

# Study on the Optimization and Stability of Single-dose Streptozotocin-induced Diabetic Modelling in Rats

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## Research

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## Abstract

**Aim:** This study was performed to optimize the experimental conditions in streptozotocin (STZ)-induced diabetic model by using Sprague-Dawley (SD) rats to evaluate the stability of the model.

**Methods:** In addition to the control group, the male and female SD rats were randomly divided into the following treatment groups (with six rats per group): STZ 45 (45 mg/kg STZ); STZ 65 (65 mg/kg STZ); STZ 85 (85 mg/kg STZ); high-fat diet with STZ 45; high-fat diet with STZ 65; and high-fat diet with STZ 85. Changes in the body weight and blood glucose were observed dynamically.

**Results:** No significant differences were found in the blood glucose or body weight between the STZ 45 and control groups in both male and female rats, whether or not the rats were on a high-fat diet. However, significant differences were found in the blood glucose between the high-dose STZ and control groups in both male and female rats, regardless of whether the rats were on a high-fat diet or not ( $P < 0.05$  or  $P < 0.01$ ). Compared with the control group, significant differences in the blood glucose levels ( $P < 0.05$  or  $P < 0.01$ ) and higher blood glucose levels were found in the male rats fed with normal diet than those of rats fed with high-fat diet.

**Conclusions:** In this study, male rats fed with ordinary feed and injected with 65 mg/kg STZ were the most stable and ideal diabetic rats.

## Implications

Diabetes is a common endocrine and metabolic disease with a wide range of effects. In 2019, there were 463 million people with diabetes worldwide, and it is expected to increase to 700 million by 2045. The treatment of diabetes and its complications has become a global problem, and clinically there is an urgent need for effective treatment to relieve the suffering of patients. Establishing a suitable animal model of diabetes is very necessary for the development of new drugs.

## Introduction

Diabetes is a chronic metabolic disease characterized by a relative or absolute lack of insulin, leading to hyperglycemia. Chronic hyperglycemia, which causes multiple complications, such as neuropathy, kidney disease, retinopathy, and increased risk of cardiovascular disease, is currently one of the most important noncommunicable diseases threatening global human health [1].

The incidence of diabetes is rising globally. According to the ninth edition of the Global Diabetes Map released by the International Diabetes Federation in 2019, 9.3% of adults currently live with diabetes, and by 2045, nearly 700 million people are predicted to be living with this disease. The prevalence of diabetes, diabetes-related deaths, and medical costs place a huge burden on the society, finance, and health care system [2]. An ideal animal model of diabetes should be established to study the pathogenesis and select the therapeutic drugs for this disease.

Many methods are currently used to establish animal models of diabetes. These models are mainly divided into models of spontaneous diabetes, such as Zucker diabetic fatty (ZDF) rats, biobreeding (BB) rats, and Goto-Kakizaki (GK) rat, and animal models of diabetes through chemical induction, such as alloxan and streptozotocin (STZ). ZDF rats were found in 1961 after Merck (M-strain) and Sherman rats were crossbred. These rats are characterized by a mutation in the leptin receptor that causes enormous appetite, and the rats become obese by 4 weeks of age. These rats also have hyperinsulinemia, hyperlipidemia, hypertension, and impaired glucose tolerance [3]. BB rats are derived from a distant relative, the Wistar rat. After puberty, the BB rats develop diabetes, with rates similar between males and females, and 90% of the rats develop this disease between 8 weeks and 16 weeks of age. The diabetes phenotype is quite severe and characterized by the development of hyperglycemia, weight loss from hypoinsulinemia, and ketouria, which requires insulin therapy to survive [4]. GK rats develop mild hyperglycemia early in life and are considered as a nonobese model, and all adult GK rats of both sexes show type 2 diabetes [5]. Alloxan and STZ are considered the most effective diabetes-promoting drugs used in diabetes research and are cytotoxic glucose analogs [6]. Although their cytotoxicity is achieved by different pathways, their selective actions on the  $\beta$  cells are the same; that is, both drugs cause insulin deficiency. The STZ pathway is targeted  $\beta$ -cell apoptosis with chemical that induces DNA alkylation, while alloxan is targeted  $\beta$ -cell destruction with chemical that induces oxidative stress [7–9]. Compared with alloxan, STZ is more stable and the best choice for repeatedly inducing diabetes in the experimental animals [10]. However, the physicochemical properties and related toxicity of STZ remain major obstacles for researchers who use STZ to treat diabetes in animals.

