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Effects of low-deuterium liquor on glucose metabolism and pancreatic islet cells and their functions in diabetic rats

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Abstract:Objective To investigate the effects of low-deuterium liquor intervention on fasting blood glucose level and pancreatic islet cells and their functions in diabetic rats. Methods The diabetic Wistar rat model was established by intraperitoneal injection of streptozotocin (STZ). According to the different intervention methods, the diabetic rats were divided into model group (normal saline gavage, drinking ordinary water), low-deuterium water group (low-

deuterium water gavage, drinking low-deuterium water), ordinary liquor group [1 unit (low amount) or 6 units (high amount) liquor + distilled water gavage, drinking ordinary water), low-deuterium liquor group (low or high amount of

liquor + low-deuterium water gavage, drinking low-deuterium water), 10 rats in each group; 10 rats without modeling were used as normal control group (normal saline gavage, drinking ordinary water); the daily gavage liquid volume

of rats in each group was 0.01 mL/g. At the end of the fourth week after intervention, blood samples were collected from the orbital vein of each group, and then the rats were killed by cervical dislocation, and pancreatic tissue

specimens were removed and prepared. Fasting blood glucose and insulin levels were measured by glucose oxidase method and radioimmunoassay, respectively; pancreatic tissue specimens were stained with HE to observe the pathological changes of pancreatic islets in each group. Results The fasting blood glucose level was as follows: ordinary liquor high-dose group > model group > ordinary liquor low-dose group > low-deuterium liquor high-dose group > low-deuterium water group > low-deuterium liquor low-dose group. The low-deuterium water group and low-deuterium liquor low-dose group were significantly different from the other groups ($P<0.05$). The fasting insulin level was as follows: low-deuterium liquor low-dose group > low-deuterium liquor high-dose group > ordinary liquor low-dose group > low-deuterium water group > ordinary liquor high-dose group > model group. Except for the ordinary liquor high-dose group, the other groups were significantly higher than the model group ($P<0.05$). Islet histological observation showed that compared with the normal control group, the number of islet cells in the model group and the ordinary liquor group was reduced, the volume was enlarged, the cytoplasm was vacuolated, and the cells were arranged in disorder. The islet cells in the low-deuterium water group and the low-deuterium liquor group were less damaged. Conclusion Low-deuterium liquor intervention can significantly reduce the fasting blood glucose level and increase

the plasma insulin level in diabetic rats; it can also reduce the islet cell damage caused by excessive drinking. Keywords: low-deuterium

water; liquor; diabetes; fasting blood glucose; fasting insulin level; islet cells DOI:10.3969/j.issn.1674-8115.2010.10.005 Chinese Library Classification Number:R587.1Document code: A Effectsofdeuterium-depleted Chinese liquor on glucose metabolis mandis let cell sand their function of diabetic rats ZHOU Zhen-yu 1 SHENCai-hong 2 AO Zong-LUZhong-hua 2 CONG ming 2Feng-song (.School of SONGLi-Agriculture hua 1and Biology, Shanghai 3

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Abstract:Objective To investigate the effects of deuterium-depleted Chinese liquor on fasting blood glucose (FBG) and is let cells and their function of diabetic rats. Methods Wister rat diabetic models were established by streptozotocin (STZ)one-time in traperitoneal injection. The experimental rats were divided into following groups (n=10) according to the means of intervention: model group (intragastric administration of normal saline, fed with normal water), deuterium-depletedwater (DDW)group(intragastric administration of DDW, Fed with DDW), normal Chinese liquor group [intragastricadministrationofoneunit(low)orsixunit(high)Chineseliquoranddistilledwater, fedwithnormalwater] , deuterium-depletedChineseliquorgroup(intragastricadministrationofloworhighdoseofChineseliquorandDDW, fed withDDW), andcontrolgroup(withoutSTZinjection, intragastricadministrationofnormalwater, fedwithnormalwater). Anoraldailyadministrationofliquorsolutionwas0.01 mL/g(volume/bodyweight).At the end of 4 weeks of intervention, the blood was collected from the orbital vein followed by killing the rats with the method of cervical dislocation. Then, Pancreatic tissues were removed to make samples. The FBG as measured by glucose oxidase, and the fasting insulin levels (FINS) were analyzed by radio immune assay. The pathology structure of pancreaticis lets was observed by HE staining.

