Antidiabetic effects of Tangnaikang on obese Zucker rats and the mechanism

Xiang-yu GUO1,2, Ying DUAN1, Juan-e LI1,2, Li-xia YANG1, Lian-sha HUANG1,2, Zhi-cheng WANG1, He-yao WANG1, Tong-hua LIU1,2
1. Beijing University of Chinese Medicine, Beijing 100029, China
2. Department of Endocrinology, Dongfang Hospital, Beijing University of Chinese Medicine, Beijing 100078, China
3. Department of Traditional Chinese Medicine, Beijing Tongren Hospital, Beijing 100730, China
4. The Third Laboratory of Pharmacology, Shanghai Institute of Materia Medica, Chinese Academy of Sciences, Shanghai 201203, China

Objective: To observe the effects of Tangnaikang (TNK), a compound traditional Chinese herbal medicine, on glucose metabolism and insulin resistance in obese Zucker rats.

Methods: Twelve male obese Zucker rats, 6 weeks old, were randomly divided into control group and TNK group (3.24 g/kg) after being fed for 2 weeks. All rats received high-fat diet and 4-week treatment. Body weight and blood glucose were tested every week. Oral glucose tolerance test (OGTT) was performed and fasting insulin level was tested on days 0, 14 and 28. Triglyceride, cholesterol, low-density lipoprotein cholesterol (LDL-C), high-density lipoprotein cholesterol (HDL-C) and free fatty acids (FFA) were tested on day 28. Glucose infusion rate (GIR) was tested by hyperinsulinemic-euglycemic clamp from day 29. The protein expressions of protein kinase B (Akt), phospho-Akt (p-Akt) (Thr308) and glucose transporter protein 4 (GLUT4) in skeletal muscle and GLUT4 in adipose tissue were measured after hyperinsulinemic-euglycemic clamp test.

Results: Compared with the control group, the fed blood glucose level and glucose level of OGTT at 120 min had a significant decline in TNK group on day 28, and TNK caused no alteration of the fasting serum insulin, and the GIR increased significantly in hyperinsulinemic-euglycemic clamp study. Furthermore, TNK increased Akt and p-Akt (Thr308) protein expressions in skeletal muscle and decreased the protein expression of GLUT4 in white adipose tissue. Body weight, and triglyceride, cholesterol, LDL-C and FFA contents were slightly decreased in the TNK group, but there were no statistically significant effects.

Conclusion: TNK increases the protein expressions of Akt and p-Akt (Thr308) of the signal transduction pathway to influence the translocation of GLUT4 in skeletal muscle and improves glucose metabolism by reducing insulin resistance.

Keywords: compound (TCD); diabetes mellitus, type 2; insulin resistance; glucose transporter protein 4; glucose clamp technique; obese Zucker rats

Open Access

糖耐康对肥胖 Zucker 大鼠血糖的影响及其机制

郭翔宇1,2, 段颖1, 李娟娟1,2, 杨丽霞1, 黄敏华1,2, 王志林1, 王忠平1, 刘锦华1,2
1. 北京中医药大学，北京 100029
2. 北京中医药大学东方医院内分泌科，北京 100078
3. 北京同仁医院中医科，北京 100730
4. 中国科学院上海药物研究所第三药理实验室，上海 201203

基金项目：北京市教育委员会共建项目（No. BJSJW2008001）

Correspondence: Tong-hua LIU, MD, Professor; Tel: 010-64286642; Fax: 010-64286642; E-mail: thliu16@163.com
目的：观察中药复方糖耐康对肥胖 Zucker 大鼠糖代谢和胰岛素抵抗的影响。
方法：6 周龄雌性肥胖 Zucker 大鼠 12 只，随机分为对照组和糖耐康组 (3.24 g/kg)，所有大鼠给予高脂饲料喂养，疗程为 4 周。每周检测体重和血糖；入组前和治疗 14, 28 d 时行口服葡萄糖耐量实验 (oral glucose tolerance test, OGGT) 并检测空腹血糖和胰岛素水平；第 28 天时检测空腹胰岛素水平 4 项和血浆游离脂肪酸 (free fatty acids, FFA) 水平；第 28 天时进行高胰岛素正葡萄糖钳夹实验检测平均葡萄糖输注率 (glucose infusion rate, GIR)；实验结束后处死大鼠并取材，检测骨骼肌中蛋白激酶 B (protein kinase B, PKB/Akt)，磷酸化蛋白激酶 B (phospho-Akt, p-Akt/Thr308)，葡萄糖转运蛋白 4 (glucose transporter protein 4, GLUT4) 的表达和脂肪组织 GLUT4 的蛋白表达。
结果：与对照组相比，治疗 4 周后，糖耐康组血清胰岛素水平没有变化，餐后血糖和 OGGT 中 120 min 时的血糖水平显著下降；糖耐康组高胰岛素正葡萄糖钳夹实验后 GIR 显著升高；糖耐康能显著增加骨骼肌 Akt，p-Akt(Thr308) 的蛋白表达，减少脂肪组织 GLUT4 的蛋白表达；糖耐康有降低脂质量、血脂 (三酰甘油、胆固醇、低密度脂蛋白) 和 FFA 含量的趋势，但两组间比较差异无统计学意义。
结论：糖耐康可能是通过增加骨骼肌 Akt 和 p-Akt(Thr308) 的表达，增强 GLUT4 的葡萄糖转运能力来实现降低血糖，改善外周胰岛素抵抗的功效。
关键词：中药复方；糖尿病，2 型；胰岛素抵抗；葡萄糖转运蛋白 4；葡萄糖钳制技术；肥胖 Zucker 大鼠

