Effect of a High-sucrose Diet on Abdominal Aortic Aneurysm Development in a Hypoperfusion-induced Animal Model

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Abstract: Abdominal aortic aneurysm (AAA) is a vascular disease that results in rupture of the abdominal aorta. The risk factors for the development of AAA include smoking, male sex, hypertension, and age. AAA has a high mortality rate, but therapy for AAA is restricted to surgery in cases of large aneurysms. Clarifying the effect of dietary food on the development of AAA would be helpful for patients with AAAs. However, the relationship between dietary habits and the development of AAA is largely unknown. In our previous study, we demonstrated that adipocytes in vascular wall can induce the rupture of AAA. Therefore, we focused on the diet-induced abnormal triglyceride metabolism, which has the potential to drive AAA development. In this study, we have evaluated the effects of a high-sucrose diet on the development of AAA in a vascular hypoperfusion-induced animal model. A high sucrose diet induced high serum TG level and fatty liver. However, the AAA rupture risk and the AAA diameter were not significantly different between the control and high-sucrose groups. The intergroup differences in the elastin degradation score and collagen-positive area were insignificant. Moreover, matrix metalloproteinases, macrophages, and monocyte chemoattractant protein-1-positive areas did not differ significantly between groups. These results suggest that a high-sucrose diet does not affect the appearance of vascular adipocyte and AAA development under the vascular hypoperfusion condition.

Key words: abdominal aortic aneurysm, high sucrose, TG, adipocyte, rupture

1 Introduction

Abdominal aortic aneurysm (AAA) is a disease that involves progressive dilation of the abdominal aorta. The risk factors for AAA include age, male sex, smoking, and hypertension. Preventing AAA rupture is an issue of high importance, as it is associated with a very high mortality rate. However, AAA progression cannot be prevented medically. Patients at an increased risk of rupture must undergo surgery, including open repair with prosthetic graft replacement or endovascular stent graft placement. Patients with an aneurysm that is >55 mm in diameter or exhibits a growth rate of >1 cm/year are usually surgical candidates. However, some small aneurysms are at risk of rupture. No method can currently predict AAA rupture. Clarifying the effect of dietary food on the development of AAA would be helpful for AAA patients. However, the relationship between dietary habits and the development of AAA is largely unknown.

The pathology of AAA is characterized by increased oxidative stress, chronic inflammation, arterial media thinning, and extracellular matrix degradation. Previous studies have reported that AAA is closely associated with inflammation-induced vascular wall weakening. This event is caused by the infiltration of inflammatory cells such as monocytes and macrophages and a subsequent increase in proteases and inflammatory cytokines, including matrix metalloproteinases (MMPs) and monocyte chemoattractant protein (MCP)-1, which are increased in the aortic wall of patients with AAA. Degradation of the arterial wall accompanied by high blood pressure is a predisposing factor for the development of AAA. The activation of MMP-2 and MMP-9 is reportedly associated with aneurysm formation in humans.

In addition to the above-mentioned established patholo-
2 Experimental

2.1 Animals

All animal experiments were approved by the Kindai University Animal Care and Use Committee and performed according to the Kindai University Animal Experimentation Regulations (approval number: KAAG-25-001). Six-week-old male Sprague-Dawley rats (SHIMIZU Laboratory Supplies Co., Ltd., Kyoto, Japan) were provided with food and water ad libitum in a humidity-controlled room with a 12-hour light/dark cycle. The room temperature was maintained at 25 ± 1°C. The rats were divided into the control-diet group (0% sucrose) and the high-sucrose-diet group (66% sucrose). Diet composition is shown in Table 1. After a 1-week habituation period, the abdominal aorta was ligated over an inserted catheter in all rats to induce an AAA. After 4 weeks, the aortic diameters were measured and rats were sacrificed. When a rat died of AAA rupture, the dilation of the abdominal aorta was measured and the abdominal aorta was immediately isolated. AAA was identified when the abdominal aorta dilation was measured and the abdominal aorta and rats were sacrificed. When a rat died of AAA rupture, abnormalities of adipocytes in the vascular wall can induce AAA rupture.

