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Stability of a type 2 diabetes rat model induced by high-fat diet feeding with low-dose streptozotocin injection

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Abstract: Objective: The present study aims at determining the stability of a popular type 2 diabetes rat model induced by a high-fat diet combined with a low-dose streptozotocin injection. Methods: Wistar rats were fed with a high-fat diet for 8 weeks followed by a one-time injection of 25 or 35 mg/kg streptozotocin to induce type 2 diabetes. Then the diabetic rats were fed with regular diet/high-fat diet for 4 weeks. Changes in biochemical parameters were monitored during the 4 weeks. Results: All the rats developed more severe dyslipidemia and hepatic dysfunction after streptozotocin injection. The features of 35 mg/kg streptozotocin rats more resembled type 1 diabetes with decreased body weight and blood insulin. Rats with 25 mg/kg streptozotocin followed by normal diet feeding showed normalized blood glucose level and pancreatic structure, indicating that normal diet might help recovery from certain symptoms of type 2 diabetes. In comparison, diabetic rats fed with high-fat diet presented decreased but relatively stable blood glucose level, and this was significantly higher than that of the control group (P<0.05). Conclusions: This model easily recovers with normal diet feeding. A high-fat diet is suggested as the background diet in future pharmacological studies using this model.

Key words: High-fat diet; Stability; Streptozotocin; Type 2 diabetes mellitus
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1 Introduction

Type 2 diabetes mellitus (T2DM) is an increasingly common disorder characterized by chronic hyperglycemia and marked dyslipidemia (Vinson and Zhang, 2005; Bhattacharya et al., 2007). The World Health Organization (WHO) estimates that the diabetic population will increase to 360 million people by 2030, which is 4.5% of the global population (Rathmann and Giani, 2004; Shaw et al., 2010). T2DM manifests itself by a progressive decline in insulin action (insulin resistance) in the early stage, followed by the dysfunction of β-cells to properly secrete insulin to compensate for insulin resistance (Srinivasan et al., 2005).

Animal models are useful tools for investigating mechanisms and pharmacological therapies for T2DM. Although a surplus of animal models (spontaneous as well as induced) is available for the study of T2DM, the initiation and development of the disease in these models do not accurately mimic the clinical situation in humans (Schnedl et al., 1994; Luo et al., 1998; Shafrir, 2003). T2DM in humans is usually a consequence of multiple gene polymorphisms in combination with environmental factors, such as surplus energy intake and sedentary lifestyle. A new T2DM model has become increasingly popular in recent years, and this is developed by high-fat diet (HFD)

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feeding to induce insulin resistance, followed by low-dose streptozotocin (STZ) injection to cause mild dysfunction in β-cells without completely compromising insulin secretion. This model closely mimics the natural development of the T2DM (from insulin resistance to β-cell dysfunction) as well as its metabolic features, and has been used for both investigating the mechanisms involved in T2DM and evaluating potential therapies (Watts et al., 2005; Sahin et al., 2007; Shatwan et al., 2013). Many researchers have tried to determine the high-fat feeding time and the optimal STZ doses to induce the T2DM models (Reed et al., 2000; Srinivasan et al., 2005; Mansor et al., 2013). However, few focus on the stability of the model, as well as the impact of different background diets on the stability of the model. Since it is unreliable to test the therapies on an unstable model, considering the stability of the model is important before testing potential therapies for T2DM. In a study by Reed et al. (2000), the fasting blood glucose (FBG) of diabetic rats fed with HFD dropped from 27.3 to 23.4 mmol/L within only 3 d after STZ injection. The authors doubted the stability of glycemia in the fat-fed/STZ model over the longer term. Other studies lack information about the stability of the model since they only presented the biochemical parameters at the end of the intervention period while no data were presented on the status right after the induction of the model (Wang C et al., 2009; Wang Y et al., 2011). Therefore, the purpose of the present study is to determine the stability of the model induced by HFD feeding combined with a low-dose STZ injection, with the background diet of either HFD or normal diet. Establishing the stability of the model will benefit the application of this model in future pharmacological studies.

2 Materials and methods

2.1 Experimental animals

Thirty male Wistar rats weighing 190–210 g were purchased from the Vital River Laboratory Animal Technology Co., Ltd. (Beijing, China; Certificate SCXK (Beijing) 2016-0001) and housed in a temperature-controlled room at (23±1) °C, with (50±5)% humidity and a 12-h dark/12-h light cycle. All the animals were humanely treated in accordance with the guidelines for animal care of the National Institute of Health (Institute of Laboratory Animal Resources Committee, 1996), and the protocols were approved by the Ethics Committee of the Beijing Key Laboratory of Functional Food from Plant Resources (permit number: A330-5).

2.2 Induction of diabetes in rats

After one-week adaptation period, the rats were randomly divided into two groups: the control group (NG, n=6) and the HFD group (n=24). The compositions of the diets are shown in Table 1. Rats were fed on their corresponding diets for 8 weeks before injection of STZ. At the end of week 8, the HFD-feeding rats were randomly divided into four groups (n=6): (1) 25STZ-ND, 25 mg STZ/kg with normal diet; (2) 25STZ-HFD, 25 mg STZ/kg with HFD; (3) 35STZ-ND, 35 mg STZ/kg with normal diet; (4) 35STZ-HFD, 35 mg STZ/kg with HFD. Before injection, the rats were fasted overnight and given a single intraperitoneal injection of STZ in a citrate buffer (pH 4) the next morning, and the NG rats received only the citrate buffer. The HFD feeding was continued for a further week (or normal diet for controls). Groups 25STZ-ND and 35STZ-ND were fed with normal diet and 25STZ-HFD and 35STZ-HFD continued HFD

