Original Article

Effects of the Chinese Medicine TSJN on Insulin Resistance and Hypertension in Fructose-Fed Rats

Yi LI*, Katsuhiro HIGASHIURA, Nobuyuki URA, Takaaki TORII, Jun AGATA, Ling WANG, Nobuhiko TOGASHI, and Kazuaki SHIMAMOTO

The aim of this study was to determine the effect of Tang-Shen-Jiao-Nang (TSJN), a Chinese medicine used to treat diabetes mellitus, on insulin resistance and hypertension in fructose-fed rats (FFR). Six-week-old male Sprague-Dawley rats were fed either normal rat chow (control) or a fructose-rich chow (FFR) for 6 wk. For the last 2 or 4 wk of a 6-wk period of either diet, the rats were treated by gavage with gum arabic solution as a vehicle (control or FFR) or TSJN (800 mg/kg/d; FFR+TS), and then we performed the euglycemic hyperinsulinemic glucose clamp technique to estimate insulin sensitivity. Systolic blood pressure was measured weekly for 6 wk. At the end of the glucose clamp, the soleus muscle was dissected out for determination of muscle fiber composition by ATPase methods. Systolic blood pressure was elevated at 2 wk after the start of the fructose-rich chow feeding and persisted thereafter throughout the study. Systolic blood pressure during the glucose clamp in the FFR group was significantly higher than that in the control group.

Although there was no effect on systolic blood pressure in rats treated with TSJN for the last 2 wk of their 6-wk diet, those treated with TSJN for the last 4 wk of their 6-wk diet had lower systolic blood pressure than did the rats in the FFR group. The average rate of glucose infusion during the glucose clamp, as a measure of insulin sensitivity (M value), was significantly lower in the FFR than in the controls (10.9 ± 0.6 and 15.4 ± 0.4, mg/kg/min, for FFR and controls, respectively; p < 0.01). Treatment with TSJN for 2 wk significantly improved the M value compared to that of the control level (15.1 ± 0.5 mg/kg/min). The composite ratio of type I fibers in the soleus muscle was significantly decreased in the FFR compared to controls (75.0 ± 1.7 and 81.7 ± 1.5%, for FFR and controls, respectively; p < 0.01), and treatment with TSJN for 2 wk led to a recovery composite ratio of type I fiber to the same level as that of the control group (78.7 ± 1.7% in FFR+TS). The M value was significantly correlated with the compositions of type I and type II fibers (for type I fibers, r = 0.45, p < 0.01, for type II fibers, r = −0.44, p < 0.05). These results suggest that the Chinese medicine TSJN may improve insulin resistance, lower the systolic blood pressure, and modulate muscle fiber composition in hypertensive and insulin-resistant fructose-fed rats.

(Hypertens Res 2000; 23: 101-107)

Key Words: insulin resistance, hypertension, muscle fiber composition, Tang-Shen-Jiao-Nang, fructose-fed rats

From the Second Department of Internal Medicine, Sapporo Medical University School of Medicine, Japan, and *Department of Traditional Chinese Medicine, Beijing Hospital, Rui Dong Diabetes Integrated Chinese and Western Medicine Research Centre, China. Address for Reprints: Katsuhiro Higashiura, M.D., Second Department of Internal Medicine, Sapporo Medical University School of Medicine, South 1, West 16, Chuo-ku, Sapporo, 060-8543, Japan. Received July 12, 1999; Accepted in revised form November 1, 1999.
Introduction

Insulin resistance and accompanying hyperinsulinemia have recently been reported to play an important role in the onset and persistence of essential hypertension, dyslipidemia, and arteriosclerosis (1–4). Insulin resistance has also been reported in several animal models for hypertension, including the spontaneously hypertensive rat and the fructose hypertensive rat. Fructose-fed rats (FFR) show an acquired form of hypertension, in which the rise in blood pressure is not genetically determined but is diet-induced (5). Although it has been speculated that the rise in blood pressure is secondary to the development of insulin resistance and hyperinsulinemia, the precise mechanism of insulin resistance in FFR remains to be clarified.

