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Research Article

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C57BL/6J mouse-model of non-insulin-dependent diabetes mellitus

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ABSTRACT

The study was to develop a mouse model of non-insulin-dependent diabetes mellitus (NIDDM) that similar to human clinical pathogenesis. Fed three week-old C57BL/6J mice with chow enriched in sugar and fat for 3 weeks, then injected those mice with Streptozotocin (STZ, 125mg/kg) and continued feeding for 4 weeks. After 7 weeks high fat diets, the blood glucose concentration of mice in high-fat-STZ group reached the standard of NIDDM mouse.

Key words: Diabetes, non-insulin-dependent, mice model

INTRODUCTION

Non-insulin dependent diabetes mellitus, namely the II-type diabetes, is the most common form of human diabetes. More than 90% patients with diabetes are non insulin dependent diabetes in the world. Therefore, to establish an ideal animal model of NIDDM is significant in studies of pathogenesis, drug action and drug screening. This research, in which we used high fat and sugar homemade feed and Streptozotocin (STZ) to induce NIDDM on mice, is reported below.

EXPERIMENTAL SECTION

The study was approved by the animal ethics committee of life science and engineering college,Southwest Jiaotong university.

1.1 Reagent
Streptozotocin (STZ, SIGMA 2Aldrich Inc. USA), batch No.053 k1569; Citric acid (Chengdu Honghe chemical reagent factory), batch No.20040821; Insulin kit (Beijing north biotechnology research institute), batch No.0403053; Glucose GOD2PAP method detection kit (Sichuan Mike technology Co., LTD), batch No. 0404071.

1.2 Animal
Clean level C57BL/6J mice were obtained from Shanghai Slack experimental animals co., LTD at the age of 3 weeks, qualified number: SCXK (Shanghai) 2003-0003.

1.3 Chow
Chow (regular chow) was provided by the Sichuan academy of medical sciences test animal research institute. High fat and sugar chow was made according to the formulation from Chengdu phargentech co., LTD. and J. Luo [1], component content (%) was shown in table 1:

Table 1 Chow formulation of high fat and sugar

Component	Percentage (%)
Sucrose	10
Malto-dextrin	28
Lard	30
Casein (75%)	27.5
Choline chloride	0.12
Salt	0.1
Methionine	0.24
Multidimensional	0.04
Mineral additives	4

1.4 Instruments

SN2695B intelligent radioimmunoassay meter (Shanghai nuclear institut Sihuan photoelectric instrument co., LTD.), sensitivity: 1um/mL BASIC semi-automatic biochemical analyzer (France SECOMAM company); ACCULAB electronic balance (Beijing Dolly instrument system co., LTD); HANGPING2JA5003 type electronic balance (Shanghai balance instrument factory).

1.5 Methods

1.5.1Animal feeding

All mice were reared in IVC barrier system, 5 mice in each box, temperature: 22±3℃, relative humidity: 50%~80%, illumination: 12hours in light/12hours in dark (illumination intensity: 150 ~300Lux). The mice were fed by sterile water and irradiated chow.

1.5.2 Blood glucose determination

In non-fasting condition, by tail-cutting method, under constant temperature (range 25~28□), gathered 200~300μL blood by 1000μL micro sampler. Placed it in 500μL plastic centrifuge tube and separate serum with centrifuge at speed of 12000round/min for 5 minutes, then put 10μL serum into a glass tube and added 1000μL glucose detection reagent, then put the tube along with its rack into electric-heated thermostatic water bath at 37□ for 10 minutes. After the steps above, we tested the blood glucose value with the semi-automatic biochemical analyzer by manual injecting 500uL sample.

1.5.3 Insulin determination

Through the Radioimmunoassay, use the serum that rest from the 1.5.2 to determine the serum insulin at intelligent radioimmunoassay meter.

1.5.4 Citric acid preparation

Placed 210.14mg citric acid weighed by electronic balance in a 50ml breaker and added 20ml saline by using suction pipette, then mixed evenly and filtered the citric acid to gain the target citric acid solution(concentration: 0.05mol/l, pH: 4.5).

