• Basic Research •

Establishment of blood glucose control model in diabetic mice

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Abstract

● **AIM:** To explore the established method of the diabetic mouse blood glucose control model and preliminary observation of its influence on the retinas of diabetic mice.

● **METHODS:** The db/db BKS-DB ($Lepr^{ko/ko}$) mice were randomly divided into two groups: the poor blood glucose control group (PG group, *n*=18) and the stable blood glucose control group (SG group, *n*=12), with BKS-DB ($Lepr^{wt/wt}$) as the normal blood glucose control group (NG group, *n*=18). According to the blood glucose values for 5 intervals which were monitored during the period of adaption, the PG group was injected with insulin aspart twice daily, fasted for 2h and then returned to normal. The SG group was injected with insulin aspart twice and insulin glargine once daily and fed with a quantitative ration. Fundus images were collected after eight weeks. The glycosylated hemoglobin (HbA1c), mean blood glucose level (MBG), standard deviation of blood glucose (SDBG), coefficient of variation of blood glucose (CVBG), and mean amplitude of glycemic excursion (MAGE) in each group were examined and calculated.

● **RESULTS:** The HbA1c, MBG, SDBG, CVBG, and MAGE levels in the PG group were significantly higher than those in the NG and SG groups (all *P*<0.05). MBG, SDBG, CVBG, and MAGE levels in the SG group were higher than those in the NG group (all *P*<0.05). There was no significant difference in HbA1c levels between the NG and SG groups (*P*>0.05). Preliminary observation of fundus images in the PG group and SG groups showed scattered retinal bleeding spots, while bleeding was more obvious in the PG group.

● **CONCLUSION:** The blood glucose control model of type 2 diabetes mellitus mice can be successfully established by subcutaneous injection of insulin aspart insulin glargine and rationed food, which is valuable for studying the mechanism of blood glucose fluctuations in diabetic complications *in vivo*.

● **KEYWORDS:** type 2 diabetes; blood glucose control; animal models

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INTRODUCTION

Type 2 diabetic mellitus (T2DM) is an epidemic that affects more than 246 million people worldwide[1]. Diabetic retinopathy (DR) is a common microvascular complication of diabetes[2]. The risk of microvascular complications is significantly higher in uncorrected glycemic control than in stable control[3]. In our clinical work, we have found that many patients with diabetes have poor blood glucose control and large fluctuations in blood glucose levels. Moreover, the results of the United Kingdom Prospective Diabetes Study showed that there was a significant microvascular benefit in reducing glycosylated hemoglobin (HbA1c) to 7% after hypoglycemic treatment. A follow-up study also found that the risk of macroangiopathy and microangiopathy in the hypoglycemic group was significantly reduced, indicating a benign metabolic memory effect[4]. It was formerly reported that many animal models of T2DM have been established, including spontaneous diabetic mouse models, experimental type 2 diabetic mouse models and genetic engineering diabetic mouse models[5-9]. However, previous animal models have been inconsistent with the characteristics of blood glucose fluctuations in patients and the purpose of treatment. Most animal studies on diabetes have focused on the effects of chronic persistent or acute hyperglycemia. Few studies have focused on models of blood glucose fluctuations in diabetic mice[10-12]. In addition, most animal models are type

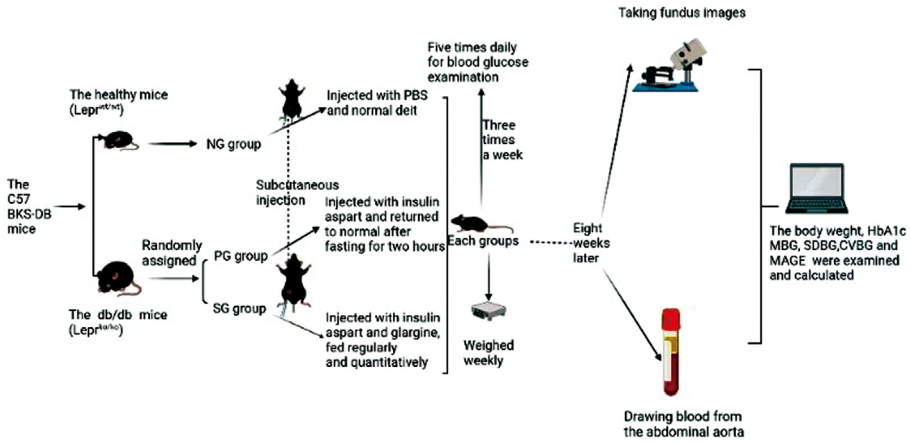

Figure 1 A brief description of the experimental design for an animal model.