The importance of skeletal muscle in insulin-mediated glucose metabolism has been established by a number of investigators (6–8). Euglycemic hyperinsulinemic glucose clamp studies have demonstrated that skeletal muscle accounts for more than 80% of glucose disposal under hyperinsulinemic conditions in humans (8). Several animal studies have demonstrated that substantial differences exist between muscle groups in insulin-mediated glucose uptake, which may relate to muscle fiber composition (9–11). Moreover, it has been shown that insulin action is greater in the red type I (slow twitch, oxidative) and type IIa (fast twitch, oxidative/glycolytic) fiber types than in the white type IIb (fast twitch, glycolytic) fiber type (11). We previously reported that the composite ratio of type I fibers in the soleus muscle is lower in fructose-fed rats (12). This suggests that changes in muscle fiber composition may be an important mechanism in insulin resistance in FFR.

Some Chinese medicines that are of botanical origin, such as Qing-Xin-Lian-Zi-Yin (QX), are thought to be effective for treating diabetes mellitus, especially for non-insulin-dependent diabetes mellitus (NIDDM). Although there has been no concept of insulin resistance in Chinese medicine, it has recently been reported that QX improved insulin-stimulated glucose uptake in peripheral tissues in streptozotocin-induced diabetic rats (13). However, the mechanism by which QX, a mixture of crude extracts of Chinese medicinal plants, improves insulin sensitivity is not yet understood. It has also been reported that long-term treatment with thiazolidinediones not only ameliorated the insulin sensitivity but also lowered the blood pressure in an animal model (14). On the other hand, while Chinese medicines for diabetes mellitus, such as Tang-Shen-Jiao-Nang (TSJN), which is of botanical origin, have been used in China to treat patients with NIDDM, the mechanisms by which blood glucose levels are lowered have not yet been clarified. To our knowledge, there has been no report on whether TSJN improves insulin sensitivity. Therefore, this study was designed to clarify the effect of TSJN on insulin resistance and hypertension, and to investigate the mechanisms by which TSJN improves insulin sensitivity by examining muscle fiber composition in FFR.

Methods

General Protocol

Six-week-old male Sprague-Dawley rats (Charles River Japan, Inc., Yokohama, Japan) were used for the experiments. The care of the animals was in strict accordance with the guiding principles of the Physiological Society of Japan. Prior to any manipulation, all rats were fed standard rat chow containing 60% vegetable starch, 5% fat, and 24% protein (Oriental Yeast Co., Tokyo, Japan). They were maintained on a 12-h light/dark cycle and given water and chow ad libitum. The rats were acclimated to handling prior to randomization, then divided into two groups at the start of the study: those fed a standard chow and those given a fructose-rich chow containing 60% fructose, 5% fat, and 20% protein (Teklad, Madison, WI) for 6 wk. The latter group was treated with a vehicle (2.5% gum arabic solution) for either the last 2 wk (FFR, n = 18) or the last 4 wk (FFR-4w, n = 11) or with 800 mg/kg, intraperitoneal, Abbott Laboratories, North Chicago, IL) for either the last 2 wk (FFR + TS, n = 21) or the last 4 wk (FFR + TS-4w, n = 11). TSJN contains Panax ginseng, Rheum rhubarb, Rehmannia glutinosa libosch, and Chinese cinnamon. The control group was also treated with the same vehicle by gavage for either the last 2 wk (control, n = 20) or the last 4 wk (control-4w, n = 11). At termination of treatment, insulin sensitivity was assessed in all conscious rats by the euglycemic hyperinsulinemic glucose clamp technique. Thereafter, bilateral soleus muscles were dissected out under anesthesia with sodium pentobarbital (50 mg/kg, intraperitoneal, Abbott Laboratories, North Chicago, IL) to evaluate muscle fiber composition.

