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Effect of insulin in combination with selenium on blood glucose and PI3K-mediated GLUT4 expression in skeletal muscle of streptozotocin-induced diabetic rats

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Abstract. – *Objectives:* **We evaluated the effect of low doses of insulin (1 U/kg/day) and selenium (180 µg/kg/day) in combination on general physiological parameters, and on PI3K and GLUT4 levels in skeletal muscle of streptozotocin (STZ)-induced diabetic rats.**

Materials and Methods: **Diabetic rats were treated with insulin, selenium, and insulin and selenium in combination for four weeks. The levels of blood glucose and hemoglobin A1c were estimated, and the levels of PI3K and GLUT4 in skeletal muscle were examined by immunoblotting and immunohistochemistry.**

Results: **Insulin in combination with selenium could significantly lower blood glucose and HbA1c levels, and restore the disturbances in PI3K and GLUT4 levels in skeletal muscle. Treatment with insulin was only partially effective in the restoration of diabetic alterations.**

Conclusion: **We conclude that there was cooperation between insulin and selenium, and that treatment of diabetic rats with combined doses of insulin and selenium was effective in the normalization of blood glucose and correction of altered PI3K and GLUT4 distribution in skeletal muscle of diabetic rats.**

Key Words:

Streptozotocin-diabetes, Insulin, Sodium selenite, Phosphatidylinositol-3 Kinase, Glucose transporter 4, Skeletal muscle.

Introduction

Skeletal muscle accounts for nearly 40% of body mass and is the main tissue involved in the insulin-induced stimulation of glucose uptake. Since glucose transport in skeletal muscle occurs mainly through GLUT4, the reduction in the GLUT4 levels results in decreased uptake of glucose. PI3K, the pivotal kinase in insulin signal transduction pathway, is necessary for insulin stimulated glucose transport and mediates glucose transport by stimulating the recruitment of GLUT4 to the cell surface. Therefore, restoration of PI3K and GLUT4 levels in skeletal muscle is very important to achieve normoglycemia.

It is well known that insulin has a beneficial effect on hyperglycemia in diabetes, but administration of insulin for controlling hyperglycemia may produce hyperinsulinemia, which is considered to be the cause of diabetes-induced coronary heart disease, hypoglycemia and allergy.

Selenium (Se) is a trace element that could improve glucose homeostasis[1] and has a protective role against lipid peroxidation and diabetes-inducing injury in several tissues[2,3]. High doses of selenium are required for this insulin mimetic and for antidiabetic activity[4], and may cause several toxic effects[5,6]. Lower doses of selenium are relatively safe, but fail to effectively decrease blood glucose levels of diabetic rats[7,8]. To date, there is no information regarding the effect of combined doses of insulin and selenium on blood glucose levels, and on PI3K and GLUT4 levels of skeletal muscle in diabetic rats.

Normally, the glycemic control in diabetes is estimated by measuring blood glucose levels. However, due to wide variations in the circulating glucose concentrations, the blood glucose measurement does not give clear data for overall glycemic control. It is very necessary that an additional parameter should be added to demonstrate the better con-

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trol of glycaemia. The amount of HbA1c reflects the average level of blood glucose over previous 1-2 months and is, therefore, a commonly used laboratory test for assessing long-term diabetic control[9]. It has been reported that the animal model used here, STZ-diabetic rats, exhibits high HbA1c values[10].

We used low doses of insulin in combination with selenium and evaluated their effect on these parameters in diabetic rats. We hoped that this would provide new insights for the clinical management of diabetes and its complications.

