The use of High-Fat/Carbohydrate Diet-Fed and Streptozotocin-Treated Mice as a Suitable Animal Model of Type 2 Diabetes Mellitus

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Summary

This study defined a mouse model of type 2 diabetes that closely simulated the development and metabolic abnormalities of the human disease. Male C57BL/6J mice were fed with diet enriched in fat and simple carbohydrate for 6 weeks and then injected with streptozotocin (STZ, 150 mg/kg intraperitoneally) to develop type 2 diabetes. High-fat/carbohydrate-fed mice showed similar blood glucose concentrations to chow-fed mice, but higher insulin concentrations ($P<0.01$). Hyperglycemia (17.6±3.27 mmol/L) was observed in these mice after STZ injection, and the insulin concentrations decreased to the level comparable to, or still higher than, the normal. The model mice showed impaired glucose tolerance in the oral glucose tolerance test (OGTT), and insulin resistance in the insulin tolerance test (ITT). Moreover, these animals had lower glycogen storage ($P<0.001$), higher serum free fatty acid ($P<0.001$), and higher triglycerides ($P<0.05$) levels compared with control mice. Furthermore, the model mice were sensitive to the glucose lowering effect of metformin. In conclusion, this mouse model could be considered as one of the suitable animal models for type 2 diabetes mellitus, and hence can reasonably be used for type 2 diabetes pathophysiological research and therapeutic-compound evaluation.

Introduction

Type 2 diabetic patients show complex syndromes with insulin resistance, increased hepatic glucose production, and pancreatic ß-cell function defect. Although precisely how the three factors interact to produce the overt diabetes is unknown, it is believed the final way to type 2 diabetes is the failure of the pancreatic ß-cells to compensate for insulin resistance. However, hyperglycemia in type 2 diabetic patients is not associated with absolute hypoinsulinemia. In fact, circulating insulin concentrations in these patients are comparable to the values seen in normal persons (*Cavaghan et al., 2000; Reaven et al., 1993; Thomas, 2003*).

The ideal pathological animal models should simulate the developing process and metabolic characteristics of human type 2 diabetes. Whereas some commonly used type 2 diabetic models were developed through genetic disruption, these have less relevance with the human diabetic pathological process (*Asakawa et al., 2003; Cheng et al., 1996; Houseknecht & Portocarero, 1998*). Moreover, feeding of these gene-mutational animals requires a strict environment and is costly. The high-fat, high-simple carbohydrate diet-fed C57BL/6J mice model, which was developed by Surwit and coworkers, closely simulated the human diabetes developing process. This model was developed through exposing mice to the high-fat, high-simple carbohydrate diet for several months. The model mice showed significant hyperinsulinemia resulting from the diet and the hyperglycemia emerged when the insulin secretion compensation could not maintain the normoglycemic state (*Surwit et al., 1988*). However, it took much time before overt diabetes appeared because of the long-term maintaining of

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insulin secretion compensation (*Luo et al., 1998; Storlien et al., 1993*).

The diet-induced and streptozotocin (STZ)-treated mice is another important and relevant type 2 diabetes pathological model. It was obtained by inducing insulin resistance and hyperinsulinemia with a specially devised diet, and impairing the pancreatic ß-cells with the appropriate dose of STZ, to give overt type 2 diabetes (*Luo et al., 1998*). This model is more cost-effective and timesaving. Based on the metabolic characteristics of type 2 diabetic patients, and previous work of others, we improved and characterized this kind of type 2 diabetes mouse model. In our work, we devised an improved diet, which is effective and easier to induce insulin resistance. The mouse model, which was successfully established exhibited typical diabetic symptoms found in patients, e.g. hyperglycemia, dyslipidemia, impaired glucose tolerance, decreased glycogen storage, and insulin resistance. The model mice showed overt type 2 diabetes symptoms within 8 weeks. In this study, we aimed to develop and characterize this kind of type 2 diabetes mouse model for pathophysiology research and new treatment development, and we illustrated its validity by metformin administration.

Materials and Methods

Diet

The regular diet (standard rats/mice chow, Slaccas, Shanghai) consisted of 21% protein, 4.0% fat, 5.0% fibre, 8.0% ash, and 47% complex carbohydrate.

The diet developed to induce insulin resistance is characterized by high fat and high simple carbohydrate content and was made by mixing the pulverized regular chow with 2.5% glucose, 21.3% corn oil, and 0.3% cholesterol and pelleting. The pellets were baked at 55 °C for 7 hours before use.