Table 1 Compositions of regular diet and HFD

Group	Corn starch (g/100 g)	Whole wheat flour (g/100 g)	Vegetable oil (g/100 g)	Soybean meal (g/100 g)	Casein (g/100 g)	Mountain flour (g/100 g)	Lard (g/100 g)
Regular diet	25.0	24.5	2.0	20.0		1.6	
HFD				6.5	11.0	0.6	23.0

Group	Sodium cholate (g/100 g)	Refined wheat flour (g/100 g)	Fish powder (g/100 g)	Calcium bicarbonate (g/100 g)	Fructose (g/100 g)	Vitamin and mineral mix (g/100 g)	Energy (kcal/g)
Regular diet		21.9	2.0	1.0		2.0	3.3
HFD	0.2	40.0		1.0	15.7	2.0	4.8

HFD: high-fat diet

from week 10 for a period of 4 weeks. At week 13, all the rats were fasted overnight and sacrificed under deep anesthesia. The liver, spleen, and kidney, along with the epididymal, perirenal, and retroperitoneal fat pads were dissected and weighed immediately. The organ indices were calculated as follows: organ indice= organ weight/body weight×100% (Wang et al., 2015). Sections of hepatic tissue and pancreatic tissue were fixed in 10% formalin for histological analysis. During the whole experimental period, rats had ad libitum access to food and drinking water. Body weight was measured weekly, and food and water consumption was monitored daily.

2.3 Biochemical parameters

Blood samples were taken from the orbital vein in 12 h-fasted rats under anesthesia at weeks 8, 9, and 13. The blood samples were kept at 4 °C for 2 h and centrifuged at 3500*g* for 10 min to obtain serum. The concentrations of serum total triglyceride (TG), total cholesterol (TC), low-density lipoprotein cholesterol (LDL-C), high-density lipoprotein cholesterol (HDL-C) and uric acid (UA), and the activity of aspartate transaminase (AST), alanine transaminase (ALT) and lactic dehydrogenase (LDH) were measured using commercial kits (Biosid Biotechnology and Science Inc., Beijing, China) on Alcyon 300 automatic analyzer (Alcyon, USA). The concentrations of malondialdehyde (MDA) and free fatty acid (FFA), and the activity of superoxide dismutase (SOD) of the serum were determined with the kits purchased from Nanjing Jiancheng Bioengineering Inst. (Nanjing, China). Fasting blood insulin (FBI) levels were determined by enzyme-linked immunosorbent assay (ELISA) kits from Beijing Sino-UK Institute of Biological Technology (Beijing, China). FBG was collected from tail and measured with a glucometer (Life Scan Inc., Milpitas, CA, USA). The homeostasis model of assessment for insulin resistance index (HOMA-IR) was calculated as follows (Liu et al., 2014): HOMA-IR=FBG×FBI/22.5.

2.4 Oral glucose tolerance test

Oral glucose tolerance test (OGTT) was measured at week 6. The rats were fasted overnight and received glucose gavage at 2 g/kg body weight. Tail vein blood was collected at 0, 30, 60, 90, and 120 min using a glucometer (Life Scan Inc., Milpitas, CA, USA). Total area under the curve (AUC) of OGTT was calculated using a trapezoidal method.

2.5 Histological analysis

Sections of hepatic tissue and pancreatic tissue were fixed in 10% formalin for 7 d and processed paraffin embedding. The tissues were sliced and stained with hematoxylin and eosin (HE). All sections were observed with a BA-9000L microscope (Osaka, Japan).

2.6 Statistical analysis

All data are presented as the mean±standard error of mean (SEM). The Student's *t*-test was used for analyzing the data between two groups. One-way analysis of variance (ANOVA) followed by Duncan's multiple-comparisons test was performed by SAS v8.2 (SAS Institute Inc., Cary, NC, USA) when there were more than two groups. A difference with $P<0.05$ was considered significant. A difference with $P<0.01$ was considered extremely significant.

3 Results

3.1 Effect of HFD feeding on glucose tolerance and biochemical parameters in rats before injection

As shown in Fig. 1, 6 weeks of HFD feeding resulted in a significant increase in AUC of OGTT, indicating impaired glucose tolerance in rats ($P<0.01$). The FBG showed a slight increase in HFD-fed rats compared to NG rats, but the difference was not statistically significant ($P>0.05$).

Table 2 illustrates that the 8 weeks of HFD feeding resulted in a significant increase in FBG, FBI, as well as HOMA-IR index ($P<0.05$), which suggests insulin resistance in HFD-fed rats. The HFD-fed rats also developed hyperlipidemia as indicated by significantly higher serum TG and FFA levels and lower HDL-C levels compared to NG rats ($P<0.05$). The elevated MDA and UA levels in HFD-fed rats indicated oxidative stress and hyperuricemia ($P<0.05$). The HFD-fed rats also presented dramatically increased activity for ALT and LDH ($P<0.05$), which are indicators of hepatic damage.