2.2 Induction of hypoperfusion-induced AAA

Hypoperfusion of the abdominal aortic wall was induced as previously described. First, the infra-renal aorta was exfoliated from the perivascular tissue. Vessels branching from the abdominal aorta were ligated with a 5-0 silk string (Akiyama-seisakusyo, Tokyo, Japan) to block the blood supply. A plastic catheter (Medikit, Tokyo, Japan) shortened to 9 mm in length was inserted via a small incision adjacent to the renal artery branches and the incision was repaired with a 6-0 monofilament string (Alfresa Pharma, Osaka, Japan). The abdominal aorta was ligated with a 5-0 silk string and the plastic catheter.

2.3 Sample Collection

The diameter of the abdominal aorta was measured using digital calipers (A&D, Tokyo, Japan). The dilation ratio was calculated according to the following formula: dilation ratio = maximal aneurysm diameter (sac)/non-dilated vascular diameter (neck). Isolated tissues were fixed in 4% paraformaldehyde (Nacalai Tesque, Kyoto, Japan), soaked in sucrose (10%, 15%, and 20%), and embedded in O.C.T. Compound (Sakura Finetek Japan Co., Ltd., Tokyo, Japan). The livers were harvested, cryopreserved in liquid nitrogen, and stored at −80°C until use.

2.4 Serum analysis

Blood samples were collected from the rats for 4 weeks after AAA induction. Serum glucose (Glu), TG, and total cholesterol concentrations were measured using a glucose kit, triglyceride kit, and total cholesterol kit (Wako Pure Chemical industries, Osaka, Japan), respectively.

2.5 Histological analysis

Isolated aorta and harvested liver cross-sections (10 μm thick) were prepared using a cryostat (CM1850; Leica Microsystems, Wetzlar, Germany) and mounted on glass slides. The aortic walls were stained with hematoxylin-eosin, Elastica van Gieson, Picrosirius Red, Oil Red O, and

Table 1 Diet composition of the control and high-sucrose diets.

<table>
<thead>
<tr>
<th></th>
<th>Control diet (0% sucrose)</th>
<th>High Sucrose diet (66% sucrose)</th>
</tr>
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<tbody>
<tr>
<td>Casein</td>
<td>200</td>
<td>200</td>
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<tr>
<td>Cystine</td>
<td>3</td>
<td>3</td>
</tr>
<tr>
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</tr>
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<td>0</td>
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<tr>
<td>Sucrose</td>
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<td>700</td>
</tr>
<tr>
<td>Cellulose</td>
<td>50</td>
<td>50</td>
</tr>
<tr>
<td>Soybean oil</td>
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<td>25</td>
</tr>
<tr>
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</tr>
<tr>
<td>AIN-93G mineral</td>
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<td>32</td>
</tr>
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<td>Dibasic calcium phosphate</td>
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<tr>
<td>AIN-93 vitamin mix</td>
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<td>Choline chloride</td>
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</tr>
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<tr>
<td>kcal/g</td>
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immunohistochemical stains. Liver tissues were stained by Oil Red O staining. The quantitative analysis of histological staining was performed using ImageJ software (National Institutes of Health, Bethesda, MD, USA). Areas within 100 μm of an adipocyte were defined as "areas with adipocytes." Elastic fibers were categorized into four grades: grade 1, intact elastic fibers; grade 2, lack of wave form; grade 3, thinning of wave from or/and partial disappearance of elastic fibers; and grade 4, complete disappearance of elastic fibers.

