Amelioration of high fructose-induced metabolic derangements by activation of PPARα

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Nagai, Yoshiio, Yoshihiko Nishio, Takaaki Nakamura, Hiroshi Maegawa, Ryuichi Kikkawa, and Atsunori Kashiwagi. Amelioration of high fructose-induced metabolic derangements by activation of PPARα. Am J Physiol Endocrinol Metab 282: E1180–E1190, 2002. First published January 22, 2002; 10.1152/ajpendo.00471.2001.—To elucidate molecular mechanisms of high fructose-induced metabolic derangements and the influence of peroxisome proliferator-activated receptor-α (PPARα) activation on them, we examined the expression of sterol regulatory element binding protein-1 (SREBP-1) and PPARα as well as its nuclear activation and target gene expressions in the liver of high fructose-fed rats with or without treatment of fenofibrate. After 8-wk feeding of a diet high in fructose, the mRNA contents of PPARα protein and its activity and gene expressions of fatty acid oxidation enzymes were reduced. In contrast, the gene expressions of SREBP-1 and lipogenic enzymes in the liver were increased by high fructose feeding. Similar high fructose effects were also found in isolated hepatocytes exposed to 20 mM fructose in the media. The treatment of fenofibrate (30 mg·kg⁻¹·day⁻¹) significantly improved high fructose-induced metabolic derangements such as insulin resistance, hypertension, hyperlipidemia, and fat accumulation in the liver. Consistently, the decreased PPARα protein content, its activity, and its target gene expressions found in high fructose-fed rats were all improved by fenofibrate treatment. Furthermore, we also found that the copy number of mitochondrial DNA, the expressions of mitochondrial transcription factor A, ATPase-6 subunit, and uncoupling protein-3 were increased by fenofibrate treatment. These findings suggest that the metabolic syndrome in high fructose-fed rats is reversed by fenofibrate treatment, which is associated with the induction of enzyme expression related to β-oxidation and the enhancement of mitochondrial gene expression.

Peroxisome proliferator-activated receptor-α; sterol regulatory element binding protein

Epidemiological studies have clearly demonstrated the existence of a clinical condition accumulating several risk factors for atherosclerosis such as dyslipidemia, hyperinsulinemia with insulin resistance, glucose intolerance, and hypertension at the same time (27). This state is referred to as metabolic syndrome and is taken seriously as the clinical target for the prevention of atherosclerosis. In an animal model, environmental factors such as a westernized diet cause conditions like metabolic syndrome. It was reported that a diet high in fructose induced metabolic derangements such as hyperinsulinemia, dyslipidemia, hypertension, and endothelial dysfunction at the same time in humans and rats (4, 33, 34). In this diet-induced metabolic syndrome model, metabolic derangements in the liver may play a central role as a cause of this syndrome. However, the precise molecular mechanisms about how a diet high in fructose induces the abnormalities in the liver are not fully understood.

Several lines of evidence have suggested that transcription factors, sterol regulatory element binding proteins (SREBPs), especially SREBP-1, have a central role for the induction of gene expression of lipogenic enzymes (30). The transcripational activity of SREBP-1 is dependent on its nuclear abundance (15, 46) and is determined by the rate of production of its precursor (21, 43). The synthesis of SREBP-1 precursor depends on its mRNA level (45). Many factors, including fasting, diabetes, carbohydrate refeeding, and insulin, affect the mRNA expression of SREBP-1 in liver (7). Furthermore, in a cultured hepatocyte cell line, it was reported that the mRNA content of SREBP-1 was increased by insulin and glucose but not by fructose (13). Although the exact molecular mechanism for the elevation of SREBP-1 expression remains to be determined (32), reversibility of the increased SREBP-1 expression by the treatment of insulin resistance is also a topic to be clarified.