Results: FBG in every group followed the order: normal Chinese liquor (high) group> model group> normal Chinese(low) liquor group>deuterium-depleted Chinese liquor(high)group>DDW group>deuterium-depleted Chinese liquor(low) group. And FBG of DDW group and deuterium-depleted Chinese liquor (low) group had significant difference toother groups (P<0.05). FINS of every group followed the order: deuterium-depleted Chinese liquor (low) group>deuterium-depleted Chinese liquor (high) group > normal Chinese liquor (low) group>DDW group>normal Chinese liquor(high) group > model group. And FINS of other groups had significant differences to model group (P<0.05) except for the normal Chinese liquor (high) group. The observation of histological examination revealed that compared to control group, in model group and normal Chinese liquor group, thecellamountofpancreaticisletsdecreasedandcoupledwithvolumeincrease, vacuolarcyto plast, and disorder. While in DDW and deuterium-depleted Chinese liquor groups, pancreatic is let cells had mild damage. Conclusion Thedeuterium-depleted Chinese liquor has significant effects in decreasing FBG of rats, increasing FINS, and reduces the is let cells damage caused by excessive drinking.

Keywords: deuterium-depleted water; Chinese liquor; diabetes mellitus; fasting blood glucose; fasting insulin levels; is let cell

Diabetes has become the second largest disease in the world after malignant tumors and cardiovascular and cerebrovascular diseases. Vascular disease is the third most serious threat to human health [3-5]. Studies have ÿ 2 found that moderate drinking can increase insulin sensitivity and reduce the risk of type diabetes; while long-term, excessive drinking can cause insulin resistance, thereby increasing the risk of type 2 diabetes.

Chinese liquor has a close relationship with water. Choosing appropriate fermentation water or adding paddles to reduce the alcohol content to improve the quality of the liquor and reduce the harmfulness of liquor to the human body has always been a concern. In nature, water is composed of 2 hydrogen atoms and 1 oxygen atom, of which hydrogen has 3 isotopes: protium, deuterium and tritium. In surface water, the ratio of deuterium to protium (D/H) is about 1:6,600, that is, the volume fraction of deuterium in water is. Water with a deuterium volume 0.015% [6] fraction of less than 0.015% is usually called low-deuterium water. Previous studies on the role of deuterium in organisms have mainly focused on In terms of heavy water In recent years, research It was found that deuterium-depleted water has the effects of reducing the expression of oncogenes, inhibiting the growth of cancer cells, anti-radiation and immune protection. Previous studies in our also laboratory have confirmed that deuterium-depleted water has the effects of inhibiting lung cancer cell proliferation and promoting cell apoptosis. In this study, a diabetic rat model was established by a single intraperitoneal injection of streptozotocin (STZ) to observe the effects of deuterium-depleted water, ordinary liquor and deuterium-depleted liquor on fasting blood glucose, insulin levels and pancreatic islet cells in diabetic rats, providing theoretical and experimental basis for the application of deuterium-depleted water in the liquor industry.

1 Materials and methods

1.1 Materials 1.1.1

Experimental animals Male Wistar rats, weight (180 ± 10) g, were provided by the Experimental Animal Center of the School of Pharmacy, Shanghai Jiao Tong University. The animal production and use license numbers are SCXK (Shanghai) 2003-0003 and SYXK (Shanghai) 2007-0025, respectively. The animals were kept at room temperature of 20ÿÿ25ÿ and relative humidity of 40%ÿ70% and were allowed to eat freely. 1.1.2 Main reagents and instruments Deuterium-depleted water (volume fraction 0.0050%) (Shanghai Chitian Ultralight Water Bioengineering Co., Ltd.); 52% liquor (Luzhou Laojiao Group Co., Ltd.); STZ (Sigma); insulin radioimmunoassay kit (China Institute of Atomic Energy). AB104-N electronic analytical balance (Mettler-Toledo Instruments Shanghai Co., Ltd.); 2000 FCA microplate reader (BD); Nova16 automatic biochemical analyzer (Nova). 1.2 Methods 1.2.1 Induction of diabetic rat model After 2 weeks of adaptive feeding, Wistar rats were fasted for 12 h; a single intraperitoneal injection of 2% STZ (50 mg/kg) was used to induce modeling; 72 h later, blood was collected from the rats' eye sockets to measure fasting blood glucose levels; if fasting blood glucose > 16.7 mmol/L, the model was considered to be successfully established. 1.2.2 Grouping and drug administration The diabetic rats with successful modeling were randomly divided into model, low-deuterium water, high- and low-dose groups of ordinary liquor, ÿ

