Sucrose and a High-Fat Diet Enhance Visceral Fat Tissue Gain and Lipid Deposition in the Aorta of Spontaneously Hypertensive Hyperlipidemic Rats

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Abstract

Hyperlipidemia, hyperglycemia, and hypertension can increase the risk of cardiovascular disease. We established a combined hyperlipidemia, hypertension, and hyperglycemia animal model and investigated the effects of sucrose (Suc) and a high-fat diet (HFD) on adipose tissue and lipid deposition in the aorta. Four-month-old male spontaneously hypertensive hyperlipidemic rats (SHHR) and Sprague-Dawley rats (SD) were administered N⁰-nitro-L-arginine-methyl ester for 1 month from 4 months of age and then fed the HFD + Suc for 2 months. Plasma glucose, insulin, and cholesterol levels were measured. Visceral fat weight and lipid deposition in aorta were also measured. Plasma glucose and insulin levels in the Suc + HFD groups of SD and SHHR were significantly increased compared with those in the control and HFD-alone groups of SD and SHHR. The plasma total cholesterol level in the Suc + HFD-fed SHHR group was also significantly increased compared with that in control SHHR. The visceral fat tissue weight in the Suc + HFD-fed SHHR was significantly increased over that in control SHHR. Marked lipid deposition in the aortic endothelium in Suc + HFD-fed SHHR was observed. These results suggest that feeding SHHR with Suc and the HFD is associated with visceral fat gain and marked lipid deposition in the aorta.

Key words

hyperglycemia, hyperlipidemia, hypertension, metabolic syndrome, rats

Introduction

Hyperlipidemia, hypertension, and hyperglycemia are important underlying causes of cardiovascular disease. Animal models of disease are widely used in cardiovascular research. Spontaneously hypertensive rats (SHR) are commonly used as an animal model of hypertension, and Watanabe heritable hyperlipidemic rabbits and apoE knockout mice are accepted animal models of hyperlipidemia. However, there is no animal model combining hypertension, hyperlipidemia, and hyperglycemia.

Lifestyle-related diseases in humans are increasing along with the incidence of hyperlipidemia, hypertension, and diabetes. We previously developed a spontaneously hypertensive hyperlipidemic rat (SHHR) model⁶. In addition, feeding with nitric oxide synthase (NOS) inhibitor and a high-fat diet (HFD) results in lipid deposition in the aorta of SHHR over a short term⁵. A HFD and sucrose (Suc) are major factors in the development of hyperglycemia, insulin resistance, and visceral obesity⁸. A HFD and high Suc intake also contribute to the development of glucose intolerance and hyper-

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lipidemia⁴,⁵. In particular, a Suc-rich diet induces glucose tolerance associated with hyperinsulinemia, changes similar to those seen in type 2 diabetes mellitus⁶. In this study, we first established an animal model combining hypertension, hyperlipidemia, and hyperglycemia and then investigated the effects of Suc and a HFD on adipose tissue and lipid deposition in the aorta of SHHR and Sprague-Dawley rats (SD).

Materials and Methods

Animals and experimental protocols

Four-month-old male SHHR and SD were used. SD rats were purchased from Nihon SLC Co. Ltd. (Shizuoka). SHHR was developed in our laboratory⁷. All studies were performed according to the Guiding Principles for the Care and Use of Laboratory Animals of the Japanese Pharmacological Society. The rats were housed in a semibarrier system under controlled room temperature (23 ± 1°C), humidity (55 ± 5%), and lighting (light from 06:00 to 18:00). The NOS inhibitor N⁰-nitro-L-arginine methyl ester (L-NAME, 100 mg/l in drinking water, Nakarai Tesque Co. Ltd., Kyoto, Japan) was administered for 1 month from 4 months of age. After the administration of L-NAME, the animals of HFD groups were placed on a HFD (2% cholesterol, 1% cholic acid, 5% coconut oil, CLEA, Tokyo, Japan) for 2 months from 5 months of age. The animals of Suc + HFD groups were placed on a HFD with 30% Suc (in drinking water) for 2 months from 5 months of age. The concentration of 30% Suc was selected based on the report by Amamoto et al.⁸. At the end of the experimental period, the animals were killed by cervical dislocation under pentobarbital anesthesia (35 mg/kg, i.p.).

Systolic blood pressure

Systolic blood pressure (SBP) was measured using the tail-cuff method (PS-100, Riken Kaihatsu Co., Tokyo, Japan) in conscious rats placed on a hotplate (37°C). Six to seven SBP readings were obtained for each rat and the results were averaged.

