Original Article

Novel High-Fat Diet Formulation and Streptozotocin Treatment for Induction of Prediabetes and Type 2 Diabetes in Rats

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Abstract

Background: The previously established methods for type 2 diabetes (T2D) have mainly concentrated on overt diabetes model development. Here, our intention was to create an animal model passing through distinct phases such as obesity with insulin resistance, prediabetes, and gradual progress to the overt diabetes stage. A high-fat high-carbohydrate diet formulation was prescribed combined with multiple low-dose streptozotocin (STZ) injections after obesity establishment**. Materials and Methods:** Sixteen male Wistar rats were separated randomly into two groups and fed a normal diet for 1 week after which the body weight and biochemical indices of each rat were measured and recorded. Subsequently, one group (n = 8) switched to the high-fat high-carbohydrate diet formulated by us for 10 weeks, whereas the other group (n = 8) continued with the normal diet. Body weight and biochemical indices of the rats in the high-fat diet (HFD) group were measured at the end of 10 weeks, and each rat received 30 mg/kg intraperitoneal STZ injections with 1-week intervals in two steps and was continued on a high-fat high-carbohydrate diet. The differences between the groups were analyzed using the Student's t-test or one-way analysis of variance and by *post hoc* multiple comparisons. **Results:** A significant change in weight, fasting blood glucose, and triglyceride was observed in rats fed with a HFD after 10 weeks. The HFD rats showed typical characteristics of T2D mellitus (T2DM) such as insulin resistance and hyperglycemia following 30 mg/kg STZ**. Conclusions:** The novel high-fat high-carbohydrate formulation we used, along with multiple low doses of STZ, can mimic peculiar characteristics of T2DM development.

Keywords: *High-fat diet, streptozotocin, type 2 diabetes mellitus rat model, type 2 diabetes*

Introduction

Diabetes is one of the most common metabolic disorders that is caused by a defect in the insulin production, secretion, and function.[1] The number of diabetic patients has doubled in the last decade. Based on the reports, there are 382 million diabetic patients in the world and the number is expected to increase to 592 million patients by 2035.[2] Among the various forms of diabetes, type 2 diabetes (T2D) is the most prevalent type of the disease. Due to its high incidence and fast spread, we need to find better prevention and treatment methods to reduce the burden and prevalence of diabetes.[2] For this purpose, finding an appropriate *in vivo* model is necessary for the disease etiology, pathogenesis, genetic studies, and environmental factor interactions as well as evaluation of the new treatment methods.[3]

T2D, in comparison with type 1 and other known types of diabetes, is more complicated. Type 2 diabetes and obesity are each characterized by the strong interaction of genetics and environment over time.[4] Obesity is an essential primary event in T2DM development. To show their interrelationship sometimes the term "Diabesity" is used.[5] The most important molecular defect in T2D initiation and progression is defective insulin signaling pathway that leads to insulin resistance when target tissues remain nonresponsive to insulin, which results in glucose uptake failure.[6] Under such circumstances, hyperglycemia, hyperinsulinemia, and hypertriglyceridemia are evident, and this course of events ultimately leads to pancreatic beta-cell insufficiency and T2D.[7]

As a multifactorial disease, T2D development is highly influenced by environmental factors, most prominently lifestyle and diet combination. Therefore, a relevant animal model that can replicate the

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disease development course as it happens in T2D patients would be of immense value for evaluation of the disease causes or new treatment methods. The animal models for T2D have been developed either by various genetic manipulations to produce transgenic animals or through feeding modified diets to normal animals of the choice.[8-10] Complications due to T2D such as cardiovascular diseases, nephropathy, retinopathy, and neuropathy are important instances that the animal models proved considerably helpful for understanding their course of development and therapy opportunities. Unfortunately, the high cost of production and maintenance of the transgene animal models, their limited life span, and current distribution restrictions from the countries of T2DM transgene animal production to the rest of the countries make the use of these models limited, especially in developing countries.[10,11] However, nongenetic models are much easier to produce by diet manipulations, are economical, and are more similar to the human T2D disease with regard to the initiation and progression phases.[9,12-14]