Animal care and a sample collection

Male C57BL/6J mice from the Shanghai Experimental Animal Center of the Chinese Academy of Sciences were obtained at 4 weeks of age and were originally housed five per cage in a temperature (23±2 °C) and humidity (60%±10%)-controlled room under a 12 h light (0600-1800) and 12 h dark (1800-0600) cycle. Diets and tap water were provided *ad libitum*. Sterilized bedding was provided by Shanghai SLAC laboratory animal Co. Ltd. Cages were changed once a week. All animals were fed the regular diet before the start of an experiment.

Three days after arrival, the animals were divided into two groups and were fed either the high-fat/carbohydrate diet or the regular diet described above. After exposure to the respective diet for 6 weeks, the high-fat/carbohydrate-fed mice and a subset of chow-fed mice were fasted for 6 hours and injected intraperitoneally with STZ (Sigma, St Lours, MO) 150 mg/kg body weight. The STZ was dissolved in 0.1mol/L citric acid buffer (pH=4.5), sterile filtrated before use and the STZ solution kept on ice during the experiment. All the animals were continued on their original diets for the duration of the study. For the sake of convenience of observation, the animals injected with STZ were housed separately. The cages of these animals were changed every other two days. At the 10th day after the STZ injection, the mice were fasted overnight, and blood was drawn from the tail vein. The serum was used for the measurement, as below, of fasting insulin and fasting blood glucose. After the serum insulin and glucose concentrations had been determined, the hyperglycemic animals without hypoinsulinemia were selected from the mice given the high-fat/carbohydrate diet and STZ-injection. This group of animals were regarded as model mice and were subjected to further research described as below. The chow-fed mice without STZ-injection were designated as controls. The body weights of animals were recorded weekly by an electroblance. At the end of the experiment, various tissues and serum samples were obtained from these mice and stored at –70 °C for determining hepatic glycogen, free fatty acid, and serum triglycerides.

Metformin treatment

A subset of model mice were divided into the met-

formin group (n=8) and the diabetes group (n=8) according to their serum glucose concentrations. In the metformin group, mice were administered metformin (Hengshan, Shanghai) by gastric gavage at a dose of 300 mg/kg per day for 9 days. The diabetes group and the control group were administered with vehicle (water). Before the first dose and after the final dose, blood samples of 6-h fasted mice were collected from the tail vein and the blood glucose concentrations were determined.

The oral glucose tolerance test (OGTT)

The mice to be tested were fasted for 24 hr and then given 20% D-glucose (AMRESCO) solution at a dose of 2 g/kg body weight by gavage. Blood samples were collected before (time 0) and 15, 30, 60, and 120 min after glucose administration from the tail vein. Following the glucose tolerance test, the animals were returned to their cages.

The insulin tolerance test (ITT)

For the insulin tolerance test (ITT), the mice were fasted for 5 hr and the given 0.75 U/kg body weight insulin (Sigma, St Lours, MO) by intraperitoneal administration. The blood glucose concentrations were measured at 0 (baseline), 15, 30, 60, 90, and 120 min after injection.

Biochemical assays

The insulin concentrations were determined via competitive radioimmunoassay according to the protocol of the kit (Shanghai immunoassay Technology Company.). During the OGTT and the ITT, blood glucose concentrations were determined with a Glucometer (ONE TOUCH II, Jonson). The serum glucose concentrations mentioned above were determined by the glucose oxidase method. Serum triglycerides were determined using a kit from Roche. Serum free fatty acid (FFA) was measured using a nonesterified fatty acid kit (Jiancheng biology, Nanjing). The hepatic glycogen content was measured by the glycogen kit (Jiancheng biology, Nanjing).

Statistics

All results were expressed as means±SD. The data were analyzed by Student's *t*-test. A P-value of < 0.05 was considered to be statistically significant.

Results

Effect of STZ injection in chow-fed and high-fat/carbohydrate-fed mice

We injected a group of chow-fed mice and a group of high-fat/carbohydrate mice with the same dose of STZ. The fasting serum glucose and fasting insulin concentrations were determined before and after the STZ injection (table 1). Before the STZ injection, the insulin level of the high-fat/carbohydrate-fed mice elevated significantly compared with chow-fed group ($P<0.01$) whereas the serum glucose levels of the two groups were similar. In response to STZ treatment, the serum insulin concentrations decreased and the blood glucose levels increased in both groups. However, the serum insulin concentration was higher in the high-fat/carbohydrate-fed group after the STZ injection com-

Table 1. Effect of STZ injection in high-fat/carbohydrate-fed mice and chow-fed mice.