and high- and low-dose groups of low-deuterium liquor, with 10 rats in each group. In addition, 10 rats without modeling were used as the normal control group. ÿNormal control group: gavage with saline and drinking ordinary water; ÿModel group: gavage with saline and drinking ordinary

water; ÿDeuterium-depleted water group: gavage with deuterium-depleted water and drinking deuterium-depleted water; ÿLow-dose ordinary liquor group: gavage with 1 unit (a 60 kg adult drinks 50 mL of 52-degree Luzhou Laojiao per day as 1 unit) of liquor + distilled water and drinking ordinary water; ÿHigh-dose ordinary liquor group: gavage with 6 units of liquor + distilled water and drinking ordinary water; ÿLow-dose deuterium liquor group: gavage with 1 unit of liquor + deuterium-depleted water and drinking deuterium-depleted water; ÿHigh-dose deuterium liquor group: gavage with 6 units of liquor + deuterium-depleted water and drinking deuterium-depleted water. The daily gavage liquid of each group was 0.01 mL/g, and the drug was administered for 4 consecutive weeks. 1.2.3 Observation and detection ÿ Sampling and specimen preparation: After the last administration, fast for 12 h and weigh the body weight; anesthetize with 10% chloral hydrate, collect blood from the orbital vein, separate the serum and store it in a -20 ÿ refrigerator for testing. After blood sampling, the animals were killed by cervical dislocation, and the complete pancreatic tissue was quickly removed, fixed with 4% neutral formaldehyde, dehydrated, embedded in paraffin, and sliced at 4 ÿm. ÿ Fasting blood glucose determination: Determined by glucose oxidase method using Nova automatic biochemical analyzer. ÿ Fasting insulin level: Determined by radioimmunoassay. ÿ Histological observation: Paraffin sections of pancreatic tissue were obtained, stained with HE, sealed, and the pathological changes of pancreatic islet tissue were observed under an optical microscope.

1.3 Statistical methods

SPSS 11.5 statistical software was used for statistical analysis.

The data were expressed as x ± s, and the t test was used for comparison between P<0.05 groups. The differences were statistically significant.

2 Results

2.1 Changes in body weight of rats in each group

At the end of the 4th week after the intervention, the body weight of diabetic rats was significantly less than that of normal control rats (P<0.05). Among diabetic rats, the body weight of low- deuterium water group and low-deuterium liquor (low and high) group was significantly greater than that of model group (P<0.05); the body weight of low-deuterium liquor high-dose group was significantly greater than that of ordinary liquor high-dose group (P<0.05); there was no statistically significant difference in body weight between ordinary liquor (low and high) group and model group (P>0.05) (Table 1). 2.2 Changes in fasting blood glucose and insulin levels in rats in each group 2.2.1 Fasting blood glucose level At the end of the 4th week after the intervention, the fasting blood glucose level of diabetic rats in each group was significantly higher than that of normal control group (P<0.05). In diabetic rats, the fasting blood glucose levels of low-deuterium water group and low-deuterium liquor low-dose group were significantly lower than those of model group (P<0.05); the fasting blood glucose levels of low deuterium liquor low-dose group were significantly lower than those of ordinary liquor low-dose group (P<0.05); there was no significant difference in fasting blood glucose levels among model group, ordinary liquor (low and high) group and low-deuterium liquor high-dose group (P>0.05) (Table 1). 2.2.2 Fasting insulin level At the end of the 4th week after intervention, compared with the normal control group, the fasting insulin levels of diabetic rats in model group and ordinary liquor high-dose group were significantly reduced (P<0.05); the fasting insulin levels of diabetic rats in low-deuterium liquor (low and high) group were significantly increased (P<0.05). In diabetic rats, the fasting

insulin levels in the low-deuterium water group, the low-dose ordinary liquor group, and the low-deuterium liquor (low and high) groups were significantly higher than those in the model group (P<0.05); the fasting insulin level in the low-dose low-deuterium liquor group was significantly higher than that in the low-dose or

