledge the support by National Institutes of Health Grants HL35018, HL63709, and TW01236 to T.W.K., by grant 1M6837605002 from the Ministry of Education of the Czech Republic to M.P., grant AV0Z 50110509 to the Institute of Physiology, and by grant 7403-3 from the Ministry of Health of the Czech Republic to L.K. Michal Pavonec is an international research scholar of the Howard Hughes Medical Institute.

**References**

Qi et al  Rat Model of Fatty Liver and Metabolic Syndrome 1011

A New Transgenic Rat Model of Hepatic Steatosis and the Metabolic Syndrome
Nathan R. Qi, Jiaming Wang, Vaclav Zidek, Vladimir Landa, Petr Mlejnek, Ludmila Kazdová, Michal Pravenec and Theodore W. Kurtz

Hypertension. 2005;45:1004-1011; originally published online April 4, 2005; doi: 10.1161/01.HYP.0000161995.64192.2b
Hypertension is published by the American Heart Association, 7272 Greenville Avenue, Dallas, TX 75231
Copyright © 2005 American Heart Association, Inc. All rights reserved.
Print ISSN: 0194-911X. Online ISSN: 1524-4563

The online version of this article, along with updated information and services, is located on the World Wide Web at:
http://hyper.ahajournals.org/content/45/5/1004

Permissions: Requests for permissions to reproduce figures, tables, or portions of articles originally published in Hypertension can be obtained via RightsLink, a service of the Copyright Clearance Center, not the Editorial Office. Once the online version of the published article for which permission is being requested is located, click Request Permissions in the middle column of the Web page under Services. Further information about this process is available in the Permissions and Rights Question and Answer document.

Reprints: Information about reprints can be found online at:
http://www.lww.com/reprints

Subscriptions: Information about subscribing to Hypertension is online at:
http://hyper.ahajournals.org/subscriptions/