Effects of Pioglitazone on Increases in Visceral Fat Accumulation and Oxidative Stress in Spontaneously Hypertensive Hyperlipidemic Rats Fed a High-Fat Diet and Sucrose Solution

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Abstract. We examined oxidative stress and metabolic characteristics of the spontaneously hypertensive hyperlipidemic rat (SHHR) when it was fed a high-fat diet and sucrose solution (HFDS) after N\textsuperscript{G}-nitro-L-arginine methyl ester ingestion to develop a rat model of metabolic syndrome. This study was carried out to assess the effects of pioglitazone on levels of lipid peroxide (LPO), Cu,Zn superoxide dismutase (Cu,Zn-SOD), catalase (CAT), glutathione peroxidase (GPx), and non-esterified fatty acids (NEFA) in the plasma and liver tissue in HFDS-SHHR compared with Sprague-Dawley rats (SD). In the HFDS-treated groups, levels of LPO, CAT, GPx, and NEFA were elevated and levels of Cu,Zn-SOD were reduced in the plasma and liver tissue, with a marked accumulation of visceral fat. The changes induced by HFDS feeding were severe in the SHHR model that had essential hypertension and hyperlipidemia, when compared with SD that did not have these essential risk factors. Subcutaneous injection of 10 mg/kg per day of pioglitazone for 2 months significantly restored levels of LPO, CAT, GPx, Cu,Zn-SOD, and NEFA in the HFDS-SHHR group, and visceral fat accumulation was reduced. These results suggest that HFDS-SHHR is a suitable model of metabolic syndrome and that pioglitazone treatment can improve oxidative dysregulation in this rat model.

Keywords: visceral fat accumulation, metabolic syndrome model, oxidative stress, pioglitazone, anti-oxidant enzyme

Introduction

Metabolic syndrome is a cluster of several risk factors for cardiovascular disease and diabetes, and it is associated with insulin resistance, obesity, dyslipidemia, inflammation, endothelial dysfunction, and hypertension (1 – 4). Global lifestyle changes, including an increase in the caloric intake and a reduction in physical activity, have increased the number of patients with metabolic syndrome, and their chance of a healthy life has been compromised; this is apparent even in younger people (5, 6). The aforementioned metabolic risk factors have complex pathophysiological relationships and the key role of each risk factor in the progression of cardiovascular disease has been debated. In 2005, new definitions for metabolic syndrome were proposed by the American Heart Association/the National Heart, Lung, and Blood Institute (AHA/NHLBI) and by a committee representing eight scientific societies in Japan (7, 8). Moreover, the International Diabetes Federation (IDF) has also proposed a new definition for the clinical diagnosis of metabolic syndrome (9). All of the newer definitions of metabolic syndrome are consistent in that the accumulation of visceral fat is a common factor and that adipocyte biology is considered to be highly important (8, 10).

To date, there is no suitable animal model of metabolic syndrome. Kumai et al. demonstrated that sponta-
neously hypertensive hyperlipidemic rats (SHHR) treated with \(N^\infty\)-nitro-L-arginine methyl ester (L-NAME) and a high-fat diet showed a marked deposition of lipids and thickening of the endothelium of their aortas (11). We have previously reported that SHHR has a systolic blood pressure that is higher than 150 mmHg, a plasma cholesterol concentration that is greater than 150 mg/dl, and apparent hyperfibrinogenemia (12, 13).

In this study, we have attempted to develop a rat model of metabolic syndrome with an accumulation of visceral fat by feeding the SHHR a high-fat diet and sucrose solution (HFDS) after L-NAME ingestion to inactivate nitric oxide (NO). Metabolic syndrome is accelerated by atherosclerosis derived from vascular endothelial injury, and NO inactivation may contribute to the initiation and progression of atherosclerosis (11, 14). So, we pretreated the SD (Sprague-Dawley) rats and SHHR with L-NAME before HFDS treatment and evaluated the pathophysiological features under the combination of hyperlipidemia, hypertension, and abnormal glucose metabolism.

It is inevitable that metabolic syndrome exacerbates oxidative stress and leads to cardiovascular disease due to insulin resistance and endothelial dysfunction (14). Many studies have determined the effects of oxidative stress in animal models with a single risk factor such as hypertension, dyslipidemia, diabetes, or obesity (15–17). However, few studies have used complex animal models with multiple essential risk factors including a combination of hypertension, hyperlipidemia, and obesity. In this study, we aimed to develop a suitable animal model of metabolic syndrome and to study oxidative stress and the anti-oxidative levels in the HFDS-SHHR as a model of metabolic syndrome.

