ISSN 1671-4083
Shanghai Institute of Materia Medica
Chinese Academy of Sciences
http://www.ChinaPhar.com

Effects of konjac extract on insulin sensitivity in high fat diet rats

MAO Cai-Ping[1], XIE Mei-Lin, GU Zhen-Lun

Department of Pharmacology, Faculty of Pharmacy, Soochow University, Suzhou Institute of Chinese Materia Medica, Suzhou 215007, China

KEY WORDS konjac extract; drugs; Chinese herbal drugs; insulin; lipids; rats

ABSTRACT

AIM: To evaluate the effects of konjac extract (KE) on insulin sensitivity in insulin resistance (IR) rats induced by high fat diet (HFD). **METHODS**: Wistar rats were fed on HFD for 4 weeks, then treated with KE 1.5, 3.0 $g \cdot kg^{-1} \cdot d^{-1}$ and metformin (Met) 0.1 $g \cdot kg^{-1} \cdot d^{-1}$ for 4 weeks, respectively. The effects of KE on intake of food and drink, body weight, and excretion were investigated. Serum insulin was measured by double-radioimmunoassay. Blood glucose, total cholesterol (TC), triglycerides (TG), and high-density lipoprotein-cholesterol (HDL-C) were measured by enzyme methods, respectively. Low-density lipoprotein-cholesterol (LDL-C) was calculated. Tissue glycogen was determined by modified anthracene ketone method and tissue TG by glycerin phosphor sour oxidation enzyme method. Insulin sensitivity was measured by modified glucose-insulin tolerance test (*K* value). **RESULTS**: HFD caused IR after 4 weeks (*K* value: 5.2±0.9 *vs* 8.3±0.7, $P<0.01$), the levels of blood insulin, TG, and LDL-C increased, while HDL-C, glycogen in liver and skeletal muscle decreased. The storage of TG in liver and skeletal muscle increased. After HFD rats were treated with KE 1.5 and 3.0 $g \cdot kg^{-1} \cdot d^{-1}$ for 4 weeks, respectively, the fasting blood glucose (FBG) was decreased from 6.4±0.4 to 6.05±0.26, 6.0±0.3 ($P<0.01$). Serum TC, TG, LDL-C were decreased, while HDL-C/TC was increased as compared with HFD rats. There was no significant effect on insulin level. KE 1.5, 3.0 $g \cdot kg^{-1} \cdot d^{-1}$, and Met 0.1 $g \cdot kg^{-1} \cdot d^{-1}$ could improve insulin sensitivity (*K* values were 6.1±0.5, 5.9±0.6, and 6.5±0.8 *vs* 5.2±0.9, $P<0.05$), elevate glycogen, and decrease TG in liver and skeletal muscle. **CONCLUSION**: KE could promote glycogen syntheses and adjust blood lipid metabolism so as to improve IR in HFD rats.

INTRODUCTION

Insulin resistance (IR) which widely exists in type II diabetic patients, is a risk factor which can cause complications. Now the ways to increase insulin sensitivity include: diet, exercise, and drugs such as metformin (Met), they can clearly improve IR, protect the function of β-cell and control blood glucose for a long term[1]. Konjac extract (KE) was refined from *Amorphophallus konjac* K Koch, a kind of Chinese herbs. KE is a kind of white crystal grain obtained

[1] Correspondence to Mao Cai-Ping MD. Phn 86-512-512-5270. Fax 86-512-519-0599. E-mail szzys@publicl.sz.js.cn
Received 2001-08-27 Accepted 2002-06-06

from its tuber. Its main component is Konjac glucomannan[2] which is a kind of excellent edible fiber. It was reported[3,4] that this polysaccharide could decrease total cholesterol(TC) and blood glucose, lose fat, and excretion. Recent studies indicated that KE could obviously improve the glucose tolerance in diabetic patients and animals[5]. High fat diet (HFD) could induce IR by migrating triglycerides (TG) to muscles[6-8]. The object of our study was to investigate the effect of KE on insulin sensitivity in HFD rats and its mechanism.

MATERIALS AND METHODS

Rats Male Wistar rats (9-10 weeks old, 190-230 g, Grade II, Certificate № 97040 were provided by Animal Breeding Center of Soochow University.

Reagents KE was provided by the Institute for Drug Control of Nanjing Military Command, the content of Konjac glucomannan was 82.4 %, Met was bought from Shuanghe Pharmacy Factory (Kunshan, China). Insulin was bought from Sigma, USA. Lipid measured reagent kits (Dongou Biology Project Company, China). Insulin measured reagent kits (China Institute of Atomic Energy). All other chemicals were of analytical grade and commercially available.