Another major challenge for STZ-induced diabetes models is how to maintain the suitability, repeatability, and inductivity of diabetes with minimal animal mortality. The lack of appropriate use of STZ has been associated with increased mortality and animal suffering [11]. Therefore, several factors, such as the preparation method, stability, appropriate dose, diet plan, animal species (related to age, weight, and gender), and target blood glucose level representing hyperglycemia should be considered when STZ is used in animals.

In this study, the effects of the gender of Sprague-Dawley (SD) rats, dose of STZ injection, and dietary conditions on the body weight, blood glucose, modeling rate, and mortality were compared.

## Material And Methods

## 2.1 Materials

Chow diet and high-fat diet (HFD; 45% fat supply ratio) were purchased from Guangzhou University of Traditional Chinese Medicine (University Town) Animal Center. Accu-Chek Active glucometer was purchased from Roche Diagnostic Corporation, Mannheim (German), while STZ was bought from Sigma.

## 2.2 Animals

Adult male and female SD rats (body weight: 180–220 g) were purchased from Guangdong Experimental Animal Center (Certificate: SCXK2018–0002) and housed in the Experimental Animal Center of Guangzhou University of Chinese Medicine (Certificate: SYXK2018–0085). The animals were housed in a specific pathogen-free animal laboratory of this Center. All experimental procedures were performed according to the ethical principle guidelines approved by the National Research Council. The animals were maintained at 12 h of light and dark cycles at room temperature (24–26°C) and relative humidity of 50–70%. Food and water were given ad libitum.

## 2.3 HFD + STZ protocols

After the acclimation period, the rats were fed a standard chow ad libitum diet ( $n = 48$ ) or HFD ( $n = 48$ ) for 4 weeks. Each group was randomly divided into four subgroups ( $n = 6$  each). After 12 h of fasting, the rats underwent intraperitoneal injection of three single doses of STZ (45, 65, and 85 mg/kg), while the control rats received the vehicle citrate buffer. The groups were as follows: 1, Control  $\square$  (male + ad-libitum food); 2, STZ 45  $\square$  (male + ad-libitum food + 45 mg/kg STZ); 3, STZ 65  $\square$  (male + ad-libitum food + 65 mg/kg STZ); 4, STZ 85  $\square$  (male + ad-libitum food + 85 mg/kg STZ); 5, HFD + STZ 45  $\square$  (male + high-fat diet + 45 mg/kg STZ); 6, HFD + STZ 65  $\square$  (male + high-fat diet + 65 mg/kg STZ); 7, HFD + STZ 85  $\square$  (male + high-fat diet + 85 mg/kg STZ); 8, Control  $\square$  (female + ad-libitum food); 9, STZ 45  $\square$  (female + ad-libitum food + 45 mg/kg STZ); 10, STZ 65  $\square$  (female + ad-libitum food + 65 mg/kg STZ); 11, STZ 85  $\square$  (female + ad-libitum food + 85 mg/kg STZ); 12, HFD + STZ 45  $\square$  (female + high-fat diet + 45 mg/kg STZ); 13, HFD + STZ 65  $\square$  (female + high-fat diet + 65 mg/kg STZ); and 14, HFD + STZ 85  $\square$  (female + high-fat diet + 85 mg/kg STZ).