3.2 Changes in body weight, food and water consumption before and after injection

As shown in Fig. 2a, the HFD-fed rats dramatically increased in body weight compared to the NG rats after 5 weeks ($P<0.05$). By week 8, the HFD-fed rats gained 54 g more weight than the NG rats and showed characteristics of obesity. However, after

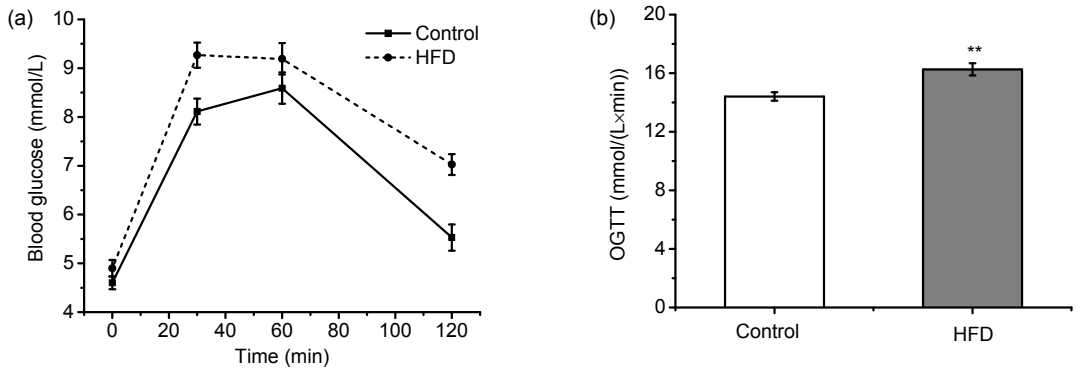

Fig. 1 Effect of HFD on glucose tolerance in rats at week 6

OGTT was conducted on overnight fasted rats at week 6. Blood glucose at 0, 30, 60, and 120 min was measured. (a) Blood glucose levels in OGTT; (b) AUC of OGTT. Values are expressed as mean±SEM (n=6 for control group, n=24 for HFD group). HFD: high-fat diet; OGTT: oral glucose tolerance test; AUC: area under the curve. ** P<0.01, vs. control

Table 2 Effect of HFD on biochemical parameters before injection

Group	FBG (mmol/L)	FBI (mmol/L)	HOMA-IR	TG (mmol/L)	TC (mmol/L)	HDL-C (mmol/L)	LDL-C (mmol/L)
Control	4.19±0.15	22.91±1.91	4.23±0.32	0.83±0.14	1.68±0.15	1.51±0.08	0.94±0.04
HFD-fed rats	4.86±0.08**	29.40±1.41*	6.34±0.34**	1.40±0.10**	1.85±0.09	1.26±0.06*	0.88±0.05
Group	FFA (mmol/L)	MDA (nmol/L)	SOD (U/ml)	UA (μmol/L)	LDH (U/L)	AST (U/L)	ALT (U/L)
Control	4.22±0.27	7.96±0.36	63.50±2.68	168.94±12.88	222.01±18.95	12.40±0.69	2.84±0.24
HFD-fed rats	5.37±0.34*	9.70±0.44*	68.26±2.84	199.09±5.92*	295.35±15.16*	14.49±0.71	5.95±0.61**

Values are expressed as mean±SEM (n=6 for control group, n=24 for HFD group). HFD: high-fat diet; FBG: fasting blood glucose; FBI: fasting blood insulin; HOMA-IR: homeostasis model of assessment for insulin resistence index; TG: total triglyceride; TC: total cholesterol; HDL-C: high-density lipoprotein cholesterol; LDL-C: low-density lipoprotein cholesterol; FFA: free fatty acid; MDA: malondialdehyde; SOD: superoxide dismutase; UA: uric acid; LDH: lactic dehydrogenase; AST: aspartate transaminase; ALT: alanine transaminase. * P<0.05, ** P<0.01, vs. control

STZ injection, the body weights sharply decreased during the first week, then mildly increased in the 25 mg/kg groups and slightly decreased in the 35 mg/kg groups from week 9 (Fig. 2b).

Although the food consumption in model rats was significantly lower than that in the control rats before injection (P<0.01, Fig. 2c), the model group had significantly higher energy intake than the control rats (data not shown). The injection of STZ caused a considerable increase in food intake as well as water consumption (P<0.05, Fig. 2d). More regular diet was consumed than the HFD under the same STZ doses (P<0.05). Consumption of regular diet was significantly higher in the 35STZ group than in the 25STZ group (P<0.05), and this was probably due to greater impairment of glucose utilization and higher energy demand in the 35 mg/kg STZ group (Leedom and Meehan, 1989).

Water consumption increased dramatically after injection. This is also a typical characteristic of T2DM. The 35STZ-ND rats exhibited as high as three times the water consumption of the control rats, and also significantly higher water consumption than the 35STZ-HFD rats (P<0.05, Fig. 2f). A similar trend was found in food intake (Fig. 2d).

3.3 Changes in FBG and FBI levels after injection

As shown in Table 3, the STZ injection caused a dramatic increase in FBG. Three-fold and 4-fold increases in FBG were observed in the 25 mg/kg STZ and 35 mg/kg STZ groups, respectively, compared to the control group (P<0.05). Surprisingly, after 4 weeks of normal diet feeding, both the 25STZ-ND and 35STZ-ND groups showed dramatically normalized FBG, in which the 25STZ-ND rats presented no difference from the control rats (P>0.05). The