Preparation of HFD rats model All rats were fed with about 300 kJ/d quantity of calorie value. Normal control rats were fed standard feedingstuff (calorie value of carbohydrate 62 %, calorie value of fat 12 %). Other rats were fed HFD confected temporarily (calorie value of carbohydrate 20 %, calorie value of fat 61 %)[7,8]. The main component of this fat is cooked lard. The rats were randomly selected in control rats (n=10) and HFD rats (n=10) to make glucose-insulin tolerance test when the rats were fed HFD for 4 weeks. IR was confirmed to form by measuring K value (5.2±0.9 *vs* 8.3±0.7, P<0.01), which was compared with glucose insulin clamp technique (Clamp), being highly-relative and able to give reproducible results[9]. Then the rats were divided into 5 groups: control group ; HFD model group ; Met 0.1 $g \cdot kg^{-1} \cdot d^{-1}$ group ; KE 1.5 $g \cdot kg^{-1} \cdot d^{-1}$ group; KE 3.0 $g \cdot kg^{-1} \cdot d^{-1}$ group. The control group and HFD model group were administered distilled water. During 4 weeks experimental period, all rats were allowed to drink water freely.

The body weights of all rats were measured weekly, the effects on quantity of food and drink and excretion by drugs were investigated. Whole blood was collected from orbital veins, fasting blood glucose (FBG) was analyzed by glucose-oxidize enzyme method. TC, TG, and HDL-C were measured by enzyme methods according to reagent kits explanation. LDL-C was calculated by formula LDL-C=TC–(TG/2.2+HDL-C). Serum insulin was measured by double-antibody radioimmunoassay.

Evaluation of insulin sensitivity Insulin sensitivity was evaluated by K value[1,9]. Rats were anaesthetized at non-limosis state, and catheter was inserted by jugular vein. Then glucose was injected (700 $mg \cdot kg^{-1} \cdot d^{-1}$) in the vein, exogenous insulin 0.175 U/kg was injected immediately, blood were collected at 0, 3, 6, 9, 12, 15, and 18 min after administration of exogenous insulin. Taking time as abscissa, blood glucose value by transforming nature logarithm as vertical coordinate. K value gained by liner was expressed as insulin sensitivity (K =-100 r). Liver, and skeletal muscle were taken out rapidly for measurement of glycogen, and stored at -20 ℃.

Measurement of glycogen and TG in liver and skeletal muscle Glycogen in liver muscle and skeletal muscle was measured by modified anthracene ketone method[1]. TG of liver and skeletal muscle was measured by glycerin phorsphor sour oxidation enzyme method, according to improved Storlien method[10].

Statistical analysis Data were expressed as mean±SD, student' s t test was used for the statistical evaluation of the results.

RESULTS

Effect on common index During the experimental period, the quantity of food and drink in KE 1.5, 3.0 $g \cdot kg^{-1} \cdot d^{-1}$, and Met 0.1 $g \cdot kg^{-1} \cdot d^{-1}$ treated groups were less than control group, especially in KE 3.0 $g \cdot kg^{-1} \cdot d^{-1}$ group. Body weights in KE 1.5, 3.0 $g \cdot kg^{-1} \cdot d^{-1}$, and Met 0.1 $g \cdot kg^{-1} \cdot d^{-1}$ groups were markedly decreased as compared with HFD model group (P<0.05). Excretion in HFD model group and KE 1.5, 3.0 $g \cdot kg^{-1} \cdot d^{-1}$ and Met 0.1

g·kg^{-1}·d^{-1} groups were watery, and in KE 1.5 and 3.0 g·kg^{-1}·d^{-1} groups increased accordingly as compared with control group.

Effect on the blood lipid The levels of TC, TG, LDL-C of KE 1.5, 3.0 g·kg^{-1}·d^{-1} , and Met 0.1 g·kg^{-1}·d^{-1} groups were lower than those of HFD model group, while HDL-C was higher than that in HFD model group, but it was only raised slightly. The ratio of HDL-C/TC in KE 1.5, 3.0 g·kg^{-1}·d^{-1} was higher than that in HFD model group (Tab 1).

Effect on FBG and insulin FBG and insulin in HFD model group were markedly increased (P<0.01 *vs* control group), and FBG of KE 1.5 and 3.0 g·kg^{-1}·d^{-1} groups were lower than that of HFD model group (P<0.05) (Tab 2).