Blood Pressure Measurement

Systolic blood pressure was measured in all conscious rats at each week during the experiments using the indirect tail-cuff method on a 37°C preheated plate for about 20 min. The rats were preconditioned to the experimental procedure before actual measurements were conducted. The equipment included a blood pressure sensor/cuff, a blood pressure amplifier, and a digital recorder (Natsume Seisakusho Co., Ltd., Tokyo, Japan). An average of six such recordings was taken as the individual systolic blood pressure. This method correlates highly with direct cannulation measurements (15).
Euglycemic Hyperinsulinemic Glucose Clamp Technique

At the end of the treatment period, rats were anesthetized with sodium pentobarbital (50 mg/kg, intraperitoneal). The left common carotid artery and the left jugular vein were exposed and then cannulated with a polyethylene tube (PE50, Becton Dickinson and Co., Sparks, MD) for collection of blood samples and administration of insulin and glucose. The technique for glucose clamping was as described previously (12). Briefly, on the day following an overnight fast (approximately 12 h), each conscious rat was placed in a foam plastic jacket, which allowed movement of all four limbs and forward vision. At the start of the glucose clamp, fasting blood glucose measurements were obtained and the initial load of insulin (25 mU/kg of humalin R, U-40, Shionogi Pharmaceutical Co., Osaka, Japan) was infused in a bolus, followed by an infusion of insulin at a rate of 4 mU/kg/min for 150 min. During the glucose clamp, 12.5% glucose solution was infused as needed to maintain blood glucose at the preinfusion level. Ten microliters of arterial blood was sampled at 7-min intervals for the determination of blood glucose level. At the end of the glucose clamp, 1.5 ml of blood was withdrawn for measurement of the plasma insulin level. The average of the rate of glucose infusion for the last 35 min was taken as the index of insulin sensitivity (M value) of each rat. Figure 1 shows the algorithm of the glucose clamp.

Determination of Muscle Fiber Composition

The dissected soleus muscles were immediately frozen in liquid nitrogen. Ten-millimeter sections sliced by a microtome were stained with 4 mM adenosine-triphosphatase and 18 mM CaCl₂ at pH 9.5 for 45 min at room temperature after preincubation at pH 10.4, 4.6, and 4.3 (16). Muscle fiber composition was then determined under a low-powered microscope. Only type I fibers are characterized by dark staining following preincubation at pH 4.3. Type I and type IIb fibers are sensitive to dark staining after preincubation at pH 4.6, and type IIA and IIb fibers are sensitive to preincubation at pH 10.4. The composite ratio of type IIb fibers was calculated by the subtraction of type I fibers from the total number of type I + type IIb fibers, and the composite ratio of type IIA fibers was calculated by counting the number of fibers in which inhibition of staining following preincubation at both pH 4.3 and pH 4.6. A minimum of 400 fibers were counted by two investigators individually after coding preparations to minimize individual bias.

Biochemical Measurements

Blood glucose levels were measured by the glucose oxidase method in an Exact 2A glucose analyzer (MediSense, Inc, Waltham, MA). Plasma insulin levels were assayed by a double antibody radioimmunoassay technique using human insulin standards (Otsuka Assay Lab, Tokushima, Japan).
Statistical Analyses

All data were expressed as mean ± SEM. Changes within each group over time were assessed by two-way ANOVA, and a comparison of the three groups was done by one-way ANOVA. Regression analyses were used to compare the relationship between M values and muscle fiber composition. Values of p < .05 were considered to be statistically significant.

Table 1. Characteristics of Each Group for 2 Weeks Treatment with Vehicle or TSJN at Age of 12 Weeks

<table>
<thead>
<tr>
<th>Group</th>
<th>Control, n=20</th>
<th>FFR, n=18</th>
<th>FFR+TS, n=21</th>
</tr>
</thead>
<tbody>
<tr>
<td>Weight (g)</td>
<td>362±6</td>
<td>335±6*</td>
<td>344±4*</td>
</tr>
<tr>
<td>Systolic blood pressure (mmHg)</td>
<td>142±2</td>
<td>155±3*</td>
<td>155±2*</td>
</tr>
<tr>
<td>Heart rate (per min)</td>
<td>342±6</td>
<td>380±8*</td>
<td>367±6*</td>
</tr>
<tr>
<td>Fasting blood glucose (mg/dl)</td>
<td>97±3</td>
<td>95±2</td>
<td>85±3</td>
</tr>
</tbody>
</table>

FFR indicates fructose-fed rats, FFR+TS indicates fructose-fed rats treated with TSJN. Values are mean±SEM. *p < 0.01 vs. control, †p < 0.01 vs. control and FFR.

