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Gerbils (*Meriones unguiculatus*) as a new experimental model of obesity induced by diet

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Background. Currently, the number of obese people in the world has reached alarming proportions. During the expansion of adipose tissue, a number of functions such as activation and release of cytokines and hormones may be affected. This causes the body to take a pro-inflammatory pattern, which may affect the proper functioning of many tissues. Thus, studying the mechanisms by which obesity induces physiological disorders is necessary, and may be facilitated by the use of animal models, in particular rodents. We sought to investigate whether the gerbil (*Meriones unguiculatus*) would be a good experimental model for the study of obesity and metabolic changes resulting from a diet rich in fats and sugars. **Methods.** 14 gerbils were divided according to weight into two experimental groups and received a a standard diet with 4,15kcal/g (CT; n = 7) or a diet rich in simple carbohydrates and fats with 5,86kcal/g (OB, n = 7) for 11 weeks. The animals had free access to water and food. The weight of each animal and food consumption of each cage were measured weekly and at the end of the experiment, blood, adipose tissue and liver were collected. The following parameters were determined: cholesterol (COL), triglycerides (TGL) and glycemia (GLI) in the plasma; cytokines (IL-6, IL-10 and TNF-α) and hormones (adiponectin and leptin) in adipose tissue; activity of superoxide dismutase (SOD) and catalase (CAT), extraction and differentiation of fat in the liver and liver histology. **Results.** The consumption of a diet rich in simple fats and carbohydrates led to an increase of total body weight, relative weights of liver and adipose tissue, glucose and triglycerides levels, and TNF-α concentration in adipose tissue. Animals of this group also showed a significant increase of total fat, cholesterol and triglyceride content in the liver, contributing to higher intensity of hepatic steatosis. On the other hand, depletion in the enzyme activity of SOD and CAT in the liver, as well as reduction of IL-10 and adiponectin levels were found in adipose tissue of these animals. **Conclusion.**

Diet consumption consisting of an excess in saturated fat and simple carbohydrates establish the gerbil as an experimental model for the study of obesity and metabolic and liver abnormalities resulting from this disease.

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Short title: Gerbils as a model of obesity

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Abstract

Background. Currently, the number of obese people in the world has reached alarming proportions. During the expansion of adipose tissue, a number of functions such as activation and release of cytokines and hormones may be affected. This causes the body to take a pro-inflammatory pattern, which may affect the proper functioning of many tissues. Thus, studying the mechanisms by which obesity induces physiological disorders is necessary, and may be facilitated by the use of animal models, in particular rodents. We sought to investigate whether the gerbil (*Meriones unguiculatus*) would be a good experimental model for the study of obesity and metabolic changes resulting from a diet rich in fats and sugars.

Methods. 14 gerbils were divided according to weight into two experimental groups and received a a standard diet with 4,15kcal/g (CT; n = 7) or a diet rich in simple carbohydrates and fats with 5,86kcal/g (OB, n = 7) for 11 weeks. The animals had free access to water and food. The weight of each animal and food consumption of each cage were measured weekly and at the end of the experiment, blood, adipose tissue and liver were collected. The following parameters were determined: cholesterol (COL), triglycerides (TGL) and glycemia (GLI) in the plasma; cytokines (IL-6, IL-10 and TNF-α) and hormones (adiponectin and leptin) in adipose tissue; activity of superoxide dismutase (SOD) and catalase (CAT), extraction and differentiation of fat in the liver and liver histology.

Results. The consumption of a diet rich in simple fats and carbohydrates led to an increase of total body weight, relative weights of liver and adipose tissue, glucose and triglycerides levels, and TNF-α concentration in adipose tissue. Animals of this group also showed a significant increase of total fat, cholesterol and triglyceride content in the liver, contributing to higher intensity of hepatic steatosis. On the other hand, depletion in the enzyme activity of SOD and CAT in the liver, as well as reduction of IL-10 and adiponectin levels were found in adipose tissue of these animals.

Conclusion. Diet consumption consisting of an excess in saturated fat and simple carbohydrates establish the gerbil as an experimental model for the study of obesity and metabolic and liver abnormalities resulting from this disease.

Introduction

In past decades, malnutrition was the main nutritional disorder requiring multi-professional care. Currently, much of the world's population has experienced the opposite problem, and the number of obese people in the world, has reached alarming proportions. The change in dietary pattern of the world population, making it rich in calories from fats and sugars, associated with physical inactivity, significantly increased prevalence of obesity not only in developed countries but also in developing ones (WHO, 2016). This fact has drawn much attention since obesity is strongly associated with increased risk of developing metabolic syndrome, characterized by central obesity, insulin resistance, dyslipidemia, increased blood pressure and nonalcoholic fatty liver disease (NAFLD) (Lumeng & Saltiel, 2011).

