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Effect of *Lycium barbarum* Polysaccharide on the Improvement of Insulin Resistance in NIDDM Rats

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Lycium barbarum is one of the traditional oriental medicines. It has been reported to reduce blood glucose levels. In this study, the effect of *Lycium barbarum* polysaccharide (LBP) on the improvement of insulin resistance and lipid profile was studied in rats, a model for non-insulin dependent diabetes mellitus (NIDDM). The rats were divided into three groups: control, NIDDM control, and NIDDM+LBP. Diabetes model groups were made by feeding high-fat diet and subjecting to *i.p.* streptozotocin (50 mg/kg). LBP treatment for 3 weeks resulted in a significant decrease in the concentration of plasma triglyceride and weight in NIDDM rats. Furthermore, LBP markedly decreased the plasma cholesterol levels and fasting plasma insulin levels, and the postprandial glucose level at 30 min during oral glucose tolerance test and significantly increased the Insulin Sensitive Index in NIDDM rats. In the present study, we have tested that LBP can alleviate insulin resistance and the effect of LBP is associated with increasing cell-surface level of glucose transporter 4 (GLUT4) in skeletal muscle of NIDDM rats. Under insulin stimulus, GLUT4 content in plasma membrane in NIDDM control rats was significantly lower than that of control ($p<0.01$), and GLUT4 content in the plasma membrane in NIDDM+LBP rats was higher than that of NIDDM control rats ($p<0.01$). In conclusion, LBP can ameliorate insulin resistance, and the mechanism may be involved in increasing cell-surface level of GLUT4, improving GLUT4 trafficking and intracellular insulin signaling.

Key words——*Lycium barbarum* polysaccharide (LBP); insulin resistance; glucose transporter 4; NIDDM; skeletal muscle plasma membrane

INTRODUCTION

Originated from China and now widely planted in warm and subtropical countries such as Japan, Korea and other southeastern Asian and European countries,[1] *Lycium barbarum* (Chinese Name: Gou qi zi, Medical Name: Fructus Lycii, Latin Name: *Lycium barbarum L*, of the family Solanaceae) is regarded as an important and a good foodstuff. In China, it is a popular health tonic since ancient times and is widely used in many health-building diet formulas. *Lycium barbarum* polysaccharide (LBP) extracted from the traditional Chinese herb *Lycium barbarum*, is found to have bioactivities such as anticancer, antioxidant and hypoglycemic activities.[2] Hypolipidemic effect of LBP was more significant than those of water decoction and purified polysaccharide fractions (LBP-x).[3] Control of diabetes mellitus normally involves exercise, diet and chemotherapy. The increasing interest has been attracted for development and utilization of antidiabetic plants. The plant kingdom is a wide field to search for natural effective oral hypoglycemic or hypolipidemic agents those have less side effects. The present study was performed to examine the therapeutic effects of LBP on the improvement of insulin resistance in NIDDM rats. In order to choose the optimal dose of LBP in the present study, we had measured the hypoglycemic effect of LBP at the dose of 1.25 mg/kg·d, 2.5 mg/kg·d, 5.0 mg/kg·d and 10 mg/kg·d respectively in our preliminary experiments. The result indicated the dose of 10 mg/kg·d is the best, so the dose was used in the present study.

The majority of patients with NIDDM are obese, and it is generally accepted that obesity represents one of the major risk factor in the pathogenesis of NIDDM.[4] One of the most prominent metabolic perturbations associated with the obesity syndrome consists of a pronounced insulin resistance of insulin-stimulated glucose uptake into skeletal muscles.[5] The peripheral insulin resistance, which can lead to hyperinsulinemia and impaired glucose tolerance, is a

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significant pathogenesis of NIDDM and predisposes obese subjects for the development of cardiovascular complications.[6,7] Whole body glucose homeostasis depends upon a delicate balance between hepatic glucose output and glucose utilization by insulin-dependent tissues (adipocytes and skeletal muscle) and insulin-independent tissues (brain and splanchnic organs).[8] Skeletal muscle accounts for at least 80% of glucose disposal during glucose and insulin infusion, whereas adipose tissue accounts for much less.[9–11] Thus, skeletal muscle is quantitatively the most important tissue involved in maintaining glucose homeostasis under insulin-stimulated conditions. Insulin resistance results largely from an impairment of insulin-stimulated glucose uptake into skeletal muscle. Glucose transporter 4 (GLUT4) in plasma membrane, the main insulin-sensitive glucose transporter, mediates trans-membrane transport of glucose (the rate-limiting step of glucose metabolism) in skeletal muscle. Insulin induces translocation of GLUT4 from the intracellular pool to the plasma membrane and thereby it increases glucose uptake in peripheral tissues.[12] Recently, LBP has been described to protect islet cells,[13] but the mechanism that improve insulin resistance has not been elucidated as yet. In this study, we evaluated the effects of LBP on the improvement of insulin resistance. In addition, we examined the effects of LBP on the insulin-induced translocation of GLUT4 in skeletal muscle of NIDDM rats and to provide scientific evidence for development of *Lycium barbarum* as a potential natural oral hypoglycemic and hypolipidemic agent for functional food.