The thiazolidinedione pioglitazone is a high-affinity ligand for the peroxisome proliferator-activated receptor-\(\gamma\) (PPAR\(\gamma\)), and it is a member of a new class of anti-diabetic drug that increases sensitivity towards insulin (18). It was thought that PPAR\(\gamma\) was only associated with metabolic diseases such as obesity, diabetes, and atherosclerosis, but now it is thought to play a role in vascular pathophysiology (19). El Midaoui et al. reported that increases in blood pressure, aortic \(O_2^\cdot\) production, blood glucose, or insulin levels and in insulin resistance were found in aortic and heart tissues in chronically glucose-fed rats (20). In addition, chronic treatment with pioglitazone prevented the marked increase in \(O_2^\cdot\) production in cultured aortic smooth muscle cells that were chronically treated with high insulin with or without high glucose levels (20). Other studies have shown that pioglitazone can relieve oxidative stress in diet-induced obese rats and alloxan-induced diabetic rabbits (21, 22). We postulated that feeding SHHR with HFDS is a suitable model of metabolic syndrome that involves a mild increase in blood pressure and plasma lipids, abnormalities in glucose tolerance, and an increase in oxidative stress. Therefore, we examined lipid peroxide (LPO) as the indicator of oxidative stress, Cu,Zn superoxide dismutase (Cu,Zn-SOD) content, and the activities of catalase (CAT) and glutathione peroxidase (GPx) as anti-oxidative enzymes in plasma and liver tissue in the HFDS-SHHR and SD rats to indicate whether HFDS-SHHR is a valid model of metabolic syndrome. In addition, the effect of pioglitazone against oxidative stress in HFDS-SHHR was investigated to assess the effectiveness of pioglitazone as a therapeutic agent for metabolic syndrome.

Materials and Methods

Animals and experimental groups

Seven-week-old male spontaneously hyperlipidemic rat (HLR) that originated from SD and female spontaneously hypertensive rat (SHR) that originated from Wister Kyoto rats (WKY) were interbred. Selected siblings were inbred to obtain offsprings with both high blood pressure (>150 mmHg) and high plasma cholesterol (>150 mg/dl); SHHR were generated in the breeding colony by brother-sister mating (12). In this study, four-month-old male SHHR and SD were used, both of which were divided into three groups: a control group fed on a regular diet (CE2; CLEA Japan, Tokyo), a high-fat diet and sucrose fed group (HFDS), and a pioglitazone-treated HFDS group. We used SD related to HLR with a genetic hyperlipidemic factor because the accumulation of visceral fats was one of the higher risk factors of metabolic syndrome. The composition of the regular diet was 8.9% water, 25.4% protein, 4.4% fat, 4.1% fiber, 6.9% carbohydrate, and 50.3% nitrogen-free extracts; the energy was 342.2 kcal/100 g. The high-fat diet (HFD) consisted of 8.2% water, 23.4% protein, 11.0% fat, 3.8% fiber, 6.3% carbohydrate, and 46.3% nitrogen-free extracts; the energy was 378.0 kcal/100 g. Each group contained 6 to 10 rats. Until the age of 4 months, the regular diet was available to all groups ad libitum. The animals except the SD-control and SHHR-control were administered \(N^\infty\)-nitro-L-arginine methyl ester (L-NAME; Nacalai Tesque Co., Ltd., Kyoto) in drinking water (100 mg/L) for 1 month, and then fed HFD with 15% sucrose solution ad libitum for 2 months from 5 to 7 months of age. The PPAR\(\gamma\)-specific ligand pioglitazone was generously provided by Takeda Pharmaceutical Co., Ltd. (Osaka). Our preliminary study showed no apparent changes of oxidative stress after 3 mg/kg per day of pioglitazone, which was equivalent to the human dose. Then subcutaneous injection of
10 mg/kg per day of pioglitazone was co-administered during 2 months on the HFDS to half the number of rats (pioglitazone-treated HFDS group). In the present experiments, we determined systolic blood pressure of SHHR and confirmed that blood pressure was over 150 mmHg before sacrificing the animals using the tail-cuff method (PS-100; Riken Kaibatsu, Tokyo). All studies were conducted according to the “Guiding Principles for the Care and Use of Laboratory Animals” of The Japanese Pharmacological Society. The rats were housed in a semi-barrier system under controlled room temperature (23 ± 1°C), humidity (55 ± 5%), and lighting (lights on from 6 a.m. to 6 p.m.). The experimental groups were 6 in total: SD-control, HFDS-SD, pioglitazone-treated HFDS-SD, SHHR-control, HFDS-SHHR, and pioglitazone-treated HFDS-SHHR.