1.5.5 STZ preparation

Placed 33mg and 36mg STZ weighed by electronic balance in two 25ml breakers respectively, and added 6ml prepared citric acid solution by using suction pipette into each breaker, then mixed evenly and filtered the STZ to gain the target STZ (concentration: 110mg/kgBW and 120mg/kgBW). Through the same way with 43mg, 51mg, 60mg STZ and 10ml citric acid solution we gained the target STZ(concentration : 130 mg/kgBW, 153mg/kgBW and 180mg/kgBW)[2-3].

1.5.6 STZ dose screening

Streotozotocin (STZ) is a broad-spectrum antibiotic, which has anti-tumor activity, antibacterial activity and the side effect of inducing diabetes. Moreover, it has highly selective toxicity to the islet beta cells of experimental animal. Although there are many reports about the killing effect of STZ to beta cells, the mechanism is not entirely clear. Blondel and his partner observed through the light microscope and found that STZ can destroy islet beta cells directly, the mechanism of which may be as follows: injecting Wistar mouse with 65mg/kgBW STZ, and many necrotic islet bata cells can be seen after 7 hours. The number of beta cells was significant reduced. The residual beta cells are almost completely degranulated. Due to the large number of beta cells' destruction, the synthesis and secretion of insulin are reduced, which cause the disorder of glucose metabolism[4].

According to the literature, the dosage of STZ is a key point in developing a model. The purpose of injecting STZ is to damage the beta cells selectively so that the secretion of insulin is not enough to maintain the balance of blood glucose. High dosage of STZ can damage the insulin beta cells completely and leads to a type I diabetes, while low

dosage may not damage the beta cells and cause the lack secretion of insulin [5].

According to the pre-experiments, the doses of intravenous injection in mice are 110mg/kgBW, 120mg/kgBW, 130mg/kgBW, 153mg/kgBW and 180mg/kgBW.

We randomly divided 105 C57BL/6J male mice according to their body weight into 7 groups ，which were 5 dosage groups, 1 citric acid group and 1 control group (each group contains 15 mice), and injected them with STZ according to their body weight in non-fasting condition. When the concentrations of STZ were 110mg/KgBW and 180mg/KgBW, the injected dose should be 0.2ml/10gBW. When the concentrations of STZ were 130 mg/KgBW, 153 mg/KgBW and 180 mg/KgBW, the injected dose should be 0.3ml/10gBW. The injected dose of citric acid group should be 0.2ml/10gBW. Taking blood by tail-cutting and testing the non-fasting glucose in each group on the 24h, 7st and 14th day, respectively.

1.5.7 The development of animal model with II-type diabetes

75 C57BL/6J mice (at the age of 3 weeks) were adaptively fed for a week and randomly divided into 5 groups according to their body weight. Each group contains 15 mice, where A, B groups were given normal diet, C, D groups were given high fat and high sugar diet (by ^{60}Co irradiation). All mice drink normally. Three weeks later, took blood by tail-cutting and tested the non-fasting glucose. When mice were 7 weeks old, we applied intraperitoneal injections with STZ 125 mg/kg (0. 05 mol/L lemon acid buffer solution configuration) to group B, vehicle (0.05 mol/L, citric acid, pH4. 5, same volume of group B) to group A, STZ 125 mg/kg BW to group D, vehicle (same volume of group D) to group C. Group E, the mice in which were fed by regular chow was normal control group[6]. Then all mice drank normally for 4 weeks, then took blood by tail-cutting and tested the non-fasting glucose in each group at the1st, 2nd and 4th weekends of the 4 weeks mentioned above.

1.5.8 Experimental statistics

All the date was showed by mean ± standard deviation ($\overline{X}$ ±S), and analyzed the variance with statistics software SPSS10.0. Then we used the q test to reveal the comparison between groups. When $P < 0.05$, the difference was significant, when $P < 0.01$, there was extremely significant difference.

1.5.9Animal welfare

The mice were killed by cervical vertebra dislocation, through which the suffering can be ameliorated..

