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Comparison of fasting overnight and 6 h in diagnosing diabetic mice

Hua Luo[1, 2], Feng Xue[3*]

[1]Department of Orthopaedics, Taizhou Hospital of Zhejiang Province affiliated to Wenzhou Medical University, Taizhou, Zhejiang 317000, China, [2]Department of Laboratory Medicine & Central Laboratory, Fengxian District Central Hospital, Shanghai 201499, China, [3]Department of Orthopaedics, Fengxian District Central Hospital, Shanghai 201499, China

xuefengfxzx@163.com

Abstract. **Objectives:** Diabetes mellitus has been one of the most common human diseases, causing a great number of complications, has been studied widely. Diabetic animal models are essential to understand the pathophysiological mechanisms and treatment of diabetes. This study set out to assess the different fasting times in diagnosing diabetic mice. **Methods:** The diabetic mice were made by injecting intraperitoneally with STZ. The intraperitoneal glucose tolerance tests were used to identify. The blood glucose was measured separately after fasting overnight and 6 h. All data were analyzed by Statistical Product and Service Solutions and $P<0.05$ was considered to be significant. **Results:** After injecting STZ, the mice appeared diabetic symptom, the IPGTTs and immunofluorescence confirmed the diabetic mice model made successfully. The fasting blood glucose after fasting 6 h was higher than fasting overnight ($P<0.05$), and the diagnostic accuracy of fasting for 6 hours was higher than that of fasting overnight ($P<0.05$). **Conclusions:** Fasting 6 h is more suitable for diabetes diagnosis than fasting overnight. It may be used as a criterion of fasting blood glucose in diabetic mice.

Keywords. Fasting blood glucose; fasting time; diagnosis; diabetic mice

Introduction

Diabetes mellitus, a growing non-communicable disease with significant complications, poses enormous challenges to the healthcare industry worldwide, especially in the current millennium [1-3]. The management of diabetes and its complications enforce an enormous economic burden on nations and their healthcare systems [4-6]. According to the International Diabetes Federation Diabetes Atlas of 2017, the number of adult diabetes patients worldwide has reached 451 million [7]. This number will increase globally due to population growth, aging populations, urbanization and sedentary lifestyle and high prevalence of obesity [8]. The occurrence of various acute and chronic complications has also increased significantly, leading to an increase in disability and a significant decrease in quality of life [9].

At present, diabetes has attracted many researchers' attention. They study the mechanism of diabetes, treatment, prevention of complications, etc. [10]. Limitations of human research, many researchers choose the diabetic animal model. In the study of diabetic animals, we have

to confirm whether the animals are successfully modeled after modeling. Fasting blood glucose is commonly used to diagnose diabetes, and usually animals are fasting overnight [11-13]. However, the mice are nocturnal feeders [14], an overnight fast can result in lower blood glucose. There is insufficient experimental evidence to explore the best fasting time.

In this study, we compared the accuracy of fasting overnight and fasting 6 h to diagnose diabetes. We specifically hypothesized that fasting 6 h is more suitable than fasting overnight for diagnosing diabetes.

Material and Methods

Experimental diabetic mice.

A total of 18 four-week-old C57BL/6 male mice weighing 18-20 g were obtained from Shanghai Jie SiJie Laboratory Animal Co. Ltd. (Shanghai, China). The mice were housed in polypropylene cages at a 40–50% relative humidity and an ambient temperature of 23±2 ℃. A 12-hour on/off light cycle was maintained in the room. The Institutional Animal Ethics Committee approved all animal care and experiments of Taizhou Hospital (Approval number: tzy-2019063).

Induction of diabetic mice

After 1 week acclimatizing to the new vivarium, the mice were injected intraperitoneally with 50 mg/kg of streptozotocin (STZ; Sigma-Aldrich St. Louis, MO) dissolved in 0.1 M sodium citrate buffer for 5 consecutive days. Non-diabetic control mice received an intraperitoneal injection of sodium citrate buffer alone. Blood glucose was measured using a blood glucose meter (FreeStyle, Abbot, USA), and diabetes was defined as fasting blood glucose ＞11.1 mmol/L.

Intraperitoneal glucose tolerance tests (IPGTTs)

IPGTTs were performed to diagnose diabetes at 2 weeks later after treatment. Mice were fasted for at least 6 h before the test, and basal blood samples were measured. Glucose load of 2 g/kg, i.p. were administered immediately. Blood samples were obtained from the tail vein at different time points (30, 60 120 min) after glucose loading. Meanwhile, blood glucose was measured by using a blood glucose meter.

Fasting blood glucose (FBG)

All the diabetic mice were studied at 1 p.m. after a fast of 6 h (food withdrawn at 7 a.m.). The blood samples for FBG were obtained by tail cut method and estimated immediately by FreeStyle blood glucose meter. The diabetic mice were then fasted overnight (from 7 p.m. to 7 a.m.) to measure their FBG after they had free access to food and water for 3 days.

