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The development of obesity and prediabetes under conditions of long-term consumption of fructose solution in rats

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ABSTRACT

The purpose of this study was to assess the development of obesity and prediabetes in rats by determination of key indicators of lipid and carbohydrate metabolism and the development of insulin resistance after prolonged consumption of 10% fructose solution. This study was carried out on rats with initial weighing of 160-200 g. Rats of the control group have been fed with a standard food and water for 10 weeks. Rats of the experimental group have been fed with a standard food and 10% fructose solution instead of water ad libitum for 10 weeks. Levels of biochemical parameters were measured. The development of insulin resistance in experimental animals was determined by measuring the sensitivity of peripheral tissues to the action of insulin via insulin-tolerance test. Studies have shown that 10-week consumption of fructose solution leads to imbalance of lipid metabolism disorders and the development of imbalances in carbohydrate metabolism, as evidenced by rising blood glucose, glycosylated hemoglobin, uric acid, decreasing levels of insulin and the development of insulin resistance. Long-term consumption of 10% fructose solution may be a cause of obesity and prediabetes, which in turn can lead to the development of metabolic syndrome and type 2 diabetes mellitus.

INTRODUCTION

The development of obesity, as a complex disease, is caused by a numerous factors, among which the use of food and beverages enriched with monosaccharaides should be mentioned (Karpovets *et al.*, 2014). Studying the impact of fructose on development of obesity is of considerable interest, since this monosaccharide is often used in food industry as a substitute for sucrose. According to current literature, fructose refers to lipogenic sugars, excessive use of which enhances the metabolic disorders in the liver and can lead to obesity, insulin resistance, dyslipidemia, hypertension and others (Malik *et al.*, 2010). Fructose enhances the processes of inflammation (Vlassara *et al.*, 2002), induces ROS production (Francini *et al.*, 2010) and resistance to leptin (Havel, 2005). Today, there are several hypotheses about the impact of excessive consumption of fructose in the pathogenesis of obesity and its related complications that have been discussed in the literature, but the molecular and biochemical mechanisms of this effect remain poorly understood and require detailed consideration. Great interest to fructose is due to its use as sweetener in diabetic patients. It is important to note that many studies have analyzed the biological effects of metabolism of fructose-sucrose mixtures, as well as features of high levels of fructose (60% by weight of the feed) as mononutrient (Zaman *et al.*, 2011). Therefore, we have chosen for our research a model of obesity induced by consuming the 10% fructose solution as an effective replacement of the models, which based on the consumption of high concentrations of fructose in the diet (Sanchez-Lozada *et al.*, 2007). The aim of this study was to investigate the effect of long-term consumption 10% fructose solution for the development of obesity and prediabetic condition as possible precursors of type 2 diabetes mellitus in rats.

MATERIALS AND METHODS

Experiments were carried out on white nonlinear female rats with initial weighing of 160-200 g in compliance with the standards of the Convention of Bioethics of the Council of Europe in 1997, European Convention for the protection of vertebrate animals that used for experimental and other scientific purposes, the general ethical principles of animal experiments approved by first National Congress of Bioethics of Ukraine (September 2001) and other international agreements and national legislation in this field.

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Animals and housing conditions

Studies conducted on 20 Wistar rats and divided to two groups of 10 animals each. The animals of each experimental group were individually housed in polypropylene cages in an environmentally controlled clean air room, with a temperature of 22±3C, a 12 h light/12 h dark cycle and a relative humidity of 60±5%.

Animals and diet

During the first week, all rats received standard food «Purina rodent chow» and water ad libitum. On the 8th day the animals were randomly divided into groups.

Rats of group 1 (NC) were given water ad libitum and were fed by a standard chow during 10 weeks of the experimental period. Food and water consumption were measured daily at the same time (09:00 to 10:00 h) and body weights were determined once a week. The (Fr10) group was fed by standard Purina chow and received 10% fructose in drinking water ad libitum during 10 weeks of the experimental period (Sanchez-Lozada *et al.*, 2007). Food and water consumption were measured daily at the same time (09:00 to 10:00 h) and body weights were determined once a week.

Biochemical determinations

Blood glucose concentration was measured using "HLYUKOFOT II" (Ukraine) glucometer, according to its instruction manual. Biochemical analysis of serum (triglycerides, total cholesterol, low density lipoproteins (LDL), high density lipoproteins (HDL) concentration) were carried out using semi-automatic biochemical analyzer Microlab 300 (Vital Scientific, The Netherlands).