RESULTS

2.1 STZ dose screening

The results are shown in table 2 and figure 1

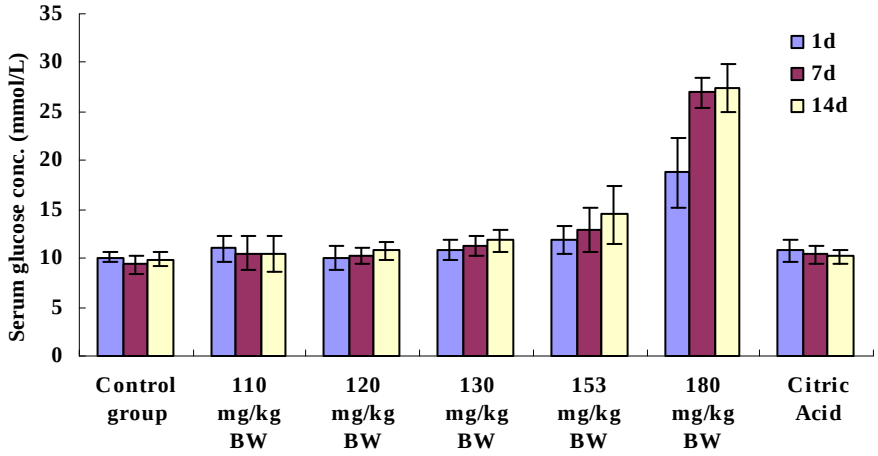

Figure 1 Serum glucose concentration before and after STZ injection（mmol/L）

Table 2 Serum glucose concentration before and after STZ injection（mmol/L）

	n	1d	7d	14d
Control group	15	10.12±0.57	9.35±0.86	9.85±0.72
110 mg/kg BW	15	10.99±1.31	10.48±1.72	10.53±1.85
120 mg/kg BW	15	10.02±1.16	10.23±0.89	10.84±0.92
130 mg/kg BW	15	10.86±0.97	11.27±1.05	11.83±1.12
153 mg/kg BW	15	11.86±1.35	12.95±2.27	14.45±3.02
180 mg/kg BW	15	18.73±3.56	26.92±1.47	27.36±2.45
Citric acid	15	10.82±1.12	10.35±0.86	10.14±0.68

From the results of this experiment and the blood glucose of the mice, we can see that the dose with130mg/kgBW is a little bit high, so that it may cause the damage of beta cell of islet. In the formal experiment we injected the mice with 125mg/kgBW STZ. The collocation is as follows: Placed 41.67mg STZ weighed with electronic balance in a 25ml breaker, and added 10ml prepared citric acid solution by using suction pipette into each breaker, then mixed evenly and filtered the STZ.

2.2 The changes of blood glucose levels before and after injection of STZ

At the end of the experiment 10 mice were dead. The results are shown in table 3 and figure 2.

Table3 Rat Serum glucose concentration before and after injection with STZ

Treatment	n	3rd Weekend (Pre.-STZ)	4th Weekend (1 week after i.p. STZ)	5th Weekend (2 weeks after i.p. STZ)	7th Weekend (4 weeks after i.p. STZ)
Control group	15	9.86±0.92	9.94±1.03	10.15±1.21	9.89±1.11
Normal- citric acid	14	9.94±0.89	10.11±1.07	9.61±1.61	9.82±1.13
Normal-STZ	12	10.04±1.27	10.37±1.14	11.15±1.47	10.72±1.25*
High-fat -citric acid	13	10.28±1.30*	10.73±1.24	10.55±1.07	10.27±1.45
High-fat-STZ	11	11.21±1.66*	11.47±2.94*	12.47±2.12*	12.92±2.91**