Another important molecule for the regulation of lipid metabolism in the liver is a peroxisome proliferator-activated receptor (PPAR)-α (18), which belongs to the superfamily of ligand-activated nuclear hormone receptors and regulates the expressions of fatty acid oxidation enzymes (28). Its ligands, fibrates, are used for the treatment of hypertriglyceridemia (8, 40). PPARα has been shown to regulate genes involved in triglyceride metabolism, such as apolipoprotein A-I (3, 42), acyl-CoA synthetase (29), and carnitine palmitoyl-
MATERIALS AND METHODS

The expression of PPARα in rat liver is upregulated by glucocorticoid, polyunsaturated fatty acids, and fasting (19, 35) and is downregulated by insulin and feeding (36). However, the expression of PPARα in the liver of insulin-resistant animals has not been reported. Interestingly, it was reported that PPARα agonists improved not only hyperlipidemia but also insulin sensitivity of high fat-fed rats (48) and Zucker obese rats (11) and high fructose with high fat-fed rats (22). However, the molecular mechanism has not been investigated. Because PPARα agonists stimulate both peroxisomal and mitochondrial β-oxidation of fatty acid, mitochondrial function may be modified by the PPARα activity. In fact, recent reports showed that PPAR agonists induced uncoupling protein (UCP)-2 expression in liver (38), which can modify the efficiency of ATP production, possibly leading to an influence on glycolysis or fatty acid oxidation activities of the cells. Furthermore, it has been shown that PPARα can bind to PPAR-γ coactivator-1 (PGC-1; see Ref. 41), which is related to the expression of nuclear respiration factors (NRFs). In turn, NRFs may regulate the mitochondrial gene expression such as ATP synthase subunit-6 (ATPase-6) through the induction of mitochondrial transcription factor (mtTFA; see Ref. 44). Therefore, it is speculated that mitochondrial gene expression is regulated through PPARα activation, although there is no report indicating such regulation.

Thus, in the present study, we have studied 1) the influence of dietary fructose on the expression of PPARα SREBP-1 and their target genes in liver, 2) in vitro high-fructose effects on PPARα and SREBP-1 mRNA expression in isolated hepatocytes, which were incubated in the presence of 20 mM fructose, and 3) the effects of fenofibrate treatment on nuclear gene expression and mitochondrial gene expression, mRNA and protein content of mtTFA, and the mRNA and protein content of UCP-3 in the liver of high fructose-fed rats to elucidate the improvements of hepatic lipid metabolism and insulin resistance by PPARα activation.

MATERIALS AND METHODS

Materials. Fenofibrate was provided from Kaken Pharmaceutical (Tokyo, Japan). Anti-PPARα and anti-UCP-3 antibodies were obtained from Santa Cruz Biotechnology (Santa Cruz, CA). Anti-mtTFA antibody was a kind gift from Dr. H. Inagaki (National Institute of Advanced Industrial Science and Technology, Tsukuba, Japan). All other materials were reagent grade and purchased from Nacalai Tesque or Sigma Chemical, unless otherwise indicated.

Animals. Six-week-old male Sprague-Dawley rats (Japan SLC, Shizuoka, Japan) were housed in an environmentally controlled room with a 12:12-h light-dark cycle. The animals were divided into the following four groups: 1) a normal diet (N), 2) a normal diet with fenofibrate treatment (NF), 3) a diet high in fructose (F), and 4) a diet high in fructose with fenofibrate treatment (FF). The rats of each group were fed for 8 wk. The normal diet (Oriental Yeast, Tokyo, Japan) consisted of 58% carbohydrate (no fructose), 12% fat, and 30% protein (energy percent of diet). The diet high in fructose (Oriental Yeast) contained 67% carbohydrate (98% of which was fructose), 13% fat, and 20% protein. Rats were pair-fed and treated for 8 wk once daily by gavage with fenofibrate (30 mg·kg⁻¹·day⁻¹) or vehicle.

The day before the experiment, food was withdrawn from all animals at 8:00 PM. Rats were refed in the dark from 6:00 AM to 8:00 AM, and then the diet was removed again. After 4 h of food deprivation, rats were anesthetized, and the liver, skeletal muscle, and epididymal fat were removed and immediately frozen in liquid nitrogen and stored at −80°C until use. RNA and protein were extracted from the frozen samples. All experiments were approved by Shiga University of Medical Science Animal Care Committees.

