

## Experimental model of glucocorticoid-induced insulin resistance<sup>1</sup>

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DOI: <http://dx.doi.org/10.1590/S0102-865020160100000001>

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### ABSTRACT

**PURPOSE:** To evaluate metabolic effects in experimental model of glucocorticoid-induced insulin resistance.

**METHODS:** Twenty Wistar male rats were randomly divided into two groups, which were treated with intraperitoneally injected dexamethasone 1mg/Kg/day for ten days consecutively (Group D; n=10) and placebo (Group C; n=10). The variables analyzed were: from the first to the 10th day - body weight (before and after treatment); food and water daily consumption; on the 10th day - glycemia, insulinemia, HOMA-beta and HOMA-IR. The blood samples for laboratory analysis were obtained by intracardiac puncture. Also on the 10<sup>th</sup> day liver fragments were taken for analyzing glycogen and fatty.

**RESULTS:** Group D animals compared to group C had: weight reduction (g), (D=226.5±24.7 vs C=295.0±25.4; p=0.001); increased glycemia (mmol/l) (D=19.5±2.1 vs C=14.2±3.1; p=0.0001); diminished insulinemia (mU/l) (D=0.2±0.1 vs C=2.0±0.4; p=0.0001); reduced HOMA-β (D=0.2±0.1 vs C=4.2±1.7; p=0.0002); diminished HOMA-IR (D=0.2±0.1 vs C=1.3±0.4; p=0.0002). Histological examination of the liver showed that 100% of group D and none of group C had moderate fatty. (p=0.2).

**CONCLUSION:** Animals treated with glucocorticoid, in this experimental model, expressed hyperglycemia, hypoinsulinism and decreased peripheral insulin sensitivity.

**Key words:** Dexamethasone. Insulin Resistance. Diabetes Mellitus, Experimental. Liver Glycogen. Fatty Liver. Rats.

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## Introduction

Glucocorticoids are amongst the most used drugs worldwide and they are efficient on the treatment of innumerable inflammatory and immunological diseases. Despite its therapeutic action, they induce insulin resistance in humans and can lead to steroid-induced diabetes or worsen a previously diagnosed diabetes. Insulin resistance is characterized by the reduction of the cellular ability in increasing the transportation and or using glucose as a response to insulin's action<sup>1</sup>.

Many authors showed metabolic changes, on in vivo experimental models, after using glucocorticoids. The reduction of weight in rats<sup>2,3</sup>. The increase on serum glucose and serum insulin<sup>4,6</sup>. Loss in muscle mass associated to liver hypertrophy<sup>7</sup>. Alteration in protein profile<sup>8</sup> and in lipid panel<sup>9</sup>. However the effects over insulinemia are still controversial. Studies show that dexamethasone synthetic glucocorticoid reduces insulin secretion by pancreatic cells due to the oxidative stress caused on them<sup>10-12</sup>. Thus this research aimed at evaluating metabolic effects on an experimental model of glucocorticoid-induced insulin resistance.

## Methods

All procedures with animals have been approved by Universidade Vale do Sapucaí Ethics Committee on Animal Usage (CEUA), by 167/12 protocol.

Experimental study conducted from March to July of 2015. Twenty male *Wistar* rats, which were 3 month-old, were utilized; they were provided by UNIVAS *vivarium*. Animals had free access to water and to rat's food (Nuvilab<sup>®</sup>) until the eve of exam collection, when they were kept on 8 hour fasting. The animals remained on isolated cages during ten consecutive days, under a temperature range from 21° to 25°C, alternating light/dark cycles.

Animals were randomized into two groups: Control (C; n = 10) and dexamethasone (D; n = 10). Group D animals received intraperitoneal dexamethasone injection (Decadron<sup>®</sup>) 1mg/Kg/day for ten days sequentially. Group C animals received intraperitoneal distilled water injection (placebo) during the same period of time. The analyzed variables were: weight (before and after treatment with placebo or dexamethasone, according to the group to which the animal belonged). Animal's food and water consumption, from 1st to 10th days. Glycemia, insulinemia, HOMA- (Homeostasis

Model Assessment)  $\beta$  e HOMA- IR (Insulin Resistance) on 10th day. The blood sample for laboratory analysis was obtained by intracardiac puncture. Colorimetric enzymatic method was used for dosing serum glucose. Plasma insulin concentrations were determined by an automated immunoassay (Access; Beckman Instruments, Fullerton, CA). For calculating HOMA-  $\beta$  e HOMA-IR equations were used:

HOMA- $\beta$ :  $(20 \times \text{fasting insulin (mU/l)}) / (\text{fasting glucose (mmol/l)} - 3.5)$ .