Materials and Methods

Animals and Model

Thirty-five male Sprague-Dawley rats (180-220 g) provided by the Medical Experimental Animal Center of Xi'an Jiaotong University were used. Animals were housed seven per cage in an environmentally controlled laboratory (temperature, 22±1°C; relative humidity, 50±2%) in the Medical Experimental Animal Center of Xi'an Jiaotong University. Rats received water and food *ad libitum*. After one week of acclimatization, rats were randomly divided into two groups. Streptozotocin (STZ) (50 mg/kg body weight, dissolved in 0.02 M sodium citrate, pH 4.5; Sigma-Aldrich, St. Louis, MO, USA) was injected (i.p.) into the first group. Models were regarded as successfully established if blood glucose concentration was >16.7 mmol/L and accompanied with hyperdiuresis or other diabetic symptoms after 7 days. Control rats (n = 7) were injected (i.p.) with a corresponding volume of citrate buffer. Animals were grouped into control (C) diabetic (D), insulin-treated diabetic (D+In), selenium-treated diabetic (D+Se) and diabetic treated with selenium and insulin (D+Se+In). The selenium treatment group involved administration (p.o.) of sodium selenite (180 μg/kg per day); the insulin treatment group involved injection (s.c.) of insulin (1 U/kg per day); and the selenium in combination with insulin treatment group were given both treatments. At the end of the experiment, rats were anesthetised by sodium pentobarbital (25 mg/kg) and blood samples were collected. After blood collection, skeletal muscle was removed, rapidly frozen in liquid nitrogen and kept at –80°C until further analysis. Experiments were carried out with the approval of the Animal Research Committee of the University of the Xi'an Jiaotong University (Xi'an, PR China).

Materials

Insulin was from Novo Nordisk (Copenhagen, Denmark) and sodium selenite and STZ were purchased from Sigma. Trihydroxymethyl aminomethane (Tris), glycine, sodium dodecyl sulfate (SDS), acrylamide and bisacrylamide were purchased from Amresco (Cochran Road Solon, OH, USA). Rabbit anti-rat β-actin polyclonal antibody was purchased from Santa Cruz Biotechnology (Santa Cruz, CA, USA). Anti-GLUT4 polyclonal antibody and Anti-PI3K polyclonal antibody were purchased from Upstate Biotechology Incorporated (Lake Placid, NY, USA). BCA™ protein assay kit, BlueRanger® prestained protein molecular weight marker mix, and Super-Signal® West Pico chemiluminescent substrate were purchased from Pierce Chemical Company (Pierce, Rockford, IL, USA). Radioimmunoprecipitation assay (RIPA) was purchased from Bioteke Corporation (Beijing, PR China).

Detection of Blood Glucose, Hemoglobin A1c (HbA1c) and Body Weight

The fasting blood glucose of each group was estimated every week using One Touch SureStep Blood Glucose meter. HbA1c and the body weight of each group were measured after four weeks of treatment.

Immunoblotting

Total protein and plasma membrane protein of skeletal muscle was extracted using RIPA agents[11,12] and quantified with BCA protein assay kit (Beyotime, Haimen, Jiangsu, China). Equal amounts of protein were separated by 10% sodium dodecyl sulfate-polyacrylamide gel electrophoresis (SDS-PAGE) and transferred onto microporous polyvinylidene fluoride membranes in running buffer with 20% methanol. After nonspecific sites were blocked with 5% milk-Tris-buffered saline Tween-20 (TBST), membranes were incubated with anti-PI3K antibody, anti-GLUT4 antibody or anti-β-actin. Membranes were washed in TBST. A horseradish peroxidase-linked antibody was employed as a secondary antibody, and the bands of interest were detected using an enhanced chemiluminescence technique. Band densities were analyzed by a computer equipped with image software (GEL DOC gel 2000) (BioRAD Laboratories, Hercules, CA, USA).

Immunohistochemistry

Briefly, 4-µm sections were treated with 2% hydrogen peroxide to quench endogenous peroxide for 30 min and washed with phosphate-buffered saline (PBS). After several washes with PBS, sections were blocked with 10% normal goat serum in PBS, followed by overnight incubation with rabbit anti-PI3K antibody or anti-GLUT4 antibodies at 4°C overnight. Sections were processed using the ABC staining method. Nickel-intensified biotinylated diaminobenzidine (DAB) was used to visualize the signal.

Data Analysis

Values were calculated as mean ±SD. A repeated measures ANOVA was used to compare blood glucose among the control, diabetic and treated groups. A one-way ANOVA was used to analyze differences in all other parameters among the control, diabetic and treated groups. Significance was considered at $p<0.05$.

Results

Effect of Insulin in Combination with Sodium Selenite on Body Weight, Blood Glucose and Hemoglobin A1c of Diabetic Rats

Body weight was significantly lowered in the diabetic group (29%, $p<0.01$). Treatment with either insulin or selenium did not improve weight loss when compared with diabetic rats (157±7.5 vs 150±12.9, 155±10.6 vs 150±12.9 g, $p>0.05$; respectively), whereas insulin and selenium in combination resulted in a modest but significant increase in body weight as compared with that in diabetic rats (167±8.5 vs 150±12.9 g, $p<0.01$).