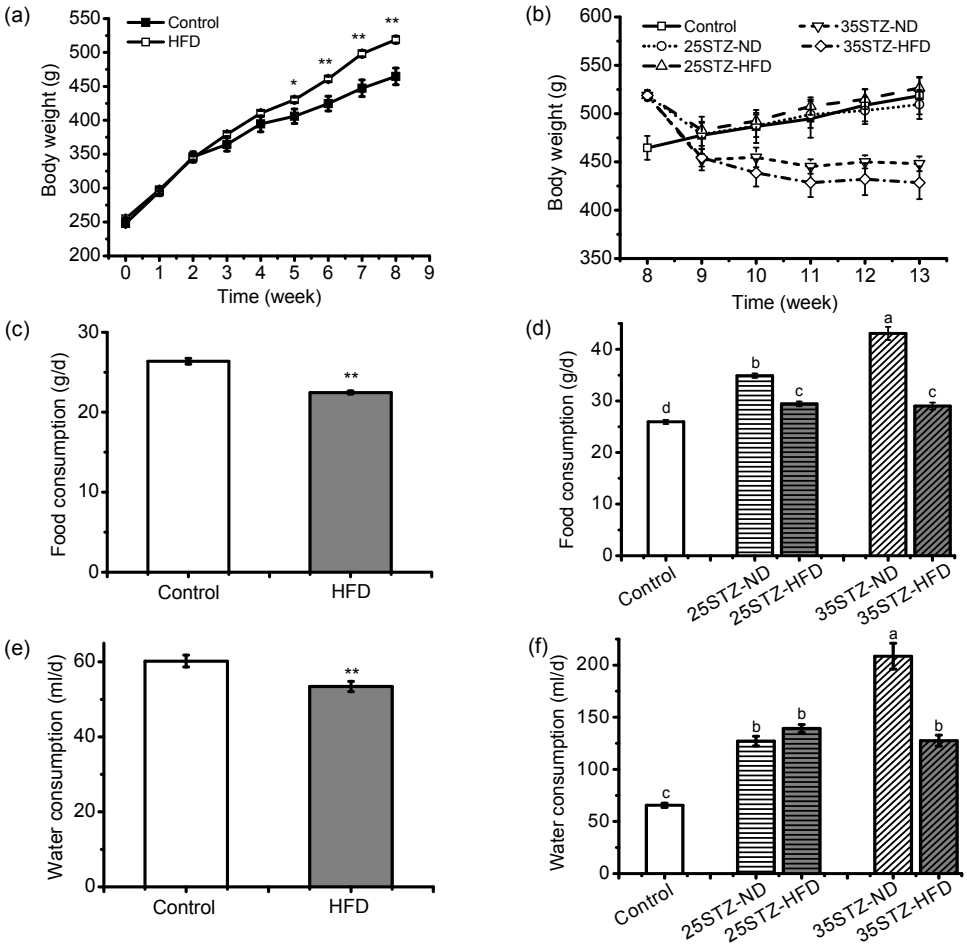

Fig. 2 Changes in body weight, food intake, and water consumption before and after STZ injection

Body weight (a), food intake (c), and water consumption (e) before STZ injection (n=6 for control group, n=24 for HFD group). Body weight (b), food intake (d), and water consumption (f) after STZ injection (n=6 for each group). Values are expressed as mean±SEM. 25STZ-ND: 25 mg/kg STZ with normal diet; 25STZ-HFD: 25 mg/kg STZ with high-fat diet; 35STZ-ND: 35 mg/kg STZ with normal diet; 35STZ-HFD: 35 mg/kg STZ with high-fat diet. Mean values with different letters are significantly different (P<0.05). * P<0.05, ** P<0.01, vs. control

FBG also experienced significant reductions with continuing HFD feeding (P<0.05). However, they are still significantly higher than the regular diet feeding groups (P<0.05), indicating a relatively stable model with HFD feeding.

As indicated in Table 3, STZ injection only slightly decreased in FBI in the 25 mg/kg groups (compared with the data from Table 2), and this was not different from the control group (P>0.05). By comparison, the dose of 35 mg/kg induced a more significant decline in FBI level than the control (P<0.05). The FBI changed little during the following 4 weeks of either HFD or regular diet feeding. Only the 25STZ-ND rats showed significantly higher FBI compared with the control group at week 13 (P<0.05).

3.4 Changes in serum lipid profiles after injection

Compared with the parameters at week 9, a significant increase in TG levels was observed in HFD feeding groups at week 13 (Table 3, P<0.01). The TC levels were also very elevated in all diabetic groups (P<0.05), among which 25STZ-HFD and 35STZ-HFD groups showed as high as 2- and 3-fold higher TC levels compared with those at week 9, respectively (P<0.01). HDL-C levels were also increased dramatically in all diabetic groups (P<0.01). The groups with 35 mg/kg STZ showed significantly higher HDL-C levels than the control group (P<0.05). The LDL-C levels did not differ among any groups at week 9. After 4 weeks of HFD/regular diet feeding,

the 25STZ-ND rats showed significantly reduced LDL-C level (P<0.05). In comparison, dramatically increased LDL-C levels were observed in the 35STZ-HFD rats (P<0.01). Similar results were found in FFA levels. Only 35STZ-HFD rats showed dramatically increased FFA level (P<0.01).

3.5 Changes in serum oxidative status and uric acid level after injection

MDA levels and activity of SOD are both indicators of oxidative stress. No significant changes in MDA levels were observed among all these groups during the 4 weeks of HFD/regular diet feeding (Table 3, P>0.05). There were similar results in the activity of SOD and UA levels (Table 3, P>0.05).

3.6 Changes in hepatic function after injection

As indicated in Table 3, the activity of LDH and ALT was dramatically increased in the diabetic rats at week 9 (P<0.05). Compared to 25 mg/kg STZ, 35 mg/kg STZ injection caused significantly higher LDH activity (P<0.05). The activity of LDH changed little during the 4 weeks of HFD/regular diet feeding. In comparison, very increased activity of ALT was observed after 4 weeks of HFD/regular diet feeding. Furthermore, the 35STZ-HFD rats showed almost as high as a 4-fold increase in ALT activity (P<0.01), indicating that HFD feeding tended to induce a greater deterioration in hepatic function.

