Role of Angiotensin II in High Fructose-Induced Left Ventricular Hypertrophy in Rats

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Recent studies suggest the linkage of hypertension and insulin resistance. High fructose diet is known to induce hyperinsulinemia and hypertension in rats. In a previous study, however, high fructose (66%) diet failed to elevate blood pressure but increased left ventricular weight in Sprague-Dawley rats. In the present study, we investigated the precise mechanism of high fructose diet–induced changes in the cardiovascular system in rats. Intake of fructose-enriched diet for 2 weeks increased serum insulin and plasma angiotensin II levels. Urinary excretion of sodium and norepinephrine was not changed. Blood pressure measured directly through an indwelling catheter was not increased, but left ventricular weight and protein content were increased by high fructose diet. To further elucidate the role of the renin-angiotensin system, an angiotensin II type 1 receptor antagonist, TCV-116, was given orally at 1 mg/kg per day with either normal or high fructose diet. Concomitant administration of TCV-116 did not affect plasma glucose or serum insulin levels. Plasma angiotensin II was increased, but neither urinary sodium nor norepinephrine was changed by TCV-116. TCV-116 similarly decreased blood pressure in rats on normal and high fructose diets. Increase in left ventricular weight induced by high fructose diet was prevented by the concomitant administration of TCV-116. On the other hand, left ventricular weight in control rats was not changed by TCV-116. In conclusion, increased plasma angiotensin II may account for the left ventricular hypertrophy induced by high fructose diet, whereas hemodynamic change, sodium retention, and the sympathetic nervous system do not play an important role. (Hypertension 1993;21:1051–1055)

KEY WORDS • angiotensin II • receptors, angiotensin • fructose • hypertrophy, left ventricular • hyperinsulinism

Recent epidemiological studies have demonstrated the high coexistence of hypertension and insulin resistance. Previous experimental studies have demonstrated that dietary supplementation of sucrose or fructose induces hyperinsulinemia, insulin resistance, and hypertension. Inhibition of insulin secretion by a somatostatin analogue or reduction of insulin resistance by exercise prevents hypertension, suggesting an etiological role of hyperinsulinemia in the elevation of blood pressure. In a preliminary study, we showed that high fructose diet failed to increase blood pressure but induced left ventricular (LV) hypertrophy in rats. Serum insulin and plasma angiotensin II (Ang II) were increased by high fructose diet, suggesting a possible role of either insulin or Ang II in LV hypertrophy. However, the precise mechanism is not clear.

The present study examined the mechanism of the development of LV hypertrophy induced by high fructose diet. Insulin causes sodium retention by increasing proximal tubular reabsorption and activating the sympathetic nervous system, which may induce LV hypertrophy. We therefore evaluated the role of these factors in cardiac hypertrophy induced by high fructose diet. We also assessed the role of the renin-angiotensin system using an Ang II type 1 receptor (AT₁) antagonist, TCV-116, because this drug specifically inhibits the action of Ang II at its receptor site.

Methods

Animal and Experimental Protocol

Male Sprague-Dawley rats weighing 160–180 g (Charles River Japan, Inc., Atsugi, Japan) were individually housed in metabolic cages and given standard rat chow containing 24.6 g protein, 5.6 g fat, 0.26 g sodium, and 0.85 g potassium per 100 g chow (Oriental Yeast Co. Ltd., Tokyo) ad libitum. After 7 days, rats were divided into two groups according to diet. Control diet was continued for a further 2 weeks in one group, and high fructose diet containing 66% fructose was given to the other group. The content of minerals, protein, fat, and vitamins was matched in the control and high fructose diets. In addition, both groups were given vehicle or AT₁ receptor antagonist, TCV-116, from the beginning of special diet administration (n = 8 in each group). TCV-116 was suspended in 0.5% gum arabic (Sigma Chemical Co., St. Louis, Mo.) solution and was given at 1 mg/kg per day by gastric gavage in the
morning (9–11 AM). The same amount of gum arabic solution (0.5 mL) was given as vehicle. TCV-116 was donated by Takeda Chemical Industries, Ltd., Osaka, Japan.

Urinary Collection
Daily urine was collected to measure sodium (n = 4 in each group) and norepinephrine excretion (n = 4 in each group) with 1 mL of 6N hydrochloric acid. Norepinephrine was measured by high performance liquid chromatography.

Blood and Tissue Sample Measurements
Blood was obtained by decapitation in the morning after rats had fasted 2–3 hours. Plasma Ang II and serum insulin levels, using rat insulin as a standard, were measured by radioimmunoassay. Glucose was measured by the glucose oxidase method.