Diabetes is a serious health problem around the world. Over 90% of patients with diabetes have type 2 diabetes. Islet dysfunction and peripheral insulin resistance are both present in type 2 diabetes and are both necessary for the development of hyperglycemia. It has already been demonstrated by epidemiological studies that hyperglycemia is one of the principal causes of complications as cardiovascular and cerebrovascular diseases. Complications lead to poor life quality, as well as high morbidity and mortality. Effective blood glucose control is the key to preventing or reversing type 2 diabetes.

Diabetes is called wasting-thirst disease in traditional Chinese medicine (TCM), characterized by polydipsia, polyphagia, polyuria, and emaciation. In TCM theory, wasting-thirst is considered a result of yin deficiency with dryness-heat. The treatment of diabetes should focus on nourishing yin to clear away heat from the body. This study sought to determine whether the mixture of aqueous and ethanol soluble extract of Tangnai-kang (TNK), a compound traditional Chinese medicine, could normalize hyperglycemia in an animal model with gene mutation, obese Zucker rat model. The model exhibits obesity and high insulin resistance, such as hyperglycemia, glucose intolerance, and hyperinsulinemia, which resembles human type 2 diabetes. We also explored the mechanisms responsible for glucose transporter 4 (GLUT4) translocation by measuring upstream protein expression level of signal transduction pathway.

1 Materials and methods

1.1 Materials TNK was composed of Spica Prunellae Vulgaris (Xiakuaocao), Folium Psidii Gravatae (Fanshihuaye), Fructus Ligustri Lucidi (Nuzhenzi), Herba Saururi (Sanbaicao) and Radix Ginseng (Renshen) and prepared according to the proportion of 2 : 2 : 1 : 1 : 1/2. All herbal drugs were obtained from Shenyang Pharmaceutical Group Corporation. The process was as follows. Firstly, the first four plants were mixed and then extracted by 8 times amount of 75% ethanol twice (1 h each time) using reflux condenser. The recovered ethanol solution (60 to 70 °C) was lyophilized which resulted in 13.83% of the original extract. Secondly, 6 times amount of water was added into the residue and the mixture was filtrated for 1 h, producing 5.49% extract. Lastly, Panax Ginseng fine powder, the two extracts, and excipients were mixed together to produce dried granule for future use. Preparation process was performed by Sichuan Medicinal Pharmaceutical. It is confirmed in Pharmacopoeia of the People’s Republic of China (2000 ed) with high-performance liquid chromatography (HPLC) analysis that the total concentration of Re and Rg1 in the ginsenoside was 0.35%, while spectrophotometry analysis confirmed that the total flavonoids in the extract was 4.23%, the total triterpene acids was 1.99%, and the total triterpenoid saponin was 3.67%. Before each experiment, TNK (3.24 g/kg, about 4.5 g crude herbal drugs) was suspended in distilled water (10 mL) for oral administration. Rats in control group were administered with distilled water. No noticeable adverse effects were observed in any rats after the treatment.

1.2 Animals The study protocol was approved by the Institutional Animal Care and Use Committee of the Beijing University of Chinese Medicine. Twelve male obese Zucker rats, 6-week old, weighing (188.98 ± 17.88) g, were obtained from Professor Wang Heyao of Shanghai Institute of Materia Medica. All rats were housed in environmentally controlled conditions with a
12-h light/dark cycle and they had free access to rodent pellet high-fat diet, including 68.5% basic diet, 15% pork fat, 15% sucrose, 1% cholesterol and 0.5% cholate, except when fasted before some experiments. After being fed for 2 weeks, all rats were randomly divided into TNK group and control group by body weight and received oral administration once a day for 4 weeks.