## 2.4 Rat weights and fasting blood glucose (FBG)

The rats were weighed on the same day and time of each week. At the end of the acclimation week, the rats were fasted for 12 h, and a tip of the tail was snipped with sharp scissors and gently squeezed for a drop of blood. Then, the plasma glucose concentrations were assessed by using a glucometer (Accu-Chek Active; Roche Diagnostic Corporation, Mannheim, German). The FBG was determined every week on the same day and time during the experiment. The glucometer was calibrated by using calibrators provided by the manufacturer.

## 2.5 Statistical analysis

SPSS 20.0 and GraphPad 5.0 were used to process all experimental data, which were presented as the means  $\pm$  standard error of the mean ( $\bar{x} \pm \text{SEM}$ ) was used to represent the experimental data. ANOVA was performed to test the significant difference between two groups, and  $P < 0.05$  was used to indicate the significant difference between two groups.

## Results

### 3.1 Effect of STZ on the weight and serum glucose of male rats

Typical features of diabetes are weight loss and a persistent increase in blood sugar. The FBG and body weights of the rats were measured on days 0, 7, 14, 21, and 28 after the animal modeling. Compared with the control group, the male rats injected intraperitoneally with 65 mg/kg STZ lost weight on days 7, 14, and 28. The modeling rate was 1/6 (dead rats/group), and no rats died. Significant weight loss was observed on days 7, 14, 21, and 28 in male rats treated with 85 mg/kg STZ. The death rate and incidence of diabetes in these rats were 1/6 and 5/6 (dead rats/group), respectively. The blood glucose of the male rats treated with 65 mg/kg or 85 mg/kg were all greater than 8.3 mmol/L at five time points. The blood glucose and body weight of the male rats treated with 45 mg/kg STZ showed no significant difference compared with the control group, in which the mortality rate was 2/6, and all the rats were modeled (Fig. 1 and Tables 1 and 2).

Table 1  
Weight (g) of male and female SD rats

	male						female						
	Control (n = 6)	STZ 45 (n = 6)	STZ 65 (n = 5)	STZ 85 (n = 4)	HFD + STZ 45 (n = 6)	HFD + STZ 65 (n = 4)	HFD + STZ 85 (n = 3)	Control (n = 6)	STZ 45 (n = 6)	STZ 65 (n = 6)	STZ 85 (n = 3)	HFD + STZ 45 (n = 5)	HFD + STZ 65 (n = 4)
0d	223.00 ± 3.85	206.58 ± 4.43	211.14 ± 5.25	205.50 ± 6.18	232.80 ± 3.48	232.68 ± 2.39	236.90 ± 4.57	197.07 ± 2.63	200.65 ± 6.27	207.5 ± 4.02	197.53 ± 8.86	205.30 ± 3.66	205.20 ± 6.03
7d	278.93 ± 6.10	261.07 ± 12.13	223.10 ± 11.49*	210.53 ± 17.11**	306.07 ± 7.51	280.08 ± 15.55	251.03 ± 8.89	244.20 ± 5.04	230.73 ± 9.74	200.62 ± 9.35*	178.83 ± 13.69**	220.96 ± 4.54*	206.73 ± 4.31**
14d	312.27 ± 6.77	264.93 ± 16.15	216.88 ± 24.53**	197.98 ± 25.74**	346.72 ± 10.82	306.35 ± 24.38	263.03 ± 12.78	254.28 ± 5.18	246.25 ± 11.49	210.18 ± 9.20*	167.80 ± 13.42**	234.06 ± 5.49	196.35 ± 6.70**
21d	320.17 ± 6.54	299.00 ± 19.37	237.80 ± 26.66	212.75 ± 28.85*	357.32 ± 11.56	297.83 ± 35.36	256.80 ± 8.48	263.75 ± 5.14	240.87 ± 14.27	209.57 ± 11.01*	163.63 ± 14.94**	235.94 ± 6.12*	208.45 ± 8.39**
28d	352.53 ± 8.12	309.95 ± 24.99	240.60 ± 33.27*	238.40 ± 34.37*	376.67 ± 15.37	301.00 ± 35.12	249.70 ± 6.37*	277.08 ± 6.38	251.37 ± 16.69	209.25 ± 12.78**	156.53 ± 18.29**	240.24 ± 4.53*	219.95 ± 12.76**
Data are presented as means ± standard error of the mean ( $\bar{x} \pm \text{SEM}$ ). Significance is denoted by * $P < 0.05$ , ** $P < 0.01$ difference from the control group.													
Abbreviation: STZ 45, 45 mg/kg STZ; STZ 65, 65 mg/kg STZ; STZ 85, 85 mg/kg STZ; HFD + STZ 45, 45 mg/kg STZ and high-fat diet (HFD); HFD + STZ 65, 65 mg/kg STZ and HFD; and HFD + STZ 85, 85 mg/kg STZ and HFD.													

