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Original Article

Effect of fasting time on measuring mouse blood glucose level

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Abstract: This study aims to investigate the effect of fasting time on measuring blood glucose levels in mice. Fasting blood glucose levels were analyzed after 12 h daytime fasting or 12 h night fasting. The results from four mouse models demonstrate that the variation of fasting blood glucose in daytime fasting was smaller than fasting overnight. Our results demonstrate that conducting daytime-fasting versus nighttime-fasting may result in more precise scientific analyses on measuring fasting blood glucose.

Keywords: Fasting blood glucose, streptozotocin, *ob/ob* mice, high fat diet

Introduction

Blood glucose level is regulated by organisms as part of metabolic homeostasis. Disorders of glucose metabolism could manifest in disorders such as diabetes, obesity and/or other metabolic diseases [1], thereby decreasing the quality of life of those affected. Although there are several measured indicators of altered glucose hemostasis, measuring the fasting blood glucose (FBG) level is the most important, and convenient, indicator of glucose metabolic state.

Although standard protocol suggests an 8-15 hour fast prior to measuring the fasting blood glucose in mice, there are very few studies that investigate the most beneficial time-frame to begin the fasting period. Millions of archives that relate to fasting blood glucose have been screened, but none could highlight the benefit from daytime versus nighttime fasting.

This study aims to compare two different fasting times (daytime fasting, and night fasting) before measuring FBG level in four mouse models to determine the effect of each on blood glucose levels.

Animal and care

C57BL/6J mice, about 8 weeks old, were purchased from Center for Pilot Animal of Jilin University. Obese *ob/ob* mice, bred in our lab, about 4 months old were also used for the experiment. The animals were housed at room temperature (22 ± 2°C) with relative air humidity of 45% to 55% with a 12-hour light/dark cycle. They were submitted to free access to a standard diet normal chow or high fat diet and water. All procedures were conducted by Institute for Experimental Animals of Jilin University in strict accordance with the PR China legislation on the use and care of laboratory animals and were approved by the university committee for animal experiments.

Experimental design

Normal chow fed mice

20 male mice, C57BL/6J, 8 weeks old, fed with normal chow, were randomly divided into two groups, 10 mice per group. Daytime Fasting Group (DFG): fasting during the daytime (6:00-18:00), then measured the FBG levels. Night Fasting Group (NFG): fasting during the night (18:00-6:00), then measured the FBG levels.

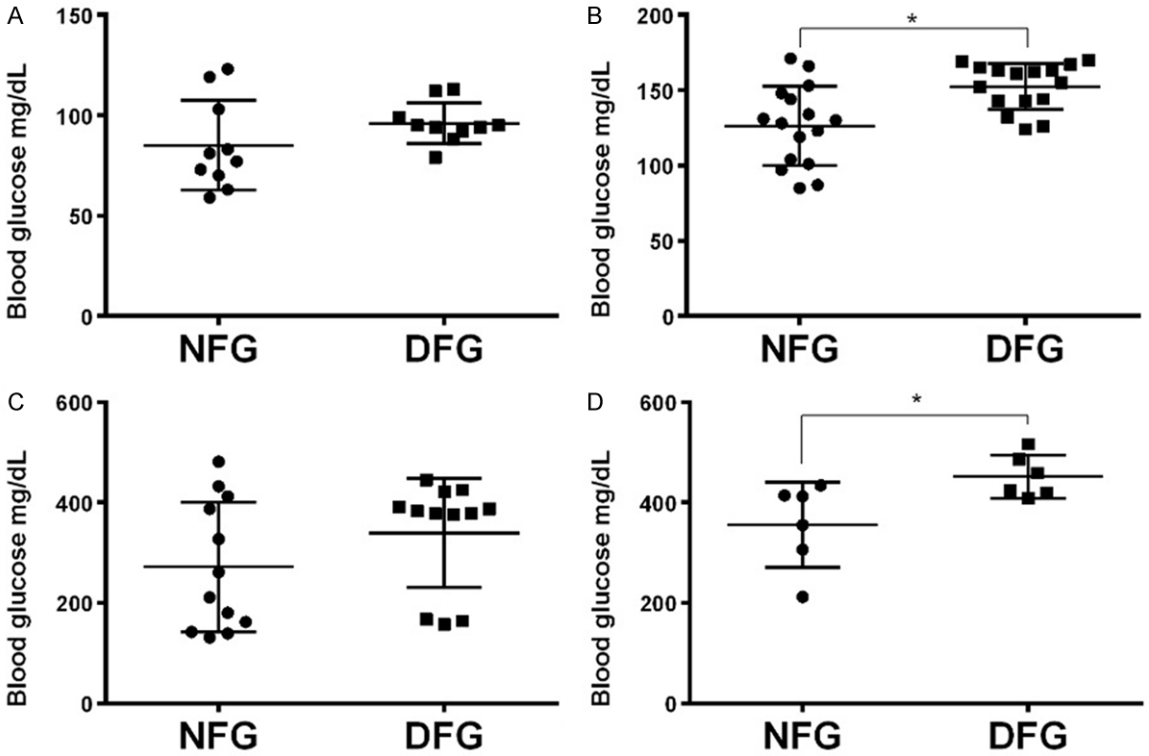

Figure 1. Effect of different fasting times on FBG level in mice Four mouse models, normal chow fed mice (A), high fat diet fed mice (B), STZ-induced diabetic mice (C) and ob/ob mice (D), were randomly divided into two groups, DFG: fasting during the daytime (6:00-18:00); NFG: fasting during the night (18:00-6:00), then FBG levels were measured. *Means P<0.05.

