Anesthesia Does Not Affect Development of Diabetes in ZDF Rats

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1 ABSTRACT

The obese Zucker diabetic fatty (ZDF) rat develops diabetes over time and is an excellent model of late stage type 2 diabetes. The development of diabetes is diet dependent and can be influenced by many environmental factors. Anesthesia has been associated with transient changes in blood glucose in rodents. However, long-term effects of anesthesia on regulation of blood glucose levels and development of the diabetic phenotype is not known in rodents. The purpose of this study was to determine whether or not administration of anesthetic agents would alter the development of the diabetic phenotype in these animals. Thirty 8-week-old male ZDF obese rats were distributed to three groups of 10 animals (ketamine/xylazine, isoflurane and control). Diet and water were provided *ad libitum* for this IACUC-approved study. Blood glucose (BG) was measured from tail nick blood samples using a handheld glucometer. Anesthesia was induced by intraperitoneal injection of ketamine (75 mg/kg) and xylazine (6 mg/kg), or inhalation of isoflurane using a chamber (2 to 4%) and followed by a 15-minute maintenance period (0.5 to 2%). Control animals experienced identical cage changes as the other groups. Immediately post-anesthesia, food was returned to half of the animals in each group, while the remaining animals were fasted through the acute sampling period. Acute BG was measured every 30 minutes for 4 hours. Chronic BG was measured once every week for 11 weeks (until 20 weeks of age). Fasted glucose was estimated 16 hours following collection of the non-fasting sample. All the animals tolerated the study well and showed similar weight gains post-anesthesia. Ketamine/xylazine increased BG immediately following anesthesia in both fasted and non-fasted animals, followed by a decrease compared to controls. BG in isoflurane animals was lower in fasted animals only relative to the control group. Long-term assessment of both fasting and non-fasting BG demonstrated no effect on the conversion to the diabetic phenotype in the animals. Based on these data, we conclude that ketamine/xylazine or isoflurane acute exposure does not affect the onset and development of diabetes in male ZDF obese rats.

2 INTRODUCTION

The incidence of obesity and diabetes is an epidemic worldwide. According to Centers for Disease Control and Prevention, an estimated 29.1 million men, women and children in the United States have diabetes (2012 data). That is an astounding 9.3% of the US population.

Studies in appropriate animal models are very important in gaining a better understanding of obesity and diabetes. Historically, rodents have been a mainstay of diabetes research. Various mouse and rat models have been found to mimic different aspects of obesity and obesity-driven diabetes. The Zucker diabetic fatty (ZDF) rat is one such model. Because it is obese, hyperinsulinemic and develops diet-dependent diabetes, the ZDF rat embodies many of the facets of human obesity and diabetes.

The ZDF rat has a mutation of the fatty (*fa*) gene, develops diabetes over time and is an excellent model of late stage type 2 diabetes. The development of diabetes in the ZDF is diet dependent and can be influenced by many environmental factors. Anesthesia has been associated with transient changes in blood glucose in rodents. However, long-term effects of anesthesia on regulation of blood glucose levels and development of the diabetic phenotype is not known in rodents. The purpose of this study was to determine whether or not administration of anesthetic agents would alter the development of the diabetic phenotype in obese ZDF rats.

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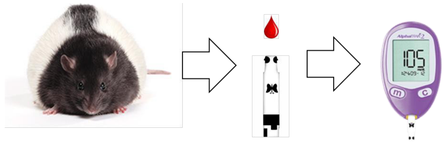
3 MATERIALS AND METHODS

Animals
Thirty 8-week-old male ZDF rats (ZDF-*Lepr^{fa}*/Crl; Charles River Laboratories, Kingston, NY) weighing between 300 and 400 grams were used. They were maintained in polycarbonate cages in a dedicated rodent surgical complex that was kept at 21 ± 2 ºC with a relative humidity of 60 ± 5% and a 12/12 hour light/dark cycle. Commercially produced, sterilized feed and water were provided *ad libitum*. All conditions of animal preparation and use were in accordance with recommendations set forth in the Guide for the Care and Use of Laboratory Animals. The animals were of a VAF/Plus® health status.

Anesthesia procedures
Ketamine/xylazine group: Ketamine (75 mg/kg) and xylazine (6 mg/kg) was injected intraperitoneally. Animals were allowed to recover in a cage placed on a heating pad.
Isoflurane group: Isoflurane was induced using a chamber (2 to 4%) and then nosecone for 15 minutes for maintenance (0.5 to 2%). Animals were allowed to recover in a cage placed on a heating pad.
Control group: Cage changes for the control group mimicked those of the treated groups.