MATERIALS AND METHODS

The fruits of *Lycium barbarum* cultivated in Ningxia Hui Autonomous Region, People's Republic of China, were purchased from a local market and were authenticated by the specialists.

High-fat Diet Composition 35.5% corn oil, 20.0% casein, 34.5% cornstarch, 5.0% cellulose powder, 1.2% vitamin mix, 3.8% mineral mix, 2247.2 energy (KJ/100 g).14)

Experimental Animals Forty male wistar rats, weighing 230～250 g, were provided by the Animal Department of Beijing Institute of Traditional Medical and Pharmaceutical Sciences. By methods in references,[15,16] forty male rats were used, in which ten were chosen out randomly as control, the rest were fed on high-fat diet. After exposure to the high-fat diets for 3 weeks, rats were intraperitoneally injected with 0.5% STZ solution at the dose of 50 mg/kg weights. 72 hours after injection, the blood sample was collected from tail vein in the fasted rats, and the level of serum glucose was determined. The serum glucose level of over 16 mmol/L was defined as diabetic model rats. Then these rats were divided into two groups of model, NIDDM control group and NIDDM＋LBP group. The NIDDM＋LBP group rats were treated by oral infusion with LBP (10 mg/kg·d) dissolved in normal saline and NIDDM control group rats received normal saline (10 mg/kg·d) for 3 weeks. All rats were individually housed in stainless cages and kept in an isolated room at a controlled temperature (18～25℃) and ambient humidity (50～80%). Lights were maintained on a reversed 12–h light/dark cycle. Body weight was determined 2～3 times per week.

Drugs Streptozotocin (STZ) from sigma Co; 0.5% STZ solution was prepared with 0.1 mol/L citric acid and sodium citric acid buffer (PH 4.4) before used.

Preparation of *Lycium barbarum* Polysaccharide The dried fruits were ground to powder, and the powder were refluxed two times (each time for 1 h) to remove lipids with petroleum ether (60～90℃), then refluxed again with 80% ethanol two times (each time for 2 h) to remove monosaccharide and oligosaccharide. The residue was extracted three times in hot water (90℃) and then combined filtrate to concentrate through decompressing using a rotary evaporator (ShangHai, China), after then, added little dose of activated carbon to decolor. After filtering, the filtrate was added 95% ethanol and deposited for one night. The precipitation was washed using 95% ethanol, 100% ethanol, acetone and aether respectively for time after time. In the end, the precipitation was collected and vacuum-dried, giving polysaccharide.

Plasma Measurement The rats were anesthetized with sodium pentobarbital (40 mg/kg) at 24 hour after last administration. The indexes of serum glucose, serum insulin, triglyceride, and total cholesterol were determined with the blood samples collected from tail vein. After blood was collected, for the insulin-stimulated study, rats received a tail vein injection of regular insulin (4 μ/100 g), after 20 min, gastrocnemius skeletal muscle from each leg was

quickly excised, weighed, clamp-frozen in liquid nitrogen, and stored at −80℃ until analysis.[17,18] The serum glucose was determined by a one touch II micro blood glucose instrument (American Life Scan Company), total cholesterol (TC) and triglyceride (TG) were measured by enzyme assay. Serum insulin was determined by radioimmunoassay method.