HOMA-IR:  $\text{fasting insulin (mU/l)} \times \text{fasting glucose (mmol/l)} / 22.5$ .

HOMA- $\beta$  evaluates the ability that pancreatic  $\beta$  cells have to secrete insulin (smaller values indicate low ability); HOMA-IR indicates sensitivity to insulin (smaller values indicate bigger insulin resistance).

Liver fragments were withdrawn for assessment on glycogen and fatty. Five  $\mu\text{m}$ -Histological cuts of hepatic tissue were stained by Hematoxylin and Eosin (HE) for quantifying fatty and were stained by periodic-acid-Schiff's reagent (PAS) for quantifying glycogen reservation. The slides were analyzed by optical microscope (Nikon E-200) on magnifications of x100 and x400. Slides evaluation was made by subjective analysis of presence and absence of glycogen and fatty of hepatic tissue. Lack of stain indicated "absence"; stain in 1/3 of the slide indicated "mild"; stain in 2/3 of the slide indicated "moderate". Slide almost fully stained indicated "plenty" of glycogen and fatty.

Data statistical analysis was performed by BioEstat *software*, version 5.0. We used D'Agostino test of normality. Numeric variables with normal distribution were compared using T test. Nonparametric data were compared by Fisher's exact test. Comparisons between groups of animals in relation to insulin were made considering the nonparametric Mann-Whitney test. It has been adopted  $p < 0.05$  for rejecting the null hypothesis.

## Results

Rats treated with dexamethasone had body weight loss, on grams, (Figure 1), when compared to control group (D=226.5 $\pm$ 24.7 vs C=295.0 $\pm$ 25.4;  $p=0.001$ ) without any reduction in providing water or food to the animals (Figures 2 and 3).

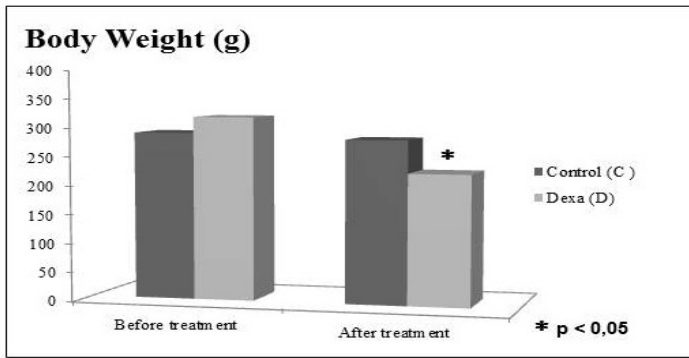


FIGURE 1 - Comparison of the Body Weight according to the group, Control (C, n=10), and Dexa (D, n=10), before and after treatment with dexamethasone.

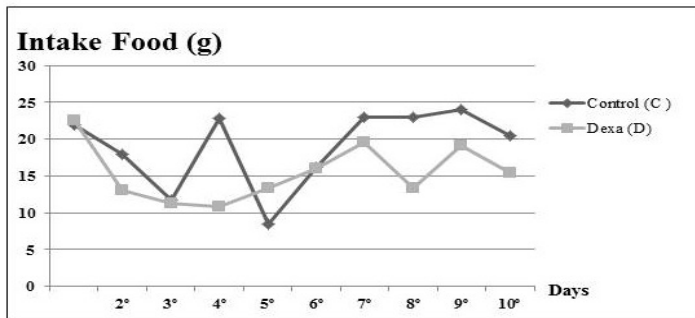


FIGURE 2 - Comparison of the Intake Water between Control (C, n=10) and Dexa (D, n=10) groups.

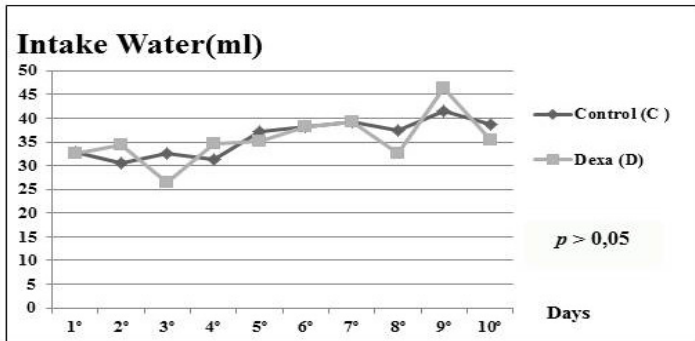


FIGURE 3 - Comparison of the Intake Water between Control (C, n=10) and Dexa (D, n=10) groups.