Effect on insulin sensitivity In glucose-insulin tolerance test, blood glucose had no difference at 3 min after injecting insulin, but at 6, 9, 12 min, blood glucose levels in KE 1.5, 3.0 g·kg^{-1}·d^{-1} , and Met 0.1 g·kg^{-1}·d^{-1} treated groups were lower than that in HFD model group. Insulin sensitivity indexes of each group were expressed as K value respectively: K value of HFD model group was decreased by 37.3 % as compared with control group (P<0.01). K value in KE 1.5, 3.0 g·kg^{-1}·d^{-1}, and Met 0.1 g·kg^{-1}·d^{-1} groups were increased by 17.3 %, 13.5 %, and 25 %, respectively (P<0.05, Tab 3).

Effect on tissue glycogen and TG After treated with KE 1.5, 3.0 g·kg^{-1}·d^{-1}, and Met 0.1 g·kg^{-1}·d^{-1} for 4 weeks, glycogens in liver and skeletal muscle were

Tab 2. Effect on FBG and insulin after treated with KE in HFD rats. n=10. Mean±SD. ^{c}P<0.01 *vs* control. ^{e}P<0.05 *vs* HFD model.

Group	FBG/mmol·L^{-1}	Insulin/mU·L^{-1}
Control	5.0±0.4	7.3±1.0
HFD model	6.4±0.4^{c}	20±4^{c}
KE 1.5 g·kg^{-1}·d^{-1}	6.05±0.26^{ce}	20±3^{c}
KE 3.0 g·kg^{-1}·d^{-1}	6.0±0.3^{ce}	20±3^{c}
Met 0.1 g·kg^{-1}·d^{-1}	6.0±0.4^{ce}	20.4±2.9^{c}

Tab 1. Effect on the blood lipid after treated with KE for 4 weeks in HFD rats. n=10. Mean±SD. ^{b}P<0.05, ^{c}P<0.01 *vs* control. ^{e}P<0.05, ^{f}P<0.01 *vs* HFD model.

Group	TC/mmol·L^{-1}	TG /mmol·L^{-1}	HDL-C/mmol·L^{-1}	LDL-C/mmol·L^{-1}	HDL-C/TC
Control	2.6±0.5	0.42±0.05	1.07±0.18	0.85±0.17	0.39±0.05
HFD model	3.6±0.3^{c}	0.88±0.17^{c}	0.8±0.3^{c}	1.20±0.09^{c}	0.23±0.06^{c}
KE 1.5 g·kg^{-1}·d^{-1}	3.30±0.19^{cf}	0.62±0.19^{cf}	1.0±0.2	0.93±0.25^{f}	0.29±0.06^{be}
KE 3.0 g·kg^{-1}·d^{-1}	3.27±0.25^{cf}	0.74±0.13^{ce}	1.0±0.3	1.05±0.19^{be}	0.30±0.07^{cf}
Met 0.1 g·kg^{-1}·d^{-1}	3.41±0.14^{c}	0.75±0.09^{cf}	0.93±0.14	0.99±0.28^{e}	0.28±0.06^{be}

Tab 3. Effect on insulin sensitivity. n=10. Mean±SD. ^{c}P<0.01 *vs* control. ^{e}P<0.05, ^{f}P<0.01 *vs* HFD model.

Group	Blood glucose/mmol·L^{-1}							
	0 min	3 min	6 min	9 min	12 min	15 min	18 min	K value
Control	5.0±0.3	12.41±0.28	9.0±0.4	6.76±0.17	5.2±0.3	4.70±0.22	4.91±0.14	8.3±0.7
HFD model	6.4±0.4^{c}	13.3±0.4^{c}	11.7±0.3^{c}	9.74±0.26^{c}	8.68±0.20^{c}	6.65±0.28^{c}	6.3±0.17^{c}	5.2±0.9^{c}
KE 1.5 g·kg^{-1}·d^{-1}	6.05±0.22^{ce}	13.2±0.4^{c}	11.56±0.22^{c}	9.4±0.3^{c}	8.05±0.17^{cf}	6.93±0.26^{c}	6.11±0.16^{c}	6.1±0.5^{ce}
KE 3.0 g·kg^{-1}·d^{-1}	5.98±0.19^{ce}	12.83±0.16^{c}	11.3±0.4^{ce}	9.08±0.25^{cf}	7.9±0.4^{cf}	6.45±0.14^{c}	5.89±0.23^{c}	5.9±0.6^{ce}
Met 0.1 g·kg^{-1}·d^{-1}	6.04±0.25^{ce}	12.39±0.09^{f}	10.02±0.22^{cf}	8.52±0.11^{cf}	7.0±0.3^{cf}	6.3±0.4^{c}	6.0±0.4^{c}	6.5±0.8^{ce}

increased, while TG contents in KE 1.5, 3.0 g·kg^{-1}·d^{-1} groups decreased as compared with HFD model group (P<0.05, Tab 4).