Changes in blood glucose levels and HbA1c levels are presented in Table I. STZ-diabetic rats showed an approximate five fold increase in plasma glucose concentration compared with that in control rats. The two combination produced normoglycemia in diabetic rats. HbA1c was significantly elevated in diabetic rats ($p<0.01$), but its levels remarkly decreased after treatment with insulin and selenium in combination. As evident from the given *table*, the combined dose of insulin and selenium was more effective than insulin and selenium alone in controlling glycaemia.

Effect of Insulin in Combination with Sodium Selenite on the Content of Pi3k Protein in Cytoplasm of Skeletal Muscle

Western blotting of total protein was done and the result analyzed using an image analyzer. The content of PI3K protein of the model group decreased by about 36% compared with that in the control groups ($p<0.01$). Treatment of diabetic rats with insulin and selenium in combination resulted in normalization of PI3K levels in the cytoplasm of skeletal muscle. Insulin only partially revived the PI3K content in cytoplasm (Figure 1).

PI3K level in control, diabetic, and treated was also measured by immunohistochemistry. The results were in accord with those obtained with immunoblotting: there was a remarkable decrease in the PI3K level of the diabetic rats. Combined treatment significantly increased the content of PI3K, but insulin alone only partially altered the

Table I. Changes in levels of blood glucose (mmol/l) in various groups: control (C), diabetic (D) and diabetic rats after each week of treatment with insulin (D+In) selenium (D+Se) and insulin and selenium in combination (D+ In + Se)

Group	Dose	FBG (mmoL/L)					HbA1c (%)
		Week 0	Week 1	Week 2	Week 3	Week 4	
C		5.4 ± 0. 8	5.5 ± 1.0	5.4 ± 0.7	5.5 ± 0.7	5.7 ± 0.7	4.9 ± 0.5
D		23.5 ± 1.7**	23.1 ± 1.0**	21.7 ± 1.3**	22.8 ± 1.4**	25.0 ± 1.6**	8.1 ± 0.3**
D+In	1U/kg/d	22.3 ± 3.5**	15.3 ± 2.4**##	15.6 ± 2.4**##	14.9 ± 2.5**##	15.0 ± 2.6**##	6.7 ± 0.3**##
D+Se	180 µg/kg/d	20.3 ± 1.8**	20.0 ± 2.1**#	19.6 ± 3.1**	17.1 ± 2.4**##	15.4 ± 2.1**##	7.0 ± 0.4**##
D+In+Se	1U/kg/d+ 180 µg/kg/d	25.5 ± 3.5**	18.1 ± 4.2**##	15.3 ± 2.8**##	10.9 ±1.3**##	7.6 ± 0.8##	5.4 ± 0.4*##

All the data were shown as the mean±SD, n=7. *Significant different from C group, $p < 0.05$; **Significant different from C group, $p < 0.01$; #Significant different from D group, $p < 0.05$. ##Significant different from D group, I < 0.01.