Preparation and biochemical determination of plasma and liver tissue samples

Blood specimens were taken from the inferior vena cava under pentobarbital anesthesia (35 mg/kg, intraperitoneally) after an overnight fast, and plasma was obtained from the citrated plasma supernatant. After the whole liver tissue and visceral fat were isolated, liver weight / body weight ratio (LW/BW) and visceral fat weight / body weight ratio (VisF/BW) were calculated. The liver tissue was homogenized in ice-cold solution containing 50 mM Tris-HCl (pH 7.5), 5 mM EDTA, and 1 mM dithiothreitol; and the homogenized samples were used for the determination of LPO, total cholesterol (TC), triglyceride (TG), and non-esterified fatty acid (NEFA) levels. The homogenized samples were then centrifuged (20,000 × g, 4°C, 30 min), and the resultant supernatant were used for the determinations of Cu,Zn-SOD, CAT, and GPx activities.

LPO level in plasma and liver tissue was estimated by the modified version of Yagi’s assay (23) with a commercially available kit (Determiner LPO; Kyowa Medex, Tokyo). Plasma and liver tissue levels of Cu,Zn-SOD were evaluated by a sandwich enzyme-linked immunosorbent assay kit (Amersham Pharmacia, Tokyo). CAT activity was determined with a Catalase Assay Kit (Cayman Chemical Co., Ann Arbor, MI, USA), which is based on the reaction of the enzyme with methanol in the presence of an optimal concentration of H$_2$O$_2$. GPx activity was determined indirectly by a coupled reaction with glutathione reductase using a commercially available kit (Cayman Chemical Co.). NEFA levels in plasma and the liver tissue were measured with a commercially available kit (NEFA C-test; Wako Pure Chemical, Osaka). Plasma and liver levels of TC and TG were determined with a commercially available kit (Cholesterol E-test and Triglyceride E-test, respectively; Wako Pure Chemical). Commercial kits were used to estimate plasma levels of glucose (Glucose CII test, Wako Pure Chemical), alanine aminotransferase (ALT) (Transaminase CII-test, Wako Pure Chemical), and insulin (Lebis Insulin Kit; Shibayagi, Gunma). Plasma level of interleukin-6 (IL-6) was determined by enzyme-linked immunosorbent assay (ELISA kit; Bio Source, Camarillo, CA, USA). Protein level in liver tissues was determined by the Bradford procedure with a Bio-Rad Protein Assay Kit (Bio-Rad Lab., Richmond, CA, USA) using bovine serum albumin as a standard; and liver levels of LPO, Cu,Zn-SOD, CAT, GPx, NEFA, TC, and TG were expressed as ratios to the protein contents.

Statistics

Data are each presented as the mean ± S.E.M. Pairwise comparisons between each group were made by Scheffe’s test following one-way ANOVA for unpaired observation. A P value of <0.05 was considered statistically significant.

Results

Background data in each experimental group

Table 1 shows the effects of pioglitazone on BW, ratios of LW/BW and VisF/BW, and ALT in SD-control, HFDS-SD, and pioglitazone-treated HFDS-SD, SHHR-control, HFDS-SHHR, and pioglitazone-treated HFDS-SHHR. There was no significant change in BW among the SD groups, BW in the SHHR groups was increased by HFDS feeding, and pioglitazone treatment in the HFDS group produced no change. In both the SD and SHHR groups, ratios of LW/BW and VisF/BW were significantly elevated after ingestion of HFDS, and pioglitazone treatment in the HFDS group significantly lowered both ratios. Compared to the HFDS-SD group, there was significant elevation of VisF/BW ratio in the HFDS-SHHR group. Plasma glucose and insulin levels in the SD and SHHR groups tended to increase by HFDS feeding, especially the HFDS-SHHR showed significant increases in these plasma levels compared to the SHHR-control, and pioglitazone treatment decreased plasma glucose and insulin levels significantly compared to the HFDS-SHHR. In the SD groups, plasma level of ALT was elevated by HFDS ingestion, and the elevation of ALT was suppressed by pioglitazone treatment. In the SHHR-control, plasma ALT was higher than that in the SD-control and pioglitazone treatment decreased significantly the elevation of ALT in the SHHR-control and HFDS-SHHR group. The plasma levels of IL-6 tended to increase in HFDS-SHHR and pioglitazone-treated HFDS-SHHR showed a decrease in the levels, but there was no significant change in the plasma levels of IL-6 by
Table 1. Effects of pioglitazone treatment on body weight (BW), ratios of liver weight per BW (LW/BW) and visceral fat weight per BW (VisF/BW), plasma glucose, insulin, ALT, and IL-6 in the SD-control, HFDS-SD, HFDS-SD + pioglitazone, SHHR-control, HFDS-SHHR, and HFDS-SHHR + pioglitazone groups