Blood glycated hemoglobin concentration was measured spectrophotometrically using a Lachema reagents kit (Czech Republic). Insulin content was determined in the serum of experimental animals by ELISA using polyclonal antibodies against insulin. Fraction of anti-insulin antibodies was obtained independently from the serum of immunized rabbits, and was purified by affinity chromatography on a protein A-sepharose and insulin-sepharose columns (Osterman, 1985).

All procedures for obtaining, cleaning and checking the specificity of antibodies were performed according to the recommendations of standard protocols (Huse *et al.*, 2002). Insulin levels are expressed in conventional units (C.U.). The development of insulin resistance in experimental animals was determined by measuring the sensitivity of peripheral tissues to the action of insulin via insulin-tolerance test (Zhang *et al.*, 2003), conducted with own modifications. Animals were anesthetized before the test via intraperitoneal injection of sodium thiopental in dose of 40 mg/kg. After determination of the basal glucose concentration in the blood, rats were intraperitoneally injected with the insulin solution ("Monodar", Ukraine) in the dose of 0.175 units/kg. Blood samples were taken and glucose concentration was measured by using the intravenous catheter and "HLYUKOFOT II" (Ukraine) glucometer in 15, 30 and 60 min after insulin administration. In order to reflect the speed of blood glucose levels normalization in response to exogenous insulin in a control group of animals and rats that consumed 10% solution of fructose, the glycemic curves were built.

RESULTS

Serum lipid profile

According to the literature, obesity is accompanied by lipid metabolism disorders, which in turn leads to excessive accumulation of adipose tissue and the development and progression of co-morbidity. Therefore, the first step of our work was to determine the parameters that are widely used in the laboratory diagnosis of metabolic disorders, including obesity, namely the total content of triglycerides, cholesterol, high density lipoproteins (HDL) and low density lipoproteins (LDL) in the serum of rats that consumed 10% solution of fructose (Table 1).

Table 1: Serum lipid profile of control animals (NC) and animals that consumed 10% solution of fructose (FR10), (M ± m, n = 10).

Parameters	NC	FR10
Cholesterol, mmol/L	2.18±0.15	1,28±0.25*
Triglycerides, mmol/L	0.56±0.06	1.17±0.2*
HDL, mmol/L	0.908±0.08	0.55±0.14*
LDL, mmol/L	0.15±0.019	0.20±0.017*

Note: * - p <0.05 differences credible with respect to the control

The studies have shown the 2 times increase of the blood serum triglycerides content in rats of the experimental group compared to the control group. 10 -week consumption of 10% fructose solution resulted in a decrease of total cholesterol in serum by 1.7 times compared to the control group. The study of LDL content in the blood serum of rats that consumed 10% solution of fructose showed an increase by 1.3 times compared with those of control group. Content of HDL in the blood serum of animals of the experimental group was reduced by 1.7 times (Table 1).

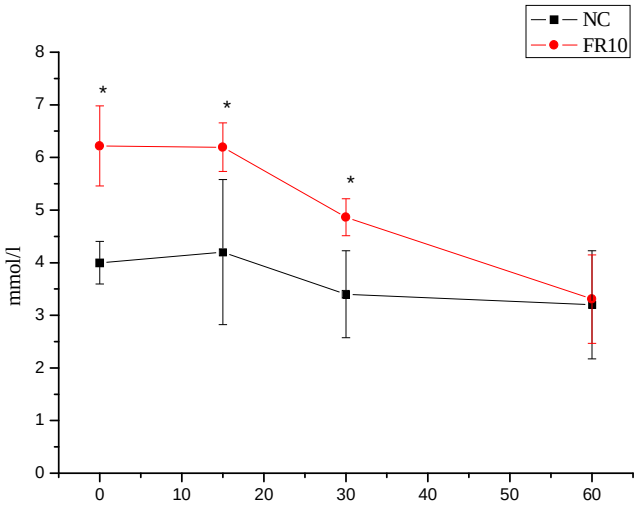

Fig. 1: Glycemic curves derived from insulin-tolerance test in a group of control animals (NC) and the group of animals that consumed 10% solution of fructose (FR10), (M ± m, n = 10).