Group	High-fat/carbohydrate-fed		Chow-fed	
	Pre-STZ	Post-STZ	Pre-STZ	Post-STZ
Insulin/μIU·mL^{-1}	25.2±6.21 [b]	14.6±1.32 [b]	12.3±3.37	8.0±2.45
Glucose/mmol·L^{-1}	8.8±1.13	20.3±4.00	5.8±1.45	26.1±5.72

[b] $P<0.01$ High-fat/carbohydrate-fed group vs. chow-fed group.
Data are illustrated as Mean±SD.
Number of mice in each group = 20

pared with the other STZ treated group (P<0.01). Furthermore, the serum insulin concentration after STZ injection in the high-fat/carbohydrate-fed group was similar to the value in the chow-fed group before STZ treatment. Meanwhile the chow-fed mice demonstrated some characteristics of type 1 diabetes after the STZ injection, such as low insulin level and hyperglycemia.

Body weight and epididymal white adipose tissue weights

At the beginning of the experiment, the body weight was 14.0±0.7 g in the group of mice which were then switched to the high-fat/carbohydrate diet and 14.1±0.9 g in the group maintained on the regular diet. In the 6- week insulin resistance developing period before STZ injection, the body weight increase in the high-fat/carbohydrate-fed mice was more rapid (the higher slope in the profiles) and larger than that in the control mice (Figure 1). The weight of epididymal white adipose tissue was higher in the model group than in the control group (P<0.01) (Table 2) and showed more severe visceral fat.

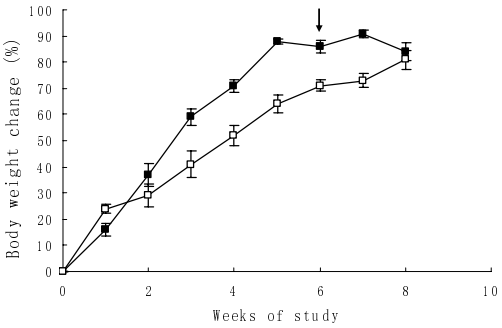

Figure 1. The body weight changes of the high-fat/carbohydrate-fed / STZ-injected mice and the control mice in the 8 weeks of the experiment. The values of both groups at the beginning of the experiment were indicated as 0 percent. ■ represents high-fat/carbohydrate-fed / STZ-injected group and □ represents control group. Arrow showed the moment of STZ injection in the high-fat/carbohydrate-fed mice. Data represent mean±SD. n=40 for each group.

Fasting blood glucose, serum insulin, FFA, and triglycerides levels

The fasting blood glucose concentrations in the model mice were higher than in control mice (P<0.001), and the model mice showed overt hyperglycemia. Fasting serum insulin concentrations in model mice were higher than in control animals, where no difference was observed. Serum FFA levels of diabetes mice were higher than that of control mice under fasting condition (P<0.001). Serum triglycerides levels in the model mice were higher than in the control mice (P<0.05) (Table 2).

Table 2. Fasting serum insulin, fasting blood glucose, FFA, triglycerides, hepatic glycogen, and epididymal WAT in C57BL/6J mice

Mice	Model	Control
Insulin/μIU·mL^{-1}	13.7±1.32	11.5±0.97
Fasting blood glucose/mmol·L^{-1}	17.6±3.27 [c]	6.2±0.42
FFA/μmol·L^{-1}	2778.5±351.6 [c]	844.7±251.1
Triglyceride/mmol·L^{-1}	0.66±0.08 [a]	0.46±0.07
Hepatic glycogen/mg·g^{-1} liver tissue	9.54±3.57 [c]	39.06±4.39
epididymal WAT/g	1.01±0.22 [b]	0.30±0.07

[a] P<0.05 model group vs. control group, [b] P<0.01 model group vs. control group, [c] P<0.001 model group vs. control group, Data are illustrated as Mean±SD. Number of mice in each group = 25