Blood glucose measurement
Blood glucose was measured using AlphaTRAK® (Abbott Laboratories) portable glucometer. Tail was wiped with alcohol and gauze pad. Lateral tail vein was punctured using lancet to collect a drop of blood and placed on glucose test strip. Blood glucose readings from glucometer were recorded.



4 EXPERIMENTAL DESIGN

The 30 rats were randomly allocated into 3 groups consisting of 10 rats each for ketamine/xylazine, isoflurane and control.

Acute effects of anesthesia: For acute blood glucose measurement, animals were further split into fasted and non-fasted, with 5 rats in each anesthesia group. BG was measured once every 30 minutes for up to 4 hours.

Chronic effects of anesthesia: Blood glucose was measured each week at the same time. Non-fasted BG was measured in the morning of day 1 of each week, then food was removed in the afternoon of day 1 and fasted BG was measured in the morning of day 2. Food was given to the animals as soon as BG measurement was completed. Animals were not fasted for more than 16 hours and water was provided *ad lib*.

Week	PRE	0	1	2	3	4	5	6	7	8	9	10	11
	BG	Anesthesia	BG	BG	BG	BG	BG	BG	BG	BG	BG	BG	BG

Note: Pre-anesthesia (PRE) blood glucose was measured the week prior to anesthesia treatment.

5 RESULTS

General observations
- All animals remained clinically healthy throughout the study.
- All animals showed similar weight gains after anesthesia (i.e., no difference between treatment groups, p > 0.05).

Acute effects of anesthesia

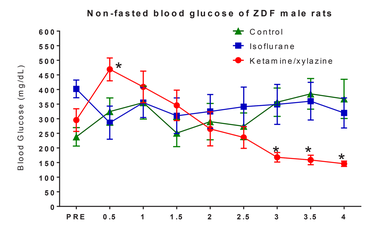


Figure 1. Acute effects of anesthesia on non-fasted blood glucose. Data is expressed as mean ± SEM. Two-way ANOVA, n = 5 in each group. *p < 0.05 ketamine significantly different from isoflurane and control.

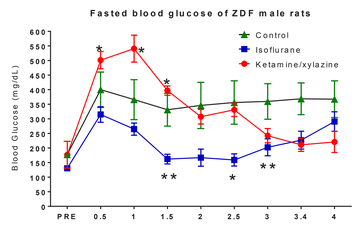


Figure 2. Acute effects of anesthesia on fasted blood glucose. Data is expressed as mean ± SEM. Two-way ANOVA, n = 5 in each group. *p < 0.05 ketamine significantly different from isoflurane. **p < 0.05 isoflurane significantly different from control.

Chronic effects of anesthesia

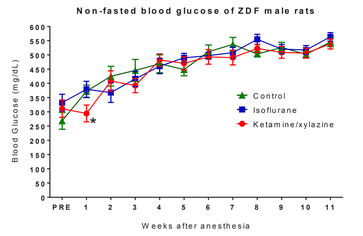


Figure 3. Long-term effects of anesthesia on non-fasted blood glucose. Data is expressed as mean ± SEM. Two-way ANOVA, n = 10 in each group. *p < 0.05 ketamine significantly different from isoflurane.

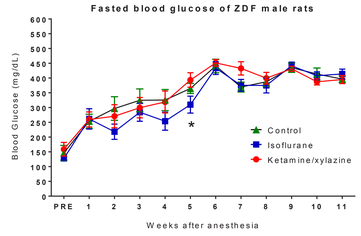


Figure 4. Long-term effects of anesthesia on fasted blood glucose. Data is expressed as mean ± SEM. Two-way ANOVA, n = 10 in each group. *p < 0.05 ketamine significantly different from isoflurane.

6 DISCUSSION

Ketamine/xylazine increased BG immediately following anesthesia in both fasted and non-fasted animals, followed by a decrease compared to controls. BG in isoflurane animals was lower in fasted animals only relative to the control group. Long-term assessment of both fasting and non-fasting BG demonstrated no effect on the conversion to the diabetic phenotype in the animals. Based on these data, we conclude that ketamine/xylazine or isoflurane acute exposure does not affect the onset and development of diabetes in male ZDF obese rats.