Note: * - p <0.05 differences credible with respect to the control

Insulin-tolerance test

The studies have shown that the lowering of the glucose rate in animals that consumed 10% solution of fructose, on 15 and 30 minutes, after administration of insulin, was slower in 1.5 and 1.4 times, respectively, compared with the control group. Figure 1 shows glycemic curves, reflecting the rate of normalization of glucose levels in response to exogenous insulin, which indicates the sensitivity of peripheral tissues to the action of the hormone in a group of control animals and rats that consumed 10% solution of fructose.

Glucose, glycosylated hemoglobin, insulin and uric acid levels in blood

The studies have shown that consumption of 10% fructose solution within 10 weeks caused an imbalance of key indicators of carbohydrate metabolism in animals of the experimental group. The data presented in Figure 2 reflect changes in blood glucose, glycosylated hemoglobin, insulin and uric acid levels in animals that consumed 10% solution of fructose compared with the control group.

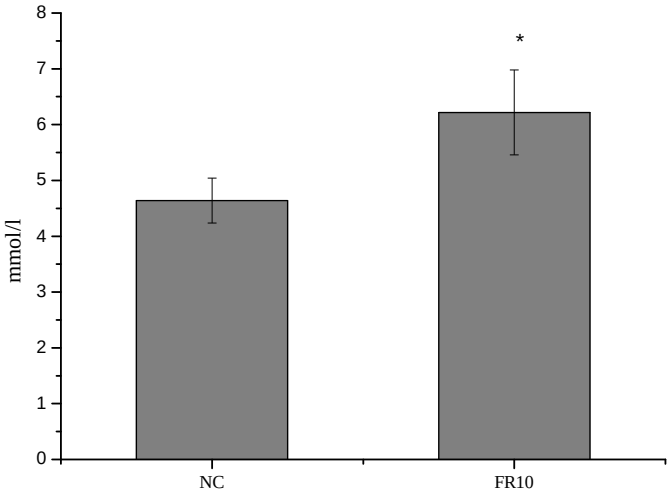

Fig. 2 A: Glucose level in the blood serum of a group of control animals (NC) and the group of animals that consumed 10% solution of fructose (FR10), (M ± m, n = 10).
Note: * - p <0.05 differences credible with respect to the control

During the studies, we have found that the concentration of glucose in the blood of animals in the control group was 4.6 ± 0.4 mmol/l. It is shown that the consumption of a 10% solution of fructose leads to the increased of fasting blood glucose almost in 1.5 times (6.2 ± 0.7 mmol/l) compared with the values of the control group (Fig. 2, A).

Our results have shown the almost 3 times increase of the blood glycated hemoglobin content in rats after consumption of 10% fructose solution compared with the control data (Fig. 2, B). We have shown the 1.8 times reduction of the insulin content in serum of rats that consumed a 10% solution of fructose in comparison with the values of the control group (Fig. 2 C). The studies of blood uric acid concentration have shown the 1.6 times increase in the blood of rats after consumption of 10% fructose solution compared with the rats of the control group (Fig. 2 D).

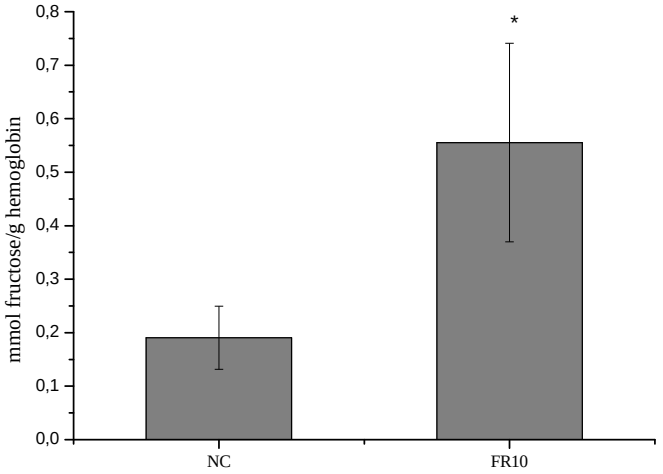

Fig. 2 B: Glycosylated hemoglobin level in the blood serum of a group of control animals (NC) and the group of animals that consumed 10% solution of fructose (FR10), (M ± m, n = 10).
Note: * - p <0.05 differences credible with respect to the control