1 diabetes mellitus (T1DM), while T2DM accounts for 90%–95% of all cases of diabetes mellitus[13]. Furthermore, some drug studies have improved glycemic control in db/db mice, but the exact level of glycemic control has not been specified[14]. Therefore, we aimed to establish a reproducible model of poor and stable blood glucose levels in diabetic mice, which is more reflective of the characteristics of blood glucose fluctuations in patients with diabetes in clinical practice. Our study provides a basis for further research on the impact and treatment of glycemic control on the underlying pathogenesis and treatment of diabetic microangiopathy.

MATERIALS AND METHODS

Ethical Approval All animal experiments strictly adhered to the provisions and general recommendations of the Chinese Laboratory Association and were fully abided by the Institutional Animal Care and Committee of Anhui Medical University.

Animals Thirty db/db mice [C57 BKS-DB ($Lepr^{ko/ko}$), male, 8 weeks old] and eighteen healthy normal control mice [C57 BKS-DB ($Lepr^{wt/wt}$), male, 8 weeks old] were obtained from Gempharmatech Co., Ltd. All the mice were housed in a well-ventilated environment. The animal room was kept on a 12-hour light/dark cycle at a constant temperature (23±3)°C and relative humidity of 55%–65% throughout the experimental period, with free access to water.

Experimental Design for Animal Model To understand the fluctuation of daily blood glucose in diabetic mice, six db/db mice and six normal mice were randomly selected five times daily (8:00, 10:00, 12:00, 16:00, and 18:00) for blood glucose examination by tail sampling using a glucometer (Roche, Germany). The db/db mice were randomly divided into two groups: the poor blood glucose group (PG group, *n*=18) and the stable blood glucose group (SG group, *n*=12), with BKS-DB ($Lepr^{wt/wt}$) as the normal blood glucose control group (NG group, *n*=18). The mice in the NG group had *ad libitum* access to a normal diet and were subcutaneously injected with phosphate buffered solution (PBS). Mice in the PG group received 6–8 U insulin aspart (Novo Nordisk, Denmark) subcutaneous injection each day at 8:00 and 16:00, and fasting for 2h. Mice in the SG group received 4–8 U insulin aspart subcutaneous injection at 8:00 and 12:00 daily, 6–10 U insulin glargine (Sanofi, France) *via* subcutaneous injection at 16:00, and were fed regularly and quantitatively (2–5 g/animal). All mice had access to water *ad libitum*. Blood glucose concentration was measured by tail sampling using a glucometer at 8:00, 10:00, 12:00, 16:00, and 18:00 three times a week. During the experiment, mice were weighed weekly. After 8wk, the mice were anesthetized with 10% chloral hydrate solution and the abdominal aorta was bled and preserved (Figure 1).

Determination of Indexes We used the mean blood glucose level (MBG), standard deviation of blood glucose (SDBG), coefficient of variation of blood glucose (CVBG), and mean amplitude of glycemic excursion (MAGE) to quantify the blood glucose stability of mice in each group. After 8wk, body weight, MBG, SDBG, CVBG, and MAGE in each group were calculated and analyzed. Fundus photos of the three groups of mice were automatically obtained using ultra-wide field scanning laser ophthalmoscopy (Optomap, Scotland, UK). The HbA1c content (expressed as absorbance per 10 g hemoglobin) of the preserved post-mortem blood was measured using an HbA1c kit (Nanjing Jiancheng Biological Engineering Research Institute, China).

Computational formula: $Mean=(x_1+x_2+x_3.....x_n)/n$, $SD=\sqrt{[\Sigma(xi-x)^2/(n-1)]}$, $CV=SD/Mean$, $MAGE=\Sigma\lambda/x$, if $\lambda/x>v$, where λ is the blood glucose change from peak to nadir, x is the number of valid observations, and v is 1 SD of the mean glucose for a 24-hour period.

