Induction of type 2 diabetes with high concentration and long term fructose intake in male Sprague-Dawley rats

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Received; 2015/02/9 revised; 2015/02/20 accepted 2015/02/20

Abstract

Introduction: High calorie diet is becoming the most important health problem in most developed western societies. Diets with high caloric contents such as high fructose intake are associated with increasing risk of type two diabetes (T2D) and insulin resistance (IR). However, large differences have been seen in the dose and duration of fructose consumption to induction of T2D. Interest to develop animal models, in which IR is induced by feeding a high caloric diet, is the main aim of this study.
Materials and methods: Male Sprague-Dawley rats were randomly sorted into three groups, control and fructose-treated (10 and 20 %) animals. After 6 and 14 weeks, fluid intake, body weight, intraperitoneal glucose tolerance test, metabolic parameters (glucose, insulin, triglyceride, cholesterol, uric acid) and homeostasis model assessment of insulin resistance were checked.
Results: There was no difference between the body weight of the fructose-treated (10 and 20 %) and control groups during the experiment. Fluid intake in fructose-treated ones (10 and 20 %) was significantly higher than the control group from third week to the end of experiment. Intraperitoneal glucose tolerance test was the same for all groups in either sixth or fourteenth weeks. Fasting plasma insulin, triglyceride, uric acid and homeostasis model assessment of insulin resistance and glucose tests showed significant enhancement in fructose-treated rats (20%) only after 14 weeks.
Conclusion: Consumption of high fructose solution (20%) for 14 weeks could induce IR, but using of low dose of fructose (10% for 6 or 14 weeks) didn't develop it.

Keywords: Fructose, Insulin resistance, Type 2 diabetes, Sprague-Dawley rat

Introduction

Type-2 diabetes (T2D) is known as a serious socio-economic problem (1). Epidemiological studies have shown that more than 100 million people in the world suffered from T2D. This type of diabetes is a complex and heterogeneous disorder is distinguished by a progressive decline in insulin action or defect in insulin signal transduction (1, 2). In this case, the tissues can't utilize glucose normally, that is associated with serum triglycerides enhancement, serum HDL reduction and sometimes LDL enhancement (3).
Appropriate animal models are required to understanding of the pathogenesis and complications of T2D and IR (4). Insulin

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resistance can be induced by injection of some chemicals like streptozotocin (STZ) (low dose of STZ in combination with high fat or high calorie diet), using transgenic animals, and special diet consumption (high fat or high calorie diet). These chemicals are commonly used, but they are inefficient to induction a close reality model of T2D. In fact, a real insulin resistance does not induce by these methods, and the insulin deficiency and hyperglycaemia are inducing primarily by direct adverse action of the chemicals on beta cells (4).

Currently, a large number of genetically modified animal models, such as transgenic animals, or tissue-specific knock-out mice, hereditary ob. /ob. mice and Sprague Dawley or Wistar rat models have been developed for the study of diabetes. These models create a more appropriate pathogenesis model for the disease, but great varieties of such models are required to study the effect of T2D on different organs. However, these models are very expensive, difficult to maintain, and are hardly found among research labs, particularly in developing countries (5, 6).

Fructose with a chemical formula ($C_6H_{12}O_6$) similar to glucose which can be found in many fruits, vegetables and beverages. Fructose intake in excess can induce moderate obesity and have several deleterious metabolic effects, including hyper-triglyceridemia and hyper-insulinaemia (7).

On the base of these evidences, some researchers attempted to induce a model of T2D using fructose. Large differences have been seen in the result of fructose consumption to induction of T2D. Collino M et al. (2010) reported that a high fructose solution (10%) for 15 weeks could induce insulin resistance (IR) (8); Also, it has been shown that the same concentration of fructose for 9weeks induced T2D in female rats (9). However, De Moura and his colleagues (2009) have shown that consumption of a high fructose solution in Wistar rats, for 8 weeks, did not change metabolic parameters and could not make T2D (7).

The primary objective of this research was to develop a suitable, non-genetic model of T2D in Sprague-Dawley rat. We proposed that this model may mimic symptoms and clinical pathogenesis which is seen in people with T2D. In the present study; we examined the effect of two different doses of oral fructose (10% and 20%), during 6 and 14 weeks in male Sprague-Dawley rats.