Sub-membrane Preparation of Skeletal Muscle in Rats As described by Barbara,19) briefly, the frozen muscle (100 mg) was pulverized with a steel mortar and pestle (cooled to −60℃) and then homogenized in ice cold buffer (contained 20 mM Tris-Hcl (PH 7.5), 330 mM sucrose, 2 mM EDTA, 0.5 mM EGTA, 1 mM phenylmethylsulfonyl fluoride (PMSF) and 25 μg/ml leupeptin). Homogenates were transferred to microcentrifuge tubes and spun at 1000×g for 10 min, and then obtained the supernatants. The supernatants were spun again at 40 000 ×g for 1 h, this time, the obtained supernatants represented the membrane component in the cell. The pellet was resuspended in the above-mentioned buffer (no sucrose, plus 1% (v/v) Triton X-100) and extracted by ultrasonic wave for 7 times (1 sec×7), then spun at 40 000×g (at 4℃) for 1 h, at last, the supernatants were the membrane ingredient including GLUT4.

Immunoblotting Proteins were separated by sodium dodecyl sulphate/polyacrylamide gel electrophoresis (SDS/PAGE) using Excel 8% gels (Sigma) and transferred to a polyvinylidene difluoride (PVDF) membrane (Sigma) in a semidry blotting apparatus. The membrane was blocked with 5% nonfat dry milk in phosphate-buffered saline (PBS), PH 7.4, and then incubated with GLUT4 polyclonal antibody (R&D systems Inc). After washing the membrane with PBS, detection was carried out by incubating the membrane with a horseradish peroxidase-conjugated rabbit anti-mice antiserum as a secondary antibody (Sigma) for 1 h at room temperature. After clear washing, the membrane was developed by using enhanced chemiluminescence (ECL) (Amersham). The relative ration of GLUT4 was quantitated by image analysis system.

Glucose Tolerance Test After 12 h fast, rats were intraperitoneally injected with glucose (2 g/kg body weight). Blood samples were obtained by cutting the tail end before glucose loading, and 0, 30, 60, and 120 min after glucose loading respectively.

Insulin Sensitive Index (ISI):

$$ISI = \ln (1/FBG \times FINS)$$

Statistical analysis Data were expressed as mean±S.E. Statistical analysis was performed by one-way analysis of variance, and differences between means were tasted using Duncan's multiple range tests. p-values of less than 0.05 were considered to be significant.

RESULTS

After thirty rats were injected with STZ, there were four rats died. All the survived rats attained diabetes standard through glucose tolerance test. During LBP treatment, there was one rat died in control group, and there were one, two rats died respectively in NIDDM+LBP group and NIDDM control group.

Effect of *Lycium barbarum* Polysaccharide on Weight Before making model, the rats had no significant difference in weight. But after LBP treatment, the NIDDM+LBP group rats weight were lower than NIDDM control group ($p<0.05$). The results were shown in Table 1. The results indicate LBP has definite weight-lowering effect on obese rats.

Glucose Tolerance Test, Fasting Plasma Insulin and Insulin Sensitive Index (ISI) The results were shown in Table 2 Before LBP treatment, the fasting plasma insulin level of NIDDM+LBP group and NIDDM control group rats was remarkably higher than control group ($p<0.01$), but ISI was remarkably lower than control group ($p<0.01$). There was not significant difference in the fasting insulin level between the two groups. The results indicate NIDDM +LBP and NIDDM control groups existed in insulin resistance in some degree. After LBP treatment, blood sugar at 0, 30, 60, and 120 min and fasting plasma insulin were determined. The results of the NIDDM control group were all higher than NIDDM +LBP groups ($p<0.05$) and they accorded with diabetes diagnosis standard. Although the blood sugar and fasting plasma insulin levels of NIDDM+LBP group were higher than control group at any time, they were lower than NIDDM control group ($p<0.05$). As shown in Fig. 1 (B), dietary LBP resulted in a significant decrease in plasma glucose level 30 min after glucose loading in NIDDM rats. For ISI, NIDDM control group rats were lower than control group ($p<0.01$). As similar, the ISI in LBP group was lower than that in control group, but it was higher than in NIDDM control group ($p<0.05$). See Table 2 The above results indicate LBP could im-

Table 1. Effect of *Lycium barbarum* Polysaccharide Treatment on Body Weight of NIDDM Rats

Groups	Body weight (g)		
	Before model	After model	After LBP
Control	240.57±5.58	260.29±9.71	282.15±9.62
NIDDM control	240.52±5.58	341±9.80[a]	394.6±9.47[a]
NIDDM+LBP	240.50±5.50	342.25±8.80[a]	367.58±8.68[a,b]

Each value represents mean±S.E, *a* Represents statistical significance vs. control ($p<0.01$), *b* Represents statistical significance vs. NIDDM control ($p<0.05$).