Results

Body Weight, Blood Pressure, Heart Rate, and Fasting Blood Glucose

The average body weight of rats in the control group was significantly higher than those of rats in the other groups. Systolic blood pressure was elevated significantly at 2 wk after the start of fructose-rich chow feeding, and the high level persisted thereafter throughout the study in the FFR. As shown in Table 1, systolic blood pressure in the FFR groups was significantly higher than that of the control group, and treatment with TSJN for 2 wk had no effect on the systolic blood pressure of the FFR. Heart rate was significantly higher in the FFR and FFR + TS groups compared to that of the control group. Four weeks of treatment of TSJN (FFR + TS-4w) significantly lowered systolic blood pressure and heart rate compared to levels in the FFR-4w group, and there was no difference in systolic blood pressure and heart rate compared to those of the control group (control-4w). The fasting blood glucose level was significantly lower in the FFR + TS group than in the other groups.

Glucose Clamp and Muscle Fiber Composition

Figure 2 shows the time course of the glucose clamp study in each group. Steady-state blood glucose levels during the glucose clamp were similar in the 3 experimental groups. The average rate of glucose infusion during the last 35 min of the glucose clamp, as an index of insulin sensitivity (M value), was significantly lower in the FFR group than in the control rats (15.4 ± 0.4 and 10.9 ± 0.6 mg/kg/min in the control and FFR groups, respectively, p < 0.01) (Fig. 3). TSJN significantly improved M value in the FFR + TS group compared to that in the FFR group (15.1 ± 0.5 mg/kg/min in the FFR + TS, p < 0.01). Four weeks of TSJN treatment improved insulin sensitivity compared to that of the FFR-4w group (Table 2). There was no difference of plasma insulin concentration among the 3 groups at the end of the glucose clamp study (99 ± 3, 101 ± 3, and 105 ± 4 mU/l for the control, FFR, and
The composite ratio of type I fibers in the soleus muscle decreased significantly in the FFR group compared to that in the control group (81.7 ± 1.5 and 75.0 ± 1.7% in the control and FFR groups, respectively, *p < 0.01), and treatment with TSJN led to recovery of the composite ratio of type I fibers in the FFR + TS group to the same level as that of the control group (78.7 ± 1.7% in the FFR + TS group) (Table 3). The composite ratio of type IIa fibers in the FFR + TS group to the same level as that of the control group. The M value was significantly correlated with the composite ratio of type I and type II fibers (for type I fibers, r = 0.45, *p < 0.05, Fig. 4A; for type II fibers, r = −0.44, *p < 0.05, Fig. 4B). No correlations were found between the M value and body weight, fasting blood glucose level, or plasma insulin level (data not shown).

Discussion

The results of this study confirmed those of previous reports that showed feeding healthy rats a fructose-rich chow results in insulin resistance and hypertension (5, 17). The reason the body weights of fructose-fed rats were lower than those of the control rats is unclear. Although we did not evaluate the amount of food intake in each group, food intake in the FFR group may have been less than that in the control rats. It is well known that insulin sensitivity is negatively correlated to body weight. Thus, food intake did not play an important role in insulin resistance in the fructose-fed rats. It is recognized that hyperinsulinemia can stimulate activation of the sympathetic nervous and renin-angiotensin systems, an increase in renal sodium retention, and the proliferation of vascular smooth muscle tissue, and it may be involved in the hypertensive mechanism (1). Although we did not evaluate insulin level fasting or post-prandial condition in this study, hyperinsulinemia has been reported in fructose-fed rats (5). These results suggest that fructose feeding leads to insulin resistance and that compensatory...
hyperinsulinemia may result in hypertension.