Immunofluorescence

All the mice were executed by cervical vertebra dislocation after the experiment. Pancreas tissues were collected and fixed in 4% paraformaldehyde fix solution (BBI, Shanghai, China) for immunofluorescence staining. Pancreatic sections (4 μm thick) were cut on glass slides, deparaffinized in toluene, and rehydrated. The standard immunohistochemical staining protocol was carried out according to the manufacturer's instructions. Briefly, the paraffin-embedded sections were rinsed with water, immersed in 3% hydrogen peroxide/deionized water for 15 min and PBS rinsing thrice for 2 min each time. Primary antibodies, guinea pig anti-insulin (1:100 dilution, abcam, Cambridge, United Kingdom) and mouse anti-glucagon (1:250 dilution, abcam), were incubated overnight at 4℃. Subsequently, slides were incubated with secondary

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antibodies. Eventually, DAPI-nuclear staining (BBI, Shanghai, China) was used. Insulin and glucagon staining were quantified respectively by Image J as mean value pixel after background subtraction.

Statistical analysis

Quantitative data are presented as means±S.D. Statistical significance was determined using paired samples t-test, and the chi-square test was used for analyzing the enumeration data. All data were analyzed using the Statistical Product and Service Solutions (SPSS, Chicago, USA) 22.0 software. P value less than 0.05 was considered to be significant.

Results

After injection of STZ, the mental state of the diabetic mice were worse than non-diabetic mice, the activity was significantly reduced, and the response was slow. On the 5th day after the injection, the diabetic mice began to show polydipsia, polyuria, and polyphagia, and the symptoms were more evident on the 7th day.

IPGTTs results confirmed the successful modeling of the diabetes model. In particular, no-diabetic mice showed islets of Langerhans are in the absence of damage, and the acini and cellular population are normal. In contrast, diabetic mice showed an induced injury of pancreatic tissue with reduced islet numbers and size. Diabetic mice significantly decreased insulin levels ($p < 0.05$) compared to no-diabetic mice (Figure 1). However, there was no significant difference in glucagon levels. This means the islet β cells were damaged and verified that the diabetes model was made successfully.

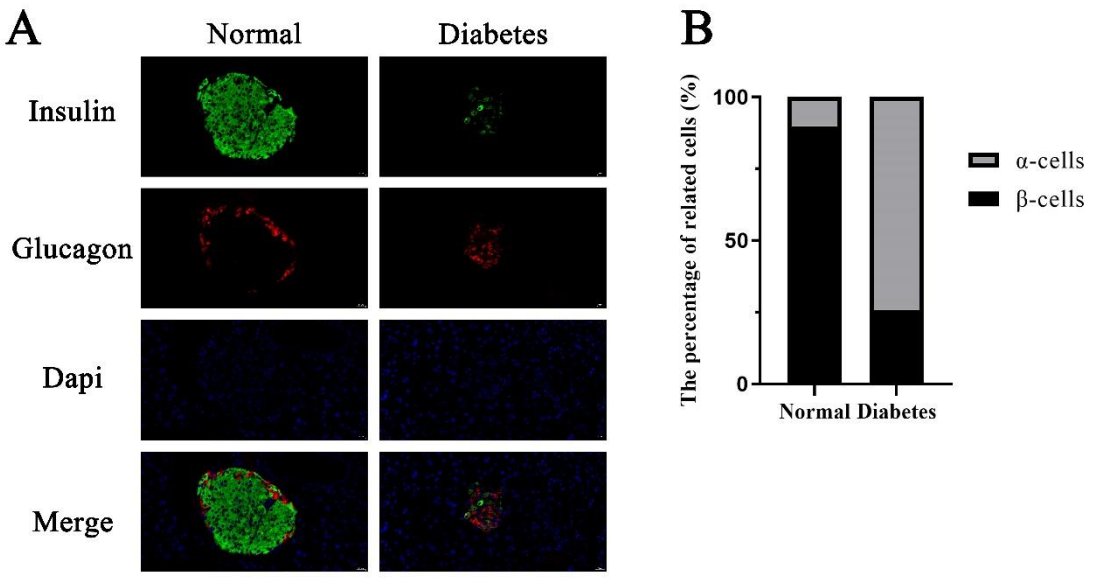

Figure 1. Immunostaining of Islets of Pancreas. (A) Immunostaining of α- (glucagon) and β-Cells (insulin) in the Islets of Pancreas after intraperitoneal injection of sodium citrate buffer or Streptozotocin (STZ) in normal, diabetic mice. (B) Quantification of α- and β-Cells staining (mean intensity). *P<0.05; original magnification ×400; scale bar = 20 μm.

The comparison of FBG

As results showed in Figure 2A, the blood glucose level after fasting 6 h in the morning was higher than fasting overnight. The difference was statically significant (P<0.05).

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Diagnostic accuracy

There was only one mouse with blood glucose higher than 11.1 mmol/L after fasting overnight. Meanwhile, all blood glucose was higher than 11.1 mmol/L after fasting 6 h. Compared to fasting overnight, fasting 6 h is more conducive to diagnose diabetes (Figure 2B). The differences were statically significant (P<0.05).