1.3 Measurements of fed glucose level, fasting serum insulin, oral glucose tolerance test, blood lipid, free fatty acids, and body weight Fed blood glucose level in tail blood samples (about 5 µL each) was measured at 4:00 P.M. on days 7, 14, 21, and 28.

Fasting serum insulin level test and oral glucose tolerance test (OGTT) were performed at 9:00 A.M. on days 0, 14 and 28. Blood lipid and free fatty acids (FFA) were only detected on day 28. On test days, blood samples (about 500 µL each) of rats (fasted for 12 hours) were taken for later tests before they received oral administration of glucose (2 g/kg) for 30, 60, and 120 minutes of OGTT (about 5 µL for each blood sample from tail).

Body weight was measured every week during the test.

1.4 Biochemical assays Blood glucose level was determined by a glucose analyzer (ACCU-Check, Roche, Switzerland), serum insulin level was assayed with radio-immunoassay (RIA) for human insulin (Furui Bioengineering Company, Beijing), lipid concentrations, including triglyceride, cholesterol, low-density lipoprotein cholesterol (LDL-C), and high-density lipoprotein cholesterol (HDL-C) were assayed with an automatic biochemical analyzer (AU400 Biochemical Analyzer, Olympus, Japan), and FFA was assayed with enzyme-linked immunosorbent assay kit (ADL, USA). All detection items were measured according to the manufacturers’ procedures.

1.5 Hyperinsulinemic-euglycemic clamp test The clamp technique was performed as developed by Attele et al[3]. Rats received TNK or distilled water for 28 days, as described above. On day 24, rats were anesthetized with hydral at a dose of 350 mg/kg. A catheter (PE50) was inserted into the left internal jugular vein, and then fixed at the vertex of the head. The catheterized rats were allowed to recover for 4 days (from day 25 to day 28) while they continued to receive the treatment until day 28. Clamp study was performed at 9:00 A.M. on day 29. Before the 240-minute hyperinsulinemic-euglycemic clamp test, rats were fasted for 12 h. During the clamp test, rats were awake and unrestrained. Both insulin (10 mU/ (kg·min), Novolin, Novo Nordisk, Denmark) and 20% glucose were administered into the same catheter implanted in the jugular vein by a two-channel microdialysis syringe pump (WZS-50F6, Smith Medical, Zhejiang), and glucose was infused at variable rates to maintain a plasma glucose at approximately 6.6 mmol/L. Blood samples (about 5 µL each) were collected from the tail every 15 minutes during the clamp test to measure the glucose level and to adjust glucose infusion rate. The average glucose infusion rate was tested at 210, 220, 225, 230, 235 and 240 minutes of clamp period for the determination of whole-body glucose disposal. At the end of the clamp study, rats were sacrificed under anesthesia. Gastrocnemius muscle from hindlimbs and epididymal white adipose tissue were removed and dissected as soon as possible, then frozen immediately with liquid N₂-cooled aluminum blocks and stored at -80°C for later analysis.

1.6 Western-blotting To check the total amount of protein kinase B (Akt), phospho-Akt (Thr308), and glucose transporter protein 4 (GLUT4) in skeletal muscle as well as the GLUT4 in white adipose tissue, the extracted protein samples were subjected to sodium dodecyl sulfate-polyacrylamide gel electrophoresis and Western blotting. Blots were probed with anti-Akt, anti-phospho-Akt (Thr308), and anti-GLUT4 (Cell Signaling Technology, Beverly, MA). Detection of immunoreactive band was performed by using the electrochemiluminescence kit (Amersham Biosciences, UK). Densitometry was performed by scanning the radiographs using the Image J 7.0 system (Media Cybernetics).

1.7 Statistical analysis Values were expressed as \( \bar{x} \pm s \). Data from each set of observations were compared by using Student’s test and ANOVA for repeated measures with SAS 8.0 software (SAS Institute Inc. Cary, NC). Differences were considered significant if \( P \) value was less than 0.05.

2 Results

2.1 Effects of TNK on fed blood glucose level Fed blood glucose level in TNK-treated obese Zucker rats was significantly lower than vehicle-treated rats on day 28 (\( P=0.039 \)).