2.6 Immunohistochemical staining
Paraformaldehyde-fixed tissue sections were rinsed in phosphate-buffered saline (PBS) with 1% Triton-X100 and incubated in 10% oxalic acid for 1 hour. For antigen activation, 0.1% trypsin in PBS was added to the tissue sections. Endogenous horseradish peroxidase in the tissue sections was blocked using 3% aqueous hydrogen peroxide in methanol for 8 minutes. After washing in PBS, the tissue sections were blocked with Blocking One Histo. The sections were incubated with the appropriate primary antibody overnight at 4°C. The histological results from the aortic wall were assessed after staining using the following antibodies: rabbit anti-matrix metalloproteinase (MMP)-2 (1:50; Thermo Scientific, San Jose, CA, USA), goat anti-MMP-9 (1:50; Santa Cruz Biotechnology, Dallas, TX, USA), rabbit anti-MMP-12 (1:100; Bioss Antibodies, Woburn, MA, USA), mouse anti-monocytes/macrophages (MAC387) (1:50; Bio-Rad Laboratories, Hercules, CA, USA), and rabbit anti-monocyte chemoattractant protein-1 (MCP-1) (1:100; Bioss Antibodies, Woburn, MA, USA). On the following day, the sections were rinsed in PBS and incubated with the appropriate secondary antibody conjugated to horseradish peroxidase. The slides were developed with 3'-diaminobenzidine (Vector Laboratories, Burlingame, CA, USA), dehydrated in ethanol (80%, 90%, and 100%), cleared in xylene, and covered with a lipid-soluble mounting medium and glass cover slips.

2.7 Statistical analysis
Values are expressed as mean ± SEM. For between-group comparisons, the Chi-square test or Fisher's exact test (for situations with small frequencies) was used for categorical variables. The statistical differences were determined using a two-sided Student's t-test. Multiple comparisons between groups were made using the Tukey-Kramer test. The statistical difference in scoring data was determined by Scheffe's test. Differences of p < 0.05 were considered significant. Statistical analyses were performed using StatView 5.0 software (SAS Institute, Cary, NC, USA).

Fig. 1  Body weight change, food intake, serum parameters, and Oil Red O staining for liver. (a) Body weight change of the rats in the control and high-sucrose groups. (b) Food intake calories of the rats in the control and high-sucrose groups. (c) Serum glucose levels and (d) triglyceride level. (e) Oil Red O staining of liver tissues. Data are expressed as mean ± S.E.M. (a) Control group (n = 11), high-sucrose group (n = 7). (b) Control group (n = 5), high-sucrose group (n = 3). *p < 0.05 versus control group. **p < 0.01 versus control group.
3 Results

3.1 Effects of the high-sucrose diet on serum parameters and liver lipid levels

The average body weight change (g) and calorie intake (kcal/day) did not differ significantly between the control and high-sucrose groups (Figs. 1a, 1b). The serum glucose level of the high-sucrose group was significantly increased compared to that of the control group at 4 and 5 weeks (Fig. 1c). The serum TG level of the high-sucrose group was significantly increased compared to that of the control group at weeks 1–4 (Fig. 1d). The serum total cholesterol level did not differ significantly between groups (data not shown). The Oil Red O staining-positive area in the liver in the high-sucrose group was significantly increased compared to that in the control group (Fig. 1e).

3.2 Effects of the high sucrose diet on AAA rupture and aortic diameter

The rupture rates of both groups are shown in Fig. 2a. The rupture rate was 27% in the control group and 29% in the high sucrose group, an insignificant difference (Figs. 2a, 2b). A formatted AAA is shown in Fig. 2c. We observed the area with the non-dilated diameter (neck) and the area with a dilated aortic diameter (sac) in both groups (Fig. 2c). The neck diameter did not differ significantly between the control and high sucrose groups (Fig. 2d). The sac was significantly increased compared with the neck in each group (Fig. 2d). The sac diameter did not differ significantly between the control and high sucrose groups (Fig. 2d). Furthermore, the dilation ratio (sac/neck) did not differ significantly between groups (Fig. 2e).