Compare with other groups: *P ＜0.05, ** P＜0.01

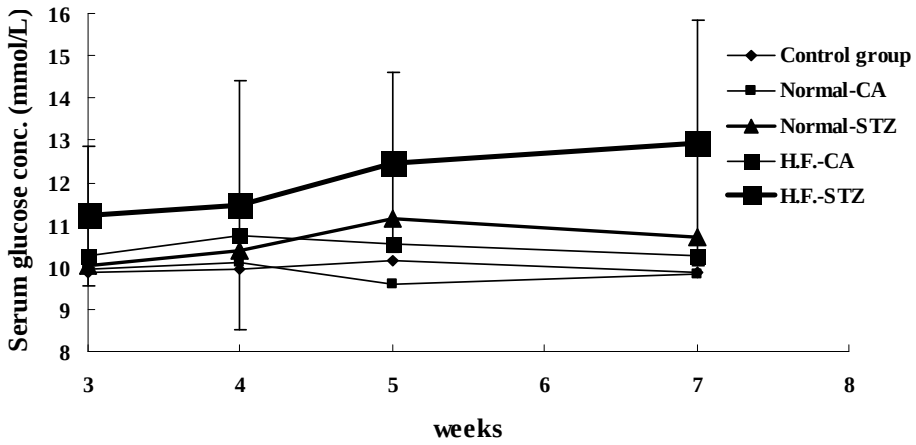

Figure 2 Change of rat serum glucose before and after i.p. STZ

At the 3rd, 4th, 5th, 7thweekends, the blood glucose values of mice in high-fat-STZ group were significantly higher than which in normal-STZ group at the same period. At the 5th and 7th weekends, the blood glucose values of mice in high-fat-STZ group were significantly higher than which in high-fat-citric acid group at the same period. At the 7th weekend, the blood glucose value of mice in normal -STZ group is significantly higher than which in the normal-citric acid group. Blood glucose value of mice in high- fat-STZ group has reached the standard of C57 mice non-fasting glucose.

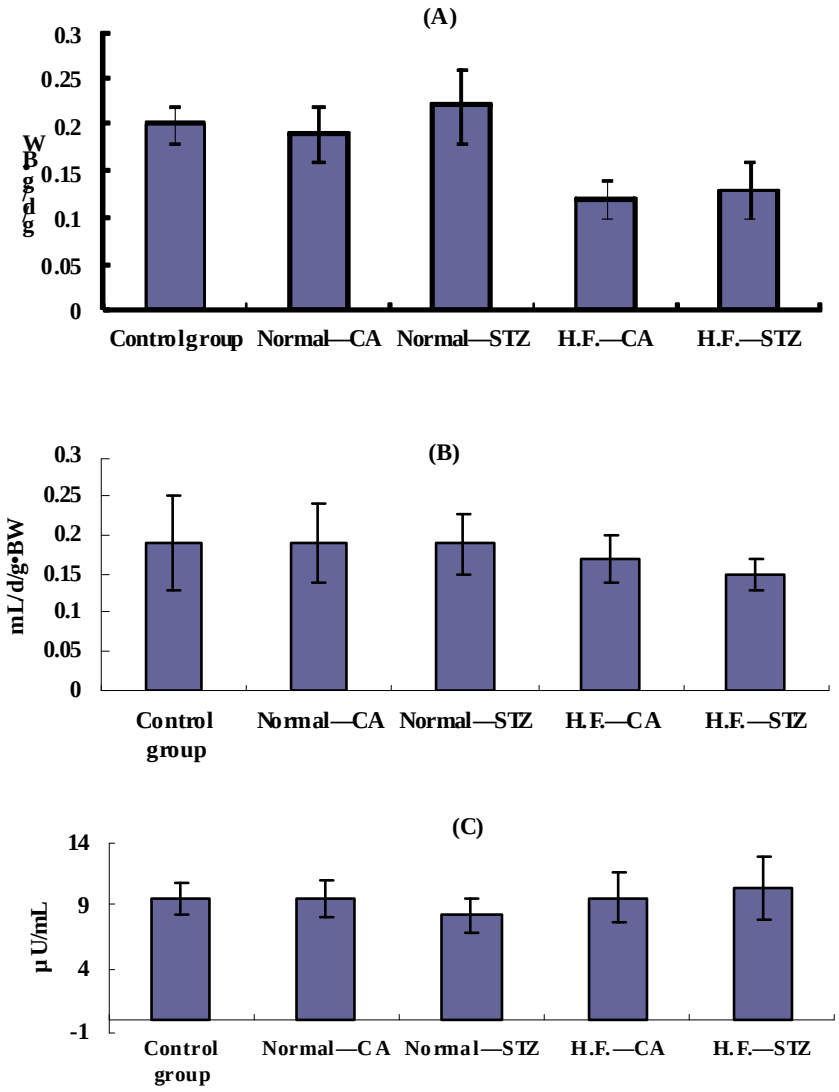

Figure 3 The intake of feed (A), water (B) and serum insulin concentration (C) of 4 groups at 7th week

2.3 The changes of food intake, water intake and serum insulin concentration
The results are shown in table 4 and figure 3.

