Brief Report

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Effects of Gosha-jinki-gan (Chinese herbal medicine: Niu-Che-Sen-Qi-Wan) on hyperinsulinemia and hypertriglyceridemia in pre-diabetic Zucker fatty rats

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ABSTRACT: The Chinese herbal medicine, Gosha-jinki-gan (GJ) (Niu-Che-Sen-Qi-Wan), has been widely used for treating patients with melalgia, lower back pain, numbness, and diabetic neuropathy. We investigated the effects of GJ on the regulation of serum insulin and triglyceride levels in obese Zucker fatty rats (fa/fa; ZFR). We administrated GJ to 6-week-old ZFR and non-obese lean rats (LR) for 12 weeks. Body weight and serum glucose, insulin, total cholesterol, and triglyceride levels were significantly increased at 18 weeks in ZFR as compared to the LR. GJ treatment in ZFR significantly suppressed elevation in serum glucose, insulin, and triglyceride levels, but no significant differences were observed in body weight and serum cholesterol levels in the ZFR group with GJ treatment compared to the ZFR group without GJ treatment. These results suggest that GJ may improve hyperinsulinemia and hypertriglyceridemia in ZFR and that GJ may be useful for preventing or delaying the onset of diabetes mellitus in a pre-diabetic state.

Keywords: Gosha-jinki-gan, obese rat, hyperinsulinemia, hypertriglyceridemia, pre-diabetic state

1. Introduction

Non-insulin-dependent diabetes mellitus (NIDDM) is a multifactorial disease caused by the interaction of environmental factors and genetic predisposition, leading to two major impairments: insulin resistance and defective β cell function. In the pre-diabetic state that precedes the onset of NIDDM, hyperinsulinemia compensates for insulin resistance (*1*). Hyperglycemia then develops with progressive beta cell dysfunction, resulting in hypertriglyceridemia (*2*). Hypertriglyceridemia is an important risk factor for coronary heart disease, especially in populations with NIDDM. Amelioration of hyperinsulinemia and hypertriglyceridemia in a pre-diabetic state can be significantly beneficial for reducing the incidence of NIDDM by using safer drugs such as Chinese herbal medicine, over a long period.

Gosha-jinki-gan (GJ) (Niu-Che-Sen-Qi-Wan), a traditional Chinese herbal complex of 10 medicinal herbs, has been widely used for treating patients with melalgia, pain in the lower back, numbness and diabetic neuropathy (*3,4*). In addition, Suzuki *et al.* reported that the antinociceptive activity of GJ was significantly greater in diabetic mice than in non-diabetic mice on the basis of nitrous oxide (NO) production (*5,6*). Further, the homeostasis model assessment of insulin resistance (HOMA-R) index of patients with type 2 diabetes showed a significant decrease after GJ treatment (*7*). However, few reports have been published on the effects of GJ on hyperinsulinemia and hypertriglyceridemia in a pre-diabetic state.

Rodent models of diet-induced hyperinsulinemia and hypertriglyceridemia are used to assess the therapeutic efficacy of drugs and nutrients that are likely to affect insulin sensitivity and lipid concentrations in the blood (*8-10*). However, the effects of GJ on ameliorating the metabolic dysregulation of spontaneously obese rats in pre-diabetic states have not been previously reported. Obese Zucker fatty rats (fa/fa; ZFR) are considered a model for pre-diabetes and are characterized by a genetic defect in the leptin receptor (*7*), which results in hyperphagia, hyperinsulinemia, and severe obesity with relatively mild hyperglycemia, hypertriglyceridemia, and hypercholesterolemia (*11*). In the present study, we investigated the effects of GJ on hyperinsulinemia and hypertriglyceridemia in obese ZFR for a period of 12 weeks.