Statistical Analysis All data were statistically analyzed using SPSS 25.0 (SPSS, Inc, Chicago, Ill, USA). Normality of the data was assessed using the Shapiro-Wilk test. All data are shown as mean±SD. For the measurement data conforming to the normal distribution, the one-way ANOVA was used for the comparison between groups, and the LSD test was used

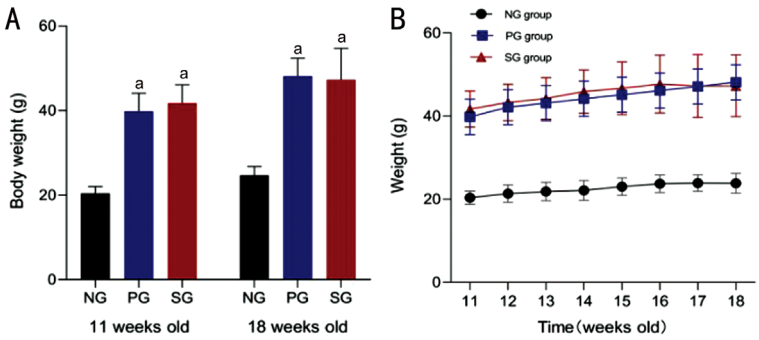

Figure 2 Changes in body weight of mice among three groups before, after and during modeling A: The mean weight at 11 and 18 weeks old; B: Evolution of body weight in grams. The results of comparison between groups showed that there was significant difference in body mass between the NG, PG, and SG groups (P<0.0001), but not between the PG and SG group (P>0.05). [a]P<0.0001. NG: The normal blood glucose control; PG: The poor blood glucose; SG: The stable blood glucose.

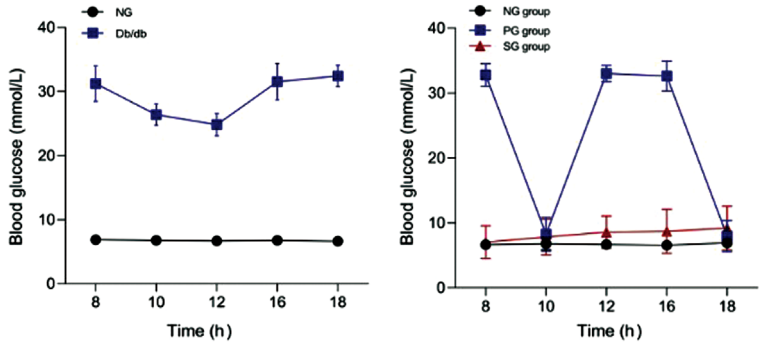

Figure 3 The daily blood glucose trend in db/db mice and controls before modeling (A) and in three groups after modeling (B). NG: The normal blood glucose control; PG: The poor blood glucose; SG: The stable blood glucose.

for multiple comparisons between groups. Differences were considered statistically significant at P<0.05.

RESULTS

General Symptoms Mice in the NG group had a normal diet, silky hair, and agile movement. Compared to the NG group, mice in the PG and SG groups showed mental depression, slow movement, negative response, dull hair, high water intake and urine output, aggravated urine smell in all mice, and urinary tract infection in some mice. Symptoms in the PG group were more obvious, and some mice showed crystal opacity. After 8wk, one mouse in the NG group, two in the PG group, and one in the SG group died.

Body Weight Initially, the mean body weight of the NG group was 20.38±1.62 g and the db/db group was 40.58±4.32 g. At the end of the study, the mean body weights were 23.85±2.38 g (NG group), 48.14±4.22 g (PG group) and 47.28±2.24 g (SG group; Figure 2A). During the experiment, the mean body weights of the NG and PG groups increased continuously (Figure 2B). In the SG group, the mean body weight increased during the first five weeks and then slowly decreased (Figure 2B).

Glycaemia Measurements Before modeling, six db/db mice and six normal control mice were randomly selected and 5 moments (8:00, 10:00, 12:00, 16:00, and 18:00) for blood glucose examination by tail sampling using a glucometer (Roche, Germany) for one week. The MBG of the NG group was 6.71±0.28 mmol/L and the db/db group was 29.28±3.75 mmol/L (Figure 3A). Blood glucose levels were high level at 8:00, 10:00, 16:00, and 18:00 in db/db mice. Blood glucose displayed a downward trend after 8:00, dropped to the lowest point at noon, and then showed a gradual upward trend. The highest level of blood glucose at 18:00 and the lowest point at 12:00. Normal control mice remained stable. During the experiment, each group of mice was treated with different insulin and diets, and blood glucose fluctuations varied (Figure 3B). After modelling, the blood glucose value of the PG group was high at 8:00, decreased to the lowest point at 10:00, increased to the highest level at 12:00, maintained until 16:00, and then decreased to the lowest level at 18:00. The blood glucose level in the SG group was the lowest at 8:00, increased to 10:00, and then maintained at a relatively stable level until 18:00. The blood glucose levels of the mice in the NG group remained stable.