3.7 Organ indices

As shown in Fig. 3, the diabetic rats showed significantly higher liver indices compared with the control rats (P<0.05). STZ with HFD remarkably increased liver indices compared with normal diet (P<0.05). The 35STZ-ND rats presented significantly higher liver indices than the 25STZ-ND rats, indicating the possibility of recovery of liver steatosis in

Table 3 Changes in biochemical parameters after injection

Group	Week	T2DM parameter		Blood lipid profile				
		FBG (mmol/L)	FBI (mIU/L)	TG (mmol/L)	TC (mmol/L)	HDL-C (mmol/L)	LDL-C (mmol/L)	FFA (mmol/L)
Control	9	4.43±0.16^{c}	19.03±2.03^{a}	0.82±0.07^{b}	2.85±0.17^{b}	1.49±0.04^{a}	0.83±0.06^{a}	4.64±0.38^{a}
	13	4.08±0.10^{c}	19.26±1.14^{b}	0.83±0.08^{c}	2.91±0.17^{d}	1.55±0.09^{c}	0.78±0.04^{b}	4.98±0.26^{b}
25 mg STZ/kg BW								
Regular diet	9	14.32±1.05^{b}	22.79±1.74^{a}	1.37±0.07^{a}	3.65±0.24^{a}	1.09±0.07^{bc}	0.86±0.06^{a}	5.44±0.50^{a}
	13	4.09±0.21^{c**}	23.84±1.18^{a}	1.61±0.10^{b}	4.63±0.16^{c**}	1.69±0.09^{bc**}	0.68±0.04^{b*}	6.07±0.56^{b}
HFD	9	16.38±0.99^{ab}	20.70±1.43^{a}	1.40±0.16^{a}	3.91±0.17^{a}	1.21±0.08^{b}	1.01±0.05^{a}	5.41±0.47^{a}
	13	11.01±1.31^{ab*}	19.38±0.97^{b}	2.38±0.15^{a**}	7.78±0.43^{b**}	1.73±0.04^{bc**}	1.08±0.11^{b}	6.19±0.51^{b}
35 mg STZ/kg BW								
Regular diet	9	17.85±1.09^{a}	12.88±1.77^{b}	1.41±0.09^{a}	3.50±0.24^{ab}	1.12±0.11^{bc}	0.99±0.11^{a}	5.61±0.20^{a}
	13	7.94±1.84^{b**}	14.95±1.65^{c}	1.69±0.13^{b}	4.45±0.20^{c*}	1.86±0.16^{ab**}	1.03±0.15^{b}	6.04±0.37^{b}
HFD	9	18.83±0.82^{a}	13.40±1.42^{b}	1.49±0.15^{a}	3.84±0.14^{a}	0.91±0.10^{c}	0.84±0.06^{a}	5.33±0.30^{a}
	13	13.15±1.55^{a**}	14.57±1.82^{c}	2.62±0.14^{a**}	11.44±0.29^{a**}	2.08±0.07^{a**}	2.64±0.34^{a**}	9.23±1.16^{a*}

Group	Week	Hepatic function			Oxidative stress		Hyperuricemia
		LDH (U/L)	ALT (U/L)	AST (U/L)	MDA (nmol/L)	SOD (U/ml)	UA (μmol/L)
Control	9	221.12±8.07^{c}	2.24±0.21^{b}	13.12±0.71^{a}	6.72±0.57^{a}	67.79±6.10^{a}	150.92±6.60^{b}
	13	197.74±16.52^{d}	2.53±0.31^{c}	12.36±1.28^{a}	7.06±0.59^{a}	70.97±5.36^{a}	157.26±6.49^{c}
25 mg STZ/kg BW							
Regular diet	9	282.37±6.19^{ab}	5.89±0.53^{a}	14.21±1.30^{a}	7.89±0.51^{a}	69.72±7.75^{a}	198.38±10.62^{a}
	13	265.83±18.55^{bc}	8.00±0.58^{b}	13.51±1.42^{a}	7.57±0.54^{a}	77.47±4.11^{a}	192.18±9.52^{bc}
HFD	9	266.07±15.84^{bc}	5.68±0.68^{a}	14.04±2.42^{a}	7.19±0.89^{a}	70.91±4.70^{a}	195.63±15.03^{a}
	13	256.07±19.51^{cd}	12.06±1.52^{b*}	12.33±1.50^{a}	8.02±0.71^{a}	80.91±3.89^{a}	213.84±11.76^{a}
35 mg STZ/kg BW							
Regular diet	9	316.51±27.51^{ab}	5.13±0.53^{a}	13.66±0.87^{a}	7.96±0.47^{a}	69.87±5.44^{a}	208.61±15.94^{a}
	13	336.79±25.12^{a}	11.21±1.25^{b**}	13.90±1.57^{a}	8.31±0.62^{a}	74.97±4.91^{a}	187.89±8.91^{ab}
HFD	9	334.20±25.38^{a}	5.98±0.49^{a}	12.82±0.93^{a}	7.88±0.88^{a}	71.74±4.05^{a}	189.95±11.67^{a}
	13	321.18±32.32^{ab}	22.45±3.48^{a**}	14.32±0.81^{a}	7.71±0.75^{a}	76.67±3.82^{a}	197.41±8.17^{ab}

Values are expressed as mean±SEM (n=6). BW: body weight; HFD: high-fat diet; T2DM: type 2 diabetes mellitus; FBG: fasting blood glucose; FBI: fasting blood insulin; TG: total triglyceride; TC: total cholesterol; HDL-C: high-density lipoprotein cholesterol; LDL-C: low-density lipoprotein cholesterol; FFA: free fatty acid; LDH: lactic dehydrogenase; ALT: alanine transaminase; AST: aspartate transaminase; MDA: malondialdehyde; SOD: superoxide dismutase; UA: uric acid. Mean values with different letters at the same week are significantly different (P<0.05). * P<0.05, ** P<0.01, vs. the corresponding value at week 9