Materials and methods

Animals: Male Sprague-Dawley rats (100-120g, 40 days age) were prepared from the animal lab of Shiraz University of Medical Sciences. All experiments (Experimental study, random sampling) done (May, 2013) in accordance with the Animal Ethics Committee (approval license No: 90-5702) of the Shiraz University of Medical Sciences guidelines. Animals were weighed and grouped randomly into three groups (n = 8). They were kept at standard temperature (22-25°C), 12h light–dark cycles, and free access to standard chow in all groups. They also had access to tap water and fructose in drinking water (10 or 20%) ad libitum in control and fructose-treated groups, respectively for 14 weeks.

Fluid intake and body weight: Changes in fluid intake and body weight were weekly assessed. Figure 1 shows the mean body weight during the 14 weeks.

Intraperitoneal glucose tolerance test (IGTT): After 6 or 14 weeks, animals were sustained while fasting for 15h and then were anesthetized with a mixture of ketamine and xylazine (70 and 10 mg/kg i.p., respectively). An unchallenged sample checked at time of zero. Thirty, 60, 90 and 120 minutes following injection of 2gr/kg glucose intraperitoneally, blood samples were checked by glucose meter (ACCU-CHEK Active, Germany) to determine the glucose concentration (10, 11).

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Metabolic parameters analysis: Before easuring the glucose tolerance test, a sample of blood (0.5 ml) is taken from the tail for analyzing the metabolic parameters (fasting glucose, insulin, triglyceride,cholesterol and uric acid). Non fasting glucose and homeostasis model assessment of insulin resistance (HOMA-IR index) were also checked.

Plasma glucose level was assayed by glucoseoxidase–peroxidase method (Pars Azmun kit, Iran). Triglyceride (TG), cholesterol and uric acid were assayed by enzymatic colorimetric end point methods (Pars Azmun kit, Iran). However; Plasma insulin was analyzed by using an ultra-sensitive rat insulin ELISA kit (Mercodia, Sweden). Fasting serum insulin and fasting blood glucose concentrations was measured and the homeostasis model assessment-estimated insulin resistance (HOMA-IR index) calculated according to the following formula (11): HOMA-IR = [fasting glucose (mM) × fasting insulin (mU/mL)] / 22.5

All data are presented as mean ± SEM. To determine the significant differencebetween test and control groups, the variance (ANOVA) was analyzed with SPSS11.5 statistical software. Significant differences between the two groups were assessed by Turkey's test ($P < 0.05$ was considered statistically significant). Figures are being drawn using Excel software.

Results

Body weight and fluid intake: Body weight did not show any significant difference between fructose-treated (10 and 20 %) and control groups in each weeks (Figure 1). But in some weeks, fluid intake in fructose-treated 10 % and 20 % was significantly higher than the control group (data not shown).

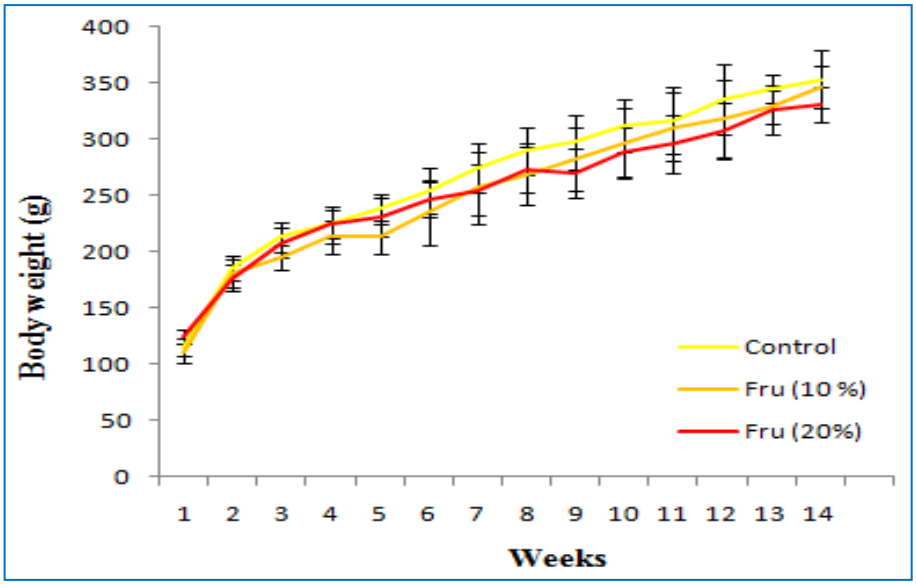

Figure 1. Mean body weight gain during 14 weeks in fructose –treated and control rats. Data are shown as the mean ± SEM (n= 8 animals).