Table 2  
Blood glucose levels (mmol / L) of male and female SD rats

	male						female						
	Control (n = 6)	STZ 45 (n = 6)	STZ 65 (n = 5)	STZ 85 (n = 4)	HFD + STZ 45 (n = 6)	HFD + STZ 65 (n = 4)	HFD + STZ 85 (n = 3)	Control (n = 6)	STZ 45 (n = 6)	STZ 65 (n = 6)	STZ 85 (n = 3)	HFD + STZ 45 (n = 5)	HFD + STZ 65 (n = 4)
3d	4.25 ± 0.44	5.18 ± 0.30	13.46 ± 2.36	17.75 ± 5.52**	5.03 ± 0.24	7.70 ± 0.93*	17.60 ± 1.80**	4.95 ± 0.28	5.55 ± 0.87	10.00 ± 1.58**	26.13 ± 2.07**	4.50 ± 0.53	8.43 ± 0.96**
7d	6.95 ± 0.23	8.72 ± 2.05	11.88 ± 2.60	20.03 ± 5.47*	5.22 ± 0.25	10.03 ± 2.48	15.77 ± 0.68**	4.70 ± 0.14	5.17 ± 0.92	7.08 ± 0.88	22.03 ± 1.03**	5.62 ± 0.35	10.63 ± 2.45*
14d	4.23 ± 0.26	10.62 ± 2.86	17.46 ± 3.78*	16.40 ± 4.66	12.23 ± 2.30	15.28 ± 3.12*	20.90 ± 4.17**	4.38 ± 0.25	7.20 ± 1.36	7.20 ± 1.06	19.53 ± 1.84**	9.50 ± 2.55	12.65 ± 3.04*
21d	4.05 ± 0.35	7.62 ± 2.69	22.02 ± 4.37**	21.05 ± 5.16*	5.53 ± 0.36	19.43 ± 4.87**	19.97 ± 1.20**	4.58 ± 0.13	7.82 ± 0.73	14.78 ± 2.69**	24.47 ± 1.96**	7.60 ± 0.90	12.55 ± 3.49*
28d	3.53 ± 0.22	7.17 ± 2.50	16.80 ± 3.05**	20.93 ± 2.07**	4.70 ± 0.19	10.25 ± 2.15**	23.13 ± 1.71**	4.53 ± 0.27	4.65 ± 0.66	15.32 ± 2.73**	20.33 ± 3.32**	6.16 ± 0.72	16.00 ± 4.07**
Data are presented as means ± standard error of the mean ( $\bar{x} \pm \text{SEM}$ ). Significance is denoted by * $P < 0.05$ , ** $P < 0.01$ difference from the control group.													
Abbreviation: STZ 45, 45 mg/kg STZ; STZ 65, 65 mg/kg STZ; STZ 85, 85 mg/kg STZ; HFD + STZ 45, 45 mg/kg STZ and high-fat diet (HFD); HFD + STZ 65, 65 mg/kg STZ and HFD; and HFD + STZ 85, 85 mg/kg STZ and HFD.													