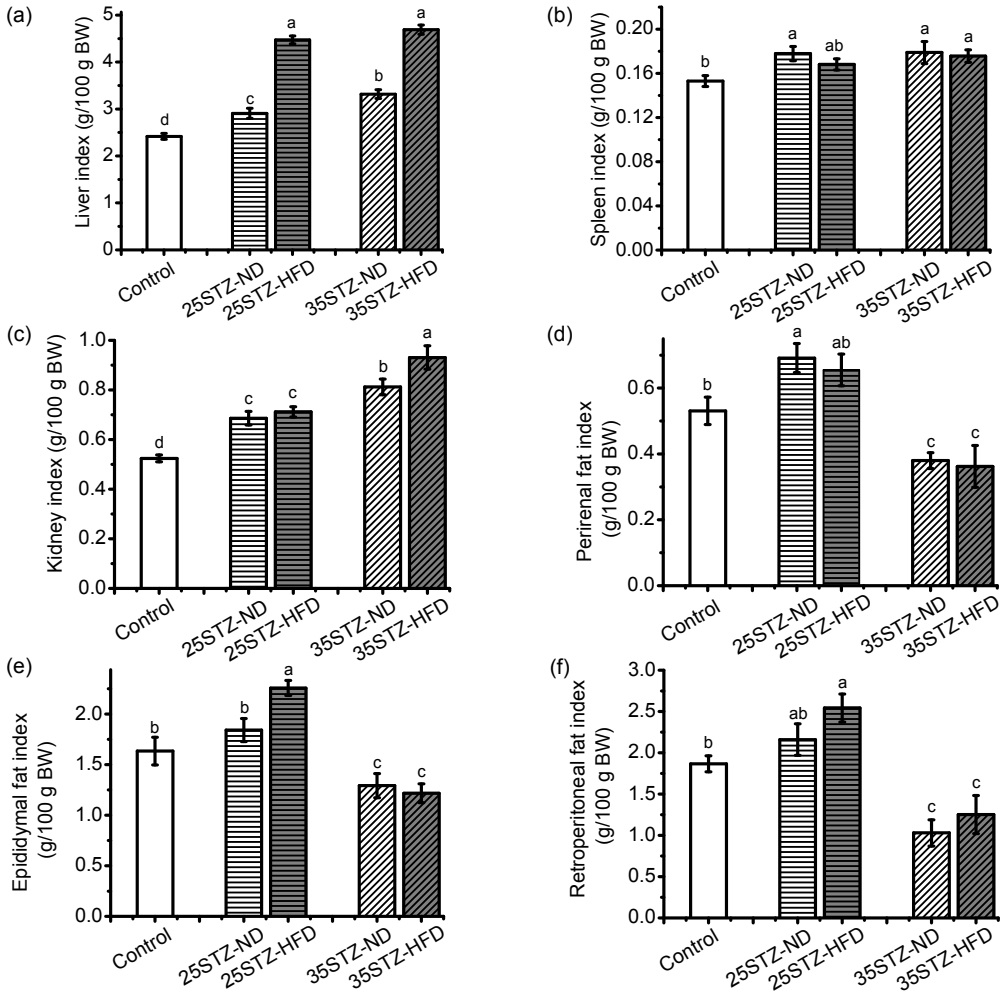

Fig. 3 Organ indices

At the end of the study, the rats were sacrificed and the liver (a), spleen (b), kidney (c), perirenal fat (d), epididymal fat (e), and retroperitoneal fat (f) were dissected and weighed immediately for the calculation of organ indices. Values are expressed as mean±SEM (n=6 for each group). 25STZ-ND: 25 mg/kg STZ with normal diet; 25STZ-HFD: 25 mg/kg STZ with high-fat diet; 35STZ-ND: 35 mg/kg STZ with normal diet; 35STZ-HFD: 35 mg/kg STZ with high-fat diet. Mean values with different letters are significantly different (P<0.05)

these rats. The spleen indices in diabetic rats were significantly higher than those in the control rats except for 25STZ-HFD (P<0.05). However, no significant differences were observed among any of the diabetic groups (P>0.05). Significantly higher kidney indices were found in diabetic rats (P<0.05), especially for the 35 mg/kg STZ groups, which were markedly higher than those in the 25 mg/kg STZ groups (P<0.05). For the fat tissue indices, all the 35 mg/kg rats showed significantly reduced fat tissue indices compared with the control rats (P<0.05), which corresponded to the body weight loss after injection (Fig. 2b). The 25STZ-HFD rats showed significantly higher epididymal and retroperitoneal fat indices than the control group (P<0.05). A significantly higher perirenal fat index was seen in the 25STZ-ND groups (P<0.05).

3.8 Histology

Examination of HE-stained liver sections revealed the typical architecture of the hepatic lobules in the control rats (Fig. 4a). In contrast, the diabetic rats showed varying degrees of degenerative changes in the hepatic lobules, characterized by fat vacuoles depositing in hepatocytes. The rats fed with regular diet after STZ injection showed mild lipid accumulation in hepatocytes, compared with dramatically larger amounts of lipid deposition in HFD-feeding rats.