<table>
<thead>
<tr>
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<th>Control</th>
<th>HFDS</th>
<th>HFDS + Pio</th>
<th>Control</th>
<th>HFDS</th>
<th>HFDS + Pio</th>
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<td>BW (g)</td>
<td>562.7 ± 23.6</td>
<td>585.3 ± 15.5</td>
<td>583.9 ± 22.1</td>
<td>530.5 ± 7.6</td>
<td>596.7 ± 16.3</td>
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<td>LW/BW (×10⁻⁵)</td>
<td>2.5 ± 0.05</td>
<td>6.1 ± 0.16⁺</td>
<td>4.3 ± 0.14⁺</td>
<td>2.9 ± 0.05</td>
<td>6.8 ± 0.15⁺</td>
<td>4.9 ± 0.11⁺</td>
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<tr>
<td>VisF/BW (×10⁻²)</td>
<td>2.3 ± 0.16</td>
<td>3.1 ± 0.15⁺</td>
<td>2.4 ± 0.18⁺</td>
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<td>Glucose (mg/dl)</td>
<td>99.5 ± 7.67</td>
<td>112.9 ± 9.59</td>
<td>99.7 ± 4.23</td>
<td>83.3 ± 16.4</td>
<td>125.7 ± 6.11</td>
<td>89.2 ± 6.94</td>
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<td>Insulin (pg/ml)</td>
<td>1637.1 ± 183.1</td>
<td>2291.5 ± 183.6⁺</td>
<td>1405.2 ± 106.1⁺</td>
<td>434.9 ± 90.2⁺</td>
<td>2141.6 ± 265.0⁺</td>
<td>1266.2 ± 131.1⁺</td>
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<td>ALT (KU)</td>
<td>37.0 ± 3.0</td>
<td>73.3 ± 9.9⁺</td>
<td>35.6 ± 7.7⁺</td>
<td>66.6 ± 9.2⁺</td>
<td>75.1 ± 12.5</td>
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<td>IL-6 (pg/ml)</td>
<td>36.6 ± 9.43</td>
<td>39.0 ± 10.38</td>
<td>46.5 ± 15.20</td>
<td>41.8 ± 14.62</td>
<td>58.7 ± 14.37</td>
<td>30.2 ± 9.58</td>
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Data are each presented as the mean ± S.E.M. ⁺P<0.05 vs SD-control, ⁶P<0.05 vs HFDS-SD, ⁷P<0.05 vs SHHR-control, and ⁸P<0.05 vs HFDS-SHHR. Pio: pioglitazone, ALT: alanine aminotransferase

Table 2. Effects of pioglitazone on plasma and liver tissue levels of total cholesterol (TC) and triglyceride (TG) in the SD-control, HFDS-SD, HFDS-SD + pioglitazone, SHHR-control, HFDS-SHHR, and HFDS-SHHR + pioglitazone groups

<table>
<thead>
<tr>
<th></th>
<th>Control</th>
<th>HFDS</th>
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<tr>
<td>SD</td>
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<td>Plasma</td>
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<tr>
<td>TC (mg/dl)</td>
<td>47.9 ± 3.7</td>
<td>93.9 ± 12.2⁺</td>
<td>124.0 ± 11.1⁺</td>
<td>135.0 ± 3.7⁺</td>
<td>530.0 ± 55.3⁺</td>
<td>472.0 ± 36.0⁺</td>
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<td>TG (mg/dl)</td>
<td>94.0 ± 4.4</td>
<td>78.8 ± 10.8</td>
<td>42.7 ± 4.0⁺</td>
<td>87.3 ± 5.4</td>
<td>91.9 ± 7.2</td>
<td>66.1 ± 3.9⁺</td>
</tr>
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</table>

Data are each presented as the mean ± S.E.M. ⁺P<0.05 vs SD-control, ⁶P<0.05 vs HFDS-SD, ⁷P<0.05 vs SHHR-control, and ⁸P<0.05 vs HFDS-SHHR. Pio: pioglitazone

HFDS ingestion and pioglitazone treatment.

