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Research Communications

Effect of dietary *Platycodon grandiflorum* on the improvement of insulin resistance in obese Zucker rats

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The effect of dietary Platycodon grandiflorum *on the improvement of insulin resistance and lipid profile was investigated in lean (Fa/−) and obese (fa/fa) Zucker rats, a model for noninsulin dependent diabetes mellitus. Dietary* Platycodon grandiflorum *feeding for 4 weeks resulted in a significant decrease in the concentration of plasma triglyceride in both lean and obese Zucker rats. Furthermore, dietary* Platycodon grandiflorum *markedly decreased both plasma cholesterol and fasting plasma insulin levels, and significantly decreased the postprandial glucose level at 30 min during oral glucose tolerance test in obese Zucker rats. Although there was no statistical significance, the crude glucose transporter 4 protein level of obese rats fed* Platycodon grandiflorum *tended to increase when compared with that of obese control rats. Therefore, the present results suggested that dietary* Platycodon grandiflorum *may be useful in prevention and improvement of metabolic disorders characterized by hyperinsulinemia states such as noninsulin dependent diabetes mellitus, syndrome X, and coronary artery disease.* (J. Nutr. Biochem. 11:420–424, 2000)

Keywords: insulin resistance; *Platycodon grandiflorum*; Zucker rats; lipid metabolism; GLUT4

Introduction

The root of *Platycodon grandiflorum* A. DC (Chinese name, *Jiegeng*; Korean name, *Doraji*; and Japanese name, *Kikyo*) has been used as an expectorant in traditional oriental medicine. Some studies on its chemical[1–3] and immunopharmacologic effects[4,5] have been done, but little is known about its clinical and dietary effects. In Korea, the root of *P. grandiflorum* grown for 4 years has generally been used as food. More recently, the root of *P. grandiflorum* grown for 22 years has been employed in folk remedies for diseases of adulthood such as hyperlipidemia, hypertension, and diabetes. The present study was performed to examine the therapeutic effects of dietary *P. grandiflorum* grown for 22 years on the improvement of insulin resistance in genetically obese animal models.

Insulin resistance, which is associated with hyperinsulinemia and impaired glucose tolerance (IGT), has been well documented in obese and noninsulin dependent diabetes mellitus (NIDDM) patients[6–8] and animal models.[9,10] Although the mechanisms for this insulin resistance remain undefined, the defective glucose transport system may play an important role in the pathogenesis of peripheral insulin resistance, because glucose transport appears to be a rate-limiting step for glucose utilization in muscle.[11]

On the other hand, previous epidemiologic studies have suggested that both hyperinsulinemia and insulin resistance contribute to the etiology of hypertension in patients with

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Table 1 Composition of control and experimental diets

Ingredients	Diets (g/100 g diet)	
	Control	Experiment
Casein	20.0	20.0
Sucrose	10.0	10.0
Corn starch	50.0	50.0
Lard	5.0	5.0
Corn oil	5.0	5.0
Mineral mixture[1]	3.5	3.5
Vitamin mixture[1]	1.0	1.0
DL-methionine	0.5	0.5
Cellulose	5.0	—
P. grandiflorum powder[2]	—	5.0

[1]This is identical with AIN-76 mixture.
[2]*Platycodon grandiflorum* grown for 22 years.

obesity and NIDDM[12,13] and are also associated with syndrome X and increased risk for coronary artery disease (CAD).[14,15] Patients with untreated hypertension, as compared with matched normotensive control subjects, are not only resistant to insulin-stimulated glucose uptake but also are hyperinsulinemic.[16–19] These patients exhibit elevated plasma triglyceride levels when compared with control subjects.[16] Based on these considerations, improvement of insulin resistance could be required to prevent development of disorders accompanied by insulin resistance. Thus, physical training[20,21] and dietary approaches[22] have been devised as means of reducing insulin resistance. Recent reports have also documented new compounds that reduce insulin resistance or potentiate insulin action in genetically diabetic and/or obese animals.[23–25]

The present study was conducted as an attempt to reduce insulin resistance, and the results obtained suggest that dietary *P. grandiflorum* lowering plasma lipids in obese Zucker rats may be effective for abnormality of lipid metabolism caused by insulin resistance.