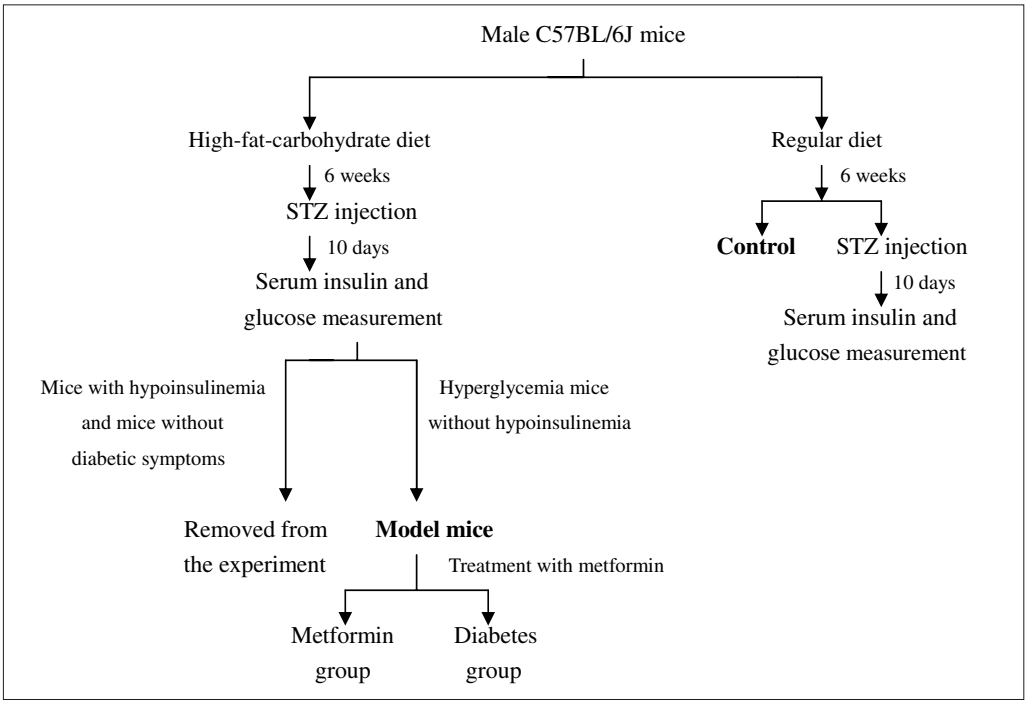

Figure 1 a. Treaments of mice in the experiment.

The oral glucose tolerance test (OGTT)

A subset (n=8) of model mice and a subset (n=7) of control mice were selected for the oral glucose tolerance test (OGTT) (Figure. 2). Blood glucose concentrations at 0 min were significantly higher in the model group compared with the control ($P<0.001$). When challenged with an oral glucose load, all of the animals showed marked rises in blood glucose concentrations, but the model mice cleared the glucose from blood less efficiently than control mice. There was a difference in blood glucose concentrations between these two groups at 120min after glucose administration ($P<0.001$). In addition, a difference was observed in blood glucose concentrations between 120min and 0min ($P<0.05$) in the model group, whereas there was no such observation in the control group.

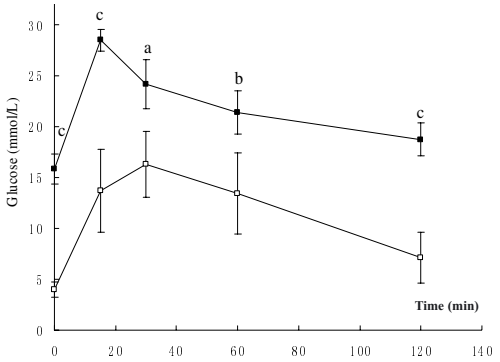

Figure 2. The oral glucose tolerance test (OGTT) in model group (n=8) and control group (n=7). After 24-h fasting, an oral glucose load (2 g/kg body weight) was given to animals. The blood glucose concentrations of both groups were determined at 0, 15, 30, 60, and 120 min. ■represents model group and □represents control group. Data are illustrated as mean±SD. [a] $P<0.05$, [b] $P<0.01$, [c] $P<0.001$ model group vs. control group.

The insulin tolerance test (ITT)

To estimate the insulin resistance of the type 2 diabetes model mice, the insulin tolerance test (ITT) was carried out on 5-h fasted mice (Figure 3). The blood glucose concentrations at 0min were higher in the model mice (n=8) than in the control mice (n=8) (19.6±4.1 vs. 7.9±0.25 mmol/L, P<0.01). After the insulin injection, the immediate decrease of blood glucose concentrations in the control mice showed a sensitive response to the administered insulin. In the model mice, however, the decrease of the blood glucose showed a distinct delay. The model and their blood glucose level did not recover as well as in the control mice.

