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Blood Glucose Reference Interval in *Mus musculus* Mice

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Abstract

The analysis of the carbohydrate status of *Mus musculus* mice is necessary for an experimental study of embryotoxicity in an animal model because to date several studies have revealed a frequent association between disorders of glycoregulation and various congenital malformations. The objective of this study is to determine the reference intervals of glycaemia in young *Mus musculus* mice intended for the study of embryotoxicity. This is an experimental study carried out at the Human Embryology Laboratory of the University of Kinshasa in May 2021 on 20 mice, *Mus musculus* adults (10 males and 10 females), in good health, weighing 20 and 30 grams in which a dosage fasting blood glucose test was performed. The effect of gender on blood glucose was assessed and reference intervals (RI) were established using a parametric method with $p < 0.05$. The rules of ethics relating to the handling of laboratory animals have been respected. The fasting blood glucose values of 20 mice ranged between 62 and 135 mg/dl. Those of female mice, between 62 and 130 mg/dl with a median of 97.10 mg/dl and those of male mice, between 64 and 135 mg/dl with a median of 99.90 mg/dl. Reference ranges for fasting blood glucose in mice were 72 - 105 mg/dl, for male mice 79 - 108 mg/dl, and for female mice 69 - 102 mg/dl ($p = 0.564$). In conclusion, the medians of fasting glycaemia of mice as well as their reference intervals are not activated by the sex of the mice. Knowledge of normal fasting blood glucose levels secures the *Mus musculus* animal model for embryotoxicity studies with impaired glycoregulation.

Subject Areas

Hematology

Keywords

Fasting Blood Glucose, Reference Intervals, *Mus musculus*, Embryotoxicity

1. Introduction

Conducting these experimental studies in humans is difficult for ethical reasons. Thus, only animal testing remains the only appropriate way to quickly obtain data on a new substance under acceptable conditions [1] [2].

Despite this possibility, the choice of the experimental animal is essential as a determining factor for the results to be reliable [3].

In our Human Embryology laboratory, the mouse, *Mus musculus*, has been acclaimed as an animal model to study the embryotoxicity of plant-based anti-malarials, in particular Manalaria® and Syrup Kilma®.

The choice of the mouse, *Mus musculus* as a model for our laboratory is based on the following arguments:

- The early embryogenesis of the *Mus musculus* mouse is similar to that of humans;
- The species *Mus musculus* is well known genetically, and is suitable for the observation of a possible dysfunction of the ontogenetic processes;
- The frequency of spontaneous malformations for this species is very low;
- The mouse, *Mus musculus* has a rapid and easy to follow germ cycle;
- The breeding of this mouse is very easy to practice.

For these many reasons [1] [4], the mouse, *Mus musculus*, is the ideal model for us to carry out embryotoxicity studies. Given that several studies to date have found a frequent association between type 1 and 2 diabetes mellitus and various malformations, we opted to examine the glycoregulation of our mice before undertaking embryotoxicity studies proper. [5]-[10]

The objective of this study is to determine the reference intervals of fasting glycaemia in *Mus musculus* mice intended for the study of embryotoxicity in order to exclude the disorder of glycoregulation in our mice to be experimented.

2. Material and Methods

This experimental study of young glycemia in mice, *Mus musculus*, was carried out at the Human Embryology Laboratory of the University of Kinshasa in May 2021. As material we used 20 adult mice (10 males and 10 females), healthy and weighing between 20 and 30 grams, a Bayer brand glucometer and Counter Neet brand strips were used to measure the blood glucose. As a method, the measurement of blood sugar was carried out with a drop of blood taken from the end of the tail after pricking it in a minimally traumatic way with a fine needle. The mice, isolated in cages and kept in a common room at an ambient temperature not exceeding 23˚C, were fed cakes produced by the Minoterie de Matadi (MIDEMA) and Alpina brand drinking water. Prior to fasting blood glucose testing, mice were starved for 8 hours. Three fasting blood glucose tests were performed on each mouse at 1 day intervals each time. At the end of these investigations, the 20 mice were all reared in our animal facility. The data collected were entered and analyzed with SPSS 25.0 software. Tukey's method was used to identify outliers before establishing IRs for blood glucose. The RI was also estab-

lished to observe the significant difference by sex. A value of $p < 0.05$ was considered statistically significant. The ethical rules for handling animals in laboratories have been scrupulously respected [11] [12] [13]. Indeed, we reduced the number of animals for the experiments to the minimum necessary (20 mice). The animals did not suffer; they were well-fed and kept in a suitable room; the blood sample at the end of the tail was carried out in a minimally traumatic manner using a needle.