Adipose tissue is not simply an energy inert deposit, but is a multifunctional organ, that exerts important endocrine and immune functions (Lumeng & Saltiel, 2011; Clemente-Postigo et al, 2011; Gregor & Hotamisligil, 2011). Adipocytes are mainly responsible for the activation and release of substances, such as cytokines IL-6, TNF-α and hormones such as leptin and adiponectin (Trujillo et al, 2004; Jung & Choi, 2014). During the expansion of adipose tissue, these functions may be affected, leading the body to a pro-inflammatory pattern, which may affect the proper functioning of many tissues (Wellen & Hotamisligil, 2005). It is believed that the inflammation induced by obesity contributes to the development of many chronic diseases, including type 2 diabetes mellitus, atherosclerosis, liver disease and some forms of cancer (Lumeng & Saltiel, 2011; Gregor & Hotamisligil, 2011; Jung & Choi, 2014).

The study of the mechanisms by which obesity induces physiological disorders may be facilitated by the use of animal models, in particular rodents (Panchal and Brown, 2011; Nilsson et al, 2012). Considering that, despite its etiology, the number of obese individuals in the world is increasing, it is suggested that environmental or behavioral factors, such as food, are the main contributors to the epidemic, rather than genetic changes (Buettner, Scholmerich & Bollheimer, 2007). Thus, whereas the experimental model should be as close as possible to the genesis of study, polygenetic animal models with diet-induced obesity have been preferably used in place of monogenetic models (Rosini, Silva & Moraes, 2012).

Due to their size, ease of handling, and similarity to patterns found in humans (Eckmann, 2003; Araújo et al, 2008), the gerbil (*Meriones unguiculatus*) is a model already widespread in many

parts of the world and well established for the study of various diseases such as giardiasis (Ventura et al, 2013), *Helicobacter pylori* infection and stomach cancer (Kodama, Murakami & Fujioka, 2004; Junior et al, 2016), hearing disorders (Abbas & Rivolta, 2015), and more recently, as a good model for the study of visceralization of *Leishmania major* (Bakirci et al., 2015). Thus, we sought to investigate whether this rodent would be a good experimental model for the study of obesity and metabolic changes resulting from a diet rich in fats and sugars.

MATERIALS & METHODS

Fourteen male (average age of 20 weeks, 60-80 g of weight) gerbils (*Meriones unguiculatus*) were obtained from the Institute of Biological Sciences (ICB) of the Federal University of Minas Gerais (UFMG). The animals were maintained throughout the experimental period in collective cages under controlled temperature and lighting, with free access to filtered water and diet. After a week of acclimatization, the animals were divided according to weight into two groups of seven animals receiving standard diet (calorie density 4,15kcal/g - CT group) or hypercaloric diet (calorie density 5,86kcal/g - group OB), as shown in Table 1. After 11 weeks of the experiment, and 12 hours fasting, the animals were euthanized under anesthesia with intraperitoneal injection of Ketamine solution (100 mg/kg) and Xylazine (12 mg/kg), and blood, small intestine, visceral adipose tissue (perirenal, epididymal and mesenteric) and liver was collected. All experiments were conducted in accordance with the guidelines of the Ethics Committee on Animal Use (CEUA) of UFMG (protocol 136/13).

Table 1. Diets composition.

Ingredients	**(g/kg)**	
	Standard	**Hyper**
Casein	200	200
Corn starch	**397,5**	**62**
Sucrose	**100**	**-**
Maltodextrin	**132**	**-**
Gooseberry syrup	**-**	**310**
Lard	**-**	**355**
Soybean oil	**70**	**20**
Powdered cellulose	50	50
Mineral Mix (AIN-93G)	35	35
Vitamin Mix (AIN-93G)	10	10
Choline bitartrate	2,5	2,5
t-butylhydroquinone	0,014	0,014
Methionine	3	3
(kcal/g)	**4,15**	**5,86**

Assessment of food intake, body weight and adiposity

Individual body weight and the total intake of each cage were recorded weekly. At the end of experiment the relative liver weight and adipose tissue were calculated (tissue weight/final body weight x 100).

Dosages of cytokines and hormones in adipose tissue

Fragments with about 100mg of the epididymal adipose tissue were homogenized in protease inhibitor solution. Enzyme linked immunosorbent assays (ELISA) of the cytokines IL-6, IL-10, TFN-α, adiponectin and leptin, were performed from portion of the supernatant following the protocol recommended by the manufacturer (R&D Systems, USA).

Dosages of total cholesterol, triglycerides and plasma glucose

All measurements were performed by enzymatic colorimetric assay (Labtest kit, Brazil) as guidelines of the manufacturer's protocols.