The fasting insulin level in the high-dose low-deuterium liquor group was significantly higher than that in the high-dose ordinary liquor group (P<0.05); there was no significant difference in the fasting insulin level between the high-dose ordinary liquor group and the model group (P>0.05) (Table 1). 2.3 Histological changes of pancreatic islets in rats in each group. Optical microscopic observation of pancreatic islet tissue HE staining showed that the number of pancreatic islet cells in the normal control group was large; the cell boundaries were clear; the cell nuclei were clear and oval with clear nucleoli; and the cells were arranged regularly. Compared with the normal control group, the pancreatic islets in the model group were atrophied; the number of pancreatic islet cells decreased and the distribution was sparse; the volume of pancreatic islet cells increased; the cytoplasm was lightly stained and the number of vacuoles increased; the cell nuclei were condensed, the chromatin was unevenly distributed and marginalized, and some cell nuclei were missing. Compared with the model group, the low-deuterium water group had different degrees of improvement in the number, volume, morphology and distribution of stained particles of pancreatic islet cells. In the ordinary liquor (low and high dose) group, the islet tissue was severely damaged, the volume of islet cells increased significantly, the cytoplasm of most cells was loose reticular or vacuolar, and the cell arrangement was obviously disordered. Compared with the ordinary liquor (low and high dose) group, the morphology, volume, and distribution of islet cells in the low-deuterium liquor (low and high dose) group were significantly improved with

dinary low-liquor dose group ordinary (P<0.05);liquor group; ÿP<0.05 compared with high-dose ordinary liquor group

Table 1 Body weight, fasting blood glucose and insulin levels of rats in each group (x±s, n=10)

ineachgroup(x ±s, n=10)

Group	Body mass (g)	Fasting blood glucose (mmol/L)	Fasting insulin (mIU/L)
Normal control	297.2±18.9	5.0±1.1	12.4±2.4
group	151.0±15.3 ÿ 30.2±4.0 ÿ 171.5±14.2 ÿÿ		5.8±0.8 ÿ
Model group	24.4±2.1 ÿÿ 29.5±2.8 ÿ 31.2±1.9 ÿ		11.5±5.6 ÿ
Low-deuterium water group Ordinary liquor low-			12.4±1.9 ÿ
dose group 166.1±29.8 ÿ Ordinary liquor high-			7.1±1.9 ÿ
dose group 142.5±26.1 ÿ Low-deuterium liquor low-dose group 173.2±25.6 ÿÿ 23.1±4.7 ÿÿÿ 24.7±1.7			
ÿÿÿ Low-deuterium liquor high-dose group 185.2±36.4 ÿÿÿ 28.2±4.1 ÿ 20.7±5.6 ÿÿÿ			

ÿP<0.05 compared with normal control group; ÿP<0.05 compared with model group; ÿP <0.05 compared