### 3.2 Effect of STZ on the weight and serum glucose of female rats

Compared with the control group, the female rats injected intraperitoneally with 65 mg/kg and 85 mg/kg STZ lost weight on days 7, 14, 21, and 28. The serum glucose levels of the female rats in the STZ 85 group were remarkably higher than those in the control group at five time points, and the

former had a mortality rate of 3/6 and modelling rate of 6/6. Compared with the control group, the female rats injected with 65 mg/kg STZ had increased serum glucose on days 3, 21, and 28. The mortality rate of STZ65 group was 16.7%, and the model formation rate was 66.7% on day 3, 33.3% on day 7, 16.7% on day 14, 66.7% on day 21, and 83.3% on week 4. In addition, the blood glucose and body weight of female rats treated with 45 mg/kg STZ showed no significant difference compared with the control group, and none of the rats in the former group were modeled or dead (Fig. 2 and Tables 1 and 2).

### 3.3 Effect of STZ on the weight and serum glucose in male rats fed with HFD

Compared with the control group, the weights of the HFD + STZ 45, HFD + STZ 65, and HFD + STZ 85 groups showed no significant differences at five time points, and the variation in weight was relatively stable. For the blood sugar, compared with the control group, the male rats injected with 65 mg/kg and 85 mg/kg STZ had increased serum glucose on days 3, 7, 14, 21, and 28. The HFD + STZ 45 group did not die and were not modeled, while the HFD + STZ 65 group has a modeling rate of 5/6 and mortality rate of 2/6. The mortality rate for the HFD + STZ 85 group was 3/6, all rats developed diabetes (Fig. 3 and Tables 1 and 2).

### 3.4 Effect of STZ on weight and serum glucose in the female rats fed with HFD

On days 7, 14, 21, and 28, compared with the control group, the weight of the HFD + STZ 45 and HFD + STZ 65 groups decreased and showed significant differences. In addition, on the same time points, compared with the control group, a slight increase in blood glucose was found in the HFD + STZ 45 group, but no significant difference was found. One of the six rats in this group died, and one was modeled. On days 3, 7, 14, 21, and 28, the blood glucose in the HFD + STZ 65 group was greater than 8.3 mmol/L, and a significant difference was found compared with the control group. The mortality rate of HFD + STZ65 group was 33.3%, and the model formation rate was 83.3% in the second week, 66.7% in the third week and 83.3% in the fourth week. After an intraperitoneal injection of 85 mg/kg STZ and HFD, all female SD rats were modeled but died within seven days (Fig. 4 and Tables 1 and 2).

## Discussion

In this study, the blood glucose levels and weights of 14 groups of male and female rat models with diet-induced and drug-induced diabetes were investigated. An ideal rat model should typically mimic the disease pattern of diabetes. Diabetes is mainly characterized by chronic high blood sugar. The model should be inexpensive and easy to access. The rat models in study included rats injected with low- or high-dose STZ [12], fed with nicotinamide and injected with STZ [13], and fed with HFD and injected with STZ [14]. A suitable nongenetic rat model that would mimic the pathogenesis and clinical features of diabetes was explored for further research.