The left ventricle was isolated and homogenized in 50 mM phosphate-saline buffer at pH 7.4 containing 2 mM ethylenediaminetetraacetic acid (EDTA). DNA was measured based on the enhancement of fluorescence seen when bisbenzimide (Hoechst 33258) binds to DNA. Protein was measured by a protein assay kit obtained from BioRad Laboratories, Richmond, Calif.

Blood Pressure Measurement
In another series of experiments, 32 rats were divided into four groups and given normal or high fructose diet and vehicle or TCV-116 similarly to the first experiment (n = 8 in each group). After 12 days of treatment, an aortic cannula was inserted via the femoral artery with rats under light ether anesthesia. Twenty-four hours after the surgery, rats were kept on sawdust in a small box for at least 30 minutes before measurements were taken. Blood pressure and heart rate were then measured directly through the catheter with the animals in a conscious, unrestricted state.

Histological Examination
Rats used for blood pressure measurements were perfused with heparinized saline followed by 10% formaldehyde in 0.2 M phosphate buffer (pH 7.4) from the heart. Cardiac tissue was isolated and further fixed in the same solution. Tissue was embedded in paraffin, and thin sections were stained with Azan.

Table 1. Effects of High Fructose Diet and Concomitant Administration of TCV-116 on Body Weight, Glucose, Insulin, and Angiotensin II

<table>
<thead>
<tr>
<th>Treatment</th>
<th>Body weight (g)</th>
<th>Glucose (mmol/L)</th>
<th>Insulin (pmol/L)</th>
<th>Angiotensin II (pmol/L)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Normal diet+vehicle (n=8)</td>
<td>335±7</td>
<td>7.4±0.2</td>
<td>250±33</td>
<td>9.4±0.8</td>
</tr>
<tr>
<td>High fructose+vehicle (n=8)</td>
<td>328±4</td>
<td>7.6±0.3</td>
<td>367±67</td>
<td>13.8±2.5</td>
</tr>
<tr>
<td>Normal diet+TCV-116 (n=8)</td>
<td>334±6</td>
<td>7.6±0.3</td>
<td>183±50</td>
<td>32.7±12.6</td>
</tr>
<tr>
<td>High fructose+TCV-116 (n=8)</td>
<td>325±5</td>
<td>7.3±0.2</td>
<td>367±50</td>
<td>63.2±12.6</td>
</tr>
</tbody>
</table>

Values are mean±SEM; probability values obtained from two-factor analysis of variance.

Results
Administration of a high fructose diet did not affect body weight and plasma glucose (Table 1). Serum insulin was increased by high fructose diet. TCV-116 did not alter these parameters. Plasma Ang II was significantly higher in rats given high fructose diet (Table 1). Treatment with TCV-116 increased plasma Ang II in rats fed either control or high fructose diet (Table 1). As shown in Figure 1, neither high fructose diet nor TCV-116 affected urinary excretion of sodium or norepinephrine. Mean blood pressure was unchanged by high fructose diet but was decreased by TCV-116 in both normal and high fructose diet groups (Figure 2). Pulse rate did not differ among the four groups. LV weight and protein content were increased, but DNA content was not changed by high fructose diet (Figure 3). Concomitant oral administration of TCV-116 prevented the increases in LV weight and protein. Histological observation showed that the content of fibrous tissue in the interstitium stained with Azan was similar in rats fed control and high fructose diets (Figure 4).

Discussion
Data obtained in the present study were similar to those reported by Reaven et al and by our group in which intake of fructose-enriched diet produced endogenous hyperinsulinemia with normal plasma glucose levels. However, Reaven et al observed an increase in blood pressure with high fructose diet, whereas we did not observe significant elevation of blood pressure. We used a similar diet composition (66% fructose) and rats (male Sprague-Dawley) but observed a relatively smaller increase in serum insulin (50%) compared with their studies, in which serum insulin more than doubled. Therefore, one possible explanation for the lack of hypertension in the present study may be the insufficient increase in serum insulin by high fructose diet. It should also be noted that Reaven et al demonstrated a mild
increase in systolic blood pressure (10–20 mm Hg) in fructose-fed rats by the tail-cuff method, whereas we performed direct measurement of blood pressure via an indwelling arterial catheter. Brands et al. studying the effect of insulin infusion on blood pressure in rats fed high sucrose (66%) diet. Using direct intra-arterial measurement, they showed that blood pressure was increased by insulin infusion but was not changed by high sucrose diet alone. Thus, another explanation for the difference in blood pressure may be the different methods of blood pressure measurement.

In the present study, high fructose diet increased LV weight and protein content but did not change DNA content, suggesting that the weight increase was due to hypertrophy. The results of histological examination further support this hypothesis, because no increase in interstitial fibrosis was observed in the left ventricle of high fructose-fed rats.