3.3 Adipocytes in the vascular wall

We evaluated adipocytes in the adventitia, as we previously reported inducing AAA rupture by adipocytes in the adventitia. Adipocytes were observed in the vascular adventitia of the AAA sac but not in the neck vascular wall in both groups (Figs. 3a-3d). These areas were stained positively by Oil Red O staining (Figs. 3e-3h). The number and size (μm²/cell) of adipocytes in the vascular wall of the AAA sac did not differ significantly between the control and high-sucrose groups (Figs. 3i, 3j).

![Fig. 2](image-url) Effects of a high-sucrose diet on abdominal aortic aneurysm (AAA) rupture and aortic diameter. (a) The effect of a high-sucrose diet on AAA rupture ratio. Data are presented as number of rats. P values of the Fisher’s exact test. (b) Kaplan-Meier curves of AAA rupture. (c) Representative images of the abdominal aorta from AAA induction in the control and high-sucrose diet groups (scale bar = 5 mm). (d) Quantitative analysis of aortic diameter. (e) Dilation ratio of the control and high-sucrose groups. Data are represented as mean ± S.E.M. (a-d) Control group (n = 11), high-sucrose group (n = 7). (e) Control group (n = 5), high-sucrose group (n = 3). Values with different letters are significantly different (p < 0.05).
3.4 Effects of the high-sucrose diet on elastin and collagen fiber degradation

The vascular wall thickness was observed using hematoxylin-eosin (Figs. 4a-4d). The vascular wall thickness in the AAA sac increased significantly compared to that in the AAA neck in both groups (Fig. 4e). However, there was no significant intergroup difference in either area (Fig. 4e). Elastin fibers in the vascular wall were observed using Elastica van Gieson staining (Figs. 4f-4i). In each area, the elastin degradation score was not significantly different between the control and high-sucrose group (Fig. 4j). The collagen-positive area/collagen fiber density in the vascular wall was measured using Picrosiris Red staining (Figs. 4k-4p). The collagen-positive area decreased significantly in the area without adipocytes compared to the area in the AAA neck (Fig. 4q). Moreover, the collagen-positive area tended to decrease in the area with adipocytes compared to the area without adipocytes (Fig. 4q). However, there were no significant differences in area between the control and high-sucrose groups (Fig. 4q).

3.5 Effects of high-sucrose diet on MMP-positive areas

After observing elastin and collagen fiber degradation, we performed immunohistochemical staining for MMPs using an extracellular matrix catabolic enzyme. Immunohistochemical staining showed the expression of MMP-2, MMP-9, and MMP-12 in each group (Figs. 5a-5r). The areas positive for MMP-2, MMP-9, and MMP-12 in the AAA sac without adipocytes significantly increased compared to the areas in the AAA neck; however, there were no significant intergroup differences in any area (Figs. 5s-5u). The areas positive for MMP-2 and MMP-9 significantly increased in the areas with adipocytes compared with the areas without adipocytes, but there was no significant difference between the control and high-sucrose groups (Figs. 5s, 5t). Moreover, the MMP-12–positive area in the AAA sac with adipo-

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**Fig. 3**  Quantification of adipocytes. (a-d) Representative images of hematoxylin-eosin-stained samples (scale bar = 200 μm). (e-h) Representative images of Oil Red O staining (scale bar = 100 μm). (i) Quantification of number of adipocytes in the control and high-sucrose groups. (j) Quantification of adipocyte area in the control and high-sucrose groups. Data are represented as mean ± S.E.M. Control group (n = 11), high-fat group (n = 7). N.D. = not detected. The arrows show the direction of intima and adventitia. In the case of enlarged figure, all layers of arterial wall were not shown.
cytes increased significantly compared to the area without adipocytes in the high-sucrose group; furthermore, the MMP-12–positive area tended to increase in the control group (Fig. 5).  