Materials and Methods

Platycodon grandiflorum

The root of *P. grandiflorum* (grown for 22 years) was obtained from Jangsang Doraji Farm (Gyeong Nam, Korea). For the purposes of this experiment it was freeze-dried, milled, and sifted through a 0.59 mm screen. The composition of the root of *P. grandiflorum* is shown in our previous study.[26]

Animals and diets

Obese (fa/fa) Zucker rats and their lean littermates (Fa/−)[27] were purchased from Tokyo Experimental Animals (Tokyo, Japan) at 3 months of age and maintained for 1 week on a laboratory chow diet consisting of 52.7% carbohydrate, 23.6% protein, 4.4% fat, 4.9% fiber, 6.6% minerals, and vitamins (CE-2, Clea Japan Inc., Tokyo, Japan) and water. They were individually housed in stainless steel cages and kept in an isolated room at a controlled temperature (23–24°C) and ambient humidity (50–60%). Lights were maintained on a reversed 12-hr light/dark cycle. Obese and Zucker rats (13 weeks old, male) were divided into two groups: *P. grandiflorum* diet and control diet groups, each with five rats, respectively. *Table 1* shows composition of the control and experimental diets. In the previous study, a supplement diet of 5% *P. grandiflorum* had been shown to be more effective than that of 10% *P. grandiflorum* in reducing the cholesterol and triglyceride concentration in serum and liver.[26] In this study, the control group received a diet with 5% cellulose, and the experimental group received a diet containing 5% (wt/wt) *P. grandiflorum* powder. Except for the oral glucose tolerance test (OGTT), the animals were allowed free access to the diet for 4 weeks. At 18 weeks of age, the animals were anesthetized via intraperitoneal injection of pentobarbital sodium (5 mg/100 g of body weight) after an overnight fast, followed by OGTT.

Oral glucose tolerance test

After an overnight fast, D-glucose (1 g/kg body weight) was given by oral tube. Blood samples were obtained by cutting the tail end before glucose loading, and 30, 60, and 120 min after glucose loading, respectively. Blood glucose levels were determined with TIDEX glucose analyzer (Miles, Slough, UK).

Plasma measurement

After anesthesia, blood was drawn from the inferior vena cava and centrifuged at 4°C. Plasma was used for measurements of glucose, immunoreactive insulin, triglyceride, and total cholesterol. After blood was collected, gastrocnemius skeletal muscle from each leg was quickly excised, weighed, clamp-frozen in liquid nitrogen, and stored at −80°C until analysis. Immunoreactive insulin was measured by radioimmunoassay as described previously[20] using human insulin as the standard. The concentrations of triglyceride and total cholesterol were measured by enzyme assay with Determiner TG and Determiner TC555 (Kyowa Medics, Tokyo, Japan), respectively.

Immunoblotting

Crude membrane fraction from gastrocnemius skeletal muscle was prepared in accordance with a previously described method.[20] The protein separated by sodium dodecyl sulphate (SDS)-PAGE was electrophoretically transferred to polyvinylidine difluoride sheets (Immobilion, Millipore, Bedford, MA, USA), and immunoblotted with an antiserum specific for C-terminal amino acid sequence of glucose transporter 4 (GLUT4) and then ^{125}I-labeled protein A as described previously.[20] To quantify GLUT4, pieces of sheet containing the GLUT4 protein were cut out and radioactivity was counted on a gamma counter.

Statistical analysis

Data were expressed as mean ± SE for five rats in each group. Statistical analysis was performed by one-way analysis of variance, and differences between means were tested using Duncan's multiple range test. *P*-values of less than 0.05 were considered to be significant.

Results

Final body weight and plasma concentrations of glucose, triglyceride, and total cholesterol in obese and lean Zucker rats are summarized in *Table 2*. Dietary *P. grandiflorum* feeding had no statistically significant effects on body weight between these animals. In the fasting plasma glucose values, there were no significant differences between obese (5.72 ± 0.28 mmol/L) and lean (5.94 ± 0.11 mmol/L) Zucker rats. However, in obese Zucker rats, *P. grandiflorum* feeding caused a 49% reduction in plasma triglyceride

Table 2 Effect of dietary *Platycodon grandiflorum* (P. g.) on body weight, plasma glucose, triglyceride, and cholesterol concentrations