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Intraperitoneal glucose tolerance test: This parameter showed no significant difference between fructose-treated and control groups after 6 or 14 weeks (Figure 2).

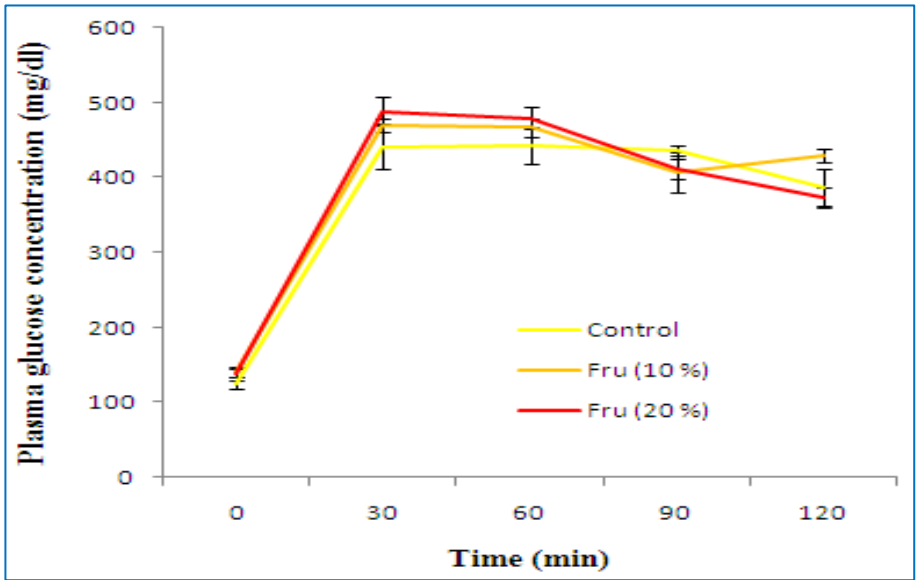

Figure 2. Intraperitoneal glucose tolerance test (IGTT) in fructose drinking and control rats after 14 weeks.

Metabolic parameters: Fasting plasma insulin (P <0.001), triglyceride (P <0.05), cholesterol (P <0.05), uric acid (P <0.01), HOMA-IR index (P <0.001) and non-fasting glucose (P <0.01) increased significantly in 20% fructose-treated compare to other groups only after 14 weeks. Metabolic parameters (in except of cholesterol) was not significantly different in control and 10% fructose treatment animals. Fasting glucose was similar in all groups (Table 1, Figures 3 and 4).

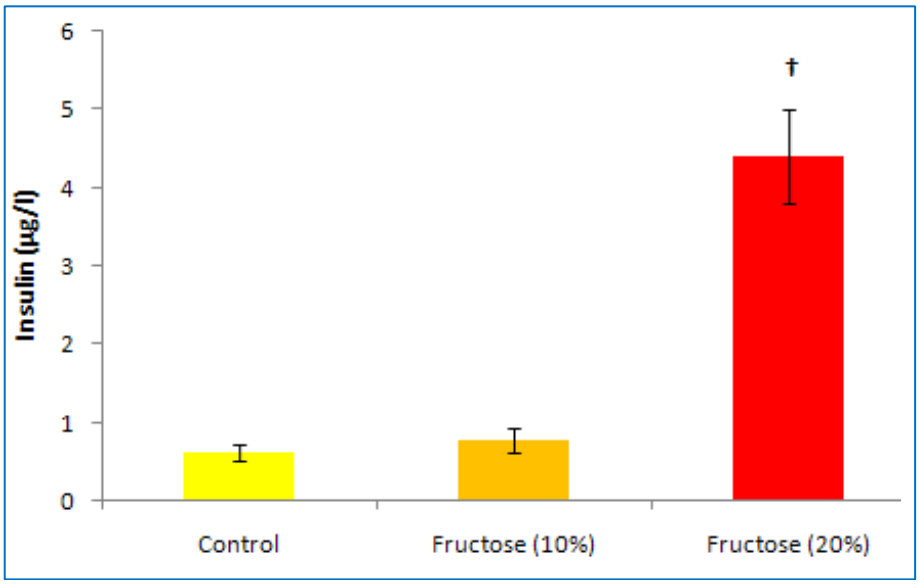

Figure 3. Fasting insulin concentration in fructose drinking compared to the control animals. (†significant difference ($p < 0.001$) compared to control group).