3.6 Effects of high-sucrose diet on monocyte/macrophage- and MCP-1–positive areas

Here we performed immunohistochemical staining for monocytes/macrophages. Immunohistochemical staining showed monocyte/macrophage expression in each group (Figs. 6a-6f). The monocyte/macrophage-positive area significantly increased in the area without adipocytes in the AAA sac compared with the AAA neck; additionally, it tended to increase in the AAA sac with adipocytes (Fig. 6g) despite no significant intergroup difference (Fig. 6g). After observing monocyte/macrophage invasion into the vascular wall, we performed immunohistochemical staining for MCP-1, which revealed MCP-1 expression in each area (Figs. 6h-6m). The MCP-1-positive area increased significantly in the area without adipocytes in the AAA sac compared with the AAA neck; additionally, it increased significantly in the area with adipocytes (Fig. 6n). However, there was no significant difference between the control and high-sucrose groups (Fig. 6n).  

4 Discussion

Here we evaluated the effects of a high-sucrose diet on AAA development and rupture using a hypoperfusion-induced AAA rat model. Thus far, studies have reported that a high-sucrose diet induces high serum TG level and TG accumulation in the livers of rats. We also observed a significant increase in TG in the serum and liver in the high-sucrose group compared with the control group. On the contrary, the rupture rate was not significantly different between the control and high-sucrose groups. The intergroup differences in aortic diameter and dilation ratio were insignificant.

The abnormal appearance of adipocytes in the vascular wall can reportedly induce AAA rupture. In this study, we observed the appearance of adipocytes in the vascular wall in both groups. The number and area of adipocytes were not significantly different between the control and...
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High-sucrose group, although the mean serum TG level in the high-sucrose group was higher than that in the control group. We previously reported that a high-fat diet promotes AAA rupture by increasing the number and area of adipocytes in the vascular wall without an increased serum TG level in a hypoperfusion-induced AAA rat model. These results suggest that a high serum TG level is not directly involved in the development of adipocytes in the vascular wall. Food composition may be associated with the development of adipocytes in the vascular wall. However, the mechanisms remain unknown. Further studies are needed to clarify the mechanisms underlying the development of adipocytes in the vascular wall under the hypoperfusion condition.

It has been reported, using the calcium phosphate-induced aneurysm mouse model, that hyperglycemia suppresses MMP-9 expression though the suppression of macrophage activity. In this study, we estimated the positive areas for MMPs, MCP-1, and monocytes/macrophages in the areas with and without adipocytes because the abnormal appearance of adipocytes in the vascular wall induces vascular inflammation, which causes elastin and collagen fiber degradation. The areas positive for MMPs and MCP-1 were increased around the areas with adipocytes in the control and high-sucrose groups. However, these vascular inflammation factors differed insignificantly between the control and high-sucrose groups. Hyperglycemia may not influence macrophage activation in a hypoperfusion-induced AAA rat model.

5 Conclusion

Our findings indicated that a high-sucrose diet did not affect AAA development and rupture in a hypoperfusion-induced AAA rat model, suggesting that AAA patients might not require strict sucrose intake control if serum TG and glucose levels and fatty liver are adequately controlled.
Fig. 6 Immunohistochemical staining for monocyte chemoattractant protein-1 (MCP-1) and mouse anti-monocytes/macrophages (MAC387+) monocytes/macrophages. Abdominal aortic aneurysm (AAA) sac areas from the two experimental groups were divided into those without adipocytes (-) (b, e, h, and k) and those with adipocytes (+) (c, f, i and l). (a-f) Representative images of immunostaining for MCP-1 (scale bar = 30 μm). (g) Quantification of MCP-1–positive areas of the vascular wall. (h-m) Representative images of immunostaining for MAC387+ monocytes/macrophages (scale bar = 30 μm). (n) Quantification of MAC387+ monocyte/macrophage–positive areas of the vascular wall. Data are represented as mean ± S.E.M. Control group (n = 11), high-sucrose group (n = 7). Values with different letters are significantly different (p < 0.05).

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Conflict of Interest statement
The authors declare that there are no conflicts of interest.

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