Comparison of Glucose Variability Parameters Among the Three Groups According to the results in Table 1, the MBG, SDBG, CVBG, and MAGE in the PG group were significantly higher than those in the SG and healthy NG groups (all P<0.0001; Figure 4A–4E). MBG, SDBG, CVBG, and MAGE in the SG group were higher than those in the NG group (all P<0.0001; Figure 4A–4E). HbA1c levels in the PG group were significantly higher than those in the SG and NG groups (P<0.05). However, there was no significant difference between the SG and healthy NG groups (P>0.05; Figure 4F).

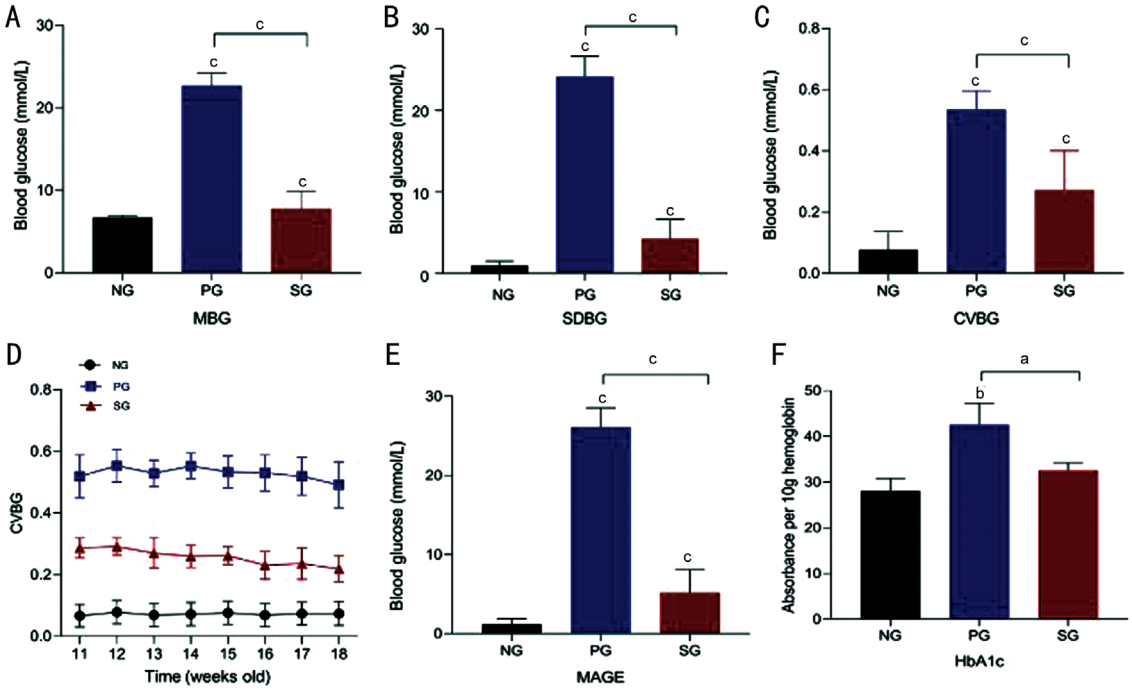

Figure 4 Comparison in glucose variability parameters among three groups of mice 8wk after modeling The MBG (A), SDBG (B), and CVBG (C) in each group and weekly fluctuation of CVBG in each group (D). E: MAGE in each group. These indexes in the PG group were significantly higher than those in the SG and NG groups (P<0.0001). MBG, SDBG, CVBG, and MAGE in the SG group were higher than those in the NG group (all P<0.0001). F: Level of HbA1c in each group. HbA1c in the PG group was significantly higher than that in the SG and healthy NG groups (P<0.05). There was no difference between the SG and healthy NG groups (P>0.05). All data are expressed as mean±SD. [a]P<0.05, [b]P<0.01, [c]P<0.0001. NG: The normal blood glucose control; PG: The poor blood glucose; SG: The stable blood glucose; MBG: Mean blood glucose level; SDBG: Standard deviation of blood glucose; CVBG: Coefficient of variation of blood glucose; MAGE: Mean amplitude of glycaemic excursion; HbA1c: The glycosylated hemoglobin.

