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Effects of hyperbaric oxygen on adiponect and glucose transporter 4 expression in type 2 diabetic rats

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Abstract: **To investigate the effect of Hyperbaric oxygen (HBO) on adiponect and glucose transporter 4 (GluT4) expression in type 2 diabetic rats. 10 healthy control subjects (group A, n=10) were randomly selected from a total of 40 male SD rats. They were fed with normal diet. The remaining 30 rats were fed with high-fat and high sugar diet, then the model of type 2 diabetic rats was induced by intraperitoneal injection with small dose of streptozotocin (STZ). The model of type 2 diabetic rats was randomly divided into diabetic control group (group B, n=11) and diabetic hyperbaric oxygen therapy group (group C, n=10). Group C was treated with hyperbaric oxygen for 30 days. After the experiment, the serum and the specimens were obtained. Serum adiponectin, fasting insulin (FINS) and GluT4 were detected by ELISA kit. Serum high density lipoprotein (HDL-C), low density lipoprotein (LDL-C), cholesterol (TC) and triglyceride (TG) were detected with a full automatic biochemical analyzer. The expression of adiponectin receptor 2 (AdipoR2) protein in liver specimens were analysised by immunohistochemical methods. Compared with group A, fasting blood glucose(FBG), 30 minutes postprandial blood sugar, 60 minutes postprandial blood glucose, 120 minutes postprandial blood glucose, TG, TC, LDL-C, FINS, AdipoR2 in group B and group C increased significantly. HDL-C, adiponectin, Glut4 expression and insulin sensitivity decreased significantly. The difference was statistically significant (P<0.05). Group C rats were treated with hyperbaric oxygen for 30 days, then compared with group B rats: FBG, 120 minutes postprandial blood sugar, FINS,TG, AdipoR2 significantly decreased HDL-C, adiponectin, Glut4 expression and insulin sensitivity ncreased significantly. Therefore, the difference was statistically significant (P<0.05). The expression level of adiponectin and GluT4 in type 2 diabetic rats was lower than that of normal rats. Hyperbaric oxygen therapy for 30 days can improve the content of adiponectin in diabetic rats, increase the expression level of GluT4, promote the oxidation of glucose in the liver increase the uptake of glucose in the cell, lower blood sugar and improve the sensitivity of insulin.**

Keywords: Hyperbaric oxygen; Diabetes type 2; Adiponectin; Glucose transporter 4

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1. Introduction

The pathogenesis of type 2 diabetes is caused by insulin resistance or/and dysfunction of the islet B cells. The disorder of insulin signal transduction is considered to be the main cause of insulin resistance. In recent years, the relationship between adipose factors and diabetes has aroused widespread concern at home and abroad. Among them, the role of adiponectin in type 2 diabetes gradually became the focus of research. Research shows: adiponectin can increase insulin sensitivity[1], increase fatty acid oxidation[2] and alleviate chronic inflammatory reaction of diabetes and so on[3,4]. Therefore, the observation of adiponectin levels will be a very good target for the detection of diabetes and metabolic diseases.Thus improving the expression of adiponectin and its receptors or enhancing their activity may become a new treatment for insulin resistance, type 2 diabetes and metabolic syndrome.

Many factors affect the expression of adiponectin, such as sex, growth hormone, glucocorticoid, chronic hypoxia, glucocorticoid, interleukin-6, endothelin -1 and so on. Among them, chronic hypoxia leads to the decrease of in adiponectin level[5]. The factors that stimulate the secretion of adiponectin, such as drugs and hyperbaric oxygen, may also be effective in treatment. Further experimental studies are needed to confirm this. Glucose transporter 4 is a glycoprotein on the membrane of the cell that is responsible for the easy diffusion of glucose. The change of GluT4 quality or quantity can lead to peripheral tissues using glucose disorder, which eventually lead to insulin resistance. Hyperbaric oxygen therapy (HBO) can improve the insulin sensitivity of diabetic patients. It has been confirmed by studies at home and abroad[6], but the specific mechanism is not clear.

In this experiment, hyperbaric oxygen was used in the treatment of type 2 diabetic rats. The changes in adiponectin level, insulin sensitivity and the expression of adiponectin receptor and GluT4 in diabetic rats were observed. To investigate the effect of hyperbaric oxygen on insulin sensitivity in type 2 diabetic rats and the related mechanisms.

2. Material and methods

2.1. Materials

2.1.1. Animals

Animal and feed 4 weeks old male SD 40 rats, weight (180-200)g, purchased from the center of Ji'nan Peng Yue experimental animal breeding Co, Ltd, Shandong, the license number of experimental animal production: SCXK (Lu) 2014 0007. High fat and high sugar feed formula: lard 10%, sucrose 10%,

cholesterol 2%, cholic acid 1%, base feed 77%.