Tab 4. Effect on tissue glycogen and TG after treated with KE for 4 weeks. n=10. Mean±SD. ^cP<0.01 *vs* control. ^eP<0.05, ^fP<0.01 *vs* HFD model.

Group	Liver glycogen /mg·g^{-1}wet tissue	Skeletal muscle glycogen /mg·g^{-1} wet tissue	Liver TG /μmol·g^{-1}wet tissue	Skeletal muscle TG /μmol·g^{-1}wet tissue
Control	18±3	5.9±0.9	2.4±0.6	2.69±0.20
HFD model	10±4^c	2.2±0.6^c	5.0±1.0^c	3.8±0.4^c
KE 1.5 g·kg^{-1}·d^{-1}	14±4^{cf}	4.1±0.8^{cf}	4.2±0.5^{ce}	3.4±0.5^{ce}
KE 3.0 g·kg^{-1}·d^{-1}	14±4^{cf}	3.9±0.5^{cf}	4.1±0.8^{ce}	3.5±0.8^c
Met 0.1 g·kg^{-1}·d^{-1}	14±4^{cf}	4.3±1.0^{cf}	4.3±0.8^{ce}	3.5±0.6^c

DISCUSSION

IR is insulin sensitivity decrement. It exists in three main parts: liver, skeleton, and fat tissue. IR in liver exhibits increment of liver glycogen outputting; IR in skeleton and fat tissue indicated that the using rate of glucose decomposition decreased. IR could cause the abnormity of serum glucose and lipid metabolism on type II diabetes. Therefore, IR played a key role in the occurrence and development of type II diabetes[11]. The biological effects of insulin is affected by the backgrounds of inheritance and environmental factors, foodstuff is an important factor affecting insulin sensitivity. It was reported[12] that the fat including saturated fatty acid and poly-non-saturated Ω-6 fatty acid could cause IR. HFD was used to establish IR model in this experiment. TG in liver and skeletal muscle was higher, glycogen in liver and skeletal muscle was lower than that of pre-treatment. It was known that glycogen synthesis was an important way to use glucose by non-oxidation approach. It was suggested that the reduction of glycogen synthesis might be related to assimilating obstruction of glucose induced by IR in HFD animals.

Research indicated that Met could improve insulin sensitivity of diabetic patients. After Met treated IR rats for 4 weeks in this study, results demonstrated that it could increase K value, improve insulin sensitivity, and enhance glycogen synthesis. In addition, Met could lower TC, TG, and LDL-C in HFD rats. These effects were beneficial to improve IR.

It is well known that improving IR would be important in curing type II diabetes and hypertension. IR causes a series of the unconventionality of glucose, lipid, and insulin metabolism. We found that KE was able to decrease glucose in normal animals, as well as in diabetic animals induced by alloxan[13,14]. The present studies discovered firstly that KE might not only improve IR and increase K value, but also lower FBG and glycogen in liver and skeletal muscle, but it had no effect on the release of insulin. The experimental results revealed that KE might improve insulin sensitivity by increasing glucose usage of non-oxidation approach, not depend on the release of insulin. In addition, KE not only decrease serum TC, TG, and LDL-C level, but also increase the glycogen and decrease TG in liver and skeletal muscle in HFD rats. Although KE had no significant effect on HDL-C, KE could increase the ratio of HDL-C/TC. From these results, KE could inhibit the occurrence and development of IR to a certain extent, increase insulin sensitivity, and improve blood glucose and lipid levels in IR rats. Its mechanism of improving IR included increment of glycogen synthesis and the improvement of lipid metabolism.

REFERENCES

1 Gao CR, Zhang JQ, Huang QL. Experimental study on berberin raised insulin sensitivity in insulin resistance rat models. Chin J Integr Trad Western Med 1997; 17: 162-4.
2 Cui X, Yao XR, Li SL. Analysis of nutritional components in three edible and medicinal plants of *Amorphophallus blume*. Acta Nutr Sin 1992; 14: 221-4.
3 Sun GZ, Huang MS, Wang X, Li Y, Chen SN. An experimental research on the anti-obesity effect of konjac flour. Acta Nutr Sin 1991; 13: 161-4.
4 Zhang MY, Huang CY, Wang X, Hong JR, Peng SS. The influence of konjac food on human lipid metabolism. Acta Nutr Sin 1989; 11: 25-9.
5 Huang CY, Zhang MY, Peng SS, Hong JR, Wang X, Jiang HJ. Effect of konjac food on the blood glucose level in diabetics.