It has been demonstrated that in vivo insulin action is significantly correlated with the histologically determined proportions of muscle fiber types (18). It has also been reported that hyperinsulinemia can lead to a change in muscle fiber composition from type I fiber to type II fiber in the soleus muscle (19). These results indicate that an interchange may occur between type I and type II muscle fibers. The results of the present study demonstrated that insulin resistance in fructose-fed rats is concomitant with changes in muscle fiber composition and that there is a correlation between insulin sensitivity and the composite ratios of type I or type II fibers in the soleus muscle. However, details of the mechanisms by which the composite ratio of type I fibers is reduced in fructose-fed rats are still unknown. We have now confirmed that both a long-acting dihydropyridine Ca channel antagonist (12) and TSJN not only improve insulin sensitivity but also lead to recovery in muscle fiber composite ratios in the FFR. Considering the results of these studies, it is possible that compensatory hyperinsulinemia may change type I fiber toward type II fiber, and the Ca channel antagonist and TSJN not only improve insulin sensitivity but also lead to recovery in muscle fiber composite ratios to the same levels as those of the control group. The mechanisms by which TSJN improves insulin sensitivity remain to be clarified.

There have been few reports on whether Chinese medicines used for the treatment of diabetes mellitus have any effect on insulin sensitivity. It has been reported that some Chinese medicines, such as Goshu-Jinki-Gan, have beneficial effects on numbness, dysuria, and residual urine feeling due to diabetic neuropathy (20). However, the mechanisms by which these symptoms are improved are also still unknown. In the present study, the Chinese medicine TSJN was found to improve insulin sensitivity in FFR, although the details of the mechanisms that lead to this improvement are still unclear. Possible mechanisms responsible for the insulin resistance in FFR are an impairment of insulin signal transduction; a change in plasma membrane fatty acid composition (21); and an increase in sympathetic nerve activity due to fructose-rich chow, which might induce a decrease in insulin sensitivity.

Although we did not assess sympathetic nervous activity in this study, elevated sympathetic nervous activity in FFR due to hyperinsulinemia, or fructose-rich chow per se, may change muscle fiber composition, and TSJN may reverse it by improving insulin resistance and canceling hyperinsulinemia. From this viewpoint, it is understandable that the systolic blood pressure and the heart rate were higher in FFR than those of the control rats, and treatment with TSJN for 4 wk significantly lowered the systolic blood pressure and the heart rate to those of control levels. TSJN contains some crude drugs, including Panax ginseng, which might have an antiatherosclerotic effect, Rheum rhubarb and Chinese cinnamon, which have been believed to have a vasodilative action, and Rehmannia glutinosa libosch, which has also been believed to have a mild blood glucose lowering action. These effects of TSJN could contribute to improving insulin resistance in FFR.

Although TSJN was found not to affect blood pressure during a 2-wk regimen, it appeared to lower blood pressure during a 4-wk treatment in the present study. It has been recognized that insulin resistance and compensatory hyperinsulinemia may result in hypertension through

![Fig. 4. The correlations between M value and composition ratio of type I fibers (A) and type II fibers (B).](image-url)
several mechanisms (1). It has also been reported that an 8-wk course of troglitazone resulted not only in an improvement in insulin sensitivity but also in a lowering of blood pressure in FFR (14). Taking these points into consideration, it is possible that 2-wk treatment with TSJN could improve insulin resistance and cancel hyperinsulinemia, while a longer treatment with TSJN could lower the blood pressure in FFR. At present, drugs such as troglitazone (14) or metformin hydrochloride (22) are known to increase insulin sensitivity. However, due to some side effects such as liver dysfunction or lactic acidosis, the use of these drugs is sometimes avoided. It was well known that Chinese medicines also have some side effects; there have been no reports whether TSJN has severe side effects. Each crude drug included in TSJN, Panax ginseng, Rheum rhubarb, Rehmannia glutinosa, and Chinese cinnamon, have been used in China, Japan and elsewhere, thus TSJN could be used to treat insulin-resistant patients.

In conclusion, insulin resistance and hypertension in fructose-fed rats are concomitant with a decrease in the composite ratio of type I fibers in the soleus muscle. The Chinese medicine TSJN improved insulin sensitivity, lowered blood pressure, and led to recovery in the composite ratio of type I fibers toward control levels. These results suggest that the fiber composition of skeletal muscles plays an important role in insulin resistance in fructose-fed rats, and that TSJN may be beneficial in treating insulin-resistant hypertensives.

References