Table 4 Food intake, water intake and serum insulin concentration

Treatment	n	Food intake (g/d/ g·BW)	Water intake (mL/d/g·BW)	Serum insulin (μU/mL)
Control group	15	0.20±0.02	0.19±0.06	9.60±1.23
Normal- citric acid	14	0.19±0.03	0.19±0.05	9.85±1.41
Normal-STZ	12	0.22±0.04	0.19±0.04	8.31±1.40
High-fat-citric acid	13	0.12±0.02	0.17±0.03	9.70±1.93
High-fat-STZ	11	0.13±0.03	0.05±0.02	10.38±2.46

Compare with other groups: *P ＜0.05, ** P＜0.01

From table 4, we can see that there was no significant difference in food intake and water intake between 4 groups and the serum insulin concentrations got no obvious deference.

2.4 The changes of body weight before and after injection with STZ
The results are shown in table 5 and figure 4.

Table 5 Body weight before and after injection with STZ

Treatment	n	3rd Weekend (Pre.-STZ)	4th Weekend (1 week after i.p. STZ)	5th Weekend (2 weeks after i.p. STZ)	7th Weekend (4 weeks after i.p. STZ)
Control group	15	23.46±0.82	24.05±0.89	24.62±1.03	26.53±1.17
Normal- citric acid	14	23.28±0.86	24.0±0.91	24.82±0.94	26.45±1.12
Normal-STZ	12	23.31±1.29	22.98±1.36	23.91±1.44	25.47±1.80
High-fat -citric acid	13	23.66±1.34	24.10±1.42	24.77±1.57	27.01±1.47
High-fat-STZ	11	23.69±1.41	23.28±1.45	24.16±1.50	25.88±1.89

Compare with other groups: *P ＜0.05, ** P＜0.01

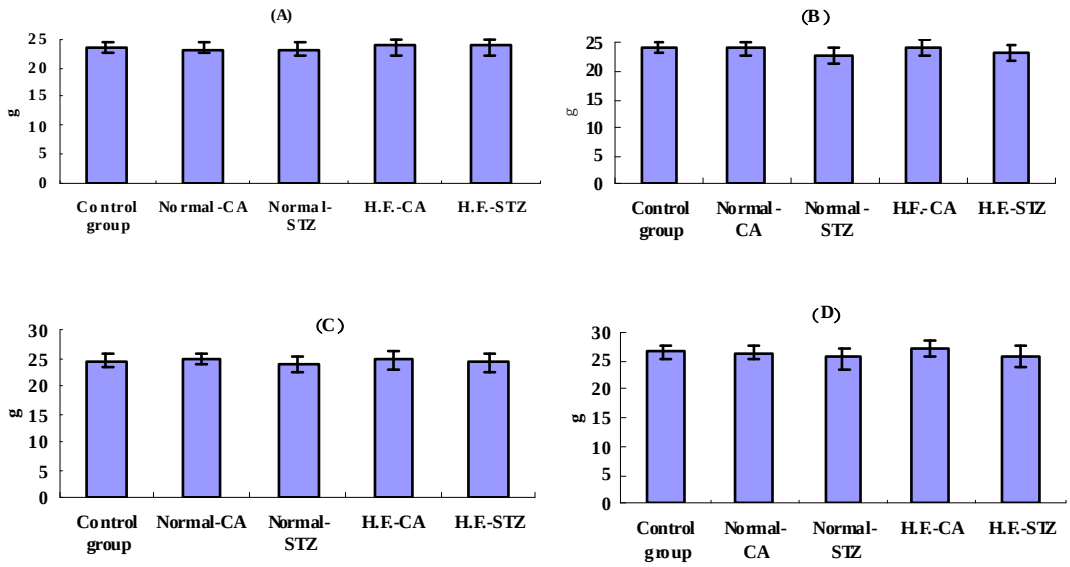

Fig.4 Body weight before and after injection with STZ. （A）3rd weekend (before i.p. STZ), （B） 4th weekend(1 week after i.p. STZ), （C） 5th weekend(2 weeks after i.p. STZ), （D）7th weekend(4 weeks after i.p. STZ)

From table 5, we can see that there was no significant difference in body weights between 4 groups. The weights of mice injected with STZ were relatively lower, but the differences were not obvious between groups.