<table>
<thead>
<tr>
<th>Table 1</th>
<th>Effects of TNK on fed blood glucose level in obese Zucker rats</th>
<th>(( \bar{x} \pm s ), mmol/L)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Group</td>
<td>n</td>
<td>Week 1</td>
</tr>
<tr>
<td>Control</td>
<td>6</td>
<td>6.32±0.61</td>
</tr>
<tr>
<td>TNK</td>
<td>6</td>
<td>6.08±0.67</td>
</tr>
</tbody>
</table>

* \( P<0.05 \), vs control group.
2.2 Effects of TNK on glucose tolerance test Glucose tolerance was evaluated by OGTT. Oral glucose load failed to return to the fasting level at 120 minutes in TNK-treated rats on days 0 and 14. On day 28, the glucose level decreased significantly in TNK-treated rats at 120 minutes, indicating a noticeable improvement of glucose disposal ($P=0.046$, Table 2).

<table>
<thead>
<tr>
<th>Table 2</th>
<th>Effects of TNK on glucose tolerance test in obese Zucker rats ($\bar{x} \pm s$, mmol/L)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Time point</td>
<td>Group</td>
</tr>
<tr>
<td>Day 0</td>
<td>Control</td>
</tr>
<tr>
<td></td>
<td>TNK</td>
</tr>
<tr>
<td>Day 14</td>
<td>Control</td>
</tr>
<tr>
<td></td>
<td>TNK</td>
</tr>
<tr>
<td>Day 28</td>
<td>Control</td>
</tr>
<tr>
<td></td>
<td>TNK</td>
</tr>
</tbody>
</table>

* $P<0.05$, vs control group.

2.3 Effects of TNK on fasting serum insulin level Fasting serum insulin level was shown in Table 3. No significant difference was found between the two groups on days 0, 14 or 28.

<table>
<thead>
<tr>
<th>Table 3</th>
<th>Effects of TNK on fasting serum insulin level in obese Zucker rats ($\bar{x} \pm s$, mU/L)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Group</td>
<td>$n$</td>
</tr>
<tr>
<td></td>
<td></td>
</tr>
<tr>
<td>Control</td>
<td>6</td>
</tr>
<tr>
<td>TNK</td>
<td>6</td>
</tr>
</tbody>
</table>

2.4 Effects of TNK on body-wide insulin-stimulated glucose disposal rate Body-wide insulin-stimulated glucose disposal rate was tested by hyperinsulinemic-euglycemic clamp as shown in Table 4. After the 28-day treatment of TNK, the rate of insulin-stimulated glucose disposal in obese Zucker rats was significantly higher than vehicle-treated rats ($P=0.025$).

<table>
<thead>
<tr>
<th>Table 4</th>
<th>Effects of TNK on body-wide insulin-stimulated glucose disposal rate in obese Zucker rats ($\bar{x} \pm s$)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Group</td>
<td>$n$</td>
</tr>
<tr>
<td></td>
<td></td>
</tr>
<tr>
<td>Control</td>
<td>6</td>
</tr>
<tr>
<td>TNK</td>
<td>6</td>
</tr>
</tbody>
</table>

* $P<0.05$, vs control group.

2.5 Effects of TNK on body weight and blood lipid and FFA contents There was a limited average body weight reduction in the TNK group over 4 weeks as compared with the vehicle ($P=0.070$, Table 5). In parallel, FFA level also decreased slightly ($P=0.11$) in the TNK group after 28-day treatment as compared with the control group. The levels of serum triglyceride, cholesterol, LDL-C, and HDL-C in the TNK group did not change significantly as compared with the control group (Table 6).

<table>
<thead>
<tr>
<th>Table 5</th>
<th>Effects of TNK on body weight change of obese Zucker rats ($\bar{x} \pm s$, g)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Group</td>
<td>$n$</td>
</tr>
<tr>
<td></td>
<td></td>
</tr>
<tr>
<td>Control</td>
<td>6</td>
</tr>
<tr>
<td>TNK</td>
<td>6</td>
</tr>
</tbody>
</table>

2.6 Effects of TNK on protein expressions in skeletal muscle and white adipose tissue We next measured the protein levels of Akt, phospho-Akt (Thr308), and GLUT4 in skeletal muscle (Figure 1) as well as protein level of GLUT4 in white adipose tissue (Figure 1) by Western blotting. TNK did not alter protein expression of GLUT4 ($0.94±0.14$ vs $1.11±0.24$, $P=0.18$) in
skeletal muscle. However, expressions of p-Akt (Thr308) (0.94±0.15 vs 0.44±0.06, \( P < 0.01 \)) and Akt (1.12±0.05 vs 0.87±0.03, \( P < 0.01 \)) proteins in skeletal muscle in the TNK group were significantly higher than those in the control group. Meanwhile, TNK significantly decreased expression of GLUT4 protein in white adipose tissue as compared with vehicle (0.52±0.08 vs 0.89±0.02, \( P < 0.01 \)).