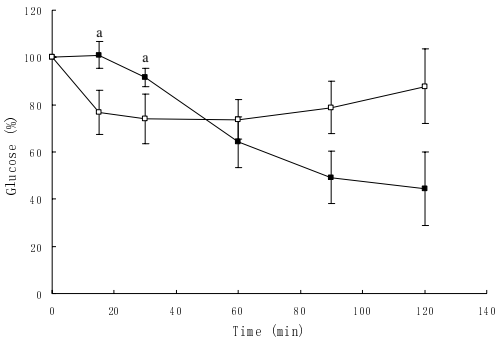

Figure 3. The insulin tolerance test (ITT) in model group (n=8) and control group (n=8). Insulin (0.75 U/kg body weight) was administered to the 5-h fasted mice and the blood glucose concentrations were determined at 0 (baseline), 15, 30, 60, 90, and 120 min. The blood glucose level was expressed as the ratio of glucose concentration at each time point to the baseline glucose, and the baseline was designated 100 percent. ■ represents model group and □ represents control group. Data are illustrated as mean±SD. [a] P<0.05 model group vs. control group.

Determination of hepatic glycogen

In the ITT curve, the model mice did not show the blood glucose recovery like the control animals. It might relate to the impaired hepatic glycogen storage of the type 2 diabetic mice. Hence the hepatic glycogen content was determined to confirm this hypothesis. The results showed that the glycogen content in the model group was lower than in the control group (P<0.001). (Table 2.)

Metformin treatment

After the 9 days' treatment of metformin, the hyperglycemia in the diabetes mice was greatly improved. There was no difference in blood glucose concentrations between the metformin treated group and the control group at the end of the treatment. (Table 3).

Table 3. Effect of Metformin treatment on blood glucose in C57BL/6J mice.

	Glucose/mmol·L^{-1}	
Group	Pre	Post
Model	23.0±5.4 [c]	20.6±4.2 [b]
Metformin	23.2±4.1 [d]	8.6±7.0
Control	6.1±3.8	6.9±1.5

[b] P<0.01 model group vs. control group
[c] P<0.001 model group vs. control group
[d] P<0.001 metformin group vs. control group
Data are illustrated as mean±SD.
Number of mice in each group = 8

Discussion

In this study, we developed the high-fat/carbohydrate diet-fed and STZ-injected mouse as a type 2 diabetes model for research and drug discovery. This mouse model showed type 2 diabetic syndrome such as hyperglycemia, dyslipidemia, impaired glucose tolerance, decreased glycogen storage, and insulin resistance.

The prediabetic state is characterized by insulin resistance and compensatory increase in insulin secretion of the pancreatic ß-cells to maintain normoglycaemia. The frank hyperglycemia and overt type 2 diabetes appear when the ß-cell dysfunction occurs and the capacity of insulin secretion is no longer able to compensate for the insulin resistance (*Gerich, 2002*). It is believed that insulin resistance and concomitant ß-cell dysfunction are two typical

features to discriminate type 2 diabetes.

Both the genotype and the environmental factors such as diet and physical inactivity are responsible for the observed insulin resistance (*Saad et al., 1991*). In this work, the C57BL/6J mouse was chosen to develop the diabetes model due to their genetic predisposition to obesity and insulin resistance when fed with a high-fat diet (*Surwit et al., 1991; Winters et al., 2003*). With the purpose of effectively inducing insulin resistance, a high-fat/carbohydrate diet was developed. Considering relevant factors of diet in the type 2 diabetic pathophysiology, the high content of fat, simple carbohydrate and cholesterol were combined together to develop a diet that could effectively induce insulin resistance in the C57BL/6J mice. Because the animal oil used by previous authors causes difficulties in the diet making process, we corn oil as the source of fat. Corn oil is easier to obtain and works better in the insulin resistance inducing diet. In our observation, the high-fat/carbohydrate diet caused weight gain and fat accumulation in obesity tissues in mice. This was confirmed by the facts that all the model mice had heavier epididymal fat pad, which indicated the visceral fat, compared with the control mice. The results in Table 1 shows that the serum insulin concentrations increased significantly in the mice exposed to the high-fat/carbohydrate diet compared with the chow-fed mice ($P<0.01$). In the absence of decreased blood glucose level, markedly elevated insulin concentrations provides presumptive evidence for the decreased insulin-mediated glucose disposal and the presence of insulin resistance. This conclusion was further supported by the insulin tolerance test.