Table 2 shows the effects of pioglitazone on plasma and liver tissue levels of TC and TG in the SD-control, HFDS-SD, pioglitazone-treated HFDS-SD, SHHR-control, HFDS-SHHR, and pioglitazone-treated HFDS-SHHR groups. Plasma and liver tissue levels of TC were elevated in the HFDS-SD and SHHR groups, and the elevation induced by HFDS feeding was significantly higher in the HFDS-SHHR group than that in the HFDS-SD group. Pioglitazone treatment in the HFDS-SHHR group suppressed TC elevation in liver tissues. Plasma levels of TG showed no significant changes in SD-control, HFDS-SD, SHHR-control, and HFDS-SHHR, but the pioglitazone-treated group showed significantly lower level of plasma TG than that in each HFDS-fed group. Liver tissue levels of TG in the SD- and SHHR-control groups were significantly elevated by HFDS feeding, although the level in the SHHR-control group was lower than that in the SD-control group, while the elevation in the HFDS-SHHR group was suppressed by pioglitazone treatment. Figure 1 shows the effects of pioglitazone on NEFA in the SD-control, HFDS-SD, pioglitazone-treated HFDS-SD, SHHR-control, HFDS-SHHR, and pioglitazone-treated HFDS-SHHR groups. Plasma level of NEFA was lower in the SHHR-control group compared to the SD-control group and was significantly higher in the HFDS-SHHR group than that in the SHHR-control group. In the pioglitazone-treated HFDS-SHHR group, plasma level of NEFA was suppressed to the same level as the SHHR-control group. Liver tissue level of NEFA was also lowered in the SHHR-control group compared with the SD- and SHHR-control groups. The increase in liver tissue level of NEFA was significantly higher in the HFDS-SHHR group than the HFDS-SD group and these increases were suppressed significantly by pioglitazone treatment.

Oxidative stress and anti-oxidative enzymes in each experimental group

To determine oxidative stress, plasma and liver tissue
LPO levels were examined in each experimental group. Figure 2 shows the effects of pioglitazone on plasma and liver tissue LPO in the SD-control, HFDS-SD, pioglitazone-treated HFDS-SD, SHHR-control, HFDS-SHHR, and pioglitazone-treated HFDS-SHHR groups. Plasma level of LPO was significantly elevated in the HFDS group, and the elevation was more marked in the HFDS-SHHR than the HFDS-SD group. In both HFDS groups, LPO level was significantly reduced by pioglitazone treatment. Liver tissue level of LPO was also elevated by HFDS feeding and the elevation was more marked in the HFDS-SHHR than the HFDS-SD group. In contrast to plasma level, LPO level in liver tissue tended to be more elevated by pioglitazone treatment in both HFDS fed rats.

Cu,Zn-SOD content and CAT and GPx activities were measured as main anti-oxidative markers. Figure 3 shows the effects of pioglitazone on Cu,Zn-SOD levels in plasma and liver tissues in the SD-control, HFDS-SD, pioglitazone-treated HFDS-SD, SHHR-control, HFDS-SHHR, and pioglitazone-treated HFDS-SHHR groups. Plasma level of Cu,Zn-SOD tended to decrease by HFDS feeding, and the decrease in the Cu,Zn-SOD content in the HFDS-SHHR group was more than that in the HFDS-SD group. In the pioglitazone-treated HFDS-SHHR group, plasma Cu,Zn-SOD level was increased compared to that in the HFDS-SHHR group. Liver tissue level of Cu,Zn-SOD was significantly reduced by HFDS feeding, and it was significantly recovered to the level in the SHHR-control group by pioglitazone treatment. Liver tissue CAT activity was significantly higher in the HFDS-SHHR group compared to the SD-control and HFDS-SD groups and the elevation in the HFDS-SHHR group was relieved by pioglitazone treatment. Liver tissue CAT activity was significantly higher in the HFDS-SHHR group compared to the
HFDS-SD group. Figure 5 shows the effects of pioglitazone on GPx activity in SD-control, HFDS-SD, pioglitazone-treated HFDS-SD, SHHR control, HFDS-SHHR, and pioglitazone-treated HFDS-SHHR groups. Plasma level of GPx activity was obviously elevated in HFDS-SHHR compared with that in the SHHR-control and HFDS-SD, and it was significantly decreased in the pioglitazone-treated HFDS-SHHR. GPx activity in liver tissue was increased in both of the HFDS groups compared with that of each control, especially in the HFDS-SHHR group, and pioglitazone treatment relieved the elevation in the HFDS-SHHR group.