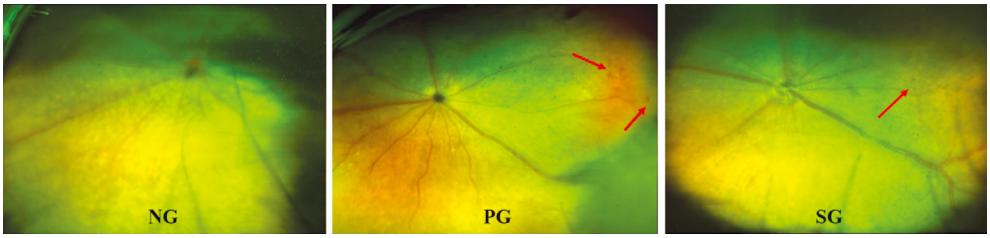

Figure 5 Scattered retinal bleeding points can be observed in the wild-field fundus photos of mice in the PG group and SG group, which is more obvious in the PG group PG: The poor blood glucose; SG: The stable blood glucose.

Table 1 Comparison of glucose variability parameters among the three groups after eight weeks of modeling

Parameters	NG (n=18)	PG (n=18)	SG (n=12)	F	P
MBG (mmol/L)	6.69±0.25	23.05±1.23	7.84±2.11	7935.68	<0.0001[a]
SDBG (mmol/L)	0.48±0.26	12.04±1.09	2.18±1.24	10199.76	<0.0001[a]
CVBG (%)	7.14±3.71	52.50±6.16	27.71±12.70	3526.48	<0.0001[a]
MAGE (mmol/L)	1.17±0.69	25.93±2.30	5.32±2.97	7274.83	<0.0001[a]
HbA1c (%)	28.06±2.71	42.54±4.6	32.53±1.67	15.43	<0.05[b]

NG: The normal blood glucose control; PG: The poor blood glucose; SG: The stable blood glucose; MBG: Mean blood glucose level; SDBG: Standard deviation of blood glucose; CVBG: Coefficient of variation of blood glucose; MAGE: Mean amplitude of glycaemic excursion; HbA1c: The glycosylated hemoglobin. [a]Multiple comparisons are tested using One-way ANOVA with LSD test, NG<PG (P<0.0001), NG<SG (P<0.0001), SG<PG (P<0.0001); [b]Multiple comparisons are tested using One-way ANOVA with LSD test, NG<PG (P=0.002), NG<SG (P=0.145), SG<PG (P=0.011).

Fundus Photography Preliminary observation of retinal lesions associated with glucose fluctuation was performed using wide-field fundus photography of the mice in each group. Mice in the PG and SG groups showed scattered retinal bleeding spots, which were more obvious in the PG group (Figure 5).

DISCUSSION

In this study, we established a model that is consistent with the characteristics of blood glucose fluctuations in patients with diabetes in clinical practice.

The db/db mouse model is the most pertinent model for early and late glycation reactions of diabetic patients[5]. The db/db mice are not only obese but also hyperglycemic and intolerant to glucose. This is consistent with the results for weight and MBG in our experiments. The MBG results of our experiments showed that, initially, the healthy group had lower body weight and lower MBG than the db/db group. Previous studies have shown that the db/db mice are ideal animal models for exploring T2DM[15-16]. During the experiment, the mean weight of the NG and PG groups increased continuously. In the SG group, the mean weight increased during the first 5wk and then decreased slowly. However, the difference in weight between the PG and SG was not statistically significant. Previously published studies have shown that insulin therapy can significantly increase the body weight of db/db mice and may lead to insulin resistance[17]. In our study, the body mass ascended and then descended during the modeling process, considering that it was due to the short period of insulin glargine treatment or possibly as a result of our controlled diet. In addition, we noticed that subjects in the PG group responded more slowly or were even unresponsive to the outside world than those in the SG and NG groups. For instance, subjects in the NG group had evident feeding movements, while those in the PG had no obvious feeding movements when fed, and those in the SG group were intermediate. However, we did not perform an objective experiment for quantification. Follow-up experiments were considered to include a tail suspension test or a forced swimming test to test whether the mice had depressive behaviors.