Measurement of insulin sensitivity. Insulin sensitivity was measured by the steady-state plasma glucose (SSPG) method described by Harano et al. (12). Rats were administered infusion containing somatostatin (120 μg·kg⁻¹·h⁻¹), glucose (1.5 g·kg⁻¹·h⁻¹), and insulin (2.0 U·kg⁻¹·h⁻¹) at a flow rate of 2.8 ml/h for 120 min. The SSPG and steady-state plasma insulin (SSPI) levels were measured at 120 min after the infusion.

Measurement of blood pressure. Blood pressure was measured the day before the experiment, and the rats were trained to the apparatus three times before measurement. Systolic and diastolic blood pressure in the tail region was measured using an electrophysiomonitor after the rats were prewarmed for 15 min.

Northern blot analysis. Total hepatic RNA was isolated with TRIzol reagent (GIBCO-BRL Life Technology, Rockville, MD), and 20-μg RNA samples were run on a 1% agarose gel containing formaldehyde and transferred to a nylon membrane (Nytran N; Schleicher & Schuell, Dassel, Germany).

The cDNA probes for Northern blot analyses were generated by RT-PCR amplification from total RNA isolated from the liver of rats. A rat cDNA fragment was amplified using the following PCR primer pairs: acyl-CoA carboxylase (ACC): sense primer 5'-GGGACTTCATGAATTTGCTGAT-TCTCATGTT and antisense primer 5'-GTCATTACCATCTCNTTACTACCTCCAATCTC (30); acyl-CoA oxidase (ACO): sense primer 5'-CAATCCAGCAATAGTTGCTGCT and antisense primer 5'-AAGGTCAGGAGGTCTTACAGCA (24); CPT I: sense primer 5'-TCCCCCCTTCAGAATGCCAGGGGTGCTCAGT and antisense primer 5'-CTTTCCGTGTGGCTACGGGTTTACC (20); fatty acid synthase (FAS): sense primer 5'-GTCCAGGCGGCCGGGTATCTCAAGCTGCCC (14); ATPase-6: sense primer 5'-TCACGGAGCCGTCGTGGGTGG and antisense primer 5'-ATGGACGAGCTGGCCTTCAG (12); PPARα: sense primer 5'-GGTCAAGGGCGGGCTTACAGGGAG and antisense primer 5'-TGACCTGACCCGGATCTGATAGCT; SREBP-1: sense primer 5'-ATGGAGAGCTCGTGGGCGTGAC and antisense primer 5'-CCAGAGGACCCAGGAGGAGCAG (31); UCP-3: sense primer 5'- AGGAAAGTCTTACCCCAAGAG and antisense primer 5'-TGTTGGGGGCTCTGGGGGCCAC (14); ATPase-6: sense primer 5'-CATGTCAGACCCCTTATGACG and antisense primer 5'-TGACCTGACCCGGATCTGATAGCT; mTFA: sense primer 5'-GCAAAATGCGTGAAGTTGGG and antisense primer 5'-TCTAGTAGAAAGCCCCAGAG. Amplification products were cloned into the TA-plasmid vector (TA Cloning Kit; Invitrogen, Carlsbad, CA) and sequenced before use. Each cDNA probe labeled with [α-32P]dCTP (NEN Life Science Products, Boston, MA) by a labeling kit (Takara, Shiga, Japan) was hybridized to ultraviolet cross-linked DNA samples. The blots were exposed to Kodak Biomax MR (Eastman Kodak, Rochester, NY) film at −80°C. The signal was quantified with a densitometer, and loading differences were corrected.
were normalized to the signal generated with a probe for 18S ribosomal RNA (37).

**Southern blot analysis.** The copy number of mitochondrial DNA was measured as previously reported (3). Total DNA was extracted from 50 mg of liver. Twenty micrograms of the DNA from each sample were digested with the *Hind* III restriction endonuclease (Toyobo), fractionated on a 0.8% agarose gel, and transferred overnight to nylon membranes. Blots were subsequently hybridized to the cDNA probes for mitochondrial DNA-encoded ATPase-6. All membranes were prehybridized at 68°C for 30 min with Perfecthyb (Toyobo), hybridized to the radioactive probe for 1 h at 68°C, and then washed at 68°C for 10 min in 2× SSC and 0.1% SDS and for 30 min in 0.1× SSC and 0.1% SDS. Membranes were then exposed to films at −80°C for 3–16 h in Kodak Biomax MR (Eastman Kodak).