Table 2. Effect of *Lycium barbarum* Polysaccharide on the Fasting Plasma Insulin Levels and ISI of NIDDM Rats

Groups	Before LBP treatment		After LBP treatment	
	FINS (mIU/l)	ISI	FINS (mIU/l)	ISI
Control	18.29±4.08	−4.45±0.21	18.97±3.01	−4.46±0.24
NIDDM Control	31.23±3.25[a]	−5.26±0.16[a]	29.65±3.21[a]	−5.23±0.17[a]
NIDDM+LBP	32.74±2.47[a]	−5.33±0.23[a]	24.25±2.28[a,b]	−4.93±0.14[a,b]

Each value represents mean±S.E., *a* Represents statistical significance vs. control ($p<0.01$), *b* Represents statistical significance vs. NIDDM control ($p<0.05$).

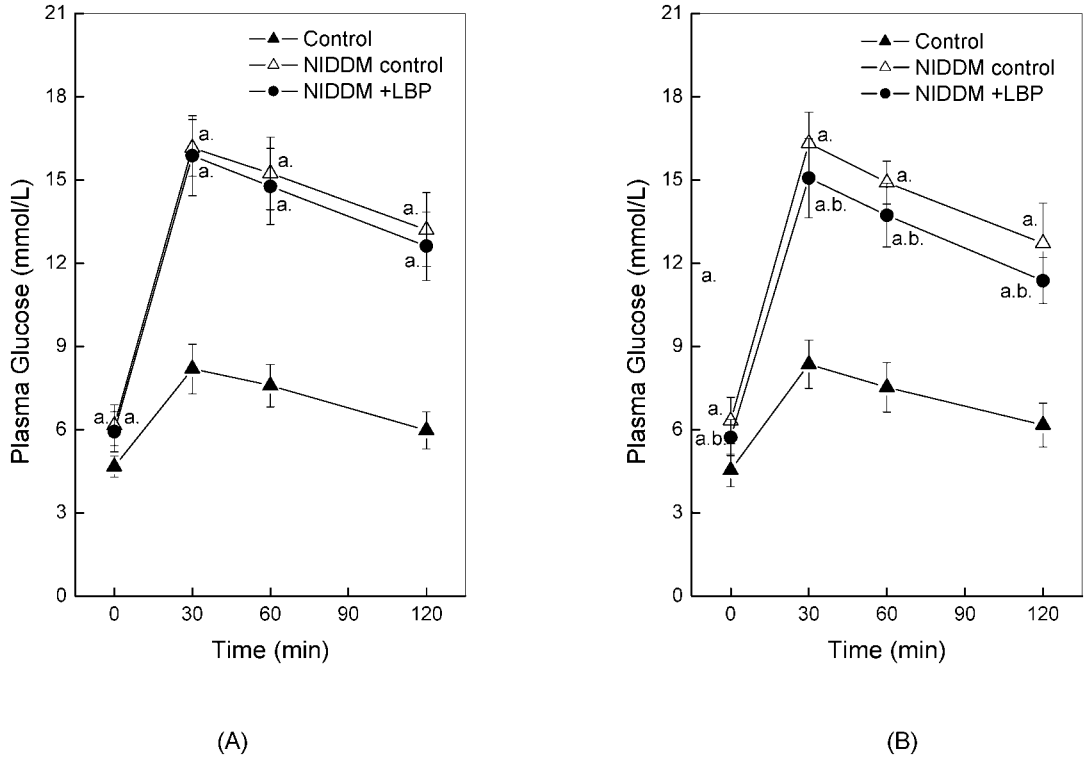

Fig. 1. (A) Before LBP Treatment, the Oral Glucose Tolerance Tests in Rats (B) After LBP Treatment, the Effect of LBP Treatment on Oral Glucose Tolerance Tests in Rats

Value represents mean±S.E.[a], Represents statistical significance vs. control ($p<0.05$),[b] Represents statistical significance vs. NIDDM control ($p<0.05$).

prove insulin resistance and abnormal glucose tolerance in NIDDM rats.

Effect of *Lycium barbarum* Polysaccharide on Blood Lipid Levels Before the rats were treated with LBP, TC and TG contents of NIDDM+LBP and NIDDM control groups were significantly higher than control group ($p<0.05$), but there wasn't significant difference between the two groups. After ad-

ministration of LBP, TC and TG contents of NIDDM +LBP group were also higher than control group, but they were lower than NIDDM control group ($p<0.05$). *See* Table 3. The results suggest that LBP has definite lipid-lowering effect on NIDDM rats.