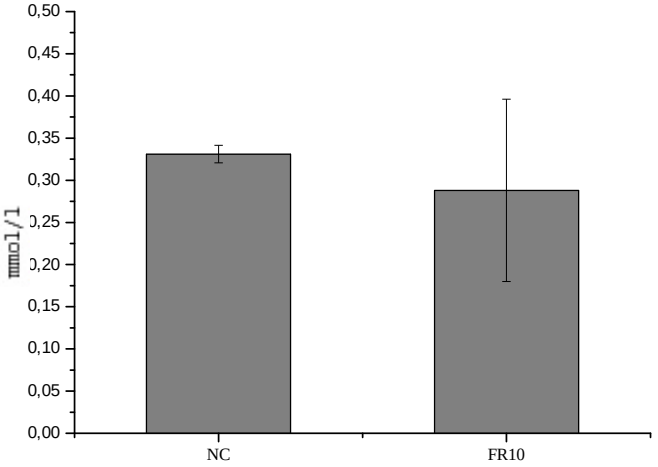

Fig. 2 C: Insulin level in the blood serum of a group of control animals (NC) and the group of animals that consumed 10% solution of fructose (FR10), (M ± m, n = 10).
Note: * - p <0.05 differences credible with respect to the control.

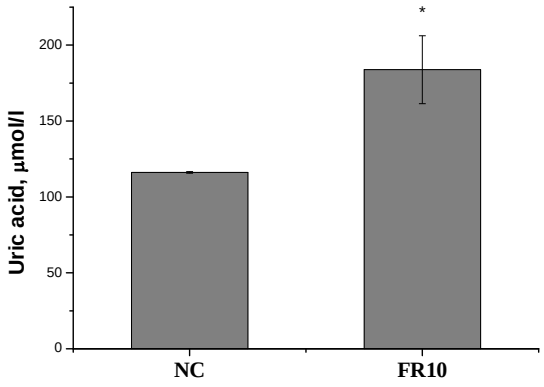

Fig. 2 D: Uric acid level in the blood serum of a group of control animals (NC) and the group of animals that consumed 10% solution of fructose (FR10), (M ± m, n = 10).
Note: * - p <0.05 differences credible with respect to the control

Statistics

Statistical analysis of data was carried out by the software package 'Statistica 7.0'. For the analysis of data distribution type, Shapiro-Wilks criterion was used. As the data were normally distributed, we used Student's t test for independent samples. Mean values (M) and standard deviations (SD) were calculated. Significant difference was considered at $p \leq 0.05$.

DISCUSSION

The development of obesity

The increase of triglycerides in the blood, that we recorded, alongside with the increasing content of LDL may indicate the development of hypertriglyceridemia - a metabolic disorder, which is typical during progression of obesity, metabolic syndrome and type 2 diabetes mellitus (Zaman *et al.*, 2011). Excessive accumulation of triglycerides is probably associated with the fructose metabolism feature, as this monosaccharide is converted to fructose-1-phosphate in the liver, which may be a direct substrate for the synthesis of the molecule backbone of triglycerides (Malik *et al.*, 2010). These results are consistent with published data which suggest that the 24-hour profile of plasma triglycerides was increased on the background of fructose and fructose-sucrose drinks consumption over the use of glucose (Stanhope *et al.*, 2008). Induction of lipogenesis increases triglycerides in the liver, adipose and muscle tissues, which leads to a weakening of insulin signaling and the development of dyslipidemia and overweight (Basciano *et al.*, 2005).

In its turn, hypertriglyceridemia can lead to coronary heart disease, myocardial infarction, atherosclerosis and thrombosis of cerebral vessels. Reduction of HDL and total cholesterol levels in rats of experimental group may indicate a breach of assimilation, or synthesis of cholesterol in the body, which on the background of the growing content of triglycerides and LDL can lead to the development and progression of cardiovascular diseases.

The development of insulin resistance and prediabetes

Today, insulin resistance is considered as a main factor in the development of insulin-independent form of diabetes, which appears in violation of the metabolic response to endogenous or exogenous insulin. According to the literature (Shpakov, 2000), the insulin resistance in obesity may develop long before the onset of clinical signs of diabetes. In response to reduced insulin sensitivity of peripheral tissues of the body, β-cells of the pancreas increases the synthesis of insulin by a compensatory mechanism that appears in an adaptive increase of circulating hormone concentration (Rafacho *et al.*, 2010).