The FBG of the male diabetic rats induced by STZ was more stable than that of the female rats. In the animal models, the physiological systems involved in the metabolic homeostasis showed sex differences, in which the sex hormones play an important role [15]. Male rodents are often used, because they show better metabolic diseases than females [16]. Here, the stability of blood glucose in the diabetic male rats induced by STZ was better than that of female rats. The male rats were also more sensitive to STZ than the female rats, and this result may be related to sex hormones. STZ could induce diabetes by causing damage to the  $\beta$  cells of the pancreas, while female sex steroids could prevent this phenomenon. Gonadal hormone 17-estradiol (E2) has been associated with the reproductive, skeletal, cardiovascular, and neuronal physiology [17]. E2 promotes the survival of mouse  $\beta$  pancreatic cells through the E2 by the estrogen receptor  $\alpha$  (ER- $\alpha$ ) [18]. E2 activates the nuclear ER through the ER element. E2 also activates the nongenomic signals through the extracellular forms of ER and G-protein-coupled ER. In the diabetic rodent model, E2 treatment can protect the pancreatic  $\beta$  cells from oxidative stress, toxicity of amyloid peptide, lipid toxicity, and apoptosis [17]. Therefore, estrogen therapy can protect  $\beta$  cells from STZ-induced apoptosis, help maintain insulin, and prevent diabetes [19, 20]. In this study, compared with the male rats, regardless of whether they were given normal diet or HFD, the blood glucose after modeling in female rats was lower than that of male rats in the same period. This result was speculated to be related to the estrogen in female rats.

In addition, the blood glucose concentrations in the fat-fed rats were not higher than those in the chow-fed rats. This conclusion is consistent with previous reports that the insulin concentrations during the fasting glucose challenge were significantly higher in high-fat rats. The insulin-mediated glucose disposal was decreased in these animals, which may have led to the lower blood glucose [21]. In Carvalho's study, compared with the STZ mice fed with low-fat diet, the STZ mice fed with HFD had lower blood glucose and higher body weight [22]. No significant difference was found between the HFD and low-fat diet. Hence, in terms of cost, the STZ-induced (65 mg/kg) model without HFD was better.

The dosage of STZ was not clearly consistent when used to model diabetes in rats. For example, Wang used single-dose intraperitoneal injection of 50 mg/kg STZ to induce diabetes [23]. In Luippold's study, male SD rats with an age of 8–9 weeks were pretreated with a single intraperitoneal dose of 60 mg/kg STZ to induce experimental diabetes [24]. To screen a better injection dose, three STZ dose groups (high, medium, and low) were selected. The cost, operability, modelling rate, and mortality were considered. In this study, the rats treated with single doses of 65 mg/kg and 85 mg/kg STZ had higher blood glucose than those in the other groups (45 mg/kg). However, a higher mortality was found in the 85 mg/kg STZ group compared with the 65 mg/kg STZ group. The single high-dose STZ-induced (65 mg/kg) diabetic group without HFD was more suitable for diabetes research. Given that the severity of diabetes in the STZ rats would gradually increase with the dose of STZ, further ongoing studies will provide more in-depth information on this phenomenon. In the future, the authors plan to study the STZ dose group with 65–85 rats with longer period of observation to determine whether the protective effect against diabetic complications persists.

## Conclusions

Comparison of the blood glucose levels and body weights showed that the male SD rat injected intraperitoneally with 65 mg/kg STZ and fed with ordinary diet was the most stable and ideal diabetic rat model.

## Abbreviations

STZ: Streptozotocin; SD: Sprague Dawley; HFD: High-fat diet

## Declarations

### Ethics approval

All experimental procedures were performed according to the ethical principle guidelines approved by the National Research Council. All SD rats were purchased from Guangdong Experimental Animal Center (Certificate: SCXK2018–0002) and housed in the Experimental Animal Center of Guangzhou University of Chinese Medicine (Certificate: SYXK2018–0085).

### Availability of data and materials

The raw data for the current study are available from the corresponding author on reasonable request.

### Author contributions

HC conceived, designed and directed this study; YZ, JZ, MH, JH, RW performed the experiments; YZ, JZ, MH, JH, RW, BT substantial contributed to analysis, interpretation of data and drafted the manuscript. HC, YZ, JZ, MH, JH, RW, BT, PH all participated in revision of the manuscript. All authors read and approved the final manuscript.

### Declaration of interest

The authors declare that they have no competing interests.