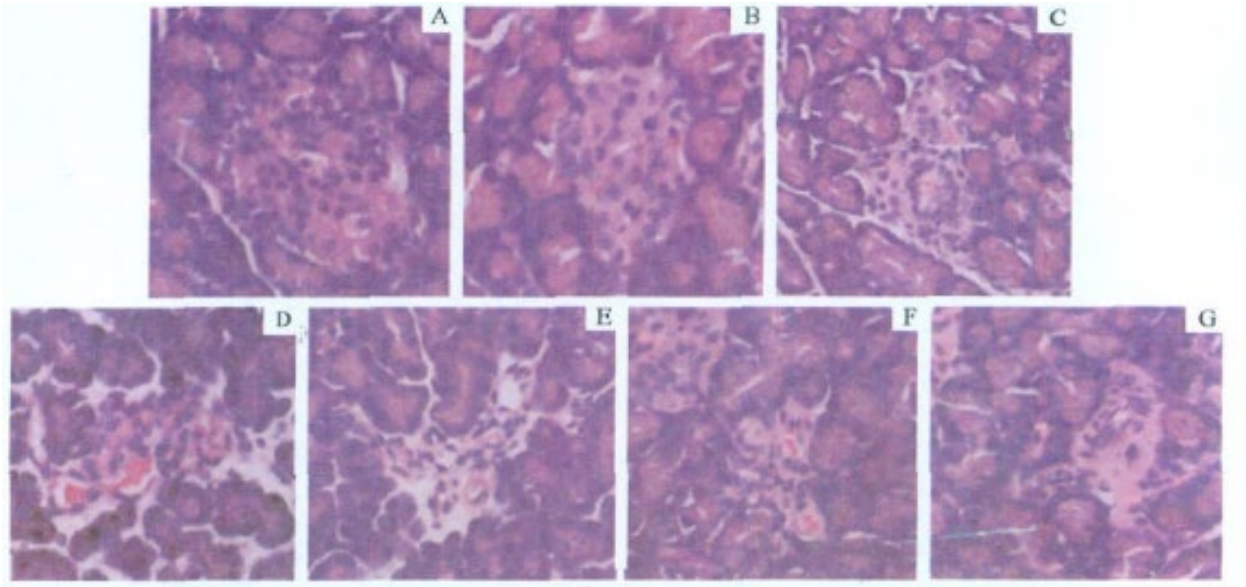

Fig.1 Pathological changes of pancreatic islet tissues in rats in each group HE staining ×200

A: normal control group; B: model group; C: low-deuterium water group; D: ordinary liquor high-dose group; E: ordinary liquor low-dose group; F: low-deuterium liquor high-dose group; G: low-deuterium liquor low-dose group

3 Discussion

The results of this study show that drinking ordinary liquor can promote the Hormone secretion; similar to Huang et al. [15] pancreatic islet.

It may be related to increased pancreatic blood flow. Some [5] S how, moderate drinking studies have shown that it can improve insulin sensitivity and reduce glycosylated hemoglobin values, which is beneficial for preventing cardiovascular complications of diabetes, while those who do not drink at all or drink heavily have a relatively increased risk of complications. This study found that the fasting insulin level of rats in the low-dose group of ordinary liquor increased significantly compared with the high-dose group. Histological observation showed that the pancreatic ÿ-cell damage in the high-dose group of ordinary liquor was more serious. Although the fasting insulin level was significantly increased, the fasting blood glucose levels of rats in the low- and high-dose groups of ordinary liquor did not change significantly compared with the model group, suggesting that insulin resistance may exist. The liver is the main organ for ethanol metabolism and an important target organ for insulin. The reduced [16] Long-term ethanol sensitivity of the liver to insulin is one of the important causes of insulin resistance. Patel et IR) decreased. [17] al. found that long-term drinking can cause insulin receptors on the surface of liver cells, and believed that the decreased insulin sensitivity of rats caused by long-term drinking may be related to the downregulation of mRNA expression of IR, insulin receptor substrate-1 (IRS-1) and insulin receptor substrate-2 (IRS-2) in the liver. In addition, ethanol may also reduce liver glycogen synthesis by affecting glucose metabolism, thereby reducing the ability of the liver and peripheral tissues to [18], absorb glucose. This experiment observed that intervention and drinking of deuterium- depleted water can significantly improve the body weight of diabetic rats, reduce fasting blood sugar, and increase

fasting serum insulin levels. Histological observations showed that deuterium-depleted water has the effect of alleviating and repairing pancreatic ÿ-cell damage. The mechanism of hypoglycemic effect of deuterium-depleted water may be related to its attenuation of streptozotocin damage to pancreatic ÿ-cells or improvement of damaged cell function, 2010, thereby 1(2):277-283. increasing insulin secretion. The results of this study showed that the rats in the low-dextrin liquor low-dose group had higher fasting insulin levels and lower fasting blood glucose levels, and the difference was statistically significant compared with the model group, suggesting that low-dextrin water and liquor have a synergistic effect in lowering blood glucose. It is speculated that the hypoglycemic effect of low-dextrin liquor may be related to its protection or repair of pancreatic islet cell damage caused by drinking, promoting pancreatic ÿ cells to secrete insulin and improving glucose metabolism. The specific molecular mechanism needs further study and exploration.

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