Animal	Body weight (g)	Plasma glucose mmol/L)	Plasma triglyceride (mmol/L)	Plasma cholesterol (mmol/L)
Obese-P. g.	578 ± 6[a]	6.27 ± 0.05[a]	2.28 ± 0.23[a]	4.14 ± 0.39[a]
Obese-Control	573 ± 15[a]	5.72 ± 0.28[a]	4.45 ± 55[b]	5.82 ± 0.16[b]
Lean-P. g.	360 ± 7[b]	5.44 ± 0.28[a]	0.47 ± 0.07[c]	1.86 ± 0.10[c]
Lean-Control	402 ± 15[b]	5.94 ± 0.11[a]	0.79 ± 0.10[d]	1.76 ± 0.08[c]

Values are mean ± SE; $n = 5$ rats. Values within a column with different superscript letters are significantly different from each other ($P < 0.05$).

concentration (2.28 ± 0.23 vs. 4.45 ± 0.55 mmol/L) and a 29% decrease in cholesterol concentration (4.14 ± 10.39 vs. 5.82 ± 0.16 mmol/L). In addition, for triglyceride values, *P. grandiflorum* feeding resulted in a 40% decrease (0.47 ± 0.07 vs. 0.79 ± 0.10 mmol/L) in lean Zucker rats.

Figure 1 shows the effect of dietary *P. grandiflorum* on plasma insulin level in Zucker rats. As expected, an excessive degree of hyperinsulinemia was exhibited in obese Zucker rats, and a normal range was observed in their lean littermates. The fasting plasma insulin level of obese Zucker rats, which was 13 times higher than that of their lean controls (2,561 ± 416 vs. 194 ± 29 pmol/L, respectively), significantly decreased in the *P. grandiflorum* diet group. In lean Zucker rats, which have been shown to exhibit normal insulin levels, the insulin level in the *P. grandiflorum* feeding group tended to increase but the difference was not significant. To determine the functional effects of dietary *P. grandiflorum*, OGTTs were performed.

As shown in *Figure 2*, there was no significant difference in fasting plasma glucose levels between obese and lean Zucker rats. However, dietary *P. grandiflorum* feeding resulted in a significant decrease in plasma glucose level 30 min after glucose loading in obese Zucker rats. From these results, when compared with control diet, dietary *P. grandiflorum* caused a significant decrease in lipid levels and improvement of insulin resistance in obese Zucker rats.

To elucidate the mechanism involved in improvement of insulin resistance, GLUT4 protein levels were determined in gastrocnemius skeletal muscle of Zucker rats by immunoblotting using a polyclonal antisera. The quantification by gamma counter is showed in *Figure 3*. There was no significant difference in GLUT4 protein levels between obese Zucker rats and their lean littermates as control

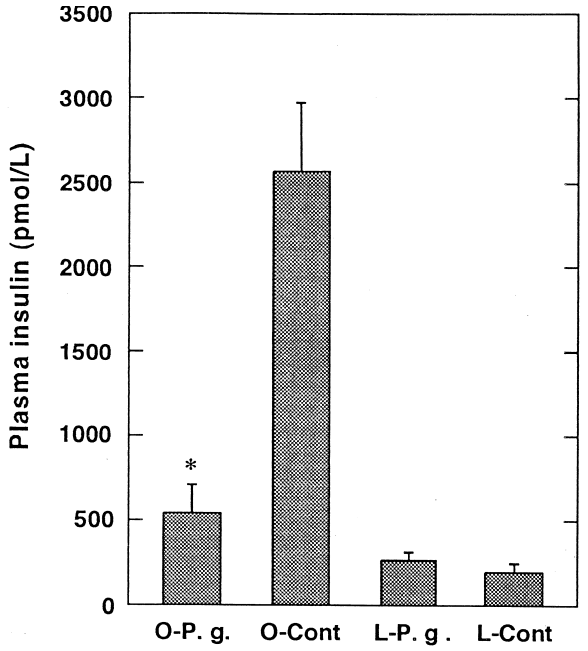

Figure 1 The effect of *Platycodon grandiflorum* (P. g.) diet on plasma insulin levels in Zucker rats. Values are mean ± SE for the five samples. *Statistically significant difference between obese Zucker rats fed P. g. diet and obese control rats fed control diet at $P < 0.05$. O-P. g., obese Zucker rats fed P. g. diet; O-Cont, obese Zucker rats fed control diet; L-P. g., lean Zucker rats fed P. g. diet; L-Cont, lean Zucker rats fed control diet.