**Western blot analysis.** Each liver was homogenized in a solubilizing buffer containing 20 mM Tris, 1 mM Na3VO4, 1 mM phenylmethylsulfonyl fluoride (PMSF), and 50 mM NaF (pH 7.5) for 30 min at 4°C. The tissue lysates were centrifuged to remove insoluble materials. For Western blot analysis, each sample (20 μg protein/lane) was denatured by boiling in Laemmli sample buffer containing 100 mM dithiothreitol and 0.2 mM EDTA buffer at 100°C for 3 min, and then stored at −80°C.

**Electrophoretic mobility shift assays.** Electrophoretic mobility shift assays were performed using radiolabeling double-stranded oligonucleotides corresponding to the PPAR consensus sequences (Santa Cruz). The protein-DNA binding reaction was performed at room temperature for 20 min in a volume of 20 μl. The reaction mixture contained 10 μg of nuclear protein extract, 1 μg of poly(dI-dC), 10 mM HEPES (pH 7.9), 60 mM KCl, 1 mM EDTA, 7% glycerol, and 100,000 counts/ min labeled probe. After the incubation, samples were loaded on 5% polyacrylamide gels in 0.25× Tris-borate-EDTA buffer and run at 150 V for 1.5 h. The gels were dried, and the bands were visualized by autoradiography. For competition assay, nonlabeled oligonucleotides were added at a 50-fold molar excess to the reaction mixture before the addition of nuclear extract protein.

**Oil red stain.** Liver tissue sections of 20 μm thickness were fixed for 24 h with 4% paraformaldehyde, 0.2% picric acid, and 0.5% glutaraldehyde in 0.2 mol/l phosphate buffer (pH 7.4) at 4°C. After being washed for 7 days with 15% sucrose at 4°C, the sections were incubated for 1 h with an oil red dye bath to stain triglyceride.

**Statistical analyses.** The values are expressed as means ± SE, unless otherwise stated. Scheffe’s multiple-comparison test was used to determine the significance of any differences among more than three groups. *P* < 0.05 was considered significant.

**RESULTS**

**Characteristics of experimental animals.** As shown in Table 1, the body weight gains of the rats fed a normal diet (N) and the rats fed a high-fructose diet (F) were similar over the study period. There were no differences in blood glucose levels among the groups, but F rats showed higher levels of plasma insulin (by 230%, *P < 0.01), total cholesterol (by 75%, *P < 0.05), and triglyceride (by 241%, *P < 0.001) than those of N rats, respectively. In addition, F rats had an increased epididymal fat weight by 53% (*P < 0.05) compared with N rats.

Systolic blood pressure of F rats was 11% higher than N rats (*P < 0.05), and the F rats exhibited a significant increase in SSPG levels by 67% (*P < 0.001), with comparable levels of SSPI, compared with N rats.

Fenofibrate treatment improved all the disorders caused by the diet high in fructose. Compared with the F rats, the plasma insulin levels were significantly lower in fenofibrate-treated F rats (FF; *P < 0.05). The serum total cholesterol and triglyceride levels of the treated rats were lower than those of F rats by 35% (*P < 0.05) and 40% (*P < 0.05), respectively. Systolic blood pressure and epididymal fat weight of F rats were normalized by the fenofibrate treatment (*P < 0.05). Consistently, histological examination of the liver from F rats showed marked lipid accumulation (Fig. 1). However, fenofibrate treatment reduced the lipid accumulation in the liver from F rats. The fenofibrate treatment induced hepatomegaly in both con-
Table 1. Characteristics of experimental animals