Effect of *Lycium barbarum* Polysaccharide on Insulin-induced Translocation of GLUT4 in Skeletal Muscle of NIDDM Rats To elucidate the mechanism involved in improvement of insulin resistance, GLUT4 content was determined in gastrocnemius skeletal muscle of tested rats. The quantification was showed in Fig. 2. Under insulin stimulus, the GLUT4 content in plasma membrane in NIDDM control rats was significantly lower than that of control ($p<0.01$), and the GLUT4 content in the plasma membrane in NIDDM+LBP rats was higher than that of NIDDM control rats ($p<0.01$).

DISCUSSION

Lycium barbarum, a well-known traditional Chinese medicinal herb, has been used to treated diabetes mellitus and related hyperlipidemia. However, its effect on insulin resistance is not well understood. Insulin resistance is a key feature of type 2 diabetes. At present, the diabetic animal model is mainly divided into three kinds: genetically type, drug type and drug plus feed type. The genetically type includes Zucker rats, ob/ob mice, OLETF rats etc. The condition of raising and cost are high, so these models are less application in domestic. The drug type made by injecting STZ is similar to insulin dependent diabetes mellitus. According to previous studies,[20] we made insulin resistance rats model through high-fat diet and combining with injecting little dose STZ to decrease the destruction of β cells. Effect of Chinese medicine on the improvement of insulin resistance was valued by ISI. The statistical significant difference between before treatment and after treatment was the assessment standard. In this test, these animals represent a well-characterized animal model of insulin resistance associated with extreme obesity, hyperinsulinemia, lipodystrophy and impaired glucose tolerance,[21] and these animals may be an appropriate model for addressing pathophysiologic question relating to the NIDDM patient.

In recent years, various plant extracts have been investigated for their antidiabetic effect. The active substances include polysaccharides. We investigated the effect of LBP on improvement of insulin resistance by using the model of obesity with insulin resistance.

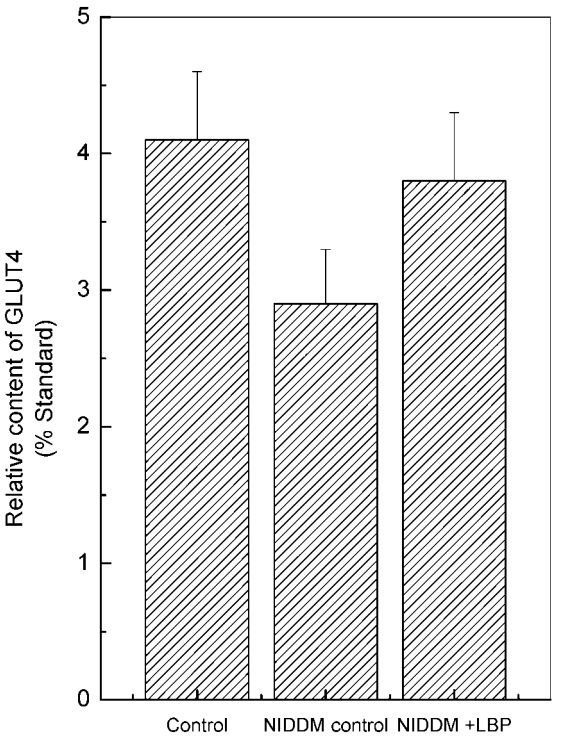

Fig. 2. The Effect of *Lycium barbarum* Polysaccharide on GLUT4 Translocation in Gastrocnemius Skeletal Muscle of NIDDM Rats

Quantification of Western blots was done by using HPIAS. 1000 Imager software. A Wistar rat gastrocnemius homogenate was run as an internal standard for quantification in each assay. A representative autoradiogram and relative amount of GLUT4 protein levels are shown. Value represents mean±S.E. NIDDM control was compared with control $p<0.01$, NIDDM +LBP group was compared with NIDDM control $p<0.01$.

Table 3. Effect of *Lycium barbarum* Polysaccharide on Blood Lipid Levels of NIDDM Rats

Groups	TC (mmol/l)		TG (mmol/l)	
	Before LBP	After LBP	Before LBP	After LBP
Control	1.83±0.12	1.81±0.19	0.59±0.11	0.61±0.11
NIDDM control	2.77±0.13[a]	2.94±0.12[a]	1.38±0.14[a]	1.61±0.28[a]
NIDDM+LBP	2.73±0.16[a]	2.41±0.11[a,b]	1.38±0.16[a]	1.11±0.19[a,b]

Each value represents mean±S.E., *a* Represents statistical significance vs. control ($p<0.05$), *b* Represents statistical significance vs. NIDDM control ($p<0.05$).