Discussion

In this study, HFDS-SHHR was found to have significant increases in VisF/BW ratio, plasma and liver contents of LPO, levels of plasma and liver TC, and liver NEFA compared to those in HFDS-SD. Therefore, HFDS-SHHR is a suitable rat model of the metabolic syndrome as it relates to oxidative stress combined with mild hypertension, hyperlipidemia, and a marked accumulation of visceral fat. In addition, we have shown that the PPARγ agonist pioglitazone could be a therapeutic agent for metabolic syndrome due to the ameliorating effects on oxidative stress and abnormal lipid and glucose metabolism.

Metabolic syndrome is a cluster of many risk factors that increase the likelihood of cardiovascular diseases. However, the way that metabolic syndrome progresses is not clear at the moment. Therefore, an appropriate animal model is required to study these mechanisms and/or to assess the efficacy of new therapies for metabolic syndrome.

The accumulation of visceral fat is the most important cause of metabolic syndrome, and excess adipose tissue reduces insulin sensitivity in metabolically responsive tissues (24). These changes are frequently associated with a cluster of cardiovascular risk factors including insulin resistance, dyslipidemia, inflammation, endothelial dysfunction, and hypertension (25, 26). We observed that the ratio of visceral fat weight to body weight was significantly elevated in the HFDS-SHHR group (+68%) compared with the HFDS-SD group, although marked visceral fat accumulation was also observed in the HFDS-SD group. These results suggest
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that the genetic predisposition of these rats to hypertension and dyslipidemia more readily causes the accumulation of visceral fat due to HFDS in the SHHR group compared with the SD group. Since visceral fat tissue secretes various physiologically active substances, we determined plasma IL-6 levels, but there was no significant increase in the HFDS-SHHR compared to the SHHR-control and HFDS-SD. Amamoto et al. reported that Wistar rats that ingested a 15% sucrose solution for 10 weeks showed a significant increase in visceral fat weight with increases in plasma blood glucose, insulin, leptin, and adiponectin, but not plasma TNF-α and IL-6 (27). Therefore, a further experiment to determine other adipocytokines is necessary.

In the HFDS-fed groups, an elevation in plasma ALT levels accompanied by a marked lipid deposition in the liver tissue was observed. There is now convincing evidence that nonalcoholic fatty liver disease is a component of metabolic syndrome and that the chronic escalation of serum ALT levels is an independent risk factor for nonalcoholic fatty liver disease (28, 29). Our results showing the increase in plasma ALT levels in the HFDS-SD and HFDS-SHHR groups agree with this concept. Moreover, NEFA derived from adipose tissue has been shown to circulate at higher concentrations in patients with nonalcoholic fatty liver disease, and even more importantly, serum levels of NEFA correlate with the severity of nonalcoholic fatty liver disease (30). In this study, plasma and liver levels of NEFA and liver levels of TG in the HFDS-SHHR group were significantly elevated compared with the SHHR-control, but NEFA levels in the plasma and liver and liver levels of TG in the SHHR-control group were lower in comparison with those in the SD-control group. Our previous study reported that plasma levels of TG in groups of animals fed a normal diet were lower in SHHR, WKY, and SHR than they were in SD and HLR, and these results also indicated that the fasting TG level in SHR tended to decrease in comparison with WKY (13). From these results, it was considered that an underlying genetic factor was responsible for the lower fasting plasma TG levels in SHR and SHHR. Van Oostrom et al. indicated that subjects with metabolic syndrome show postprandial increases in plasma levels of TG and NEFA compared with subjects without metabolic syndrome and that the fasting plasma level of NEFA showed no significant change between subjects who had metabolic syndrome and those who did not (31). Thus, plasma levels of TG and NEFA can be easily influenced by fasting. Following overnight fasting, the HFDS-SHHR group showed a greater increase in the level of NEFA in the liver tissue compared with the HFDS-SD group. It was thought that the level of NEFA increased in the liver because of the enhanced flux of NEFA from the adipose tissue to the liver, as shown in patients with metabolic syndrome (32, 33). Plasma and liver levels of TC in the HFDS-SHHR group were elevated significantly when compared with those in the HFDS-SD group after overnight fasting. These findings suggest that HFDS-SHHR accompanied by essential hypertension, hyperlipidemia, and the marked accumulation of visceral fat showed a close resemblance to familial dyslipidemic hypertension that contributes to a high risk of early coronary artery disease. Therefore, HFDS-SHHR is considered to be a valuable model of metabolic syndrome (34).