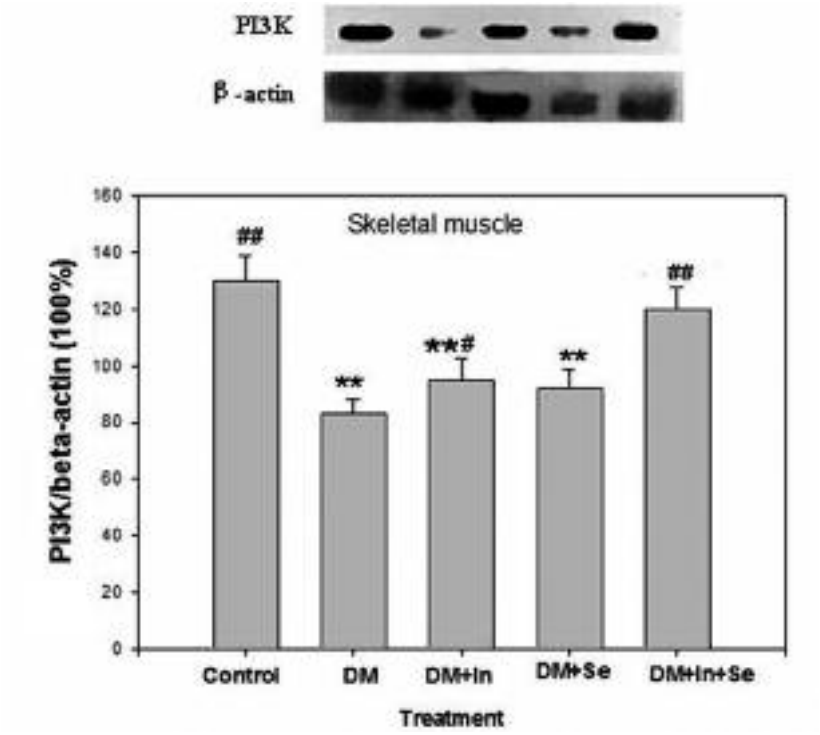

Figure 1. PI3K levels in the skeletal muscle of control-(C), diabetes-(DM) and diabetic rats treated with insulin-(DM+In), selenium-(DM+Se), Combined dose of insulin and selenium-(DM+In+Se). Results are presented as ±SD. $*p<0.05$ compared with control group, $**p<0.01$ compared with control group; $^{\#}p<0.05$ compared with DM group, $^{\#\#}p<0.01$ compared with DM group.

PI3K expression. These results demonstrate that combined treatment was more effective than insulin and selenium alone in restoring PI3K levels (Figure 2).

Effect of Insulin in Combination with Sodium Selenite on the Content of Glut4 Protein in the Membrane Fraction of Skeletal Muscle

Western blotting of membrane protein was done and the result analyzed using an image analyzer. The content of GLUT4 protein of the model group decreased by about 47% compared with that in the control groups ($p<0.01$). Treatment of diabetic rats with insulin and selenium in combination resulted in normalization of GLUT4 levels in the skeletal muscle membrane fraction. Insulin and selenium only partially revived the GLUT4 content in the membrane (Figure 3).

GLUT4 level in the skeletal muscle of control, diabetic, and treated rats was also mea-

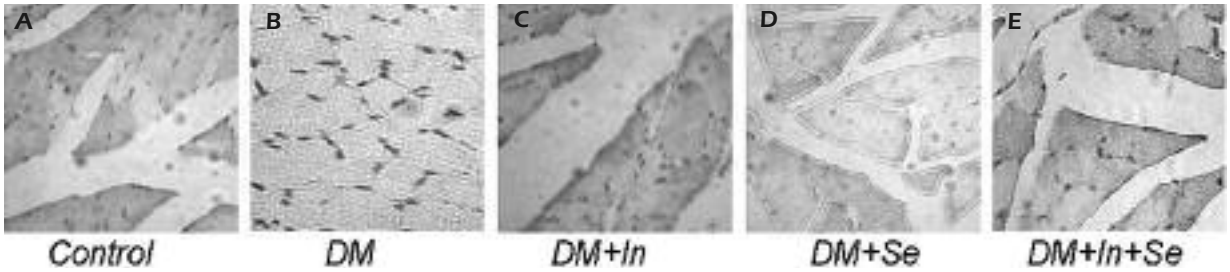

Figure 2. Immunohistochemical analysis of PI3K in the skeletal muscle of ***(A)*** control ***(B)*** diabetic rats and diabetic rats treated with ***(C)*** insulin ***(D)*** selenium and ***(E)*** insulin+selenium.