Conversion of prediabetes to frank hyperglycemia is associated with a decline in the secretory capacity of the pancreatic ß-cells, and there is extensive evidence that ß-cell function is impaired in type 2 diabetes (*Cavaghan et al., 2000; Kahn, 2001*). However, it should be noticed that the serum insulin concentrations in type 2 diabetic patients are comparable to the values seen in normal persons; that is, the impairment of insulin secretion in type 2 diabetes is not absolute but relative. Although not reduced compared with lean normoglycemic individuals, the ß-cell mass and the insulin level in type 2 diabetic patients are reduced when compared with obese, insulin resistant, normoglycemic individuals (*Thomas, 2003*). Based on these facts, an attempt was carried out to simulate this pathophysiological process. The high-fat/carbohydrate-diet fed, hyperinsulinemic mice were treated with a dose of STZ that lowered the serum insulin concentration to a level comparable to normal. From Table 1, a noticeable result was that the high-fat/carbohydrate-fed mice showed hyperglycemia after the STZ injection. However, the serum insulin concentration in these mice after STZ treatment was higher than that in the chow-fed group before STZ injection.

Streptozotocin (STZ) is a pancreatic ß-cell selective toxin that can induce diabetes in most laboratory animals. Determination of the appropriate STZ dose should be emphasized in the model developing process. The proper dose may cause partial damage of the *ß*-cell mass and produce the mild insulin deficient state required in this work (*Portha et al., 1989*), whereas an inappropriate dose may lead to gradual recovery or to deterioration into type 1 diabetes mellitus (*Arulmozhi et al., 2004*). In our previous work, we had employed different STZ doses in several groups of diet treated mice. In the group dosed at 150 mg/kg body weight, most of the mice showed hyperglycemia while the serum insulin concentration was higher than, or similar to, the normal. In the group treated with the higher dose, the mice showed hypoinsulinemia which indicated excessive damage of the ß-cells, whereas in the lower dose groups, the mice did not show hyperglycemia after STZ injection (the data are not listed here). Based on these results, 150 mg/kg body weight was determined as the optimal STZ dose to stimulate the partial insulin secretion deficiency in the diet-induced insulin resistance in mice.

The ITT was employed to estimate the insulin resistance in the model mice. After insulin administration, blood glucose levels of both model and control groups were decreased, whereas the blood glu-

cose decline in the model mice was delayed markedly. In the ITT curve, we found that the model group did not have the glucose level recovery like the control. We believed that it was only partly due to the action of insulin since the model mice showed a delayed response after insulin administration, which implicated the decreased insulin sensitivity. It showed the abnormality in the blood glucose regulation which might relate to the rapid depletion of hepatic glycogen in the model mice. Moreover, the decrease of hepatic glycogen content is also the general pathological characteristic in type 2 diabetes individuals (*Klover & Mooney, 2004*). To confirm this hypothesis, we determined the hepatic glycogen contents of mice in both model and control group under nonfasting condition. Table 2 showed that in the model mice, the hepatic glycogen contents were significantly lower than that in the control mice.

Metformin is widely used as an oral glucose-lowering drug to treat type 2 diabetes. It is accepted as an insulin-sensitizing drug that could alleviate hyperglycemia by reducing hepatic glucose production and enhancing peripheral glucose uptake (*Bailey & Turner, 1996; Stumvoll et al., 1995*). In this study, metformin was proved to work efficiently in improving hyperglycemia in the model mice. The results showed that this type 2 diabetes mice model is suitable for the evaluation of anti-diabetes treatments.

In conclusion, the mice model we developed in this study was able to simulate some metabolic abnormalities appearing in human type 2 diabetes. We employed a diet high in fat and carbohydrate to induce insulin resistance, the appropriate dose of STZ to impair insulin secretory capability. The mice model also showed impaired glucose tolerance, reduced glycogen storage, and elevated free fatty acid and triglycerides levels. All these type 2 diabetes symptoms were observed within a period of 8 weeks. This model was further evaluated by metformin administration. The results suggested that this type 2 diabetes mice model could be utilized for new drug and treatment development.

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