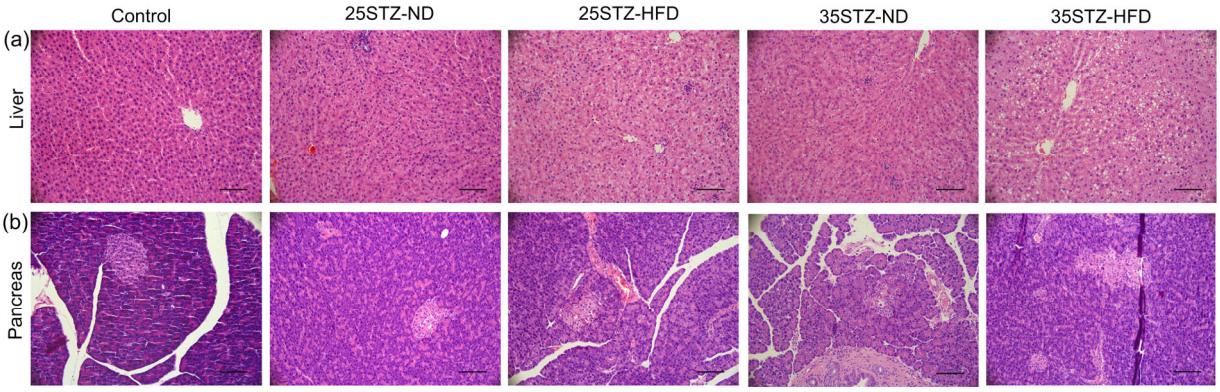

Fig. 4 Histological examination of the liver (a) and pancreas (b)

Representative samples were stained with hematoxylin and eosin (HE). The bars represent 94 μm. 25STZ-ND: 25 mg/kg STZ with normal diet; 25STZ-HFD: 25 mg/kg STZ with high-fat diet; 35STZ-ND: 35 mg/kg STZ with normal diet; 35STZ-HFD: 35 mg/kg STZ with high-fat diet

The HE staining of the pancreas was shown in Fig. 4b. The pancreatic islet in the STZ-HFD rats showed severe damage deforming its shape with angiotelectasis and inflammatory cell infiltration in the pancreatic islet. In comparison, the 25STZ-ND rats normalized pancreatic structure with no inflammatory cell infiltration in the pancreatic islet.

4 Discussion

T2DM is a complex, heterogeneous, and polygenic disorder which is characterized by a decline in insulin action (insulin resistance), followed by the inability of β-cells to secrete enough insulin to compensate for the insulin resistance (pancreatic β-cell dysfunction). To be relevant to humans, animal models must replicate the phenotype and mimic the developmental process of the disease. Thus the establishment of the T2DM model was achieved by feeding HFD to produce insulin resistance, followed by low dose of STZ injection to cause mild β-cell dysfunction (Srinivasan et al., 2005). After 8 weeks of HFD feeding, all the rats developed abdominal obesity, dyslipidemia, hyperglycemia, and insulin resistance (Table 2, Fig. 2), which mimicked the natural development of the early stage of T2DM. This is an important basis for the establishment of the T2DM model. On the basis of insulin resistance and obesity induced by 8 weeks of HFD feeding in rats, low-dose STZ injection was performed to induce mild impairment of insulin secretion which resembled the feature of the late stage of T2DM (Srinivasan et al., 2005). Research showed that many metabolic parameters including FBG and FBI displayed a dose-dependent relationship with STZ. High dose of STZ tended to induce metabolic features that more closely resembled type 1 diabetes, which is characterized by severe deficiency in insulin secretion. However, a low dose of STZ might fail to sufficiently induce T2DM (Mansor et al., 2013). Mansor et al. (2013) suggested that low doses of 15–25 mg/kg STZ induced a type 2 diabetic phenotype, and higher doses more closely recapitulated type 1 diabetes. However, in the study of Srinivasan et al. (2005), 35 mg/kg was proved to be the optimal dose for T2DM, and 25 mg/kg failed to increase blood glucose level. In some other research, the doses of 30–40 mg/kg were mostly utilized to induce T2DM combined with HFD (Wang et al., 2011; Mahmoud et al., 2012; Shatwan et al., 2013; Ji et al., 2015). In the present study, 25 and 35 mg/kg were chosen to induce T2DM in rats.

T2DM is characterized by glucose and fatty acid metabolic disorders. Higher needs of food and water intake are the typical symptoms of T2DM (Bibak et al., 2014). In the present study, the rats showed remarkably increased food and water intake after STZ injection, and tended to consume more regular diet than HFD, probably because of the satiety of HFD. The increment in food intake of diabetic rats could be explained by the decreased activity of the leptin receptor in the hypothalamus with relatively insufficient insulin (Lee et al., 1994).

The control rats constantly increased their body weight, whereas diabetic rats dropped body weight during the first week after STZ injection probably

because of decreased glucose metabolism and increased fat metabolism (Rossmeisl et al., 2003). Subsequently, body weight in 35 mg STZ/kg groups was further reduced. Considering the reduced FBI (Table 2) and body weight, the diabetes induced by 35 mg/kg STZ more resembled type 1 diabetes. The difference in body weight between 35STZ-ND and 35STZ-HFD groups might be due to about 15 g difference in food intake.

We measured the serum biochemical parameters 1 week and 5 weeks after the injection to determine the stability of the model fed with either regular diet or HFD. Our results indicated that blood glucose was not stable in this model and could be easily recovered by administering the normal diet. Although significant reductions in FBG levels were also observed by continuing HFD feeding (P<0.05), they were still significantly higher than those in the control and normal diet feeding diabetic groups (P<0.05), indicating that the model with HFD feeding was relatively stable in FBG. In the study of Ji et al. (2015), regular diets were used in the intervention period after the 7 weeks of HFD feeding combined with 30 mg/kg STZ injection. The FBG was not normalized in the diabetic rats, but the body weights experienced a dramatic decrease of more than 200 g during the intervention period of 8 weeks, which more resembled the features of type 1 diabetes. Another study used regular diet in the intervention period for 8 weeks. The diabetic rats remained extremely high FBG at the end of the study. That is probably due to the twice injections with 30 mg/kg STZ, which would cause more severe damages in pancreas. This was proved by the dramatically reduced insulin level of the diabetes group, which also resembled type 1 diabetes (Shatwan et al., 2013).