Plasma glucose level

Animals were fasted for 12 h before blood collection. Blood was collected from the abdominal aorta under pentobarbital anesthesia to obtain plasma. The plasma glucose level was determined using the mutarotase and glucose oxidase methods with a commercially available kit (Glucose C2-test Wako, Wako Pure Chemical Industries Ltd., Osaka, Japan).

Plasma insulin level

The plasma insulin level was determined using the enzyme-linked immunosorbent assay with a commercially available kit (Insulin kit for rats-T, Shibayagi, Gunma, Japan).

Plasma cholesterol level

The plasma total cholesterol level was determined using the cholesterol oxidase method with a commercially available kit (Cholesterol E-test Wako, Wako Pure Chemical Industries Ltd.).

Tissue cholesterol level

Immediately after the rats were killed, the aortas (from arch to upper thoracic aorta) were harvested by dissection from the surrounding tissue. The tissue cholesterol level was measured as described previously⁹. Briefly, the aortic tissue was homogenized with phosphate-buffered saline 2 mM and incubated with alcoholic KOH. Cholesterol was extracted with dichloromethane and measured using the cholesterol oxidase method mentioned above.

Histological examination

Immediately after the rats were killed, the aorta (from arch to upper thoracic aorta) was harvested, stored in saline on ice, and dissected from the surrounding tissues. Then, the tissue of aorta was fixed in 10% formalin neutral buffer solution (pH 7.4, Wako Pure Chemical Industries Ltd.) for the preparation of frozen section. Sections of the aorta were stained with Sudan III for visualization of lipid deposition.

Statistical analysis

Two-way ANOVA followed by Scheffe’s test was performed for statistical comparisons. A P value of less than 0.05 was considered to represent a statistically significant difference.

Results

Effects of Suc and HFD on body weight in SD and SHHR

Body weight in the control SD group was significantly greater than that in the control SHHR group (Table 1). However, there were no significant
Table 1. Characteristics of SD and SHHR with High Fat Diet and Sucrose

<table>
<thead>
<tr>
<th></th>
<th>SD</th>
<th>SHHR</th>
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<tbody>
<tr>
<td></td>
<td>Cont</td>
<td>HFD</td>
</tr>
<tr>
<td>Number of rats</td>
<td>(8)</td>
<td>(4)</td>
</tr>
<tr>
<td>Body weight (g)</td>
<td>606.4</td>
<td>644.0</td>
</tr>
<tr>
<td>±28.5</td>
<td>±14.8</td>
<td>±16.3</td>
</tr>
<tr>
<td>SBP (mmHg)</td>
<td>140.9</td>
<td>135.9</td>
</tr>
<tr>
<td>± 2.0</td>
<td>±1.9</td>
<td>±3.0</td>
</tr>
<tr>
<td>Plasma glucose (mg/dl)</td>
<td>104.6</td>
<td>116.2</td>
</tr>
<tr>
<td>±4.6</td>
<td>±0.5</td>
<td>±5.3</td>
</tr>
<tr>
<td>Insulin (µU/ml)</td>
<td>13.0</td>
<td>11.9</td>
</tr>
<tr>
<td>±1.4</td>
<td>±1.2</td>
<td>±1.4</td>
</tr>
</tbody>
</table>

Data are expressed as the mean ± S.E.M. Cont, control group; HFD, high fat diet group; HFD+Suc, HFD and 30% sucrose group; # p < 0.05 and $$ p < 0.01$ vs. respective control group, $$ p < 0.01$ vs. SD control group.

Changes in body weight among the control, HFD-alone, and Suc + HFD-fed SD groups. On the other hand, body weight in the Suc + HFD-fed group of SHHR was significantly greater ($p < 0.05$) than that in the control SHHR.

Effects of Suc and HFD on plasma glucose levels in SD and SHHR

Plasma fasting glucose levels in the Suc + HFD-fed groups of SD and SHHR were significantly increased compared with those in the control and HFD-alone groups of SD and SHHR (Table 1). Plasma fasting glucose levels in the Suc + HFD-fed SD and SHHR groups increased to approximately the same extent.

Effects of Suc and HFD on plasma insulin levels in SD and SHHR

Plasma fasting insulin levels in the Suc + HFD-fed groups of SD and SHHR were significantly higher than those in the control and HFD-alone groups of both types of rat (Table 1). However, the increases noted in plasma fasting insulin levels in the Suc + HFD-fed SD and SHHR groups were similar.

Effects of Suc and HFD on SBP in SD and SHHR

As shown in Table 1, SBP in the control SHHR group was significantly higher than that in the control SD group. However, SBP changes among the control, HFD-alone, and Suc + HFD-fed groups of SHHR showed no significant difference.