3. Results

3.1. Fasting Blood Glucose

The values of all fasting blood glucose levels performed varied between 62 and 135 mg/dl. For the 10 female mice, the fasting blood glucose values were between 62 and 130 mg/dl while for the 10 male mice, the fasting blood glucose values were in the range between 64 and 135 mg/dl.

The median fasting blood glucose levels of female mice and male mice are shown in Table 1.

3.2. RI Established for Blood Glucose

Using Tukey's method, we identified the lower limit of blood glucose levels at 62 mg/dl mg/L, and the upper limit at 130 mg/dl. Thanks to a Box-Cox conversion, the normality of the data was improved and the IRs for glycaemia were established based on the parametric method. Sex-specific IRs have also been established in Table 2.

Table 1. Median fasting blood glucose levels of 10 female mice and 10 male mice.

Sex		Dosage 1	Dosage 2	Dosage 3	Mean
Females	Median (mg/dl)	99.70	105.30	86.10	97.10
	N	10	10	10	10
	± DS	16.391	18.373	21.210	7.102
Males	Median (mg/dl)	105.70	92.80	101.30	99.90
	N	10	10	10	10
	± DS	27.301	20.896	25.781	13.295
	95% IC	−15.16 to 27.16	−31 to 6	−6.99 to 12.82	−7.22 to 12.82
	p	0.559	0.173	0.167	0.564

Table 2. Sex-specific reference ranges for blood glucose in mice.

	Total				Females				Males				*p*
	LL	IC to 95%	UL	IC to 95%	LL	IC to 95%	UL	IC to 95%	LL	IC to 95%	UL	IC to 95%	
Glycaemia	72.01	63.61 - 84.26	105.11	102.99 - 142.63	69.03	54.03 - 75.24	102.26	99.91 - 115.33	79.21	73.74 - 94.92	108.12	102.83 - 164.64	0.234

4. Discussion

The discussion of our results revolves around the following 2 points: the fasting glycaemias of female and male mice and the reference intervals of the glycaemias of the mice.

4.1. Fasting Blood Glucose Levels of Female and Male Mice

The mean fasting blood glucose levels of 3 assays in female and male mice were 97.10 ± 7.109 mg/dl and 99.90 ± 13.295 mg/dl, respectively. The comparison of these medians did not show a statistical difference ($p = 0.564$). These results suggest that for healthy mice, gender does not influence fasting blood sugar. As a result, we can use both male and female mice in our experiments.

4.2. Reference Intervals of Female and Male Mice

The reference ranges for fasting glycaemia in mice were 72 to 105 mg/dl, for male mice 79 to 108 mg/dl and female mice 69 to 102 mg/dl ($p = 0.234$).

When comparing these fasting blood glucose reference intervals, we did not observe a statistical difference according to gender.

In this study, it is interesting to note that the reference ranges of fasting glycaemia in mice are close to those of fasting glycaemia in normal humans, which vary between 63 and 110 mg/dl (6).

5. Conclusion

This experimental study enabled us to establish the reference values for glycaemia when *Mus musculus* mice were in young, and these results approach those of humans. The medians of the fasting glycaemias of the mice as well as their reference intervals are not activated by the sex of the mice. Knowledge of normal fasting blood glucose levels secures the *Mus musculus* animal model for embryotoxicity studies with impaired glycoregulation.

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Contribution of the Authors

- Kangudia Mbaya J. contributed to all stages of project execution;
- Mbanzulu Pita Nsonizau D. was the designer of the project and took part in all stages of its execution;
- Mbanzulu Nsolani N. took an active part in the discussion of the results, in typing, in text processing.
- Zosuruna Mbuy T. took an active part in the handling of animals in the laboratory.

Conflicts of Interest

The authors declare no conflicts of interest.

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