2. Materials and Methods

2.1. *Animals*

6-week-old male lean Zucker (+/+) rats and obese

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Zucker (fa/fa) rats (Japan SLC Inc., Shizuoka, Japan) were used. The rats were maintained on a standard powder diet (MF® diet; Oriental Yeast, Tokyo, Japan) for 1 week. They were allowed free access to rat chow and water and were kept in a room maintained at 22 ± 2°C with a 12-h/12-h light/dark cycle (light cycle began at 8:00 AM). All experimental procedures were conducted according to the Osaka Ohtani University Guidelines for the Care and Use of Laboratory Animals, and the study protocol was approved by the local Animal Ethics Committee.

2.2. *Drugs*

Spray-dried GJ powder was manufactured and provided by Tsumura & Co. Ltd. (Tokyo, Japan). The composition of GJ is as follows: 5 g of Rehmanniae radix (*Rehmannia glutinosa* Liboschitz); 3 g each of Achyranthis radix (*Achyranthes bidentage* Blume), Corni fructus (*Cornus officinalis Sieb.* et Zucc), Dioscoreae rhizoma (*Dioscorea batatas* Decaisne), Plantaginis semen (*Plantago asiatica*), Alismatis rhizoma (*Alisma orientale* Juzep), Hoelen (*Poria cocos* Wolf), and Moutan cortex (*Paeonia suffruticosa* Andrews); and 1 g each of Cinnamomi cortex (*Cinnamomum cassia* Blume) and Aconiti tuber (*Aconitum carmichaelii* Debeaux).

2.3. *Animal treatments and preparation of blood samples*

The lean Zucker +/+ rats (113-136 g) and obese Zucker fa/fa rats (166–196 g) were randomly divided into groups of 6. The lean Zucker +/+ rats in the 2 groups were maintained on standard chow (L+0%GJ) and standard chow containing 3% powdered GJ extract (L+3%GJ). The obese Zucker fa/fa rats in the O+0%GJ group were maintained on standard chow supplementation without GJ, whereas those in the O+1%GJ and O+3%GJ groups were fed standard chow containing 1% and 3% powdered GJ extract, respectively. The rats had access to the chow and tap water *ad libitum*. Body weights of the rats and food intake per cage were measured on a weekly basis. For 12 weeks, fasting blood samples were collected from the jugular vein and centrifuged, and serum aliquots were stored frozen on the same day.

2.4. *Assays to determine serum glucose, insulin, triglyceride, and cholesterol levels*

Serum glucose levels were determined using a commercial assay kit (Glucose CII-Test Wako; Wako Pure Chemical Industries Ltd., Osaka, Japan). Serum triglyceride and cholesterol levels were determined using the commercial lipid assay kits Triglyceride E-Test Wako and Cholesterol E-Test Wako, respectively (Wako Pure Chemical Industries Ltd.). Serum immunoreactive insulin levels were measured using a commercial assay kit (Merodia Insulin Eiken Elisa kit; Mercodia AB Co. Ltd., Uppsala, Sweden).

2.5. *Data analysis*

Experimental data are expressed as mean values with standard deviations (SD). Statistical analysis of the differences between the mean values obtained was performed using Tukey's multiple comparison test and an unpaired Student's *t*-test with a significance level of $p < 0.05$.

3. Results and Discussion

Rodent models of diet-induced hyperinsulinemia and hypertriglyceridemia are used to assess the therapeutic efficacy of drugs and nutrients that are likely to affect insulin sensitivity and lipid concentration in the blood (*8-10*). However, the effects of GJ in ameliorating the metabolic dysregulation of spontaneously obese rats in pre-diabetic states have not been previously reported.

The changes in the body weights of the rats are shown in Figure 1. These changes were significantly greater in the 3 obese-rat groups than in the lean-rat groups ($p < 0.01$). The body weight changes in the O+1%GJ and O+3%GJ rat groups administered GJ were similar to those in the O+0%GJ rat group. The food intake of the 3 obese-rat groups was greater than that in the lean-rat groups (data not shown). In our study, the body weights and intake weights were similar among the 3 groups of obese rats.