<table>
<thead>
<tr>
<th></th>
<th>Normal Diet</th>
<th>Fenofibrate-Treated Normal Diet</th>
<th>Fructose Diet</th>
<th>Fenofibrate-Treated Fructose Diet</th>
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<tr>
<td><strong>Baseline data</strong></td>
<td></td>
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<td></td>
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<tr>
<td>Weight, g</td>
<td>400 ± 21</td>
<td>400 ± 9</td>
<td>438 ± 7</td>
<td>392 ± 10</td>
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<tr>
<td>Glucose, mg/dl</td>
<td>139.3 ± 8.5</td>
<td>148.2 ± 1.9</td>
<td>146.3 ± 4.8</td>
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<td>Insulin, μU/ml</td>
<td>6.97 ± 0.46</td>
<td>9.90 ± 1.46</td>
<td>23.0 ± 2.08a</td>
<td>10.9 ± 3.15d</td>
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<td>Total cholesterol, mg/dl</td>
<td>51.0 ± 1.7</td>
<td>43.0 ± 3.5</td>
<td>89.0 ± 6.5a</td>
<td>57.7 ± 9.7d</td>
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<td>Triglyceride, mg/dl</td>
<td>78.7 ± 3.7</td>
<td>57.8 ± 7.7</td>
<td>269.0 ± 24.5c</td>
<td>161.7 ± 31.4d</td>
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<td>Ketone body, μmol/l</td>
<td>1224 ± 853</td>
<td>823 ± 196</td>
<td>1,166 ± 548</td>
<td>713 ± 56</td>
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<td>Systolic blood pressure, mmHg</td>
<td>135.7 ± 4.4</td>
<td>134.8 ± 3.3</td>
<td>150.3 ± 0.3a</td>
<td>135.0 ± 2.9d</td>
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<td>Diastolic blood pressure, mmHg</td>
<td>90.3 ± 6.9</td>
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<td>Liver weight, g</td>
<td>12.1 ± 0.98</td>
<td>18.8 ± 0.73a</td>
<td>15.4 ± 1.13</td>
<td>27.1 ± 0.88c,e</td>
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<td>Epididymal fat weight, g</td>
<td>6.52 ± 0.57</td>
<td>6.22 ± 0.75</td>
<td>10.01 ± 0.62a</td>
<td>6.88 ± 0.02d</td>
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<td>Insulin sensitivity test</td>
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<tr>
<td>SSPG, mg/dl</td>
<td>102.3 ± 10</td>
<td>105.2 ± 8.7</td>
<td>171.1 ± 12.7e</td>
<td>120.0 ± 9.7d</td>
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<tr>
<td>SSPI, μU/ml</td>
<td>515 ± 49.5</td>
<td>606 ± 127</td>
<td>564 ± 64.5</td>
<td>536.0 ± 52.3</td>
</tr>
</tbody>
</table>

Data are means ± SE; n = 9 rats in each group. SSPG, steady-state plasma glucose; SSPI, steady-state plasma insulin. *P < 0.05, bP < 0.01, and cP < 0.001 vs. normal diet-fed rats. dP < 0.05 and eP < 0.001 vs. fructose.

trol and high fructose-fed rats. However, serum transaminase levels were not different between the rats with and without fenofibrate treatment (data not shown), indicating that liver injury was not induced by the treatment. FF rats were 11% less in body weight when compared with F rats, although this difference was not statistically significant.

Fenofibrate-treated N rats (NF) did not show any significant difference in these characteristics compared with N rats.

Expression of PPARα and fatty acid oxidation enzymes in liver from experimental animals. Figure 2 shows a Northern blot analysis series of PPARα (Fig. 2A) and fatty acid oxidation enzymes ACO (Fig. 2B) and CPT I (Fig. 2C). The PPARα and CPT I mRNA contents were reduced by 50 and 80% in the liver of F rats compared with N rats, respectively. However, ACO mRNA was not reduced by a high-fructose feeding. Fenofibrate had no effect on mRNA expression of PPARα in FF rats, but the PPARα protein levels were increased to levels compatible with NF rats (Fig. 2E). This effect of fenofibrate on PPARα was confirmed by electrophoretic mobility shift assay (Fig. 2D). Consistently, these effects of fenofibrate were also observed in the mRNA contents of the target genes of PPARα (Fig. 2, B and C).