2.1.2. Major reagents and instruments

Streptozocin (STZ), which was purchased from Sigma Company in the United States, product batch number: WXBC2544V; ELISA kit, for adiponectin, FINS, and GluT4, which were purchased from Wuhan Youo Health Polytron Technologies Company; The immunized adiponectin receptor 2 antibody, which was purchased from Cloud-Clone Corp Company. Use Hitachi 7600-020 automatic biochemical analyzer to detect the biochemical indicators. The rats in group C were treated with hyperbaric oxygen animal made by Yantai moon Co. Ltd. hyperbaric oxygen.

2.2. Experimental methods

2.2.1. Preparation of experimental model

40 healthy SD male rats were fed for 1 weeks, then 10 normal control groups were randomly selected and fed with ordinary feed; The remaining rats were fed with high fat and high glucose feed for 4 weeks. After 12 hours of fasting, rats in the induced model group were injected with a small dose of STZ (30mg/kg) into abdominal cavity. (STZ is dissolved in 0.1mmol/L and PH4.5 sodium citrate solution in early ice bath to prepare 2% streptozotocin solution). After 72hours of injection, rat tail vein blood glucose was detected (Johnson whole blood glucose meter). The random blood sugar is greater than or equal to 16.7mml/L for type 2 diabetic rats model success. The type 2 diabetic rats after the model were fed with high fat and high sugar feed until the end of the experiment. Finally, 21 rats with type 2 diabetes were formed.

2.2.2. Grouping and hyperbaric oxygen intervention

21 type 2 diabetic rats were randomly divided into 2 groups. There were 11 diabetic control group and 10 diabetic hyperbaric oxygen group. The rats in each group were free to eat and drink. The diabetic animal into hyperbaric oxygen group were give consent to medical treatment by hyperbaric oxygen under the pressure of 0.1MP every day. Under the condition of steady pressure, the rats were treated with 60min for 30 days. The rest of the two groups were fed according to the requirements of different feed.

2.2.3. Collection of specimen

After the experiment, the rats in each group were fasting weighed and recorded. 3 rats in each group were randomly selected for glucose tolerance test. Glucose tolerance test: after 12 hours of fasting, the rats were intraperitoneally injected with 50% glucose solution at the dose of (2g/kg). Then, the blood glucose level of 0 hour, 0.5 hour, 1 hour and 2 hours was measured by Johnson glycemic tailing. After 30 days of hyperbaric oxygen therapy, the rats in each group were fasted for 12 hours. Then, the rats were anesthetized by intraperitoneal injection of 10% chloral 3ml/kg, every rat's blood were collected from the abdominal aorta. After centrifugation, the blood serum was taken from the rat's blood and sealed in the -80℃ refrigerator. Then, we killed all the rats and cut the liver tissue of about $1cm^3$. After that the liver tissue was flushed with normal saline. Next, we used filter paper to dry the liver tissue and fixed it in Formaldehyde Solution. Then, we embedded the liver tissue in dehydrated paraffin for conventional slicing.

2.2.4. Detection of biochemical indexes

Blood glucose and glucose tolerance test are determined by Johnson's Ultraesy glycemic apparatus and matching test paper. TC, TG, HDL-C, LDL-C were measured by automatic biochemical analyzer. Serum adiponectin, FINS, and GluT4 were measured by ELISA kit. The insulin sensitivity index formula is ISI=In[1/ (FBG x FINS)].

2.2.5. Immunohistochemical staining

The AdipoR2 index in liver tissue was carried out by SP Staining Kit. The dilution ratio of first antibody was 1:400, and the dilution ratio of second antibody was 1:200. Five horizons were randomly selected for each slice under an optical microscope. The results showed that brown yellow granules in the cytoplasm of the liver are positive. Then the mean gray value of the positive expression was measured by the image analysis software.

2.3. Statistical analysis

The experimental data were expressed as means ± SD, and SPSS 21.0 statistical software was used for data analysis. The data were compared by T test. $P<0.05$ was considered as statistically significant.

3. Results

3.1. The general situation of rats in each group

The rats in the normal control group had good growth, normal feeding, sensitive reaction, quick action and smooth and glossy hair. The rats in the diabetic control group had a significant increase in the amount of food intake, urine volume increase, listlessness, slow, dull hair withered. The general situation of hyperbaric oxygen group in diabetes was better than that of the diabetic control group.

3.2. Body mass and biochemical index of rats in each group

Compared with the A group, the TG, LDL-Cand TC increased significantly in the group B and the group C, and HDL-C decreased significantly in the

group B and the group C (P<0.05). After hyperbaric oxygen therapy, the TG in group C was significantly lower than that in group B (P<0.05), and HDL-C in group C was significantly increased in group B (P<0.05). (Table 1).