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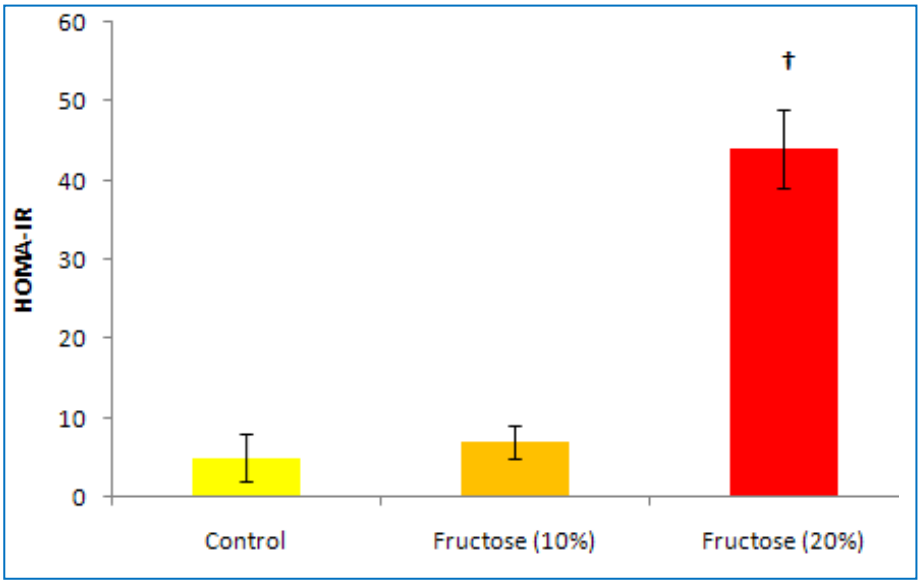

Figure 4. Homeostasis model assessment of insulin resistance (HOMA-IR) in fructose drinking compared to control animals († significant difference ($p < 0.001$).

Table 1. Metabolic parameters in fructose treated and control rats after 6 and 14 weeks.

Metabolic parameters	Groups					
	6 weeks treatments			14 weeks treatments		
	Fructose (10%)	Fructose (20%)	Control	Fructose (10%)	Fructose (20%)	Control
Fasting glucose (mg/dl)	134±4	140±16	131±6	142±8	134±7	132±5
Non-fasting glucose (mg/dl)	266±30	280±43	237±18	258±17	340±28 **	249±31
Uric acid (mg/dl)	1.08±.05	1.13±.16	0.99±.11	0.99±.07	2.93±.5 **	1.08±.05
Triglyceride (mg/dl)	66±10	83±15	70±8	71±9	134±23 *	86±6
Cholesterol (mg/dl)	72±4	108±17 *	69±6	109±11 *	126±14 *	83±9

*, ** represent $p < 0.05$ and $p < 0.01$ in comparison with control group, respectively.

Discussion

T2D is a multi-factorial disorder, usually associated with a set of pathologic symptoms such as obesity, hypertriglyceridemia, glucose intolerance and insulin resistance (IR) (3). To understanding the mechanisms which involves in pathological symptoms of T2D development; it must be made an appropriate animal model. Many attempts were done in this relation. However, the great controversy was seen in dose and duration of fructose intake in induction of T2D. As De Moura and his colleagues (2009) have shown that 6 or 8 weeks consumption of a high fructose solution didn't change metabolic parameters and could not induce T2D in Wistar or Sprague-Dawley rats (7). However; beta

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cells impairment and mortality rate was high when some drugs were used such as STZ (in low dose) in combination to fructose for T2D induction (4). Genetically modified animal models, also are too expensive, difficult to maintain and are hardly found among research labs, particularly in undeveloping countries (5, 6). For these reasons, the primary objective of this research was to develop an alternative non-genetic model in Sprague- Dawley rat for T2D, which mimic symptoms and clinical pathogenesis seen in people with IR, and on the other hands, induce of T2D as easily. In the present study, was tested the effect of two different dose of fructose (10% and 20%) during 6 and 14 weeks in male Sprague-Dawley rats on T2D induction.