Expression of SREBP-1 and lipogenic enzymes in liver from experimental animals. Figure 3, A–C, shows a Northern blot analysis series of SREBP-1 (A) and lipogenic enzymes such as acetyl-CoA carboxylase (ACC, B) and fatty acid synthase (FAS, C) in the liver. The hepatic mRNA content of SREBP-1 was elevated about twofold (P < 0.01) in F rats compared with N rats. The increased amount of SREBP-1 in the nuclei of F rat liver was also confirmed by Western blot analysis (Fig. 3D). Consistently, the mRNA contents of ACC and FAS, known as targets for SREBP-1, were also increased in the liver of F rats. Fenofibrate had no effect on the mRNA expression of SREBP-1 and its target genes ACC and FAS in FF rats. However, as shown in Fig. 3E, both control and fructose-fed rats treated with fenofibrate showed an enormous amount of unknown protein (69 kDa) expression in the nuclei. Therefore, the relative proportion of SREBP-1 to total nuclear protein was affected by the unknown protein, and then we could not compare the nuclear content of SREBP-1 between the rats without and with fenofibrate treatment exactly.

mRNA content of PPARα and SREBP-1 in primary rat hepatocytes. We studied the direct effect of fructose on mRNA content of PPARα and SREBP-1, using rat primary cultured hepatocytes. As shown in Fig. 4A, the medium containing 20 mM fructose decreased the mRNA expression of PPARα to 60 ± 6% (P < 0.05, n = 3) in rat primary cultured hepatocytes. However, the medium containing 25 mM glucose did not affect the mRNA expression of PPARα.
As shown in Fig. 4B, the media containing 20 mM fructose raised SREBP-1 mRNA expression by 2.1 ± 0.3-fold (P < 0.05, n = 3) compared with the control medium. In contrast, the medium containing 25 mM glucose did not affect the mRNA expression of SREBP-1. In the presence of 5 μg/ml actinomycin D, the half-life of mRNA of SREBP-1 was not different between the cells cultured with control and high fructose-containing media (6 h in both conditions; data not shown). These results indicated that high fructose-containing medium enhanced the mRNA content of SREBP-1 by stimulating the transcriptional activity.

Mitochondrial gene expression and mitochondrial copy number. The increase in mRNA content of enzymes for regulation of β-oxidation with fenofibrate treatment raises the possibility that not only β-oxidation but also mitochondrial electron transport is facilitated by the treatment. To test this hypothesis, we investigated the mRNA expression of both mtTFA encoded on nuclear DNA and ATPase-6 encoded on mitochondrial DNA. The mRNA contents of mtTFA and ATPase-6 were significantly increased in both NF and FF rats (Fig. 5, A and C). The elevated expression of mtTFA in fenofibrate-treated rats was also confirmed by Western blot analysis, as shown in Fig. 5B.

Southern blot analysis of mitochondrial DNA was carried out to determine the mitochondrial copy number. As shown in Fig. 5E, the amount of mitochondrial DNA corrected by the amount of nuclear DNA in NF and F rats was increased more than that of N rats. Furthermore, the mitochondrial copy number in FF rats was markedly elevated compared with the other groups.
Expression of UCPs in liver, skeletal muscle, and epididymal fat from experimental animals. We further studied the expression of UCP mRNA in liver. Hepatic UCP-2 mRNA levels in F rats were decreased by 40% ($P < 0.05$) compared with those of N rats. The fenofibrate treatment did not affect the hepatic UCP-2 mRNA levels, as shown in Fig. 6A. In contrast, the UCP-3 mRNA signals in the liver were not detectable in either N or F rats. However, the hepatic UCP-3 mRNA was slightly detectable in NF rats, and the expression was markedly elevated in F rats, which were treated with fenofibrate (Fig. 6B). The increase in UCP-3 mRNA levels in FF rats was also confirmed by the protein level, as shown in Fig. 6C. The expressions of UCP-3 mRNA in skeletal muscle and epididymal fat are shown in Fig. 6, D and E, respectively. The fibrate treatment increased UCP-3 mRNA expression in skeletal muscles in both N and F rats but not in epididymal fat tissues.