Acta Nutr Sin 1989; 11: 360-6.
6 Kraegen EW, Clark PE, Jenkins AB, Daley EA, Chisholm DJ, Storlien LH. Development of muscle insulin resistance after liver insulin resistance in high–fat–fed rats. Diabetes 1991; 40: 1397-403.
7 Pedersen O, Kahn CR, Flier JS, Kahn BB. High fat feeding cause insulin resistance and a marked decrease in the expression of glucose transporters (Glut 4) in fat cells of rats. Endocrinology 1991; 129:771-7.
8 Li CZ, Zhang SH, Shu CD, Ren W. Rat model of insulin-resistant induced by high-fat feed. Basic Med Sci Clin 2000; 20: 93-5.
9 Liang XL. Insulin sensitivity ration mensurate. Bull Med Res 2001; 30: 28-30.
10 Storlien LH, Oakes ND, Pan D, Kusunoki M, Jenkins AB. Syndromes of insulin rsistance in the rat. Inducement by diet and the amelioration with benfluorex. Diabetes 1993; 42: 457-62.
11 Xia H, Chen JW, Shen J, Li Y, Zhang JX, Li HP. Mechanism of insulin resistance in patients with impaired glucose tolerance and type II diabetes mellitus. Chin J Endocrinol Metab 1996; 12; 131-4.
12 Storlien LH, Jenkins AB, Chisholm DJ, Pascoe WE, Khouri S, Kraegen EW. Influence of dietary fat composition on development of insulin resistance in rats. Diabetes 1991; 40: 280-9.
13 Mao CP, Gu ZL, Hypoglycemic effect of puncturevine captrap (amorphophallus konjac). Chin J Modern Appl Pharm 1999; 16: 14-6.
14 Mao CP, Xu NY, Gu ZL. Hypoglycemic effects of amorphophallus konjac in alloxan diabetic rats. Chin J Modern Appl Pharm 2001; 18: 185-7.

魔芋提取物对高脂饮食大鼠胰岛素敏感性的影响

茅彩萍[1]，谢梅林，顾振纶
(苏州大学医学院药学系药理教研室，苏州中药研究所，苏州 215007，中国)

关键词　魔芋提取物；中草药；胰岛素；脂类；大鼠

目的：探讨魔芋提取物(KE)对高脂饮食大鼠胰岛素敏感性的影响．方法：利用高脂饮食(HFD)胰岛素抵抗(IR)大鼠模型，观察KE对IR的治疗作用，二甲双胍为对照药物．HFD饲养正常Wistar大鼠4周，分别以KE和二甲双胍灌胃治疗4周，观察动物的摄食量，饮水量和大便变化情况，并用葡萄糖氧化酶法测定空腹血糖，双抗体放免法测定血浆胰岛素，生化方法测定血脂(TC,TG,和HDL-C)，并计算LDL-C，采用改良蒽酮法测定组织糖原，甘油磷酸氧化酶法测定组织中甘油三酯及改良的葡萄糖-胰岛素耐量试验测定胰岛素敏感性(K值)．结果：HFD饲养4周时，与正常对照组比较，HFD组血糖，血清胰岛素，血脂明显升高，肝脏和肌肉中TG含量均明显升高($P<0.01$)，糖原含量显著下降($P<0.01$)，与正常对照组(K=8.3±0.7)比较，胰岛素敏感性下降(K=5.2±0.9，$P<0.01$)．用KE(1.5，3.0 g·g^{-1}·d^{-1})和二甲双胍(0.1 g·g^{-1}·d^{-1})治疗4周后，与HFD组比较治疗组胰岛素敏感性改善，K值分别为6.1±0.5，5.9±0.6和6.5±0.8（$P<0.05$），组织糖原含量明显增加($P<0.01$)，TC，TG，LDL-C显著降低($P<0.05$)，HDL-C升高不明显，HDL-C/TC值明显升高($P<0.05$)．结论：KE能在一定程度上阻止IR的发生和发展，具有提高HFD大鼠胰岛素敏感性的作用，其机制可能与其促进糖原合成和降低血脂有关．

(责任编辑　韩向晖)