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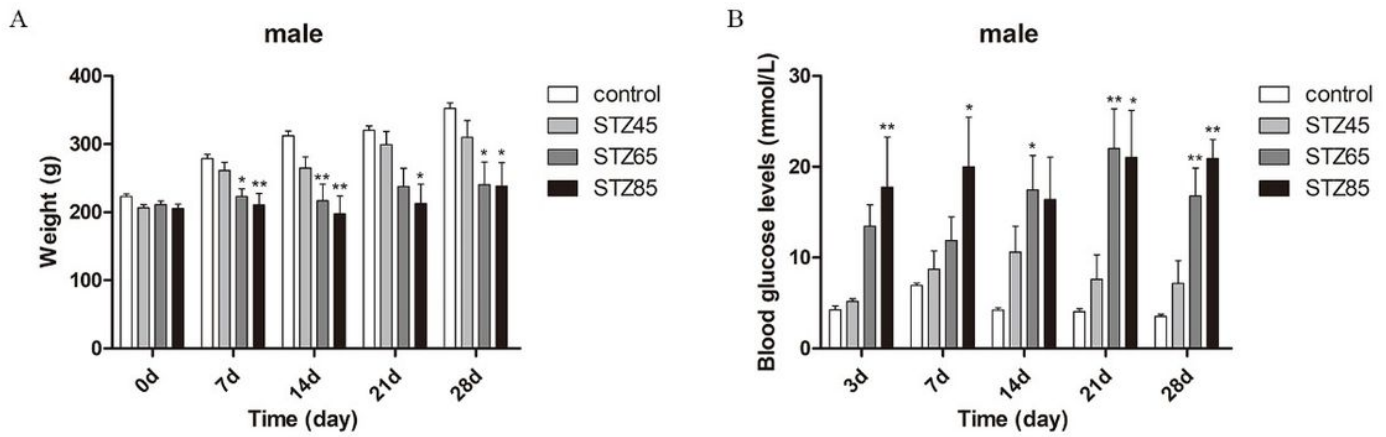
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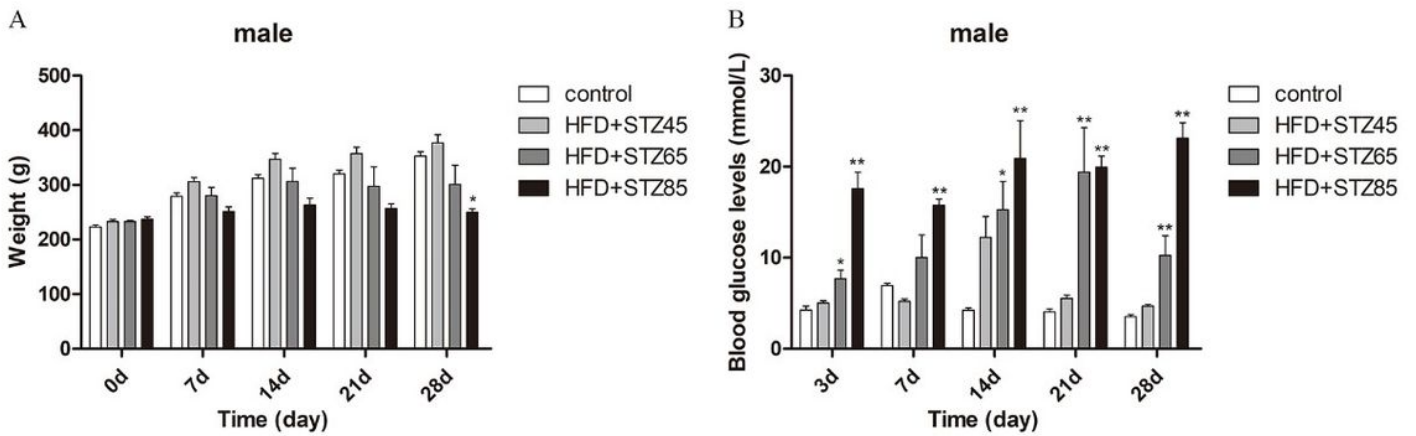
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## Figures