Table 1 Comparison of body weight and biochemical indexes (Mean±SD)

group	n	weigh(g)	TG(mmol/L)	TC(mmol/L)	HDL(mmol/L)	LDL(mmol/L)
A	10	322.50±45.73	0.71±0.36	0.71±0.12	0.85±0.92	0.36±0.01
B	11	365.55±63.30	1.56±0.20*	1.07±0.13*	0.44±0.09 *	0.80±0.29
C	10	350±41.63	1.11±0.19*#	1.06±0.16*	0.65±0.91*#	0.62±0.31*

Note: * P<0.05 compared with A group; #P<0.05 compared with group B.

3.3. Comparison of glucose tolerance and insulin sensitivity in rats of each group

Compared with group A, fasting blood glucose, postprandial 30 minutes blood glucose, postprandial 60 minutes blood glucose, postprandial 120 minutes blood glucose and FINS were significantly increased in group B and C, and the insulin sensitivity index decreased significantly in both group B and C (P<0.05). After hyperbaric oxygen therapy, the fasting blood glucose, postprandial 120 minutes blood glucose and FINS in group C was significantly decreased than that in group B (P<0.05), and the insulin sensitivity index in group C was significantly increased than that in group B (P<0.05). (Table 2).

Table 2 Glucose tolerance and insulin sensitivity index（Mean±SD）

group	n	FBG(mmol/L)	30 minutes after meal	60 minute after meal	120 minute after meal	FINS(pg/ml)	ISI
A	10	5.45±0.89	8.05±0.71	7.2±0.43	6.15±0.45	395.95±40.68	-7.67±0.94
B	11	17.32±6.20*	22.19±4.28*	24.32±8.41*	23.48±6.62*	1058.40±327.27*	-9.71±0.45*
C	10	9.02±1.19*#	20.48±4.19*	19.44±5.14 *	12.48±1.98*#	743.38±91.24*#	-8.79±0.21*#

Note: * P<0.05 compared with A group; #P<0.05 compared with group B.

3.4. Serum adiponectin level, liver AdipoR2 and GluT4 expression in each group

Compared with the A group, the expression of serum adiponectin and GluT4 decreased in both group B and group C, but the expression of AdipoR2 in liver increased in both group B and group C (P<0.05). After hyperbaric oxygen therapy, the expression of serum adiponectin and GluT4 in the C group was significantly higher than that in the B group, and the expression of AdipoR2 in the C group was significantly lower than that in the B group (P<0.05). (Table 3, Figure 1).

Table 3 serum adiponectin, liver AdipoR2 and GluT4 expression（Mean±SD）

group	n	adiponectin (ng/ml)	Gray value of liver AdipoR2	GluT4（U）
A	10	8308.24±490.68	98.00±2.16	0.37±0.04
B	11	4467.03±1189.63*	137.82±9.22*	0.15±0.04 *
C	10	6118.61±1152.28*#	116.10±8.49*#	0.20±0.01 *#

Note: * P<0.05 compared with A group; #P<0.05 compared with group B.

4. Discussion

The main cause of type 2 diabetes is insulin resistance and functional defects of the islet B cells. The important manifestations are the lower sensitivity of the liver, adipose tissue and skeletal muscle to insulin sensitivity, and a decrease in the insulin sensitivity index. The relationship between adipose tissue and metabolic diseases such as diabetes is becoming more and more important. Adiponectin secreted by adipose tissue plays an important role in the development of diabetes.

Adiponectin was discovered by American scientist Shererp in the study of 3T32L1 adipocytes in mice in 1995. It is a specific cytokine secreted by adipocytes[7]. Adiponectin monomer exists in fat cells and play a biological role. The monomer form a polymer, and it is secreted to the outside of the cell. Two adiponectin monomer is composed of two dimers through two sulfur bonds. Three adiponectin monomers combine in the spherical domain to make up the adiponectin trimer, and 4-6 trimers form a higher level structure through the colloidal domain. Adiponectin contains four regions: the amino terminal signal sequence, the carboxy terminal sphere area, the variable non collagenous sequence area, and the G-X-Y3 amino acid repeat glial area[8].

In 2003, Yamauchi et al first cloned the adiponectin receptor gene from human skeletal muscle cells. The receptor genes were named AdipoR1 and AdipoR2, respectively. The study found that AdipoRl was expressed in a large number of skeletal muscles, and AdipoR2 was mainly expressed in the liver[9]. Epidemiological studies of different races indicate that the decrease of plasma adiponectin plays an important role in type 2 diabetes, hypertension, atherosclerosis and myocardial infarction[10]. Adiponectin was negatively correlated with insulin resistance, low density lipoprotein and triglyceride, and is positively related to insulin sensitivity and high density lipoprotein[11].