The blood glucose levels were measured by blood glucose meters One Touch Ultra (LifeScan, Milpitas, CA, USA).

High fat diet induced obese mice

32 male mice, 2 months old were fed with HFD for 8 weeks. Then mice were randomly divided into two groups, 16 mice per group. Fasting blood glucose levels were measured as described above in normal chow fed mice.

STZ-induced diabetic mice

After overnight fasting, 24 male C57BL/6J mice were injected with 180 mg/kg Streptozotocin (STZ, Sigma-Aldrich, St. Louis, MO, USA) freshly dissolved in 0.1 mol/L citrate buffer (pH 4.5). Then, after 72 h feeding, mice were randomly divided into two groups, 12 mice per group. Fasting blood glucose levels were measured as described above in normal chow fed mice. Mice with fasting blood glucose levels above 200 mg/dL were considered to be diabetic mice.

Diabetic model success rate = diabetic mice number/total mice number * 100%.

ob/ob mice

12 *ob/ob* mice, male, about 4 months old, fed with normal chow, were randomly divided into two groups, 6 mice per group. Fasting blood glucose levels were measured as described above in normal chow fed mice.

Statistics

The results were analyzed for statistical significance by t-test using the Statistical Package of the Social Science (SPSS) program. All data were expressed as mean ± SD. *P*-values of less than 0.05 were regarded as significant.

Results

The results in **Figure 1A** and **Table 1** show that there was no significant difference of FBG levels between DFG and NFG in normal chow fed mice, but the deviation in daytime fasting group (Mean ± SD = 96.1 ± 10.2) is smaller than night fasting group (Mean ± SD = 85.1 ± 22.5).

High fat diet induced obese mice are commonly used as models in obesity related research.

Table 1. FBG values in four animal models

Group	NFG Glucose Values (mg/dL)		DFG Glucose Values (mg/dL)	
	Individual	Mean ± SD	Individual	Mean ± SD
Normal Chow Fed Mice	77, 73, 83, 103, 123, 81, 59, 119, 70, 63	85.1 ± 22.5	94, 88, 113, 92, 94, 95, 112, 79, 99, 95	96.1 ± 10.2
High Fat Diet Fed Mice	166, 87, 130, 144, 119, 128, 101, 171, 148, 85, 97, 104, 153, 134, 131, 123	126.3 ± 26.4	143, 132, 163, 143, 155, 167, 152, 126, 163, 161, 124, 144, 170, 169, 162, 165	152.4 ± 15.3*
STZ-induced Diabetic Mice	162, 412, 261, 131, 432, 142, 139, 180, 481, 328, 211, 387	358.7 ± 96.9	378, 445, 157, 376, 383, 164, 391, 378, 387, 167, 425, 421	398.2 ± 25.4
ob/ob Mice	212, 412, 354, 306, 434, 414	355.5 ± 84.6	512, 419, 408, 456, 486, 423	451.7 ± 42.8*

Compared with NFG values, *means P<0.05.

Figure 1B and **Table 1** demonstrates that there was a significant difference of FBG levels between DFG and NFG, and the DFG's deviation (Mean ± SD = 152.4 ± 15.3) is smaller than NFG (Mean ± SD = 126.3 ± 26.4).

STZ-induced diabetic mice are widely used as a Type 1 diabetes mellitus model to for scanning potential hypoglycemic drugs in vivo [2, 3]. The results from our study illustrate that diabetic model success rate of DFG was 75%, but NFG was only 58.3% (**Figure 1C** and **Table 1**). We then recalculated the STZ-induced diabetic mice within the two groups and found the variation of FBG value in DFG group (Mean ± SD = 398.2 ± 25.4) is much lower than NFG group (Mean ± SD = 358.7 ± 96.9).

Obese *ob/ob* mice, which cannot produce leptin, are popularly used as type II diabetes animal models. Our results from *ob/ob* mice model showed similar results as mice fed with HFD, and variation of FBG value in NFG (Mean ± SD = 355.5 ± 84.6) is higher than DFG (Mean ± SD = 451.7 ± 42.8) (**Figure 1D** and **Table 1**).

Discussion

In this study, we compared different fasting times, daytime fasting (6:00-18:00) and night fasting (18:00-6:00), before measuring mice FBG levels. Our results show that there was a significant difference between DFG and NFG in HFD-fed mouse model as well as the *ob/ob* mouse model. Moreover, the variation of FBG from DFG is consistently smaller than NFG in all four mouse models tested, which is important in data statistical analysis for animal experiment.

The reason why night fasting could result in higher variation could be explained by the fact that mice are nocturnal and are more active during the night hours. Varying degrees of physical activity of mice during through these night hours could have a more pronounced effect on blood glucose levels [4].

The FBG test is commonly used to detect or accompany a diagnosis of diabetes mellitus [5-7] in common healthcare practices. The test is done in the morning, before the patient has eaten breakfast, and has therefore undergone an overnight fast. Mice are ideal mammalian models for life science and medical research by better understanding disease processes that pose a risk to humans. But, there are some very key differences between mice and humans, which could affect the experimental results [8, 9]. One key difference is that humans are diurnal, whereas mice are nocturnal, suggesting that when carrying out fasting in mice, a daytime fast would be more appropriate. Indeed, our experimental results suggest that there is less variation when conducting daytime fasts in the mouse models examined.

Our results demonstrate that conducting daytime-fasting versus nighttime-fasting may result in more precise scientific analyses. Further work is needed, however, to better understand the relationship of these fasting differences to study-specific end points.

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Disclosureof conflict of interest

None.

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