Significant changes were detected in serum glucose levels in the O+0%GJ group rats compared to L+0%GJ group rats at week 12 (Figure 2A). Compared with the O+0%GJ group, the O+1%GJ and O+3%GJ rat groups

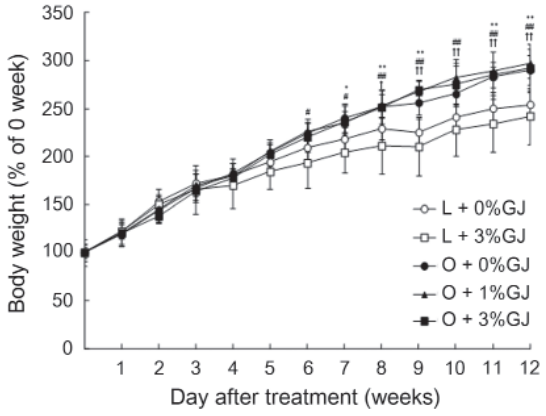

Figure 1. Effects of GJ on body weight in Zucker lean and obese rats. The mean values of O+0%GJ group rats significantly increased compared with that of the L+0%GJ group rats after the 7th week (* $p < 0.05$, ** $p < 0.01$). The mean values of O+1%GJ group rats significantly increased compared with that of the L+0%GJ group rats after the 6th week ($^{\#} p < 0.05$, $^{\#\#} p < 0.01$). The mean values of O+3%GJ group rats significantly increased compared with that of the L+0%GJ group rats after the 8th week ($^{\dagger} p < 0.05$, $^{\dagger\dagger} p < 0.01$). Data represent the mean ± S.D of values in each group ($n = 6$).

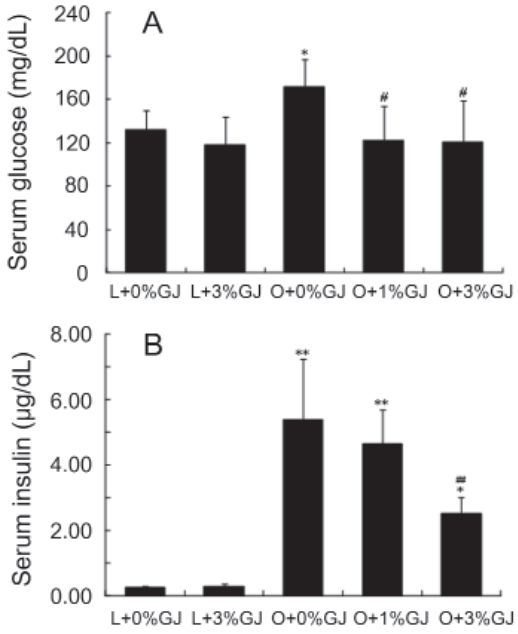

Figure 2. Effects of GJ on serum glucose (A) and insulin levels (B) in Zucker lean and obese rats. (A): The mean value of O+0%GJ group rats significantly increased compared to that of the L+0%GJ group rats (* $p < 0.05$). The mean value of O+3%GJ group rats significantly decreased compared to that of the O+0%GJ group rats ($^{\#}p < 0.05$). **(B):** The mean values of O+0%GJ, O+1%GJ and O+3%GJ group rats significantly increased compared to that of the L+0%GJ group rats (* $p < 0.05$, ** $p < 0.01$). The mean value of O+3%GJ group rats significantly decreased compared to that of the O+0%GJ group rats ($^{\#\#}p < 0.01$). Data represent the mean ± S.D. of values in each group ($n = 6$).