Insulin has been shown to stimulate Na⁺,K⁺-ATPase activity and is suggested to be related to cell growth in muscle. The presence of insulin receptors has been reported in the heart. In patients with non-insulin-dependent diabetes, the higher the postglucose serum insulin level, the greater the LV mass. In contrast, no correlation was observed between the ratio of fasting insulin to glucose and LV mass index in lean hypertensive subjects. Therefore, at present the action of insulin on cardiac myocytes is not fully understood, and further investigation is needed to clarify the direct relation between serum insulin level and LV hypertrophy.

Insulin has been reported to cause sodium retention and activation of the sympathetic nervous system, which may lead to cardiac hypertrophy. Intake of high fructose diet, however, did not affect urinary sodium or norepinephrine excretion, suggesting that these factors are not involved in the development of LV hypertrophy induced by high fructose diet.

The renin-angiotensin system is another candidate for the hypertrophic factors and was assessed in the present study by measurement of its biologically active peptide Ang II. Plasma Ang II was higher in rats fed high fructose diet than in control rats, suggesting its relation to LV hypertrophy. To further evaluate the role of Ang II in LV hypertrophy, we used the AT₁ receptor antagonist TCV-116, because it is more specific in Ang II inhibition than angiotensin converting enzyme inhibitor. TCV-116 is a nonpeptide, orally active, non-competitive antagonist for AT₁ receptor. In spontaneously hypertensive rats, once-daily oral administration of TCV-116 at 1 mg/kg per day produces sustained and effective hypotensive action (systolic blood pressure, approximately -40 mm Hg) (unpublished observations). In this study, administration of TCV-116 increased plasma Ang II more than three times, possibly through a feedback mechanism, and prevented LV hypertrophy by high fructose diet. These results suggest

![Mean Blood Pressure vs. Pulse Rate](image_url)
that an increase in Ang II is one of the factors that explains the mechanism of cardiac hypertrophy induced by high fructose diet.

Cardiac hypertrophy induced by high fructose diet was not accompanied by hypertension. TCV-116 inhibited the increase of LV mass in fructose-fed rats, whereas blood pressure was decreased in both normal and high fructose-fed rats. However, these results could not completely exclude the possible role of blood pressure reduction in the prevention of LV hypertrophy by an Ang II antagonist. Additional study using the anti-hypertensive drugs that have no inhibitory effect on the renin-angiotensin system may further clarify the role of blood pressure in LV hypertrophy.

It has been shown that TCV-116 inhibits the receptor binding and vascular action of Ang II but has no effect on the contraction induced by norepinephrine, potassium chloride, serotonin, prostaglandin F₂α, or endothelin,¹¹ suggesting that TCV-116 is a selective inhibitor of Ang II. In this study, TCV-116 had no effect on either glucose or insulin level. However, the possibility still remains that effects of TCV-116 other than Ang II antagonism, such as a central effect or direct action, may have contributed to the regression of LV hypertrophy.
In high fructose-fed rats, administration of TCV-116 prevented LV hypertrophy. Because in normal diet groups there was no significant difference in LV weight despite blood pressure reduction after TCV-116 treatment, the difference in the LV weight between the two high fructose diet groups cannot be ascribed to the similar blood pressure difference but possibly to the blockade of the action of elevated Ang II on cardiac myocytes. Actually, Simon and Altman\(^2\) reported that intraperitoneal infusion of Ang II at 200 ng/kg per minute did not increase blood pressure but stimulated protein synthesis in the aorta. Although they did not directly demonstrate the effect of a subpressor dose of Ang II on cardiac myocytes, it is possible that the dose of Ang II required to elevate blood pressure is much greater than that needed to induce cell hypertrophy.

Angiotensin converting enzyme inhibitor has been reported to suppress the cardiac renin-angiotensin system, which may be related to the regression of LV hypertrophy in spontaneously hypertensive rats.\(^21\) It is therefore possible that TCV-116 inhibited the tissue renin-angiotensin system and prevented LV hypertrophy induced by high fructose diet.

In conclusion, although administration of high fructose diet failed to produce hypertension, it caused LV hypertrophy in rats. The increase in plasma Ang II by high fructose diet and prevention of hypertension by an AT\(_1\) receptor antagonist suggest that the renin-angiotensin system is one of the mechanisms involved in LV hypertrophy induced by high fructose supplementation. However, further study is needed to investigate whether TCV-116 has actions other than AT\(_1\) receptor antagonism, which affects LV hypertrophy.

References

Role of angiotensin II in high fructose-induced left ventricular hypertrophy in rats.
R Kobayashi, M Nagano, F Nakamura, J Higaki, Y Fujioka, H Ikekami, H Mikami, N Kawaguchi,
S Onishi and T Ogihara

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