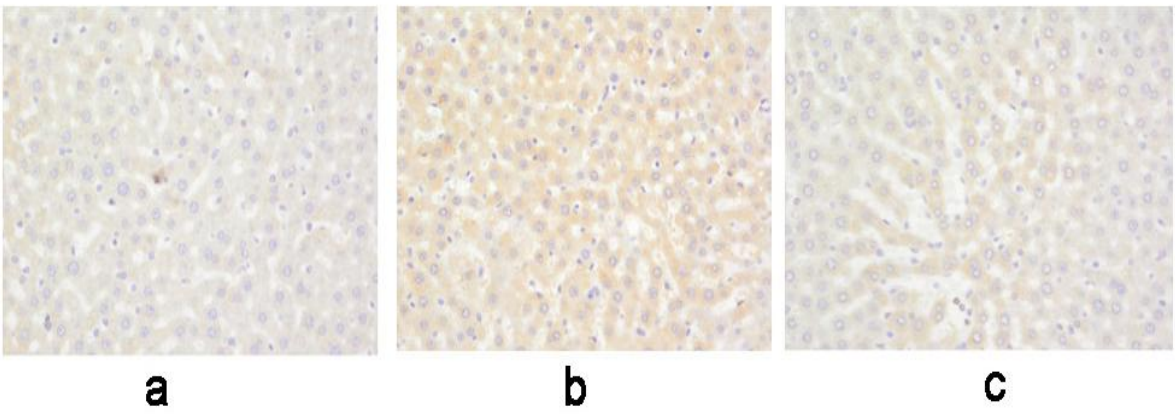

Figure 1. liver AdipoR2 protein expression (immunohistochemical staining) *400; Compared with normal rats, the expression of AdipoR2 in type 2 diabetic rats increased. 30 days after HBO, The AdipoR2 in type 2 diabetic rats is lower than that type 2 diabetic rats without HBO. It was demonstrated that HBO reduced the expression of AdipoR2 in type 2 diabetic rats.(a: Normal control group; b: Diabetes control group; c: Diabetes after HBO for 30 days group).

Adiponectin secreted by adiponectin activates adiponectin receptor 1 and adiponectin receptor 2[12]. Adiponectin also activates PPARY and increases the oxidation rate of beta cells. The rate of oxidation of beta cells is the main pathway of lipid metabolism. Adiponectin receptor 1 increases the activity of NK-k beta, TNF- alpha, IL-1, IL-4 and other genes, and indirectly regulates P13K and P13K acts on HSP90, and reduce the level of VACM1, ICAM1, and IL-8, which are involved in the inflammatory response of the body. It is worth noting that HSP90 can increase the activity of nitric oxide synthase, and there is a close relationship between nitric oxide synthase and oxidative stress. Adiponectin receptor 2 can activate APPL1 and thus up-regulates AMP active protein kinase 1, and the elevated AMPK also increases the activity of PCPCK, and eventually increases the glucose isogenesis. Adiponectin can enhance fatty acid oxidation by activating of AMP kinase[13,14] and peroxisome proliferator activated receptor–α (PPAR-α)[15-17], then improve insulin sensitivity. Studies have shown that adiponectin can improve glycolipid metabolism and insulin resistance: Adiponectin can activate APPL1 and two receptors that acts on Akt. AKt can increase GluT4 transposition and ultimately improves glucose uptake and utilization[18]. The treatment of breathing pure oxygen under the condition of higher atmospheric pressure is called hyperbaric oxygen therapy. Fife and other studies abroad show that: HBO can increase the local blood flow and oxygen content by improving the local ischemia and hypoxia state of diabetic patients, so as to improve the functional state of all important organs in diabetic patients. But the specific molecular mechanism is still unclear.

To further clarify the mechanism of hyperbaric oxygen therapy for diabetic glycometabolism. We measured changes in adiponectin level, insulin sensitivity and the changes in adiponectin receptor and GluT4 expression in diabetic rats after after 30 days of HBO treatment. The results showed that 30 days after simple hyperbaric oxygen therapy, the fasting blood glucose of type 2 diabetic rats was significantly lower than that of the control group. Meanwhile, the increase of glucose tolerance, adiponectin and insulin sensitivity increased significantly, and AdipoR2 decreased significantly. Therefore, the above differences are statistically significant. This suggests that hyperbaric oxygen has a definite effect on reducing glucose in diabetic rats，and that that hyperbaric oxygen may improve insulin sensitivity by increasing adiponectin and upregulating GluT4 expression level in insulin signaling pathway, improving glucose uptake and insulin resistance in type 2 diabetic rats. This may be one of the important mechanisms of hyperbaric oxygen hypoglycemic effect.

The mechanism that insulin signal transduction pathway involves a number of transduction pathways and a number of related signaling molecules is quite complex. The effects of other signal molecules, such

as AMPK, IRS-1, protein kinase B, etc. on insulin sensitivity, are not studied in this experiment. Furtherresearch is needed.

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