Extraction and quantification of hepatic lipids

The determination of a hepatic lipid profile was taken after extraction of the total lipids in organic solvent, as described by Folch et al. (1957). 100 mg of liver tissue were used. For determination of the total cholesterol and triglyceride levels, lipid extracts were resuspended in 500µL of isopropanol, and dosage was performed by colorimetric enzymatic assay (Labtest kit, Brazil).

Histological analysis

Liver fragments were collected and washed with a saline solution. Tissues were then fixed in 10% buffered formalin, embedded in paraffin, and then sliced to a thickness of 4µm and stained with Hematoxylin & Eosin (H&E). A representative area of 30 images of liver tissue were calculated by the KS400 software on a Carl Zeiss image analyzer.

Evaluation of oxidative stress in the liver

Fragments of tissue (approximately 100mg) were homogenized in saline solution and the supernatant was performed with the following dosages:

Dosage of antioxidant enzyme catalase

Determination of catalase activity was based on the decrease in absorbance of hydrogen peroxide by this metabolization by catalase, as described by Nelson & Kiesow (1972). The calculations were made by the difference of reading in the start and end time, divided by the sample volume (mL). The result was expressed by protein concentration (mg/mL) measured by colorimetric assay.

Dosage of antioxidant enzyme SOD

The dosage of SOD was based on its ability to clean the radical O2-, decreasing the rate of auto-oxidation of pirogallol, adapted to Dieterich et al. To calculate the result, it was considered that 1 unit (U) of SOD was able to auto-oxidation 50% of pirogallol of the standard. The result was expressed as unit per mg of SOD protein.

Data analysis

To verify the distribution of the data the Shapiro-Wilk test was used, and to compare the means or medians the Student t-test or Mann Whitney was used, respectively. All statistical analyzes were performed using the program Prism (GraphPad Software, San Diego California, USA), version 6.0. Significance level was considered at 5%.

RESULTS

Body weight (Figure 1A) and relative liver weight (Figure 1B) were significantly increased in animals that received the diet rich in simple carbohydrates and fats. These results were supported by increased adiposity (Figure 1C) of the animals of group OB and were independent of the average amount of food each group ingested per week (Figure 1D).

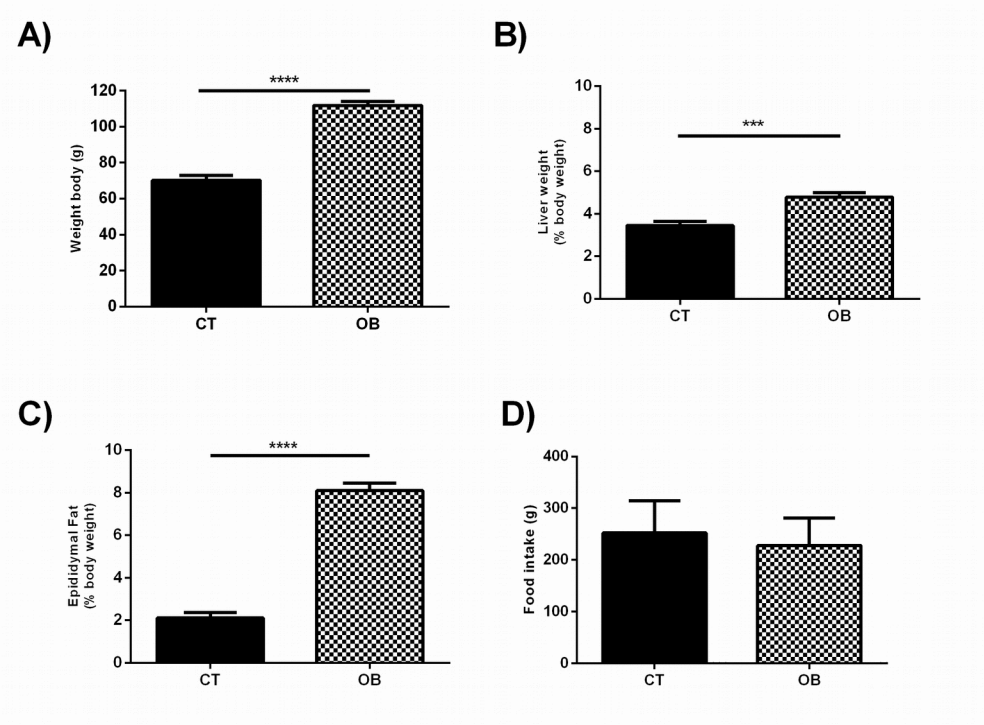


Fig. 1. (A) Body weight, (B) Liver weight (% body weight), (C) Epididymal fat (% body weight) e (D) Food intake in gerbil fed control (CT) or hiper (OB) diet during durante 11 semanas. Average ± S.E.M. n = 7 per group **** p< 0,0001 ou *** p<0,001.