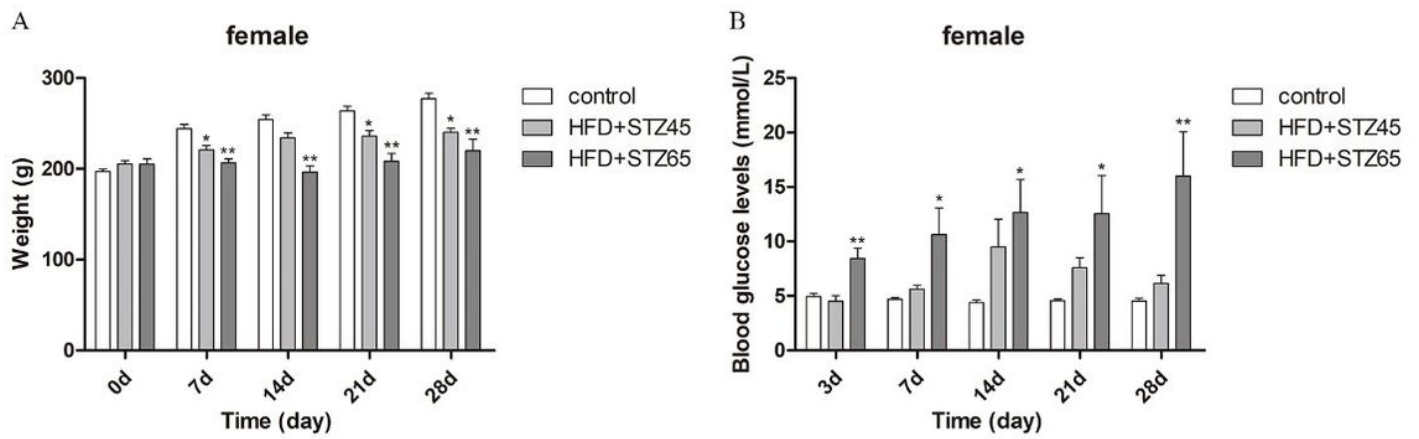


**Figure 1**  
 (A) Weight (g) and (B) blood glucose levels (mmol/L) of male SD rats (STZ 45, 45 mg/kg STZ; STZ 65, 65 mg/kg STZ; and STZ 85, 85 mg/kg STZ). Data are presented as means  $\pm$  standard error of the mean ( $\bar{x} \pm$  SEM) (n=6 for the control group; n=6 for the STZ 45 group; n=5 for the STZ 65 group; and n=4 for the STZ 85 group). Significance is denoted by \*P<0.05, \*\*P<0.01 difference from the control group.



**Figure 3**  
 (A) Weight (g) and (B) blood glucose levels (mmol/L) of male SD rats (HFD + STZ 45, 45 mg/kg STZ and high-fat diet (HFD); HFD + STZ 65, 65 mg/kg STZ and HFD; and HFD + STZ 85, 85 mg/kg STZ and HFD). Data are presented as means  $\pm$  standard error of the mean ( $\bar{x} \pm$  SEM) (n=6 for the control group; n=6 for the HFD + STZ 45 group; n=4 for the HFD + STZ 65 group; and n=3 for the HFD + STZ 85 group). Significance is denoted by \*P<0.05, \*\*P<0.01 difference from the control group.





**Figure 4**

(A) Weight (g) and (B) blood glucose levels (mmol/L) of female SD rats (HFD + STZ 45, 45 mg/kg STZ and high-fat diet (HFD); and HFD + STZ 65, 65 mg/kg STZ and HFD). Data are presented as means  $\pm$  standard error of the mean ( $\bar{x} \pm$  SEM) (n=6 for the control group; n=5 for the HFD + STZ 45 group; and n=4 for the HFD + STZ 65 group). Significance is denoted by \*P<0.05, \*\*P<0.01 difference from the control group.