T2DM models were formerly produced using a combination of high-fat diet (HFD) and streptozotocin (STZ) injection. However, there were differences in the food type, duration of the HFD, and STZ injection doses.[3] As an example, in the study of Zhang *et al.*, the rats were fed chow for 4 weeks and then received a single injection of 45 mg/kg STZ or two STZ (30 mg/kg at weekly intervals for 2 weeks) injections.[3] Other reports described feeding male Sprague–Dawley rats normal chow or a HFD for 2 weeks and then injecting 50 mg/kg STZ,[14] while in another study, a combination of a HFD for 2 weeks and then 35 mg/kg STZ as single injection was used to develop a T2D rat model.[15] However, an inexpensive animal model to investigate different aspects of prediabetes status such as insulin resistance or even the efficiency of a particular diet component to induce T2D is of great interest.[16] In this study, Wistar rats were fed a high-fat and high-carbohydrate diet for 10 weeks followed by the application of low doses of STZ for the development of a diet-induced T2DM animal model.

Materials and Methods

Sixteen male Wistar rats (6 weeks old) with 150 ± 5g weight were separated randomly into two groups and fed a normal diet (51.93 g carbohydrate, 3.03 g lipid, 20.50 g protein and 4.17 g crude fiber/100 g diet, and 3.00 kcal/g energy) for 1 week. The body weight and biochemical indices (blood sugar, cholesterol, triglyceride, low-density lipoprotein [LDL], and high-density lipoprotein) were measured before the beginning of the HFD. The conditions under which the animals were kept are as follows: 20°C–24°C, 12-h light cycle, and 70% of humidity. The study was approved by the University Ethical Committee for animal studies. At the end of the 1st week, the diet of one group (n = 8) was changed to a high-fat high-carbohydrate diet formulated by us containing high-calorie meals such as ruminator's fat and carbohydrates (5.3 kcal/g, 30% and 70% of total calories from fat and carbohydrate, respectively) as well as additional 30% refined coarse fructose in their drinking water [Table 1]. This diet was provided to the animals for 10 weeks continuously.

The body weight and serum indices of both groups were assessed weekly. Blood samples were collected after anesthetizing the rats using ether and relevant indices were measured using their blood serum. Blood glucose level was measured using a glucometer (EmpEror, ISOTECH CO., LTD), and serum insulin level (Mercodia Rat Insulin ELISA kit Cat#10-1250-01), triglyceride, total cholesterol, and LDL (Crystal Chem Rat LDL-Cholesterol Assay Kit cat# 80096, USA) were measured.

STZ 30 mg/kg prepared in 0.1 M citrate buffer (pH 4.4) was injected in two steps with a 1-week interval to provide stable pancreatic cell injuries. The animals were continued with the high-fat high-carbohydrate diet during the post-STZ administration period. Fasting blood glucose (FBG) and non-FBG of rats were daily measured using a glucometer to indicate the stability and effectiveness of the diabetes induction.

The animals received a solution of 2% glucose after 12-h fasting. The blood glucose was measured at the 0, 30, 60, 90, and 120 min time intervals after receiving the glucose solution.

Data are represented as mean ± standard deviation from the mean. The differences between the groups were analyzed using the Student's t-test or one-way analysis of variance and by *post hoc* multiple comparisons. Analytical analysis was performed using the SPSS software (version 18.0; SPSS, Chicago, IL, USA), $P \leq 0.05$ was considered statistically significant.

Results

Our results indicate that the rats fed on high-fat high-carbohydrate diet gained 20 g more weight in comparison to the control group. The serum cholesterol and LDL levels did not show a significant increase between two groups, while the triglyceride and fasting blood sugar levels were higher in the HFD group in comparison to the control group. The HFD group also showed a decreased insulin level, although the difference between two groups was not significant [Table 2].