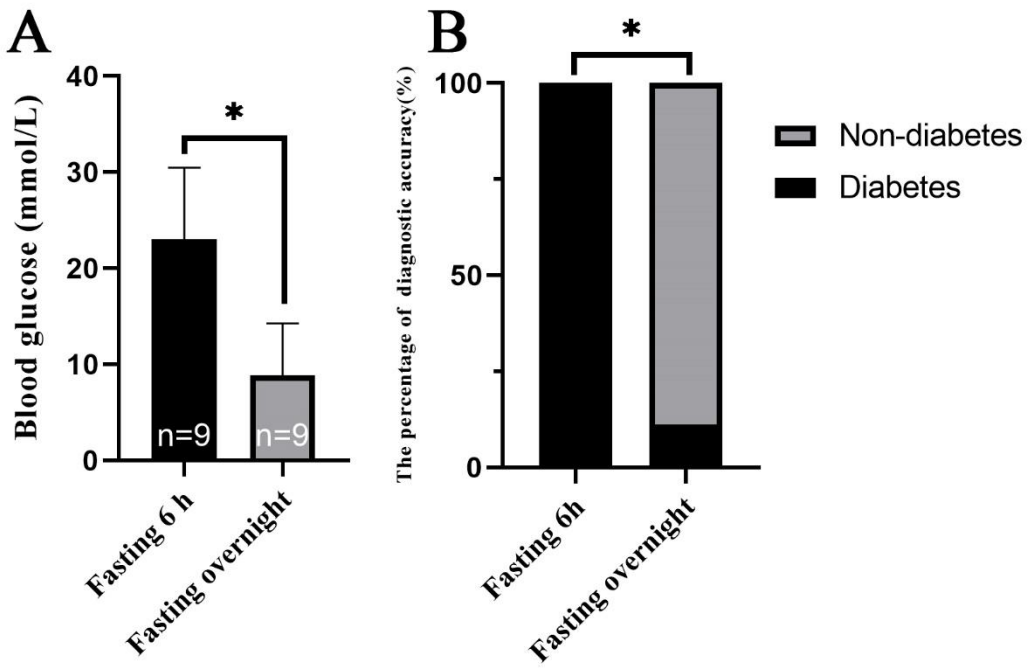

Figure 2. (A) The blood glucose level after fasting 6 h and overnight. (B) The percentage of diagnostic accuracy. *P<0.05.

Discussion

For the first time, the present study reports the effects of fasting 6 h and fasting overnight in diagnosing diabetes.

Diabetes mellitus is rising to epidemic proportions, resulting in devastating complications if not treated well [15]. Diabetes is a chronic metabolic disease caused by islet B cell function defects and insulin resistance and its pathogenesis have not yet been fully elucidated [16-18]. Unlike many clinical studies, animal models allow controlled experimental designs, clinically and standardized relevant treatment regimens, detailed organ insights and can systematically assess the effect of therapeutic interventions. The establishment of an animal model of diabetes provides an important means for studying the pathogenesis of diabetes and the development of therapeutic drugs.

Methods for modeling experimental diabetic animal models include STZ-induced, alloxan-induced, surgical removal of the pancreas, etc. [19-21]. Anyway, we need to make sure whether the diabetic animals were modeled successfully. One of the methods used widely to diagnose diabetes is blood glucose after fasting overnight. While mice are nocturnal feeders, once the overnight fasting is performed, it is equivalent to fasting approximately 24 h, which leads to the fasting blood glucose deviates from the normal value. Meanwhile, the 24 h fast can activate several physiologic counterregulatory mechanisms resulting in the reliability of glucose readings unclear and usually lower than normal [22]. Suppose the fasting blood glucose of the

animal model is lower than diabetes diagnostic criteria. In that case, it will inject STZ or other medicine again or abandon these animals that failed to model diabetes, causing a waste of research funding and violating animal welfare.

In the process of experiments, the IPGTTs is regarded as the diagnostic criteria of diabetes. However, the process of IPGTTs is cumbersome and the blood glucose test strips are expensive. Repeatedly measuring blood glucose, causing the animal pain, is not in line with animal welfare. If fasting blood glucose can be used to diagnose diabetes, it will save research costs and decrease researchers' waste of time. After injecting STZ, the blood glucose of these mice were increased continuously. We used IPGTTs to confirm whether the diabetes model was made successfully. Furthermore, the diabetic mice developed irregular morphology, reduced volume, decreased islet secretion cells, and partial nuclear pyknosis. This study demonstrates that fasting 6 h is more accurate in diabetes diagnosis than fasting overnight. Moreover, it does not cause a bias of the blood glucose level of the selected diabetic mice. To a certain extent, the welfare of animals also has been protected.

Our experiment also has several limitations. Firstly, the blood samples for FBG were obtained by tail cut method rather than oculi choroidal vein, which the amount of blood fetched lacks, prone to hemolysis and may not reflect the real blood glucose level. Secondly, the FreeStyle blood glucose meter was used to estimate blood glucose. However, the blood glucose detected by a blood glucose meter usually has 20% error. Thirdly, in this study, we only analyzed C57BL/6 mouse, more animal strains need to be studied further.

Conclusions

This investigation shows that fasting 6 h is more suitable in diabetes diagnosis than fasting overnight. It may be used as a criterion of fasting blood glucose in diabetic mice in the future.

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