DISCUSSION

In the present study, we demonstrated that the expression of PPARα was downregulated in the liver of F rats, whereas the expression of SREBP-1 was upregulated. A PPARα activator, fenofibrate, increased the protein content of PPARα and its target gene expression, such as the enzymes regulating β-oxidation. The fenofibrate treatment improved metabolic derangements induced by a diet high in fructose without affecting the SREBP-1 expression in liver. Consistent with the activation of PPARα, fenofibrate modified mitochondrial gene expression, such as increases in the mRNA expression of mtTFA, ATPase-6, and UCP-3 as well as the content of...
mitochondrial DNA, suggesting an increase in mitochondrial fatty acid degradation and energy consumption.

It is well documented that the expression of SREBP-1 is regulated by insulin and glucose (7). The findings of the present study demonstrated that a diet high in fructose also induced SREBP-1 gene expression in the liver. Although the diet high in fructose induced a hyperinsulinemic state, the induction of SREBP-1 might not be a secondary phenomenon because, despite normalization of hyperinsulinemia in FF rats, the expression of SREBP-1 was still enhanced in these rats. These findings suggest that the diet high in fructose directly stimulates SREBP-1 expression in the liver. Supporting these observations, we demonstrated that a diet high in fructose suppressed PPARα expression in the liver. Because insulin is reported to negatively regulate PPARα expression (35), it is possible that hyperinsulinemia partly contributes to the decreased expression of PPARα. However, we also found that the PPARα expression in primary cultured hepatocytes incubated in the high-fructose condition was downregulated, indicating a direct effect of fructose or its metabolites on PPARα expression. The activation of PPARα induces peroxisomal and mitochondrial β-oxidation (6). Therefore, decreased PPARα activity may lead to the reduction of lipid oxidation, resulting in lipid accumulation in the cells. In fact, the PPARα knockout mouse clearly demonstrates that the absence of PPARα activity decreases β-oxidation and then accumulates intracellular lipids in the liver (18). These findings are compatible with the present finding that F rats showed decreased mRNA expression of CPT I, which regulates the entry flux of fatty acids into mitochondria but not mRNA expression of ACO, which regulates peroxisomal β-oxidation.

The reduced expression of PPARα in the liver observed in the F rats promotes the idea that stimulation of fatty acid oxidation through the activation of PPARα reverses the abnormalities found in F rats. As expected, the fibrate treatment not only improved the hypertriglyceridemia in F rats but also increased the mRNA expression of fatty acid oxidation enzymes and the protein level of PPARα in the liver. Although the exact mechanism was not clear, the mRNA expression of PPARα is directly activated by fibrate treatment. These findings are compatible with the findings of the present study that fructose suppressed PPARα expression in the liver. Additionally, the observation that PPARα expression is decreased in the liver of F rats is consistent with the finding that fibrate treatment increased PPARα expression in the liver. These results suggest that fructose decreases PPARα expression in the liver, which may lead to decreased β-oxidation and increased lipid accumulation in the liver. These findings are consistent with the observation that fructose suppresses PPARα expression in the liver, which may lead to decreased β-oxidation and increased lipid accumulation in the liver.

Fig. 4. Amount of mRNA for PPARα (A) and SREBP-1 (B) in rat hepatocytes incubated with glucose or fructose. Hepatocytes were isolated from rat livers and incubated for 16 h with DMEM. Cells were then incubated for 6 h in medium containing the indicated concentrations of glucose (G) and fructose (F). C, control. Values are shown as means ± SE (n = 3). All values are normalized against the expression of 18S ribosomal RNA. *P < 0.05 vs. N rats.
of PPARα was not affected, but its protein level was increased by the administration of fenofibrate. One possible mechanism is that the ligand of PPARα might stabilize the PPARα protein, resulting in accumulation of PPARα protein. In contrast to the effect of PPARα activation on fatty acid oxidation enzyme expression, fibrate treatment did not affect the mRNA expression of SREBP-1 and other lipogenic enzymes in the liver. Thus, fibrate treatment accelerated the fatty acid oxidation enzyme gene expression, resulting in overcoming the lipogenic gene expression in the liver of F rats. As a result, fatty liver induced by fructose feeding was normalized by fenofibrate administration (Fig. 1). A similar improvement of fatty liver by fenofibrate treatment has also been reported in another animal model (39).