DISCUSSION

Presently, there are mainly 4 kinds of animal model of II-type diabetes, including chemical damage caused, fattening caused, spontaneous caused and STZ and fat diet caused animal model. There are some problems with the former three methods, for example: it is difficult to control the STZ's dose when developing chemical damage caused model [7-8], fattening caused model has a high animal mortality (about 70% [9]), spontaneous caused model is scarce and expensive[10-12]. These problems limited the application of these methods in the study of NIDDM.

C57BL / 6J mouse model of II-type diabetes induced by High-fat diet and STZ is numerously studied in recent years, which is regarded as an ideal animal model of II-type diabetes. C57BL/6J mice, which are inbred mice with abdominal obesity and II-type diabetes genetic predisposition, possess ob/ob genetic background and they are sensitive to blood glucose rise caused by fat. Long-term high-fat and high-sugar diets can induce II-type diabetes which is similar with human NIDDM [13-15].

This research shows that blood glucose values of mice with STZ injected and fat fed are significantly higher than those in other groups, and reached the standard of non-fasting glucose diabetes (11.0 mmol/L), whereas there are no significant changes in serum insulin level appends. Some research reported that injecting STZ could lead the level of serum insulin to decrease sharply so that it cannot maintain the balance of blood glucose and finally get a hyperglycemia during the process of its growth. But this kind of model is not identical to clinical features [16-18]. According to this experiment and previous studies, high-fat feed and STZ are necessary to induce 2- type diabetes in C57BL/J mice. High-fat diet can enhance the sensitivity of mice to STZ and increase blood glucose and have no influence on islet function. This is consistent with morphology observing results of normal pancreatic tissue [19-22]. Some reports suggested that the increase of insulin level caused by fat-feeding was to overcome insulin resistance,

whereas STZ inhibited the ability to maintain high levels of insulin in the body, so serum insulin went down to normal level and the ability to balance blood glucose decreased significantly [23].

In existing reports about this method, the serum insulin turns to be obviously increase or non-increase. This research shows us that serum insulin levels have no obvious difference between experimental groups and normal groups. The increase of serum insulin mentioned in reports above should be relevant to the prescription of the high-fat chow or the dose of STZ. If the percentage of fat was too high in chow, animal could be excessively fat and β-cells of islet could secrete insulin excessively which led a stronger resistance to insulin and finally caused a hyperinsulinemia. In this paper, the proportion of chows is as follows: fat 30%, protein 27.5%, while in mentioned reports: fat 61%, protein 45.5%, etc. If the dose of STZ was too low, serum insulin would increase. After comprehensive analysis, we suggest that the increase of serum insulin levels should happen after the middle stage of II-type diabetes, and during the early onset, the blood glucose increased while insulin levels maintained in the normal range, this is consistent with the actual onset condition of human NIDDM patient.

During the whole experiment, the blood glucose of high-fat fed and STZ injected mice rose slowly, while the increase of body weight was similar to the control group, food intake and water intake had no obvious increase. At the end of the experiment, the blood glucose levels of C57BL/6J mice were high while insulin levels stayed normal. The whole process of metabolic changes slowly and is very close to natural pathogenesis of human NIDDM patients.

CONCLUSION

High fat diet and STZ are necessary to establish a NIDDM model, the blood glucose of mice with high-fat feed and STZ injected rises slowly while the weight gain, the food and water intake having no significant changes compared to the control group. At the end of the experiment, the blood glucose of C57BL/6J mice comes to high level while insulin's normal. Besides, the whole process of metabolic changes slowly and is very close to natural pathogenesis of human NIDDM patients.

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