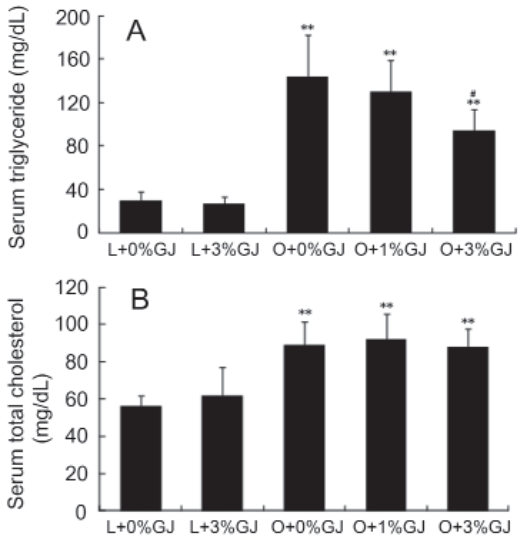

Figure 3. Effects of GJ on serum triglyceride (A) and total cholesterol levels (B) in Zucker lean and obese rats. (A): The mean values of O+0%GJ, O+1%GJ and O+3%GJ group rats significantly increased compared to that of the L+0%GJ group rats (** $p < 0.01$). The mean value of O+3%GJ group rats significantly decreased compared to that of the O+0%GJ group rats ($^{\#}p < 0.05$). **(B):** The mean values of O+0%GJ, O+1%GJ and O+3%GJ group rats significantly increased compared to that of the L+0%GJ group rats (** $p < 0.01$). Data represent the mean ± S.D. of values in each group ($n = 6$).

showed significantly decreased serum glucose levels ($p < 0.05$), similar to the L+0%GJ group rats at week 12. Figure 2B shows the changes in the serum insulin levels. In the O+0%GJ group rats, serum insulin levels were significantly higher than those in the L+0%GJ group rats ($p < 0.01$). The O+3%GJ group rats showed significantly decreased serum insulin levels as compared to the O+0%GJ group rats ($p < 0.01$). These results suggest GJ administration may prevent the development of hyperinsulinemia. We confirmed that GJ did not significantly reduce the serum glucose and insulin levels in lean rats (Figures 2). It has been reported that the nitric oxide pathway may mediate the effect of GJ on insulin action in insulin-sensitive tissues of diabetic model rats (*12*). Cinnamon extract, including cinnamomi cortex, one of the GJ components, has been shown to improve insulin action by enhancing the insulin-signaling pathway in skeletal muscles (*13*). In addition, Qin *et al.* reported that GJ administration improved impaired insulin sensitivity in rats with streptozotocin (STZ)-induced diabetes (*14*). Since GJ is a complex medical preparation in which each ingredient has a different pharmacological action, further studies are required to ascertain the molecular mechanisms underlying the effect of GJ on insulin sensitivity.

Significant increases were detected in the serum triglyceride and cholesterol levels in the 3 obese-rat groups at week 12, compared to the levels in the L+0%GJ group rats (all groups: $p < 0.01$; Figure 3). Serum triglyceride levels in the O+3%GJ group rats were significantly lower than only those in the O+0%GJ group rats ($p < 0.05$; Figure 3A), whereas serum cholesterol levels in the O+1%GJ and O+3%GJ rat groups were not significantly lower than those in the O+0%GJ group rats at week 12 (Figure 3B). Cinnamaldehyde (CA), one of the active components of cinnamon (derived from Cinnamomi cortex), has been reported to reduce plasma triglyceride and nonesterified fatty acid levels when a 40 mg/kg CA-administered group were significantly decreased (*15*). Chemical compounds of the GJ components, such as alisol A 24-acetate (in Alismatis rhizoma), may reduce blood cholesterol levels(*16*). However, this study showed that the administration of GJ did not alter serum cholesterol levels, but did reduce elevated serum triglyceride levels in obese rats.

In conclusion, the data in the present study suggest that GJ may prove useful in the amelioration and/or prevention of hyperinsulinemia and hypertriglyceridemia in a pre-diabetic state. However, further investigation will be necessary to elucidate the molecular mechanisms of GJ in long-term administration.

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