The 25 mg/kg STZ injection caused a slight decrease in FBI in the diabetic rats, and no significant variation was observed between diabetic and normal rats (P>0.05). A similar result was observed in previous research in which high-fat feeding significantly increased blood insulin, and the injection of 30 mg/kg STZ brought insulin concentrations back to control levels without seriously damaging the pancreas (Srinivasan et al., 2005). This indicated that STZ induced mild impairment instead of complete dysfunction in the pancreas, and this closely reflected the natural pathogenic process of T2DM. After the following 4 weeks of HFD/regular diet feeding, the FBI changed little in all groups. However, the FBG levels significantly recovered in the diabetic rats, especially in the normal diet-feeding rats, indicating improved insulin resistance. This could be proved by the histology results. The reduced lipid accumulation in hepatocytes in the STZ/ND rats might contribute to the improved insulin resistance in the liver (Fig. 4a) (Garg and Misra, 2002). On the other hand, the normalized FBG in 25STZ-ND rats might also be attributed to the regeneration of β-cells of pancreatic islets (Fig. 4b).

With regard to the blood lipid, all the diabetic rats presented more severe hyperlipidemia after STZ injection, especially the HFD feeding groups. This suggested that the diabetic rats were more prone to develop severe hyperlipidemia after STZ injection even when fed with normal diet. However, it seems controversial that the STZ/ND rats showed more severe hyperlipidemia, and at the same time they presented reduced liver steatosis. This might be related to the increased secretion of TG from hepatocytes, and the underlying mechanisms need to be further identified. The degree of dislipidemia in diabetic rats is related with the diet and dose of STZ, since HFD feeding or 35 mg/kg STZ injection caused more severe dislipidemia in rats. This is consistent with previous research (Srinivasan et al., 2005; Mansor et al., 2013). As a component of TC, the increase in HDL-C levels in all diabetic groups was probably due to the dramatically increased TC level. Although HDL-C is a well-known good cholesterol carrier (Marx, 1979), as a risk marker for cardiovascular disease, TC to HDL-C ratios should always been considered (Siri-Tarino et al., 2010). Higher TC to HDL-C ratio was found in STZ-HFD groups compared to STZ-ND groups, indicating higher susceptibility to cardiovascular disease in the STZ-HFD rats.

A lot of research has looked for the optimal method to establish T2DM models. Most used 2–4 weeks of HFD feeding before STZ injection. However, they rarely provided direct evidence that the rats developed insulin resistance (Zhang et al., 2010; Tan et al., 2005; Wang et al., 2011), which is the precondition of STZ injection and an important factor in the natural history of T2DM. For example, in the study of Mansor et al. (2013), 2 weeks of dietary modification failed to develop obesity in rats, and it probably did

not developed insulin resistance (Hotamisligil et al., 1993). The present study monitored the status of insulin resistance in rats during the HFD-feeding period and found that the rats did not develop insulin resistance at week 4 (data not shown). Therefore, the HFD-feeding time was continued for as long as 8 weeks to ensure the development of insulin resistance before STZ injection (Table 2).

There are certain limitations in the present study. Monitoring the model for a longer time and examining biochemical parameters at different time points could provide more information about the model, such as the plateau period over which the FBG remains relatively stable. In addition, more work should be done to uncover the underlying mechanisms of the recovery of the model.

5 Conclusions

The present study investigated the stability of T2DM rat model induced by HFD feeding combined with a low-dose STZ injection. From our data, doses of 35 mg/kg STZ (and potentially above) would be less desirable than lower doses (25 mg/kg in this case) for modeling T2DM due to the extreme systemic phenotype induced, which more resembled type 1 diabetes. Although it is more reasonable to test a therapy on this model using regular diet, in our study, 25 mg/kg STZ modeled rats followed by regular diets showed extremely unstable features including recovered FBG and normalized structures of pancreas. Therefore, compared with the normal diet, continuing HFD was more preferable in the further pharmacological study. However, further modifications of this model, such as increasing injection time and modifying composition of HFD, are still needed since the diebetic rats with HFD also showed significantly changed biochemical parameters including FBG.

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Compliance with ethics guidelines

Xiao-xuan GUO, Yong WANG, Kai WANG, Bao-ping JI, and Feng ZHOU declare that they have no conflict of interest.

All institutional and national guidelines for the care and use of laboratory animals were followed.

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中文概要

题　目：高脂饮食联合低剂量链脲佐菌素造大鼠二型糖尿病模型稳定性的研究

目　的：探讨高脂饮食联合低剂量链脲佐菌素（STZ）造大鼠二型糖尿病模型在不同饮食背景下的稳定性。

创新点：首次探讨在正常饮食和高脂饮食的背景下，该常见的大鼠二型糖尿病模型的稳定性。可为造模后期干预阶段的饲料选择提供依据。

方　法：饲喂 Wistar 大鼠高脂饮食 8 周后，注射 25 或 35 mg/kg STZ 来诱导二型糖尿病。之后每组分别饲喂正常饲料和高脂饲料 4 周，检测血液生化指标的稳定性。

结　论：不管在何种饮食下，糖尿病大鼠都出现了空腹血糖显著恢复，血脂紊乱加剧的现象。其中 35 mg/kg STZ 注射组大鼠的特征更接近一型糖尿病，而 25 mg/kg STZ 注射组大鼠表现出二型糖尿病的特征。相比高脂饮食，正常饮食更容易导致空腹血糖和胰岛结构的恢复。因此，在正常饮食下该模型稳定性欠佳，在后期的药理学实验中推荐使用高脂饮食。

关键词：高脂饮食；稳定性；链脲佐菌素；二型糖尿病