LBP significantly reduced plasma triglyceride and total cholesterol levels in obese model rats. In addition, LBP treatment resulted in the reduction of hyperinsulinemia. The hypoglycemic activity of LBP is in a dose dependent manner. These results indicate that dietary LBP is effective on abnormal glucose and lipid metabolisms associated with insulin resistance responsible for diabetic syndromes (hyperglycemia, glucose intolerance, hypertriglyceridemia, and hyperinsulinemia).

Obesity is almost invariably associated with insulin resistance and any reduction of excess body fat contributes to an improvement of insulin sensitivity.[22] Many studies have demonstrated that when weight loss occurs,[23,24] or when there is increased physical activity,[25,26] the plasma levels of insulin go down, insulin resistance is decreased, and all of the components of the metabolic syndrome are improved. Obesity elevates serum free fatty acid concentrations and enhances lipid oxidation.[27] This may cause insulin resistance via inhibition of pyruvate dehydrogenase and phosphofructokinase, directing glucose into the hexosamine pathway.[28] Fatty acids induce changes in glucose transport activity could be a result of the effects of fatty acids on the GLUT4 transporter directly or could result from alterations in the insulin-signaling cascade. Fatty acids get into the muscle cell, ultimately decreased activation of GLUT4. We show here a reduced weight gain in obese rats treated by LBP.

Many investigations reveal that hypertriglyceridemia is closely linked to insulin resistance.[29,30] In unpublished studies from our laboratory, rats with primary hypertriglyceridemia were found to be insulin resistant by the glucose-clamp technique. High serum triglyceride levels in rats with insulin resistance are due in part to overproduction of VLDL triglyceride, secondary to increased triglyceride synthesis in the liver. This study showed that the abnormality was significantly improved by treatment with LBP. It is thus conceivable that the amelioration of hyperlipidemia through dietary LBP treatment is due to the augmentation of insulin sensitivity.

GLUT4 is insulin-dependent and is responsible for the majority of glucose transport into muscle and adipose cells in anabolic conditions. Insulin stimulates increased glucose transport in these tissues (muscle and adipose) by causing the redistribution of GLUT4 from an intracellular pool to the cell surface where it acts as a favorable transporter to enhance entry of glucose into the cell.[31—34] Normally, insulin binds to insulin receptors on target organ cells, resulting in a series of cellular events that promote intracellular glucose transport and metabolism.[35,36] Insulin resistance is the inability of peripheral target tissues to respond properly to normal circulating concentrations of insulin. Skeletal muscle is the major site of insulin-stimulated glucose disposal.[37] Glucose transport in skeletal muscle is regulated primarily via GLUT4.[38] Under physiological conditions, the transport of glucose across the skeletal muscle or adipose cell plasma membrane is the first rate limiting step in the regulation of glucose metabolism.[39—42] Under basal conditions, intracellular free glucose concentrations in skeletal muscle are normally negligible. When the rate of glucose transport is excessively elevated by either hyperinsulinemia,[43] hyperglycaemia,[44] or exercise,[45] free glucose accumulates within the skeletal muscle cell, indicating that intracellular steps involved in glucose metabolism may become rate limiting. Furthermore, overexpressing GLUT4 in these tissues resulted in enhanced whole body insulin sensitivity in normal[46—48] and diabetic mice[49]. Thus, defects in insulin-stimulated glucose transport are likely to alter whole body insulin sensitivity which may result in insulin resistance.

The intracellular signaling mechanisms after insulin receptor stimulation have been investigated extensively in recent years. Since GLUT4 membrane translocation was important for its activation, in the present study, under insulin stimulus, the GLUT4 content in plasma membrane in LBP treatment rats was markedly higher than that of NIDDM control rats. The effect of LBP on the improvement of insulin resistance involved in facilitating GLUT4 translocation to the plasma membrane from an intracellular pool and increasing the GLUT4 content in the skeletal muscle membrane. However, whether the improvement is also associated with the overexpressing GLUT4 in skeletal muscle or increasing the intrinsic activity of GLUT4, the further studies need to be confirmed. In conclusion, our findings may provide information to reveal the true effect of dietary Lycium barbarum on insulin resistance in NIDDM.

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