Our results indicate that the development of insulin resistance, which plays an important role in the development of type 2 diabetes. Loss of sensitivity of liver cells, muscle and adipose tissue to insulin action is the one of the key causes of metabolic disorders of carbohydrate and lipid metabolism in type 2 diabetes mellitus and the one of the major factors in the development and progression of its complications (Balabolkin and Klebanova, 2004).

Today we know that the development of insulin-independent diabetes mellitus is accompanied by pathological changes in the processes of carbohydrate metabolism. According to the literature (World Health Organization, 2003), the main criteria, used to diagnose imbalances in carbohydrate metabolism, are: glucose, glycosylated hemoglobin, insulin and uric acid levels in blood. The one of the key causes of metabolic disorders of carbohydrate and lipid metabolism in the development of insulin-independent diabetes is a loss of sensitivity of the cells of the liver, muscle and adipose tissue to insulin action - a state of insulin resistance. Therefore, we have investigated these parameters in terms of consumption of 10% fructose solution for the diagnosis of prediabetes in animals of the experimental group.

According to the literature, normal fasting blood glucose varies within 3.5-5.5 mmol/l (American Diabetes Association, 2012). Our results may indicate the development of hyperglycemia in experimental group of animals that may develop due to the formation of glucose from fructose by activation of gluconeogenesis and by lowering insulin secretion β-cells of the pancreas and insufficient utilization of glucose by tissues of the body, due to the development of insulin resistance.

The concentration of glycated hemoglobin is an important marker of diabetes, and is used as a measure of average blood glucose level over a long period, as well as glycemic control in the treatment of antidiabetic drugs. The increase of glycated hemoglobin in the blood of experimental group animals suggests prolonged hyperglycemia in experimental group, which can lead to diabetes.

Reduction of insulin levels, on the background of prolonged hyperglycemia and reduced sensitivity of peripheral tissues to the action of this hormone, may indicate the development of the development of insulin deficiency, caused by the dysfunction of the pancreas β-cells, by which these cells lose their ability to produce insulin (Wang *et al.*, 2011).

According to the literature (Ogbera and Azenabor, 2010), metabolic syndrome is characterized by impaired carbohydrate, lipid and purine metabolism. Important factors in uric acid homeostasis are glucose and insulin, as the imbalance of carbohydrate metabolism resulting in hyperuricemia and hyperuricosuria. Relationship of uric acid with excess body weight, lipid metabolism (especially triglycerides) was observed not only in patients with gout, but also in arterial hypertension, coronary heart disease, obesity and diabetes (Karpovets *et al.*, 2014).

The increase of concentration of uric acid in the blood of experimental animals may be associated with features of the transformation of fructose in the body, as in the metabolism of this monosaccharide in the liver the adenosine-5-monophosphate is formed, which is a substrate for the synthesis of uric acid (Johnson *et al.*, 2007). In its turn, hyperuricemia leads to reduced bioavailability of nitric oxide (NO), the development of

endothelial dysfunction and, consequently, insulin resistance and type 2 diabetes mellitus (Nakagawa *et al.*, 2005).

Thus, in the course of studies have shown an imbalance of metabolism in rats that consumed 10% solution of fructose, which is accompanied by lipid metabolism disorders and the development of imbalances in carbohydrate metabolism, as evidenced by rising blood glucose, glycosylated hemoglobin, uric acid, decreasing levels of insulin and the development of insulin resistance, as reflected by the decrease in the sensitivity of peripheral tissues to the action of the hormone. Based on the results presented, it can be argued that long-term consumption of 10% fructose solution may be a cause of obesity and prediabetes, which in turn can lead to the development of metabolic syndrome and type 2 diabetes mellitus.

REFERENCES

American Diabetes Association. Diagnosis and classification of diabetes mellitus. Diabetes Care, 2012; 35:64-71.

Balabolkin MI, Klebanova EM. Role of insulin resistance in the pathogenesis of type 2 diabetes. Ter archive, 2004; 10:54-58.

Basciano H, Federico L, Adeli K. Fructose, insulin resistance, and metabolic dyslipidemia. Nutr Metab, 2005; 2:5-19.