Fenofibrate induced mitochondrial gene expression, such as mtTFA, a mitochondrial-encoded gene (ATPase-6), and the copy number of mitochondrial DNA. This is the first study to report that activation of PPARα increases mtTFA expression. mtTFA is the only known transcription factor in mitochondria (26) and is required for both transcription of mitochondrial DNA and duplication of mitochondrial DNA (17). Therefore, the increase in mtTFA expression may be a key factor for increased expression of mitochondrial DNA and the mitochondrial DNA copy number observed in fibrate-treated rats. Recently, Wu et al. (44) reported that PGC-1 increases mtTFA expression and the number of mitochondria in fat cells. It has been reported that PPARα also can bind to PGC-1 (41). This is compatible with our findings that increases in mtTFA gene expression and mitochondrial gene expression are seen in the liver of fibrate-treated rats. Furthermore, we also found the presence of an unknown mechanism, that fructose diet induced the mitochondrial gene expression without changes in the gene expression of mtTFA, as shown in Fig. 5, A-C. This unknown mechanism might explain the difference between mitochondrial DNA contents and nuclear-encoded mitochondrial protein or enzyme expression levels.

In concert with elevated mitochondrial gene expression, we found that expression of UCP-3 was also markedly induced in the liver of F rats after the fibrate treatment. It was reported that UCP-3 is expressed only in skeletal muscle and adipose tissue and not in liver (49). Its expression is influenced by fasting and serum levels of thyroid hormone and fatty acids (9), suggesting that UCP-3 gene expression is increased under conditions of increased fatty acid influx and metabolism. Although these regula-
tions are only known in skeletal muscle, the findings of the present study suggest that UCP-3 is markedly induced in the liver to the same level of UCP-2 by fenofibrate treatment. Further studies will be needed to clarify the molecular mechanism of UCP-3 expression and its physiological significance in the liver. The fenofibrate treatment improved not only hyperlipidemia but also insulin sensitivity in F rats. Because the systemic glucose disposal rate is determined by skeletal muscle, the fenofibrate treatment may have an effect on skeletal muscle and liver. Ye et al. (48) showed that the insulin-stimulated glucose disposal rate of skeletal muscle is inversely correlated with the content of long-chain acyl-CoAs and with plasma triglyceride levels in high fat-fed rats. Although the mechanisms of insulin resistance in F rats have not been fully understood, improvement of hyperlipidemia after fenofibrate treatment may be partly associated with the improvement of insulin sensitivity. Furthermore, fenofibrate treatment also enhanced the expression of UCP-3 in skeletal muscle but not in epididymal fat in the present study (Fig. 6, D and E). These findings are consistent with the previous observations that there is a positive correlation between UCP-3 expression and the insulin-mediated glucose utilization rate (16), suggesting that the up-regulation of UCP-3 in skeletal muscle by fenofibrate may also contribute to the improved insulin sensitivity in the F rats. Furthermore, our preliminary study showed that the mRNA expression of phosphoenolpyruvate carboxykinase (PEPCK), a rate-limiting enzyme of gluconeogenesis, was decreased in the fructose-fed rats compared with control rats, and fenofibrate treatment did not change the mRNA expression of PEPCK in control rats (data not shown). These results suggest that the improvement of insu-

Fig. 6. Northern blot analyses of the mRNA content of uncoupling protein (UCP)-2 (A), UCP-3 in liver (B), UCP-3 in skeletal muscle (D), and UCP-3 in epididymal fat (E). Total RNA was isolated from the liver, skeletal muscle, and epididymal fat of N, NF, F, and FF rats. Values are shown as means ± SE (n = 3). All values are normalized against the expression of 18S ribosomal RNA. *P < 0.05 and ‡P < 0.001 vs. N rats. C: representative Western blot analysis of UCP-3 of 3 separate samples in each group.
lin sensitivity by fenofibrate treatment may be independent of the regulation of hepatic glucose production.

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