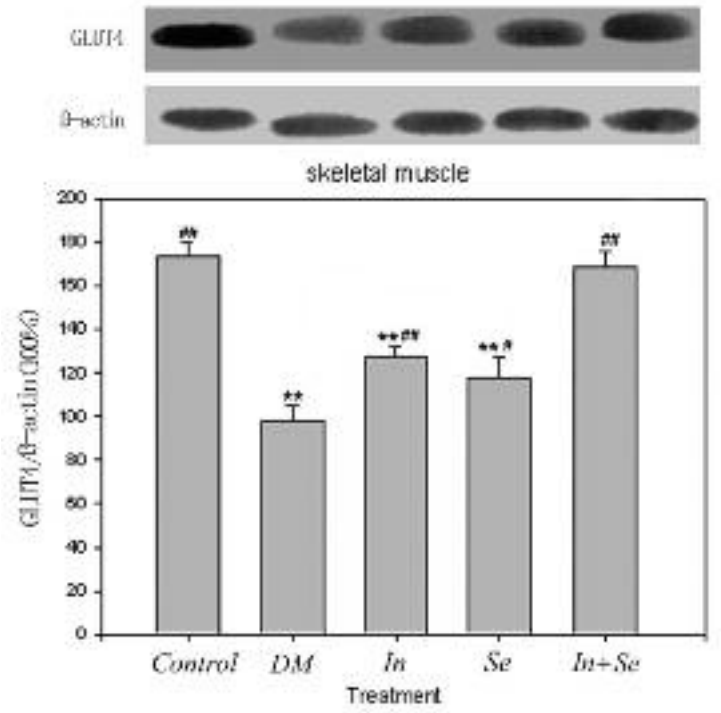

Figure 3. PI3K levels in the skeletal muscle of control-(C), diabetes-(DM) and diabetic rats treated with insulin-(DM+In), selenium-(DM+Se), Combined dose of insulin and selenium-(DM+In+Se). Results are presented as ±SD. *p<0.05 compared with control group, **p<0.01 compared with control group; #p<0.05 compared with DM group, ##p<0.01 compared with DM group.

sured by immunohistochemistry: the results were in agreement with those obtained with immunoblotting. In the diabetic state, there was a marked decrease in the GLUT4 content in the membrane. Combined treatment remarkably corrected the content of GLUT4, but insulin and selenium alone only partially altered the GLUT4 expression in plasma membrane of skeletal muscle. Combined treatment effectively restored GLUT4 content in the membrane of skeletal muscle (Figure 4).

Discussion

Diabetes mellitus is a clinical disorder of sugar and fat metabolism. This is reversed by insulin, and insulin-mimetic substances[12]. Basically, two promising insulin-mimetics, selenium and vanadate, have been studied[6,13,14]. The effect of insulin in combination with selenium on the level of blood glucose, and on the level of PI3K and GLUT4 expression in skeletal muscle of STZ-diabetic rats has not been done until now. In addi-

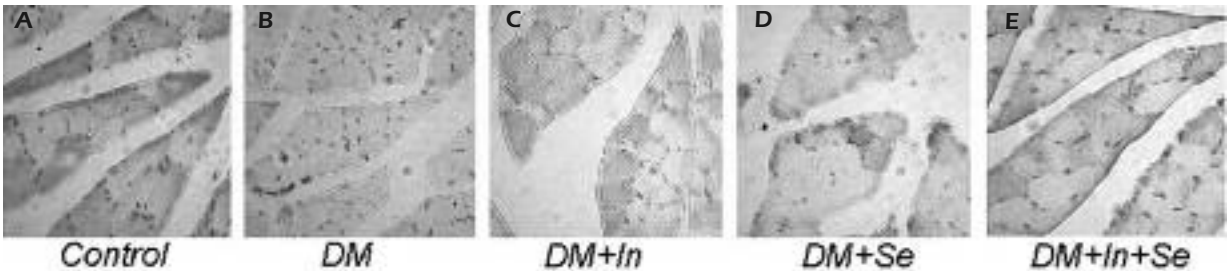

Figure 4. Immunohistochemical analysis of GLUT4 in the skeletal muscle of ***(A)*** control, ***(B)*** diabetic rats, and diabetic rats treated with ***(C)*** insulin ***(D)*** selenium and ***(E)*** insulin + selenium.

tion, HbA1c levels reflect the average concentration of glucose variation level over the preceding 4 to 8 weeks and become an important index in evaluation of long-term glycemic control for diabetes and related diseases. Therefore, we firstly studied the effect of insulin and selenium combined dose on blood glucose levels and HbA1c levels in STZ-diabetic rats. The results showed that a combined dose of insulin and selenium given to diabetic rats displayed near-control plasma glucose levels and displayed a remarkable decrease in HbA1c. Insulin- or selenium-treated diabetic rats only partially lowered blood glucose levels and HbA1c values. The two combination signified better control of blood glucose levels in diabetic rats.