Effects of Suc and HFD on plasma cholesterol level in SD and SHHR

HFD slightly increased the plasma cholesterol level in SD (Fig. 1). On the other hand, the HFD alone also slightly increased the plasma cholesterol level in SHHR. Furthermore, Suc + HFD significantly increased the plasma cholesterol level by 2.5-fold in SHHR.

Effects of Suc and HFD on visceral fat tissue weight in SD and SHHR

The HFD alone had no effect on visceral fat tissue weight in either SD or SHHR (Fig. 2). Suc + HFD feeding significantly increased visceral fat tissue weight in SHHR, but not in SD.

Effects of Suc and HFD on aortic lipid deposition in SD and SHHR
Figure 1. Effects of 30% Suc and HFD on plasma cholesterol level in SD and SHHR. Bars indicate mean ± S.E.M. Cont, control group. Number of rats is shown in parentheses.

![Graph showing plasma cholesterol levels](image)

**Figure 2.** Effects of 30% Suc and HFD on visceral fat tissue in SD and SHHR. Comparisons among groups. Bars indicate mean ± S.E.M. Number of rats is shown in parentheses.

![Graph showing visceral fat tissue weights](image)

Lipid deposition under endothelial cells and endothelial lesions were observed in the aorta of HFD-fed SHHR (Fig. 3). Further, marked lipid deposition under endothelial cells and endothelial lesions in the aorta of Suc + HFD-fed SHHR and slight lipid was observed. On the other hand, lipid deposition in SD rats of Cont and Suc + HFD groups were not observed.

**Figure 3.** Effects of 30% Suc and HFD on aortic lipid deposition in SD and SHHR. The aortic tissue section was stained using Oil red O staining. Cont, control group. Scale bar is 50 μm. (original magnification ×400)

![Images of aortic tissue sections](image)

**Effects of Suc and HFD on aortic cholesterol levels in SD and SHHR**

The HFD + Suc significantly increased the cholesterol level in the aorta in the SD group compared with the control group (Fig. 4). Feeding with the HFD alone and Suc + HFD significantly increased the cholesterol level in the aorta of SHHR.

**Discussion**

In short, we demonstrated the increased visceral fat, serum cholesterol level, and endothelial lipid deposition in the present model rats. We found that Suc + HFD enhanced visceral fat tissue gain and lipid deposition in the aortic endothelium in SHHR, but not in SD. There was no difference in the body weight of Suc + HFD-fed SD compared with control SD. On the other hand, the body...
weight of Suc + HFD-fed SHHR significantly increased compared with that of control SHHR. The visceral fat tissue weight of Suc + HFD-fed SHHR was also significantly greater than that of control SHHR. Interestingly, the HFD alone had no effect on the visceral fat tissue weight of SHHR. The increases in visceral fat tissue weight may be enhanced by the addition of Suc in HFD-fed SHHR. Furthermore, some of the body weight gain in Suc + HFD-fed SHHR may be related to the increase in visceral fat tissue weight with Suc + HFD feeding.

Plasma fasting glucose and insulin levels in Suc + HFD-fed SD and SHHR were significantly higher than those in the HFD-alone and control SD and SHHR groups. Therefore, it is thought that the addition of Suc to the diet increased plasma glucose and insulin levels in both SD and SHHR. Previous studies demonstrated that Suc and fructose loading increased plasma glucose and insulin levels\(^{12,13}\). Kawasaki et al. reported that long-term Suc administration in drinking water leads to the rapid development of glucose intolerance in normal rats\(^{12}\). Although Suc does not appear to result in acute increases in insulin levels, chronic exposure appears to cause hyperinsulinemia indirectly through other mechanisms\(^{12}\). One hypothesis proposed that the mechanisms of increases in plasma insulin and glucose levels with Suc feeding involved an excess hexammine synthesis pathway with long-term overnutrition (Suc intake), leading to long-term energy storage and type 2 diabetes\(^{13,14}\). These data suggest that hyperglycemia with hyperinsulinemia in Suc + HFD-fed SD and SHHR are caused by long-term Suc administration.