![Figure 1](image)

Figure 1  Expressions of Akt, phospho-Akt (Thr308) and GLUT4 proteins in skeletal muscle and expression of GLUT4 protein in white adipose tissue detected by Western blotting

3 Discussion

This study examined the anti-hyperglycemic effects of TNK (3.24 g/kg) on obese Zucker rat model. Homozygotes (fa/fa) with this mutation rats exhibit leptin resistance, the genetic lesion causing the obesity and whole body insulin resistance, a feature of human metabolic syndrome\(^{[9]}\). Obese Zucker rat is particularly suitable for researching antidiabetic natural products and the underlying mechanisms\(^{[10]}\).

The results of this study clearly showed that TNK produced a consistent hypoglycemic effect. The fed blood glucose level and glucose level of OGTT decreased significantly after the 28-day treatment. More importantly, treatment of TNK in obese Zucker rats also significantly increased the rate of insulin-stimulated glucose disposal in hyperinsulinemic-euglycemic clamp study. However, there was no difference on fasting serum insulin level between the two groups.

The reduction of glucose transport and metabolism in muscle and adipocytes is the early characteristic defect in developing type 2 diabetes of insulin resistance\(^{[11,12]}\). GLUT4 is the major glucose transporter isoform expressed in skeletal muscle and adipose tissue. When GLUT4 translocation is stimulated by an insulin-dependent mechanism, insulin regulates glucose transport by activating insulin receptor substrate-1-dependent phosphatidylinositol 3-kinase which, via increases in phosphatidylinositol-3, 4, 5-triphosphate, activates Akt\(^{[13]}\). Although our results did not indicate a difference in the protein expression of GLUT4, significantly higher phospho-Akt (Thr308) and Akt suggest that the antidiabetic effects of TNK may be achieved by induced stimulation of GLUT4 glucose transporter translocation and glucose transport activity in insulin-resistant skeletal muscle.

Adipose tissue and skeletal muscle play a distinct and complementary role in glucose transporter GLUT4 mediating glucose disposal states\(^{[14]}\). However, insulin resistance in young obese Zucker rats is muscle-specific and adipose tissue is hypersensitive to insulin compared to lean littermates\(^{[15,16]}\). Over-expression of GLUT4 in adipose tissue might compensate for the insulin resistance in skeletal muscle\(^{[17]}\). Down-regulation of GLUT4 and glucose transport selectively in adipose tissue can cause insulin resistance and thereby increases the risk of developing diabetes\(^{[18]}\).

Improved glucose tolerance observed in skeletal muscle in the TNK group may result in the compensatory reduction in adipose tissue, following a significant reduction of GLUT4 protein expression in white adipose tissue. When peripheral blood glucose level rises, muscle adapts to utilization of glucose, whereas liver appears to decrease glucose uptake, and glucose incorporates into fatty acids, resulting in a reduction in FFA concentrations. Thus, we conclude that the hypoglycemic effect in TNK-treated obese Zucker rats may be a result of the improvement of glucose metabolism in skeletal muscle and white adipose tissue conjunctively.

Lowering the body weight can improve the glucose metabolism in obese type 2 diabetes patients\(^{[19]}\). Serum FFA levels are usually elevated in obesity because the enlarged adipose tissue mass releases more FFAs, which directly block glucose transport and phosphorylation in skeletal muscle. Although there was no statistically significant change in body weight and serum FFA level between the two groups, the limited reduction of body weight may also contribute to the decrease of FFA in TNK-treated obese Zucker rats, which may repair the glucose uptake in skeletal muscle to some extent.

In summary, this study showed that TNK had a significant anti-hyperglycemic activity effect in obese Zucker rats. We demonstrated that oral administration of TNK can significantly improve systemic insulin sensitivity and glucose homeostasis in obese Zucker rats. This may prove to be of clinical importance to improve the therapeutic efficacy of treatment of type 2 diabetes. Further identification of effective component and mechanism of anti-hyperglycemic activity of TNK need to be carried out in the future.
Acknowledgements

The authors would like to thank Applygen Technologies Inc. for technical assistance. This project was supported by grants for Liu Tong-hua from Beijing Municipal Education Commission (No. BJSJW2008001).

REFERENCES