Insulin stimulates cellular glucose uptake in muscle tissues by inducing the translocation of GLUT4 from an intracellular pool to the plasma membrane. In the diabetic state due to the deficiency of insulin, GLUT4 translocation does not take place efficiently and GLUT4 transporters remain inside the cell where they are not functional. This results in a decreased uptake of glucose by muscle cells, which contributes significantly to the elevated glucose levels. PI3-kinase is known to be involved in vesicular trafficking through the cytoplasm, membrane ruffling, and GLUT4 translocation towards the cell membrane. Therefore, revival of PI3K and GLUT4 levels is one of the major factors for assuaging the hyperglycemic condition and could be used as a parameter to evaluate the effectiveness of an antidiabetic compound. Previous investigation showed selenium treatment alone could stimulate translocation of the glucose transporters to the membrane surface in isolated rat muscle[13]. Our study showed that the level of PI3K and GLUT4 protein significantly decreased in skeletal muscle of diabetic rats. Treatment of diabetic rats with insulin and selenium in combination restored PI3K and GLUT4 levels close to normal values. Treatment of diabetic rats with insulin only partially restored PI3K and GLUT4 levels in STZ-diabetic rats. Combined treatment was more effective in correcting alterations in the PI3K and GLUT4 protein.

Similar results were obtained by immunohistochemical analyses of PI3K and GLUT4 in skeletal muscle. In the diabetic state, PI3K and GLUT4 contents decreased drastically when compared to that in control. Treatment with the two in combination restored PI3K and GLUT4 content. Combined treatment was more effective in correcting changes in the PI3K and GLUT4 protein.

The use of insulin and selenium in combination was based on well-known antioxidant role of selenium and its insulin-like role[6,15,16]. Chronic hyperglycemia causes an imbalance between radical oxygen species and scavenging system activity, and leads to oxidative stress[17,18]. Selenium is an integral part of glutathione peroxidase and protects various cells against oxidative damage. Its insulin mimicry has been shown in the areas of glucose uptake[16,19,20], glucose metabolism[16,21,22] and signal transduction[13,23-25]. Ghosh et al. also reported an increase in the insulin levels of selenium-treated STZ-diabetic mices[20]. Insulin could rapidly revise hyperglycemia in diabetic, but administration of insulin for controlling hyperglycemia might produce side effects (such as hyperinsulinemia and hypoglycemia). Therefore, combined doses of insulin and selenium were superior to administering them alone.

In conclusion, low doses of insulin and selenium in combination could significantly lower the blood glucose and increase the PI3K and GLUT4 contents of skeletal muscle in diabetic rats. Therefore, we thought that the two in combination decreases blood glucose by upregulating the level of PI3K and GLUT4 in skeletal muscle. The data suggest that a combination of insulin and selenium may prove useful as a new therapy for diabetic patients but much more work needs to be done.

References

1) Navarro-Alarcon M, Lopez-G de la Serrana H, Perez-Valero V, Lopez-Martinez C. Serum and urine selenium concentrations as indicators of body status in patients with diabetes mellitus. Sci Total Environ 1999; 228: 79-85.

2) Karpen CW, Pritchard AK, Arnold JH, Cornwell DG, Pangamala RV. Restoration of prostacyclin/thromboxane A2 balance in the diabetic rat. Diabetes 1982; 31: 947-951.

3) Salonen JT, Salonen R, Seppänen K, Kantola M, Parviainen M, Alfthan G, Mäenpää PH, Taskinen E, Rauramaa R. Relationship of serum selenium and antioxidants to plasma lipoproteins, platelet aggregability and prevalent ischemic heart disease in Eastern Finnish men. Atherosclerosis 1988; 70: 155-160.

4) Xi-Qun. Sheng Kai-Xun Huang, Hui-Bi Xu. Influence of alloxan-induced diabetes and selenite treatment on blood glucose and glutathione levels in mice. J Trace Elem Med Biol 2005; 18: 261-267.

5) Liu Xuedong. State of study in selenite cataract. Studies Trace Elem Health 2000; 17: 68-69.

6) Wu Jianmin, Chen Wangrong, Zhang Wei, Zhang Rong. Effects of selenium on the growth of midbrain neurons. Studies Trace Elem Health 2000; 17: 3-4.

7) Wu Yuntang, Sun Zhong, Che Suping , Chang Hong, Huaqing Zhu, Xiangfen Kong. Effects of zinc and selenium on the disorders of blood glucose and lipid metabolism and its molecular mechanism in diabetic rats. J Hygiene Res 2004; 33: 70-73.