Insulin is usually adopted clinically in combination with short-acting and long-acting preparations for diabetic treatment; Therefore, we used short-acting preparations to control postprandial hyperglycemia in the SG group in the pre-experiment and maintained their blood glucose levels between meals and at night with long-acting preparations. Moreover, in the PG group, medium-and long-acting insulin can keep the subjects' blood glucose constant throughout the day while lowering the glucose, which can prevent fluctuations in blood glucose levels. Therefore, we chose short-acting insulin to establish a fluctuating blood glucose model. Our pre-experiment revealed that the blood glucose levels of subjects in the SG group could not be stably maintained by long-acting plus short-acting insulin only, and the blood glucose fluctuation of those in the PG group was slight after treatment with short-acting insulin only. The nature of the unrestrained diet in db/db mice was also considered. Therefore, we added a diet control.

To assess the variability in blood glucose levels, we selected a more representative and simple calculation of MBG, SDBG, MAGE, and HbA1c[18-24]. Wu *et al*[12] showed that blood glucose in the poor glycemic control group was higher at 10:30, with a downward trend to the lowest level at 12:30; blood glucose continued to rise to a peak at 14:30, then dropped to 16:30, and then rose again. Wu *et al*[12] and Wu *et al*[25] both formed two significant intraday peaks and troughs. This was consistent with our study of blood glucose fluctuations in the PG group. The difference is that, first, all the above researchers used the hyperglycemic group as the control group. In addition, in our study, the MBG in the PG group fluctuated greatly throughout the day, exceeding 20 mmol/L between the highest and lowest points. The fluctuation in blood glucose in the PG group was more obvious than that in the NG and SG groups. The MBG of the SG group was maintained at approximately 10 mmol/L. The results of this study showed statistically significant differences in MBG, SDBG, CVBG, and MAGE between the three groups. Furthermore, we found that HbA1c levels in the PG group were higher than those in the NG and SG groups, whereas there was no difference between the NG and SG groups. This is different from the results of previous studies[12,25]. Fundus photography of the three groups showed that there were more retinal bleeding spots in the PG group than in the NG and SG groups. This result suggests three possible explanations. First, HbA1c is the product of the non-enzymatic glycation of hemoglobin, reflecting the previous 8–12-week average blood sugar level. This indicates that although the differences in blood glucose levels between the SG and NG groups were significant in the short term, glycemic control was good and meaningful in the long term. Second, poor blood glucose control can accelerate the progression of DR. Third, the method used in this study is meaningful for establishing a model of adverse and stable blood glucose in diabetic mice.

Two main methods for generating blood sugar fluctuations in diabetes models have been reported in the literature: simple intraperitoneal injection of high-dose streptozotocin (STZ) and a high-fat diet combined with low-dose intraperitoneal injection of STZ. Its main features are as follows: First, a simple intraperitoneal injection of high-dose STZ: the STZ injection model has the advantage of lower cost and has been widely used in experiments for making a model of T1DM. In contrast, according to a previous study, only 50% of the mice developed severe diabetes approximately 3wk after STZ injection, indicating that this method has a low success rate[26]. Furthermore, some animals die rapidly after STZ treatment due to fatal hypoglycemia caused by massive islet β-cell necrosis and the sudden release of insulin. Moreover, there is no consensus on the optimal dose of STZ, and different animal

strains have different sensitivities to STZ[26]. Second, diabetes models induced by hyperlipidemia and STZ are commonly used to induce T2DM in animal models. Hyperlipidemic diets associated with a certain dose of STZ showed positive results in establishing T2DM, but there were variations in diet composition, STZ doses, animal species, and age[27]. As mentioned previously, the db/db mice are ideal animal models for the exploration of T2DM[15-16]. Our mice more closely resembled the characteristics of clinical type 2 diabetes, with high success rates and low mortality rates. In contrast, most previous studies on modeling blood glucose fluctuations were performed with intraperitoneal or oral glucose and intraperitoneal insulin injections[11-12,28-29]. The control group consisted primarily of the normal and hyperglycemia groups. Our main advantage is that we chose to control glycemic fluctuations and maintain its stability with insulin and a controlled diet, with the main idea of excluding the effect of a high-fat and high-sugar diet on the results compared to previous studies. In addition, it is more consistent with the blood glucose fluctuations in clinical patients. The results showed that blood glucose was significantly higher and more volatile in the PG group, consistent with previous studies[12,25]. The differences in HbA1c levels between the NG and SG groups were not significant. The SG group had glycemic characteristics similar with those of clinical diabetic patients who had better glycemic control after standard treatment.