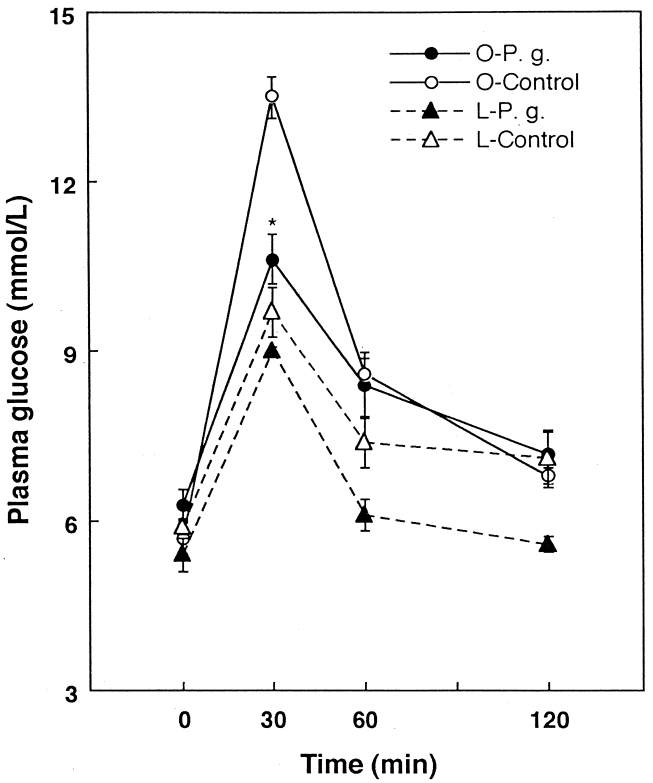

Figure 2 The effect of dietary *Platycodon grandiflorum* (P. g.) feeding on oral glucose tolerance tests in Zucker rats. Values are mean ± SE for the five samples. *Statistically significant difference between obese Zucker rats fed P. g. diet and obese control rats fed control diet at $P < 0.05$. O-P. g., obese Zucker rats fed P. g. diet; O-Control, obese Zucker rats fed control diet; L-P. g., lean Zucker rats fed P. g. diet; L-Control, lean Zucker rats fed control diet.

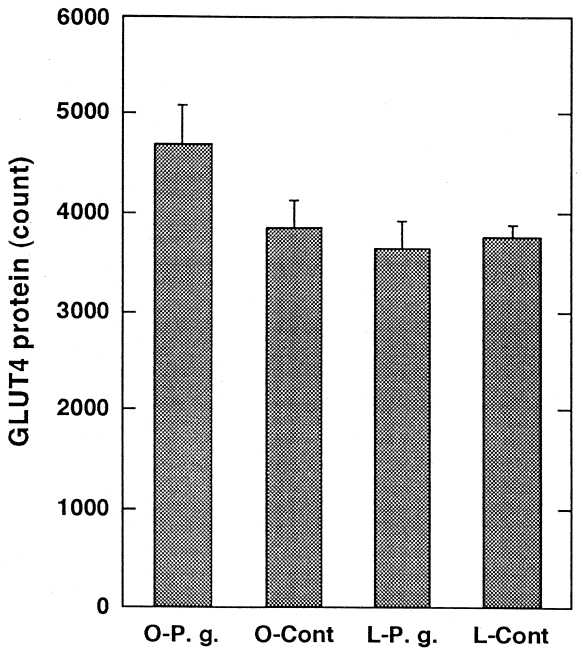

Figure 3. The effect of dietary *Platycodon grandiflorum* (P. g.) feeding on glucose transporter 4 (GLUT4) protein level in gastrocnemius skeletal muscle of Zucker rats. Protein (50 μg) from mixed gastrocnemius skeletal muscle was extracted, subjected to SDS-PAGE, transferred to immobilion membrane, and immunoblotted with polyclonal antibody to GLUT4 protein in accordance with a previously described method.[20] GLUT4 protein was measured by gamma counter obtained from immunoblotting analysis. Values are mean ± SE for the five samples. O-P. g., obese Zucker rats fed P. g. diet; O-Cont, obese Zucker rats fed control diet; L-P. g., lean Zucker rats fed P. g. diet; L-Cont, lean Zucker rats fed control diet.

groups. However, GLUT4 protein levels tended to increase in obese Zucker rats fed the *P. grandiflorum* diet when compared with obese controls.