According to our data, chronic consumption (14 weeks) of fructose solution (20%) changed the metabolic parameters and induced T2D. The same results were achieved about female Wistar rats by Zakula Z et al. (9).

The mechanism underlying the effects of fructose is the hepatic intermediary metabolism. Fructose bypasses the phosphofructokinase regulatory pathway and enters the glycolysis or gluconeogenesis cycles at the triose phosphate step which in increases hepatic triglyceride production (12). Hyper-triglyceridemia in fructose-treated animals has been proposed caused by either VLDL-TG enhancement due to an increased hepatic secretion of them or a decreased elimination of TG-rich lipoproteins from the circulation (13). Plasma triglycerides and cholesterol enhancement are the major factors of IR induction. Entrance ability of glucose to the cells and its oxidation was inhibited by fatty acids through the glucose transporters activity impairment and change of the enzymatic activity of glycogen synthesis. Thus, was occurred prolonged hyperglycemia and insulin action impairment (14). A new hypothesis provided by recent findings show that a high fructose concentration causes uric acid enhancement, which inhibits nitric oxide (NO) availability. It has been showed that insulin act through the NO for glucose uptake stimulation, and it can be assumed that fructose-induced hyperuricemia may have a pathogenic role in insulin resistance induction and metabolic syndrome development (15). Other studies have shown that fructose can induce uric acid generation, and thereby develops mitochondrial oxidative stress which stimulates fat accumulation (16). Some research had reported a significant enhancement in fasting plasma glucose level after fructose consumption (17) and the other did not show any significant changes (13). But, in contradiction with some previous findings (17-19), we found that, fructose intake for 14 weeks did not make impairment in fasting blood glucose. One possible reason for this is, in our model, pancreas is intact (did not use of chemical agents in combination to fructose intake to destruction of beta cells) and has appropriate response to blood glucose fluctuations. So we suppose that insulin enhancement keep the fasting blood glucose in normal level but response to insulin is lower than the normal so that non-fasting glucose increase with fructose consumption. Hyper-insulinemia compensate low insulin sensitivity for maintain of normal glucose homeostasis (15). Glucose-regulation did not alter in mice which was drinking 15% fructose solution for 16 weeks and also Wistar rats used high fructose solution (10%) for 8 weeks (7, 20-21). Axelsen et al. (2009) achieved similar results with male Sprague-Dawley rats in which animals were fed high cholesterol-fructose diet for 15 weeks (22). The animals' weight and fluid intake were measured weekly. Fluid intake in fructose-treated rats was greater than control group, from 3th weeks of treatment to the end of experiment. Animal's growth was normally, but their body weight did not show any significant difference in experimental groups in each

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week. The results of the present study are similar to those of Takatori et al., Van der et al. and Zamamiet al. (19, 23-24). However, Stranahanet al. and Brito et al. showed high fructose diet promotes weight gain in rodents (17, 25). High fructose consumption may results in an increase in body weight through the positive energy balance. Obesity due to positive energy balance associate with a high concentration of free fatty acids, which may diminish insulin sensitivity and an increase in blood glucose level (3). However; in our study, any difference was observed in body weight parameter between fructose-treated and control animals, proposing that obesity is not a major contributor to induction of T2D.

Amount of fructose intake was also measured in some studies, and various results were reported (4, 23). The reasons for these differences might be the different route of fructose administration as some studies used of fructose with combination to food. Another reason may be the difference of experimental rodent´s species that might make different susceptibility to fructose treatment.

This model may mimic symptoms and clinical pathogenesis are seen in people with T2D which can be developed as an easier method (low cost and simple method) with compared to several existing animal models of T2D.

Conclusion

Results of this research show that using of low dose of fructose (10% for 6 or 14 weeks) didn't develop T2D, but high fructose solution (20%) intake only in long term (14 weeks) could make it in male Sprague-Dawley rats. This animal model can be used to studies in which require to an animal model of T2D with IR.

Acknowledgments

This study was supported financially by the grant (No: 90-5702) from the vice chancellor of research of Shiraz University of Medical Sciences.

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