Based on the above description, the models in this study have the following advantages: first, the mouse models are more consistent with clinical blood glucose control. Second, the PG group had a remarkably increased and greatly fluctuating blood glucose level, whereas the SG group had better glycemic control. Third, it has lower mortality, a higher success rate, and better reproducibility. Naturally, our model has several limitations in our model, First, we only selected male mice for our experiments, but sex may influence the development of obesity in mice. And male mice tend to gain more weight and fat weight than females[30], which will be considered in future studies. Second, the mechanism by which blood glucose fluctuations influence DR remains unclear. We will do further study in follow-up experiments.

In conclusion, a blood glucose control model of T2DM mice can be successfully established by subcutaneous injection of insulin aspart, insulin glargine, and rationed food, which will be valuable for studying the mechanism of blood glucose fluctuation in diabetic complications *in vivo*. This should draw our attention in the future.

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Conflicts of Interest: Rao CH, None; **Liu L,** None; **Gao J,** None; **Du ZH,** None; **Gao C,** None.

REFERENCES

1 Tinajero MG, Malik VS. An update on the epidemiology of type 2 diabetes: a global perspective. *Endocrinol Metab Clin North Am* 2021;50(3):337-355.

2 Saeedi P, Petersohn I, Salpea P, *et al*, IDF Diabetes Atlas Committee. Global and regional diabetes prevalence estimates for 2019 and projections for 2030 and 2045: results from the International Diabetes Federation Diabetes Atlas, 9th edition. *Diabetes Res Clin Pract* 2019;157:107843.

3 Dandona P. Minimizing glycemic fluctuations in patients with type 2 diabetes: approaches and importance. *Diabetes Technol Ther* 2017;19(9):498-506.

4 United Kingdom Prospective Diabetes Study (UKPDS). 13: Relative efficacy of randomly allocated diet, sulphonylurea, insulin, or metformin in patients with newly diagnosed non-insulin dependent diabetes followed for three years. *BMJ* 1995;310(6972):83-88.

5 Guilbaud A, Howsam M, Niquet-Léridon C, Delguste F, Boulanger E, Tessier FJ. The $LepR^{db/db}$ mice model for studying glycation in the context of diabetes. *Diabetes Metab Res Rev* 2019;35(2):e3103.

6 Kowluru RA. Retinopathy in a diet-induced type 2 diabetic rat model and role of epigenetic modifications. *Diabetes* 2020;69(4):689-698.

7 Nagao M, Esguerra JLS, Wendt A, Asai A, Sugihara H, Oikawa S, Eliasson L. Selectively bred diabetes models: GK rats, NSY mice, and ON mice. *Methods Mol Biol* 2020;2128:25-54.

8 Lutz TA. An overview of rodent models of obesity and type 2 diabetes. *Methods Mol Biol* 2020;2128:11-24.

9 Pandey S, Dvorakova MC. Future perspective of diabetic animal models. *Endocr Metab Immune Disord Drug Targets* 2020;20(1):25-38.

10 Hsieh CF, Liu CK, Lee CT, Yu LG, Wang JY. Acute glucose fluctuation impacts microglial activity, leading to inflammatory activation or self-degradation. *Sci Rep* 2019;9:840.

11 Wu N, Shen HT, Liu HN, Wang YJ, Bai Y, Han P. Acute blood glucose fluctuation enhances rat aorta endothelial cell apoptosis, oxidative stress and pro-inflammatory cytokine expression *in vivo*. *Cardiovasc Diabetol* 2016;15(1):109.

12 Wu WH, Chai Q, Zhang ZY. Glucose fluctuation accelerates cardiac injury of diabetic mice via sodium-dependent glucose cotransporter 1 (SGLT1). *Arch Biochem Biophys* 2021;709:108968.