It is well known that oxidative stress is associated with metabolic syndrome and is related to the accumulation of visceral fat and nonalcoholic fatty liver disease (35, 36). Oxidative stress impairs insulin secretion from pancreatic β cells and glucose transport in the muscle and adipose tissue, and it leads to hypertension caused by endothelial dysfunction (37). Roberts et al. suggested that the diet-induced metabolic syndrome in rat models showed an increase in levels of oxidative stress in their aortas and kidneys and that the oxidative stress can
SOD catalyzes the reaction $\text{O}_2^-$ reduced to water, principally by CAT and GPx. Cu,Zn-reducing it to hydrogen peroxide, which is easily a defense mechanism by intercepting superoxide and $\text{O}_2^-$ species and result from the excessive production of reactive oxygen species (38, 39). Three anti-oxidative markers, Cu,Zn-SOD, CAT, and GPx, have been established and utilized in the evaluation of oxidative stress in animal models with hypertension, hyperlipidemia, obesity, and diabetes, but the interplay between them varies. In addition, there are no studies that have examined oxidative stress in the essentially hypertensive, hyperlipidemic rat model of metabolic syndrome, so we evaluated levels of Cu,Zn-SOD, CAT, and GPx as anti-oxidative enzymes and LPO as an oxidative stress marker in the plasma and liver tissue of the HFDS-SHHR as a model of metabolic syndrome. The results showed that the HFDS-SHHR group developed marked levels of oxidative stress as shown by the significant increase in the levels of LPO in the plasma and liver tissue when compared with the SHHR-cont and HFDS-SD groups. The marked increase in the production of LPO could be due to $\text{O}_2^-$ overload, indicating the presence of oxidative stress and a subsequent increase in the production of hydrogen peroxide (40). In this model, the animals were treated by giving them $\text{L-NAME}$ in drinking water for 1 month to reduce NO production and to increase oxidative stress for the preparation of metabolic syndrome. Therefore, we should be careful to evaluate effects of treatment for metabolic syndrome by enhancing NOS activity in the L-NAME and HFDS-treated SHHR.

Cu,Zn-SOD plays an important role in the oxygen defense mechanism by intercepting superoxide and reducing it to hydrogen peroxide, which is easily reduced to water, principally by CAT and GPx. Cu,Zn-SOD catalyzes the reaction $\text{O}_2^- + \text{O}_2^- + 2\text{H}^+ \rightarrow \text{H}_2\text{O}_2 + \text{O}_2$; and in the second step, the $\text{H}_2\text{O}_2$ is converted to $\text{H}_2\text{O}$ by CAT and/or GPx. The scavenging of $\text{O}_2^-$ by Cu,Zn-SOD protects liver cells against its deleterious effect. As shown in the present study, levels of Cu,Zn-SOD in the plasma and liver tissue were significantly decreased in the HFDS-fed groups. These decreases in the levels of Cu,Zn-SOD were marked in the plasma of the HFDS-SHHR group, although liver levels of Cu,Zn-SOD were similar in both the SD and SHHR groups. The reason for the decrease in the plasma and liver levels of Cu,Zn-SOD in the groups fed HFDS are now unknown. In HFDS-SHHR, it is possible that the hydrogen peroxide generated from $\text{O}_2^-$ directly reduces levels of Cu,Zn-SOD (41) or glomerular permeability of Cu,Zn-SOD may increase since HFDS-SHHR showed the severe glomerular injury by pathological study (data not shown). In addition, the consumptive decrease of the plasma and liver Cu,Zn-SOD against the increased oxidative stress in the groups fed HFDS might have occurred, but the concentration of Cu,Zn-SOD did not correlate with its enzymatic activity (42); therefore, the detailed mechanism of the decrease in the Cu,Zn-SOD level in HFDS groups cannot be explained from our results.