8) Wu Y, Che S, Sun Z, Chang H, Wang Y. Regulation of zinc, selenium, chromium on disorders of blood glucose and lipid metabolism in diabetic rats. Chin J Public Health 2004; 20: 1083-1085

9) Inzucchi S. Classification and diagnosis of diabetes mellitus. In: Porte D, Sherwin R, Baron A (Eds.), Elenberg & Rifkin's Diabetes Mellitus, 2003; p. 274.

10) Portha B, Giroix MJ, Serradas IP, Movassat J, Bailbe ID, Kergoat M. The noeonatally streptozotocin induced (n-stz) diabetic rats a family of NIDDM models. In: Anders, A.F., Eleazar, S. (Eds.), Animal Models of Diabetes. Harwood Academic Publishers, Amsterdam 2002; pp. 247-272.

11) Zhang P, Liang Z, Wang H, Cai H, Zhang S, Wang S. Vanadium increases GLUT4 expression in skeletal muscles of streptozotocin-induced diabetic rats. Acta Acad Med Militaris Tertiae 2004; 26: 533-536.

12) Zhang Lei, Chen Li-mei, Ni Hong-xia, Yu Chun-rong. Effects of puerarin on the expression of glucose transporter 4 in adipocytes of streptozocin induced diabetic rats. Chin J Clin Rehabil 2006; 10: 135-139.

13) Ezaki O. The insulin-like effects of selenate in rat adipocytes. J Biol Chem 1990; 265: 1124-1130.

14) Orvig C, Thompson KH, Battell M, McNeill JH. Vanadium compounds as insulin-mimics. Met Ions Biol Syst 1995; 31: 575-594.

15) Berg EA, Wu JY, Campbell L, Kagey M, Stapleton SR. Insulin-like effects of vanadate and selenate on the expression of glucose-6-phosphate dehydrogenase and fatty acid synthase in diabetic rats. Biochimie 1995; 77: 919-924.

16) Becker DJ, Reul B, Ozcelikay AT, Buchet JP, Henquin JC, Brichard SM. Oral selenate improves glucose homeostasis and partly reverses abnormal expression of liver glycolytic and gluconeogenic enzymes in diabetic rats. Diabetologia 1996; 39: 3-11.

17) Kinalski M, Sledziewski A, Telejko B, Zarzycki W, Kinalska I. Lipid peroxidation and scavenging enzyme activity in streptozotocin- induced diabetes. Acta Diabetol 2000; 37: 179-183.

18) Stefek M, Sotnikova R, Okruhlicova L, Volkovova K, Kucharska J, Gadjosik A, Gajdosikova A, Mihalova D, Hozova R, Tribulova N, Gvozdjakova A. Effect of dietary supplementation with pyridoindole antioxidant stobadine on antioxidant state and ultrastructure of diabetic rat myocardium. Acta Diabetol 2000; 37: 111-117.

19) Stapleton SR. Selenium: an insulin-mimetic. Cell Mol Life Sci 2000; 57: 1874-1879.

20) Ghosh R, Mukherjee B, Chatterjee M. A novel effect of selenium streptozotocin-induced diabetic mice. Diabetes Res 1994; 25: 165-171.

21) McNeill JH, Delgatty HLM, Battell ML. Insulin like effects of sodium selenate in streptozocin-induced diabetic rats. Diabetes 1991; 40: 1675-1678.

22) Ersöz G, Yakaryilmaza A, Turan B. Effect of sodium selenite treatment on platelet aggregation of streptozotocin-induced diabetic rats. Thrombosis Res 2003; 111: 363-367.

23) Furnsinn C, Englisch R, Ebner K, Nowotny P, Vogl Claus, Waldhausl W. Insulin-like vs non-insulin-like stimulation of glucose metabolism by vanadium, tungsten, and selenium compounds in rat muscle. Life Sci 1996; 59: 1989-2000.

24) Stapleton SR, Garlock GL, Foellmi-Adams L, Kletzien RF. Selenium: potent stimulator of tyrosyl phosphorylation and activator of MAP kinase. Biochim Biophys Acta 1997; 1355: 259-269.

25) Stapleton SR, Jivraj S, Wagle A. Insulin and insulin-mimetic selenium mediate the regulation of glucose-6-phosphate dehydrogenase gene expression via difference signal proteins. FASEB J 1998; 12: 1404.