The first 30 mg/kg STZ injection did not have any stable effect. The enhanced weight was not significant and the fasting and nonfasting blood sugar returned to the normal level in 5 days after the injection in spite of the continuation of the HFD. The second 30 mg/kg STZ injection was performed 1 week later. After second STZ

Table 1: The nutritional values of control and high-fat high-carbohydrate diet fed animals

Diet components	Control diet	High-fat diet
Energy (kcal/g)	3.00	5.3
Calorie percent		
Protein	22	5
Fat	12	60
Carbohydrate	66	35
Weight percent		
Protein	60	13.8
Fat	24	34
Carbohydrate	6.6	40
Materials	Standard chow diet	Cholesterol (Merk, Germany), coconut oil, fructose, saturated fat, corn starch, yeast, carbonate calcium

Table 2: Serum indices and weight changes in control and diabetic rats

Parameters tested	Before diet intervention		After diet intervention (10 weeks later)	
	Control (NFD)	HFD	Control (NFD)	HFD
Weight (g)	145.75±1.99	144.75±3.06	191.6±6.51	223.5±3.2*
TC (mmol/l)	1.5±0.13	1.51±0.08	1.53±0.13	1.55±0.09
TG (mmol/l)	1.23±0.16	0.88±0.08	1.18±0.10	2.14±0.29*
LDL (mmol/l)	0.9±0.34	0.9±0.36	0.91±0.33	0.88±3.7
FBG (mmol/l)	4.91±0.28	5.05±0.17	5.34±0.43	5.83±0.10*
Insulin (µg/l)	1.4±0.1	1.2±0.1	1.3±0.2	0.5±0.03

*Shows significance at <0.05. NFD: None fat diet, HFD: High-fat diet, TC: Total cholesterol, TG: Triglyceride, FBS: Fasting blood sugar, LDL: Low-density lipoprotein

dose, mean fasting blood sugar was 9.5–11 mmol/l and the mean nonfasting blood sugar was ≥19 mmol/l, also the typical signs of human T2D such as polyuria and polydipsia appeared in the high-fat high-carbohydrate diet group.

Oral glucose tolerance test result (9.5 mmol/l) show an immediate increase in the blood glucose level in both case and control groups 30 min postglucose ingestion [Figure 1]. Blood sugar level decreased in the next 60, 90, and 120 min time intervals in the control group, while it increased in the HFD group (21 mmol/l). Besides, the HFD group indicated a glucose tolerance defect that could be a sign of disability in absorbance and response to glucose (another typical sign of T2D).

Discussion

The model of high-fat feeding to C57BL/6 mice was first described in 1988.[17] High-fat feeding can lead to obesity, hyperinsulinemia, and altered glucose homeostasis due to insufficient compensation by the beta cells of the pancreatic islets.[18] Since obesity is induced by environmental manipulation rather than genes, it is thought to model the

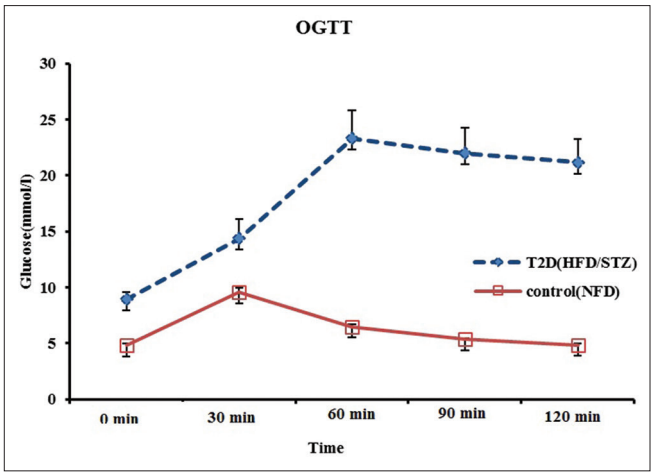

Figure 1: Oral glucose tolerance test in none fat diet and high-fat diet/streptozotocin rats. The amounts have been indicated as mean ± standard error of the mean, *n* = 8 for each group, *P* ≤ 0.05 is considered statistically significant