The plasma total cholesterol levels in Suc + HFD-fed SD and SHHR were significantly increased compared with those in the respective control groups. In particular, there was a 2.5-fold increase in Suc + HFD-fed SHHR compared with the SHHR control groups. The level in SHHR control groups was reported to be \(\geq 200\) mg/dl\(^{9}\). In the SHHR groups in this study, the HFD diet increased plasma total cholesterol levels by approximately 1.25-fold. The further increase in the plasma total cholesterol level in Suc + HFD-fed SHHR suggested that Suc enhanced the HFD-induced elevation of plasma cholesterol level, although Suc was administered for only 2 months. Insulin and glucose are known to regulate lipid synthesis and secretion directly\(^{15}\). Insulin and glucose can activate the cholesterol synthetic pathway through the control of hepatic sterol regulatory element binding protein expression\(^{16}\). Suc administration increased the plasma insulin and glucose levels of SD and SHHR in our experiments. On the other hand, the increase in the plasma cholesterol level in SHHR fed Suc + HFD was marked compared with that in the group fed the HFD alone. We previously reported that CYP7A, an enzyme catalyzing cholesterol, was decreased in the liver of SHHR\(^{16}\). Further, low density lipoprotein (LDL) was detected in the plasma of SHHR, but not of SD\(^{16}\). LDL is major storage source of cholesterol in the plasma as well as high density lipoprotein. Therefore, cholesterol may tend to accumulate in SHHR. These data demonstrate that Suc-induced increases in insulin and glucose levels may be related to dyslipidemia in Suc + HFD-fed SHHR.

The visceral fat tissue weight gain in the Suc + HFD-fed SHHR was significantly greater than that in control and HFD-alone SHHR. On the other hand, the visceral fat tissue weight did not differ significantly among control, HFD-fed, and Suc + HFD-fed SD. He et al. reported that although the
total weight of the adipose tissue was greater for fa /fa obese rats, the tissue cell density was lower than in lean rats, a consequence of the significantly greater diameter of the cells in obese rats than in the lean rats\textsuperscript{46}. The enlargement of adipocyte cell in diameter related to visceral fat accumulation. Cell diameter may influence glucose uptake by adipocytes via lipid synthetic capacity of the adipocytes\textsuperscript{47}. Hyperglycemia induced glucose uptake into adipose tissue\textsuperscript{49}. Therefore, glucose intolerance may be one of the most important factors linked to visceral adiposity. Further, impaired suppression of adipocyte lipolysis is also associated with visceral adiposity\textsuperscript{50}. CYP7A was significantly decreased in SHHR compared with that of the SD\textsuperscript{2}. It is possible that lipolytic system of SHHR may be impaired. And, Suc + HFD-fed SHHR not only develop hyperinsulinemia and hyperglycemia but also hypercholesterolemia. Therefore, the combined-increases in these risk factors involved in adiposity may be related to visceral fat tissue weight gain in Suc + HFD-fed SHHR, but not in Suc + HFD-fed SD.

Lipid deposition in the aorta of SHHR was markedly enhanced with Suc + HFD feeding compared with the HFD alone. Furthermore, the aortic cholesterol level in Suc + HFD-fed SHHR was also markedly increased compared with those in control and HFD-alone SHHR. We previously reported that lipid deposition was observed in the endothelium of HFD-fed SHHR\textsuperscript{42}. Atherosclerosis progresses with the accumulation of cholesterol on aortic endothelial lesions. In addition, diabetes is a risk factor for atherosclerosis in the clinical setting\textsuperscript{42,45}. Glucose and insulin are adipogenic factors in obesity\textsuperscript{42,45}. Suc + HFD-fed SHHR develop severe hypercholesterolemia and hyperglycemia. Although Suc + HFD-fed SD rats also develop hyperglycemia, total cholesterol levels were not as high as in Suc + HFD-fed SHHR in the present study. Therefore, the markedly high plasma total cholesterol level in the Suc + HFD-fed SHHR in the hyperinsulinemic and hyperglycemic state may be related to lipid deposition in the aorta.

Suc + HFD feeding had no effect on SBP in either SD or SHHR. It is well known that obesity or insulin resistance is linked to hypertension thorough the activation of the sympathetic nervous system. The sympathetic nervous activity in SHHR was found to be increased compared with that in normotensive control groups\textsuperscript{42}. Those results suggest that the degree and/or duration of obesity or hyperglycemia in the present study do not affect SBP in SHHR.

Recently, it has been reported that hypertension, hyperlipidemia, and hyperglycemia lead to cardiovascular disease and tissue injury. Although interventional clinical studies on lifestyle-related diseases are important, numerous patients must be studied to explain the mechanisms of their development and to establish new therapies. Although the establishment of animal models for the study of lifestyle-related disease is still at an early stage, it is necessary for the fundamental understanding of and novel clinical approaches for the treatment of metabolic syndrome\textsuperscript{45}.

In conclusion, Suc + HFD feeding results in visceral fat gain and severe lipid deposition in the aortic endothelium with the development of hyperglycemia and hypercholesterolemia in SHHR. Our animal model combining hypercholesterolemia, hyperglycemia, and hypertension will be useful in research on cardiovascular disease and lifestyle-related disease-induced tissue injury.

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