Table 1 shows that rats which received dexamethasone presented, glycemia, on mmol/l, higher than control group (D=19.5±2.1 vs C=14.2±3.1; p=0.0001); insulinemia, on mU/l, lower than control group (D=0.2±0.1 vs C=2.0±0.4; p=0.0001); HOMA-β, whose smaller values indicate diminished ability of the pancreatic β cells to secrete insulin, it was smaller than in control's group (D=0.2±0.1 vs C=4.2±1.7; p=0.0002); HOMA-IR, whose smaller values express bigger insulin resistance, it was smaller than in control's group. (D=0.2±0.1 vs C=1.3±0.4; p=0.0002).

TABLE 1 - Mean and standard deviation of the blood glucose, blood insulin, HOMA β and HOMA IR in Control (C) and Dexa (D) groups.

VARIABLES	C (n=10)	D (n=10)	P
Blood Glucose (mmol/l)	14.2±3.1	19.5±2.1	0.0001
Blood Insulin (mU/l)	2.0±0.4	0.2±0.1	0.0002
HOMA b	4.2±1.7	0.2±0.1	0.0002
HOMA IR	1.3±0.4	0.2±0.1	0.0002

HOMA = Homeostasis Model Assessment

Table 2 demonstrates that 100% of the animals treated with dexamethasone and no animal from control group had moderate presence of fatty on their liver (p=0.2), when submitted to histological exam.

TABLE 2 - Moderate hepatic glycogen and hepatic lipids in the group, Control (C) and Dexa (D).

VARIABLES	C (n=10)	D (n=10)	P
Hepatic glycogen n(%)	6 (60)	8 (80)	0.2
Hepatic lipids n(%)	0	10 (100)	0.0002

### Discussion

This experimental model indicated that the use of dexamethasone – glucocorticoid – induced hyperglycemia, which is alongside with other studies, and it can be explained by insulinemia reduction and the increase to peripheral resistance to insulin (smaller HOMA-IR)<sup>14,15</sup>.

Researches made with experimental models of peripheral resistance to insulin induced by glucocorticoids have shown controversy concerning insulinemia. Serum insulin level can be increased<sup>16</sup>, unaltered<sup>17</sup> or decreased<sup>18</sup>. The results of this study showed that there was hypoinsulinemia which, according to other researches it could have been a consequence of the decrease on pancreatic β cells action (smaller HOMA-β), due to oxidative stress caused by glucocorticoids<sup>19</sup>. Other studies demonstrated that glucocorticoids have several effects on pancreatic β cells<sup>20</sup>. Long-term exposure to glucocorticoids is associated with many diseases, including diabetes. A possible mechanism for toxicity in insulin-producing cells exposed to dexamethasone might be an increase in oxidative stress, because pancreatic β cells are particularly vulnerable and susceptible to ROS (reactive oxygen species)

toxicity<sup>21</sup>. Pancreatic beta cells are particularly vulnerable to ROS toxicity, due to their low antioxidant capacity and especially due to their low capacity to detoxify hydrogen peroxide, as these cells possess very low levels of catalase and glutathione peroxidase<sup>22</sup>. Probably the differences encountered must be related to dose and duration of administrating dexamethasone.

Several studies show that glucocorticoids induce peripheral insulin resistance, *in vivo* and *in vitro*<sup>5,8,23-26</sup>, by increasing hepatic glucose output and decreasing the peripheral glucose uptake. There are evidences that ROS<sup>7</sup> production plays a fundamental role on developing insulin resistance by being responsible for the smaller capture of glucose on muscle<sup>27</sup> and on adipose tissue<sup>28</sup>.

During quantitative analysis of liver fragments it was observed that a bigger percentage of animals treated with dexamethasone had moderate fatty amount. The increase on liver fatty has been associated to altered metabolic profiles on which insulin resistance is the prevailing characteristic<sup>29</sup>.

The reduction on body weight wasn't accompanied by decrease on feeding and drinking water. Studies indicate that injecting dexamethasone can reduce body weight without affecting food ingestion, possibly given to an increase on caloric expenditure<sup>30</sup>. It would be interesting developing future researches to evaluate energetic metabolism in this experimental model.

## Conclusion

Animals treated with glucocorticoids in this experimental model presented hyperglycemia, hypoinsulinemia and smaller peripheral sensitivity to insulin action.

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### Acknowledgements

To Ivan Lúcio de Melo and José Lopes Chave Neto for technical assistance.

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Received: Jun 21, 2016

Review: Aug 19, 2016

Accepted: Sep 18, 2016

Conflict of interest: none

Financial source: FAPEMIG

<sup>1</sup>Research performed at Experimental Surgery Laboratory, Medical School, Universidade do Vale do Sapucaí (UNIVAS), Pouso Alegre-MG, Brazil.