human situation more accurately than the genetic models of obesity-induced diabetes. Using diet manipulation strategy, we developed an ideal model of human T2D which could replicate the typical steps of insulin resistance, prediabetes, and diabetes. It has been estimated that up to 70% people with prediabetic condition will eventually become diabetes, and that they also are at a higher risk of developing cardiovascular disease as well as other diabetic complications.[19] Therefore, prediabetes would be a critical point in T2DM development, and hence in our work we paid special attention to observe the occurrence of this phase in our T2DM animal model development, and this would be regarded as one of the prominent features of our work in comparison to the other studies.

T2D is a complicated, heterogenic, and polygenic disease because of the fact that several factors are involved in its development such as insulin resistance by target organs and decreased insulin production from pancreatic beta cells.[3,16-19] Accordingly, an animal model should have the same characteristics and mimic the pathogenesis and clinical features of human T2D.

Several studies have reported that a HFD for 2–7 weeks would induce stable insulin resistance[20] and the HFD in combination with low doses of STZ induces diabetes in rats.[12-14,21] Here, in our study, despite the lack of pork fat (which is known as the essential factor for blood lipid profile enhancement and insulin resistance induction) and use of ruminator's fat as the fat resource, we observed a significant increase in the blood glucose and triglyceride levels as well as a reduced insulin level after 10 weeks.

Since there were different doses and number of STZ injections reported for the development of rat T2D model, we tried to find the best protocol and the most similar model to the human disease. As an example, Reed *et al*. used 50 mg/kg STZ for inducing T2D,[14] while

Srinivasan *et al.* used younger rats and single 35 mg/kg interperitoneal injection.[13] On the other side, the period of HFD usage was also variable. While the usual protocol used the HFD for 2–4 weeks before injection,[14] Zang *et al.* used the diet for 2 months.[3] Based on our results, 10 weks HFD decreases the insulin secretion by pancreatic cells as well as the induction of insulin resistance by target tissues.

The fructose-enriched diet decreases blood leptin and insulin (energy homeostasis hormones) and results in weight gain and its metabolic consequences. The fructose rather triggers lipid accumulations in the liver.[22] Fructose induces insulin resistance, glucose tolerance defect, hyperinsulinemia, triglyceride and blood pressure enhancement, weight gain, and T2D.[16,23] Rats fed fructose for 4 weeks showed decreased insulin sensitivity and defective insulin function, while receiving glucose for 7 days made no alteration in the insulin function.[24] Moreover, a diet that includes 10% fructose and 40 mg/kg STZ could be an alternative method for inducing T2D in a short time.[12] In the present study, we used fructose as the carbohydrate resource of the HFD and as an inducer of the diabetes. Our study indicates that the combination of HFD and fructose for 10 weeks is effective for prediabetes induction, and in combination with low doses of STZ is effective for T2D.

Since the development of transgene T2D animal models is technically complicated, expensive, and time taking and international restrictions prevent easy accession to such models from other countries, the diet manipulation would be an ideal alternative. Since the protocols presented in the literature for stable diet-induced T2DM animal models are heterogeneous with no consensus existing for a standard procedure, conducting studies in this field to reach a common procedure for T2DM animal model production is of intensive value. Accession to an easy, cost-effective, and accurate animal model would facilitate more studies on the physiopathological aspects of the disease as well as new therapeutic strategies in T2D.

Conclusions

The present study demonstrates the ability to make diet-induced prediabetes and diabetes models induced by multiple low doses of STZ domestically. Moreover, we produced an animal model of T2D that is more similar to the human disease than the single-gene and obese rat models.

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Conflicts of interest

There are no conflicts of interest.

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