Discussion

We investigated the effect of dietary *P. grandiflorum* on improvement of insulin resistance by using the rodent model of obesity with insulin resistance, the (fa/fa) Zucker rat, and its lean littermate (Fa/−). Dietary *P. grandiflorum* significantly reduced plasma triglyceride and total cholesterol levels in obese Zucker rats. In addition, *P. grandiflorum* feeding resulted in the improvement of IGT and reduction of hyperinsulinemia. These results indicate that dietary *P. grandiflorum* is effective on abnormal glucose and lipid metabolisms associated with insulin resistance responsible for diabetic syndromes (hyperglycemia, glucose intolerance, hypertriglyceridemia, and hyperinsulinemia).

Hyperlipidemia, in particular hypertriglyceridemia, is one of the most prominent features of obese (fa/fa) Zucker rats. This study showed that the abnormality was significantly improved by treatment with *P. grandiflorum*. The association of hyperlipidemia with insulin resistance has been documented: The increase in hepatic very low density lipoprotein (VLDL)-triglyceride secretion caused by hyperinsulinemia and the reduction of the rate of removal of VLDL-triglyceride from plasma that accompanies insulin resistance has been shown to cause hypertriglyceridemia.[28] It is thus conceivable that the amelioration of hyperlipidemia through feeding a *P. grandiflorum* diet is due to the improvement of hyperinsulinemia through augmentation of insulin sensitivity.

Skeletal muscle is the predominant tissue responsible for insulin-stimulated glucose disposal and a major site of insulin resistance in diabetes.[7] The genetically obese (fa/fa) Zucker rat has proven to be a useful model for the study of mechanisms of insulin resistance because of the well characterized defect in insulin-stimulated glucose uptake by skeletal muscle.[29–31] Glucose transport in skeletal muscle is regulated primarily via GLUT4.[32–34] The defect of GLUT4, which is responsible for facilitated glucose transporter in response to insulin, may contribute significantly to whole-body insulin resistance. The increase of the amount of GLUT4 could contribute to the improvement of insulin resistance. In the present study, however, despite the improvement of insulin resistance by dietary *P. grandiflorum* feeding, GLUT4 protein level was not significantly affected in skeletal muscle of obese Zucker rats. These findings suggest that, in genetically insulin-resistant rodents, the increase in the level of GLUT4 protein does not necessarily reflect the improvement of insulin resistance. This suggestion is supported by previous results obtained from obese (fa/fa) Zucker rats, in which the insulin resistance in the genetic form of obesity is not due to depletion of GLUT4 protein in skeletal muscle.[35] Some studies have reported changes of glucose uptake associated with insulin resistance without a significant change of GLUT4 protein level.[36–39] It has been demonstrated that insulin-resistant states such as obesity and NIDDM in which decreased glucose uptake into skeletal muscle cannot be explained by a decrease in GLUT1 or GLUT4.[36–38] Very recently, it was reported that metformin, an antihyperglycemic agent used in the treatment of NIDDM, reduced the extent of hyperglycemia and hyperinsulinemia, consistent with a reduction of insulin resistance without increasing either new synthesis of GLUT1 and GLUT4 or numbers of these transporters in the muscle membrane of dexamethasone-treated mice.[39] Thus, we could speculate on the other factors for reduction of fasting plasma insulin levels such as increase of translocation and/or intrinsic activity of GLUT4 and improvement of insulin sensitivity and/or responsiveness in the skeletal muscle. This may be supported from significant decrease of postprandial 30-min glucose level on OGTT.

On the other hand, it is well known that impairment of insulin-stimulated glucose uptake is associated with hyperinsulinemia, glucose intolerance, increased plasma triglyceride, decreased high density lipoprotein cholesterol concentration, and high blood pressure. This cluster of phenomena has been called syndrome X.[8] Insulin resistance may play a role in the etiology of this syndrome, and all of these proposed consequences of insulin resistance have been shown to be associated with increased risk for CAD.[14] Based on these considerations, if insulin resistance can be improved, development of this syndrome will be reduced, and the risk for CAD will be minimized. In our previous study,[26] dietary *P. grandiflorum* showed a beneficial effect in preventing hypercholesterolemia and hyperlipidemia in diet-induced hyperlipidemic rats. In the present study, it is

noteworthy that dietary *P. grandiflorum* has shown a significant lipid-lowering effect in genetically obese Zucker rats.

Finally, the present study was performed to screen the natural source for improving insulin resistance. The results suggest that dietary *P. grandiflorum* may be useful in reducing the incidence of metabolic disorders characterized by hyperinsulinemia such as NIDDM, syndrome X, and CAD.

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