Francini F, Castro MC, Schinella G, García ME, Maiztegui B. Changes induced by a fructose-rich diet on hepatic metabolism and the antioxidant system. Life sciences, 2010; 86:965-971.

Havel PJ. Dietary fructose: implications for dysregulation of energy homeostasis and lipid/carbohydrate metabolism. Nutr Rev, 2005; 63:133–157.

Huse K, Böhme HJ, Scholz GH. Purification of antibodies by affinity chromatography. J. Biochem Biophys Methods, 2002; 51:217-231.

Johnson RJ, Segal MS, Sautin Y, Nakagawa T, Feig DI. Potential role of sugar (fructose) in the epidemic of hypertension, obesity and the metabolic syndrome, diabetes, kidney disease, and cardiovascular disease. Am J Clin Nutr, 2007; 86:899–906.

Karpovets TP, Konopelnyuk VV, Savchuk OM, Ostapchenko LI. Food behavior of rats under development of obesity. RJPBCS, 2014; 5(5):253-259.

Karpovets TP, Konopelnyuk VV, Galenova TI, Savchuk AN, Ostapchenko LI. High-calorie diet as a factor of prediabetes development in rats. Bulletin of Experimental Biology and Medicine, 2014; 156(5):639-641.

Malik VS, Popkin BM, Bray GA, Després JP, Hu FB. Sugar-sweetened beverages, obesity, type 2 diabetes mellitus, and cardiovascular disease risk. Circulation, 2010; 121:1356-1364.

Muhammad-Quaid Zaman, Véronique Leray, Jérôme Le Bloc'h. Lipid profile and insulin sensitivity in rats fed with high-fat or high-fructo se diets. British Journal of Nutrition, 2011; 106:206-210.

Nakagawa T, Tuttle KR, Short RA, Johnson RJ. Hypothesis: fructose-induced hyperuricemia as a causal mechanism for the epidemic of the metabolic syndrome. Nat Clin Pract Nephrol, 2005; 1:80-86.

Ogbera A., Azenabor A. Hyperuricaemia and the metabolic syndrome in type 2 DM. Diabetol Metab Syndr, 2010; 2:24-31.

Osterman LA. 1985. Chromatography of proteins and nucleic acids. Moscow, Russia: Nauka.

Rafacho AL, Quallio SD, Ribeiro DL, Taboga SR, Paula FM. The adaptive compensations in endocrine pancreas from glucocorticoid-treated rats are reversible after the interruption of treatment. Acta Physiol, 2010; 200:223-235.

Sanchez-Lozada LG, Tapia E, Jimenez A, Bautista P, Cristóbal M. Fructose-induced metabolic syndrome is associated with glomerular hypertension and renal microvascular damage in rats. American journal of physiology. Renal physiology, 2007; 292:423-429.

Shpakov AO. Structural and functional characterization of insulin receptor substrate proteins and the molecular mechanisms of their interaction with insulin superfamily tyrosine kinase receptors and effector proteins. Membr Cell Biol, 2000; 13:455 – 484.

Stanhope KL, Griffen SC, Bair BR. Twenty-four-hour endocrine and metabolic profiles following consumption of high-fructose corn syrup-, sucrose-, fructose-, and glucose-sweetened beverages with meals. Am J Clin Nutr, 2008; 87:1194–1203.

Vlassara H, Cai W, Crandall J, Goldberg T, Oberstein R. Inflammatory mediators are induced by dietary glycotoxins, a major risk factor for diabetic angiopathy. Proc Natl Acad Sci, 2002; 99:15596–15601.

Wang YH, Li M, Gao S, Zhang XJ, Li LX. Correlations of serum alanine aminotransferase and insulin resistance, pancreatic B-cell function. Zhonghua Yi Xue Za Zhi, 2011; 27:3426-3430.

World Health Organization. Screening for type 2 diabetes. Report of a World Health Organization and International Diabetes Federation meeting, 2003: 1-48.

Zaman M.Q, Leray V, Le Bloc'h J, Thorin C, Ouguerram K. Lipid profile and insulin sensitivity in rats fed with high fat or high-fructose diets. British Journal of Nutrition, 2011; 106:206-210.

Zhang F, Ye C, Li G, Ding W, Zhou W. The rat model of type 2 diabetic mellitus and its glycometabolism characters. Exp Anim, 2003; 52:401-407.

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