Liver CAT activity was significantly increased in the HFDS-SHHR group, and GPx levels in the plasma and liver tissues were elevated in the HFDS-SHHR group when compared with the SHHR-control group. Two reasons for these increases can be considered: degradation of the increased hydrogen peroxide generated from $\text{O}_2^-$ and/or inhibition of the marked LPO increase in the plasma and liver tissue. Furukawa et al. determined the activities of the anti-oxidative enzymes, Cu,Zn-SOD, GPx, and CAT in the white adipose, liver, and muscle tissues from the mouse model of metabolic syndrome; and they showed that the levels of these enzymes differed depending on which tissue they were measured in (43). Our data showing the decrease in the level of Cu,Zn-SOD and the increase in the activities of CAT and GPx in the liver tissues were consistent with their results. It is difficult to evaluate the association and activities of these enzymes in metabolic syndrome because each risk factor that is involved in the syndrome is complex, and they are influenced by age and tissue type (43, 44). Abdilla et al. indicated that the contribution of the separate components of metabolic syndrome to the burden of oxidative stress generated by hypertension was minimal (45). However, we have shown that in SHHR, feeding with HFDS tended to accelerate changes in the three aforementioned anti-oxidative enzymes and this elevated the level of oxidative stress.

PPARγ is strongly expressed in adipose tissue where it plays a key role in adipose tissue function. Indeed, a mutation in PPARγ2 causes human obesity, so it is important for adipocyte differentiation and insulin action (46). Pioglitazone as an agonist of PPARγ is widely used in the treatment of type 2 diabetes due to its insulin-sensitizing properties, and now it is hoped that it will be a suitable therapeutic agent for metabolic syndrome. In the present study, we investigated the effect of pioglitazone on the metabolic characteristics and oxidative stress in HFDS-SHHR with marked visceral fat accumulation. This study showed that pioglitazone significantly suppressed the increases in visceral fat accumulation caused by HFDS ingestion in both SD and SHHR groups. Miyazaki et al. examined the effect of pioglitazone on abdominal fat distribution in patients with type 2 diabetes mellitus, and their results reflected those of the present study using HFDS-SHHR (47). Our data also showed that the elevation of NEFA and TG levels in the plasma and liver in the HFDS-SHHR group was suppressed by pioglitazone treatment.
It is thought that fasting hyperlipidemia is regulated by an increase in NEFA fluxes from adipose tissue (31). Therefore, we suggest that the impact of pioglitazone on TG and NEFA levels in the HFDS-SHHR group was due to improvements in the accumulation of visceral fat.

Pioglitazone treatment tempered the elevation of plasma LPO as an indicator of oxidative stress, but hepatic LPO levels tended to be higher in both pioglitazone-treated groups. Memon et al. suggested PPAR\(\gamma\) expression is up-regulated in the livers of genetically obese mice and that thiazolidinediones induce several PPAR\(\gamma\) target genes that are involved in lipid uptake and storage in the liver (48). Therefore, the cause of the elevation in liver LPO levels in the pioglitazone-treated groups could be due to the pioglitazone-activated uptake of plasma LPO by the liver. Changes in the levels of the anti-oxidative enzymes in the plasma were also tempered by pioglitazone treatment, especially in the SHHR groups. Recent studies indicated that the higher expression of PPAR\(\gamma2\) could constitute an important part of the mechanism underlying the adipogenic effect of a high-fat diet and the expression was enhanced by adipogenesis (49, 50). Since the increased accumulation of visceral fat was observed in the HFDS-SHHR group more than in the HFDS-SD group, it was postulated that PPAR\(\gamma2\) expression was increased in that HFDS-SHHR group. Therefore, more significant effects of pioglitazone in the HFDS-SHHR group were considered compared with those in the HFDS-SD group. Similarly in liver tissue, the anti-oxidative and the dyslipidemic changes caused by HFDS feeding were significantly improved in the pioglitazone-treated HFDS-SHHR group. Vidal-Puig et al. revealed that a high-fat diet increases the level of PPAR\(\gamma2\) mRNA expression in the liver, and Zhang et al. indicated that elevated rates of hepatic lipogenesis were directly linked to increased levels of hepatic expression of PPAR\(\gamma2\) (50, 51). In our study, the liver tissue levels of TC and NEFA in the HFDS-SHHR group were higher than those in the HFDS-SD group. Therefore, it was considered that in the livers from the HFDS-SHHR group, PPAR\(\gamma2\) was more strongly expressed than it was in the HFDS-SD group and thus significant effects of pioglitazone were produced.

In conclusion, the results obtained in the present investigation suggest that HFDS-SHHR is a suitable model of metabolic syndrome especially in relation to the development of oxidative stress demonstrated by the increase in levels of LPO in the plasma and liver tissue and the changes in levels of the anti-oxidative enzymes accompanied by the marked accumulation of visceral fat and increase in NEFA. In addition, pioglitazone treatment significantly restored oxidative stress regula-

References


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