13 Holman N, Young B, Gadsby R. Current prevalence of Type 1 and Type 2 diabetes in adults and children in the UK. *Diabet Med* 2015;32(9):1119-1120.

14 Kim HK, Jeong J, Kang EY, Go GW. Red pepper (capsicum annuum L.) seed extract improves glycemic control by inhibiting hepatic gluconeogenesis via phosphorylation of FOXO1 and AMPK in obese diabetic db/db mice. *Nutrients* 2020;12(9):2546.

15 Guest PC, Rahmoune H. Characterization of the db/db mouse model of type 2 diabetes. *Methods in Molecular Biology*. New York, NY: Springer New York, 2018:195-201.

16 Wang BX, Chandrasekera PC, Pippin JJ. Leptin- and leptin receptor-deficient rodent models: relevance for human type 2 diabetes. *Curr Diabetes Rev* 2014;10(2):131-145.

17 Ye X, Qi J, Wu Y, Yu D, Xu P, Li S, Zhu S, Wu Q, Ren G, Li D. Comparison of PEGylated FGF-21 with insulin glargine for long-lasting hypoglycaemic effect in db/db mice. *Diabetes Metab* 2015;41(1):82-90.

18 Zheng MY, Luo YT, Lin W, Khoja A, He Q, Yang SQ, Zhao X, Hu P. Comparing effects of continuous glucose monitoring systems (CGMs) and self-monitoring of blood glucose (SMBG) amongst adults with type 2 diabetes mellitus: a systematic review protocol. *Syst Rev* 2020;9(1):120.

19 Silver B, Ramaiya K, Andrew SB, Fredrick O, Bajaj S, Kalra S, Charlotte BM, Claudine K, Makhoba A. EADSG guidelines: insulin therapy in diabetes. *Diabetes Ther* 2018;9(2):449-492.

20 Chehregosha H, Khamseh ME, Malek M, Hosseinpanah F, Ismail-Beigi F. A view beyond HbA1c: role of continuous glucose monitoring. *Diabetes Ther* 2019;10(3):853-863.

21 Sun B, Luo ZY, Zhou JC. Comprehensive elaboration of glycemic variability in diabetic macrovascular and microvascular complications. *Cardiovasc Diabetol* 2021;20(1):9.

22 Piona C, Marigliano M, Mozzillo E, *et al*. Relationships between HbA1c and continuous glucose monitoring metrics of glycaemic control and glucose variability in a large cohort of children and adolescents with type 1 diabetes. *Diabetes Res Clin Pract* 2021;177:108933.

23 Rodbard D. Glucose variability: a review of clinical applications and research developments. *Diabetes Technol Ther* 2018;20(S2):S25-S215.

24 Kovatchev BP. Metrics for glycaemic control—from HbA1c to continuous glucose monitoring. *Nat Rev Endocrinol* 2017;13(7):425-436.

25 Wu YJ, Wu YB, Zheng SG, Wang YF, Liu XL, Zhang XJ, Lv MG. Experimental research on establishing a blood glucose fluctuation model in diabetic rats. *Nan Fang Yi Ke Da Xue Xue Bao* 2014;34(6):771-776.

26 Furman BL. Streptozotocin-induced diabetic models in mice and rats. *Curr Protoc* 2021;1(4):e78.

27 Atanasovska E, Tasic V, Slaninka-Miceska M, *et al*. Six week follow-up of metabolic effects induced by a high-fat diet and streptozotocin in a rodent model of type 2 diabetes mellitus. *Pril* (*Makedon Akad Nauk Umet Odd Med Nauki*) 2014;35(1):169-179.

28 Wang B, Smyl C, Chen CY, Li XY, Huang W, Zhang HM, Pai VJ, Kang JX. Suppression of postprandial blood glucose fluctuations by a low-carbohydrate, high-protein, and high-omega-3 diet via inhibition of gluconeogenesis. *Int J Mol Sci* 2018;19(7):1823.

29 Ying CJ, Liu T, Ling HW, *et al*. Glucose variability aggravates cardiac fibrosis by altering AKT signalling path. *Diab Vasc Dis Res* 2017;14(4):327-335.

30 Nishikawa S, Yasoshima A, Doi K, Nakayama H, Uetsuka K. Involvement of sex, strain and age factors in high fat diet-induced obesity in C57BL/6J and BALB/cA mice. *Exp Anim* 2007;56(4):263-272.