The animals with excess body weight also presented metabolic alterations characterized by increased glucose and increased circulating triglycerides and ectopic fat deposition in the liver (Table 2).

Table 2. Fasting glucose, blood lipid profile and ectopic (liver) lipid concentration in gerbil fed control or hiper diet

Parâmetro	CT	OB
Fasting glucose (mg/dL)	133,5 ± 3,26	182,0 ± 14,17*
Blood cholesterol (mg/dL)	82,75 ± 2,91	89,08 ± 3,45
Blood triacylglycerol (mg/dL	180,2 ± 14,49	240,4 ±16,61*
Liver lipids (mg/g)	52,43 ± 3,66	210,0 ± 16,45****
Liver cholesterol (mg/g)	4,39 ± 0,28	7,76 ± 0,57***
Liver triacylglycerol (mg/g)	3,28 ± 0,39	93,89 ± 5,90**

Average ± S.E.M. n = 7 per group. *p<0.05, ** p<0.01, *** p<0.001 e ****p< 0.0001.

This hepatic lipid deposition in the animals of group OB generated histopathological changes with intense micro and macrovesicular hepatic steatosis, as shown in Figure 2 (A and B). These changes were confirmed by morphometric analysis (Figure 3), with greater involvement of the liver of the animals in the group OB by steatosis, compared to the control group (407811 ± 30903 μm^2 vs 29906 ± 18009 μm^2, respectively).

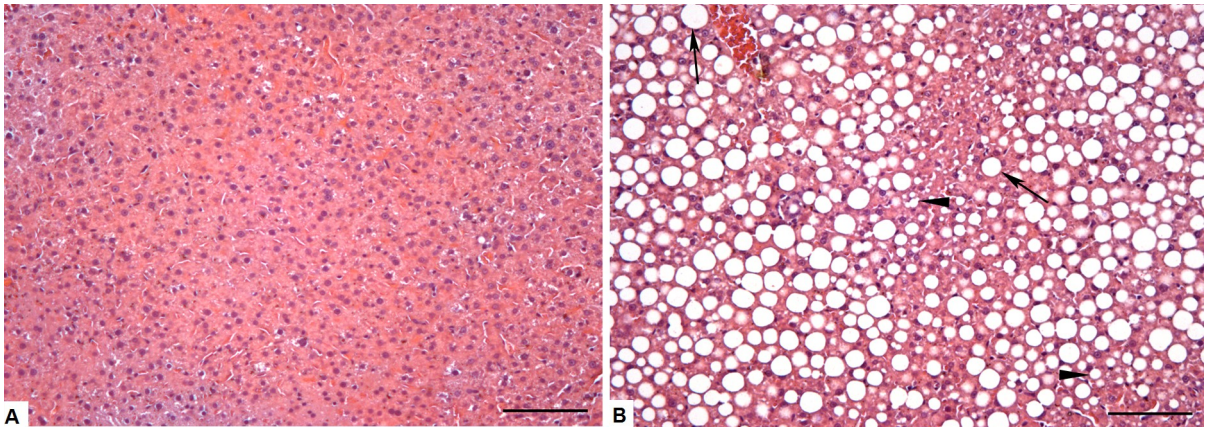


Fig 2. Photomicrograph of the liver tissue CT and OB groups A) Control group showing normal liver parenchyma.. **B)** Obese group. Microvesicular (arrowheads) and macrovesicular (arrow) steatosis and hepatocyte ballooning (setas). Hematoxylin & eosin. Bar = 100 µm.

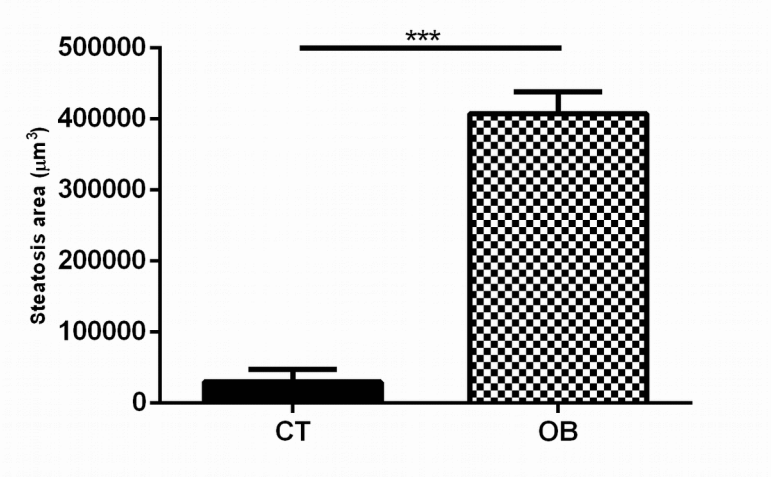
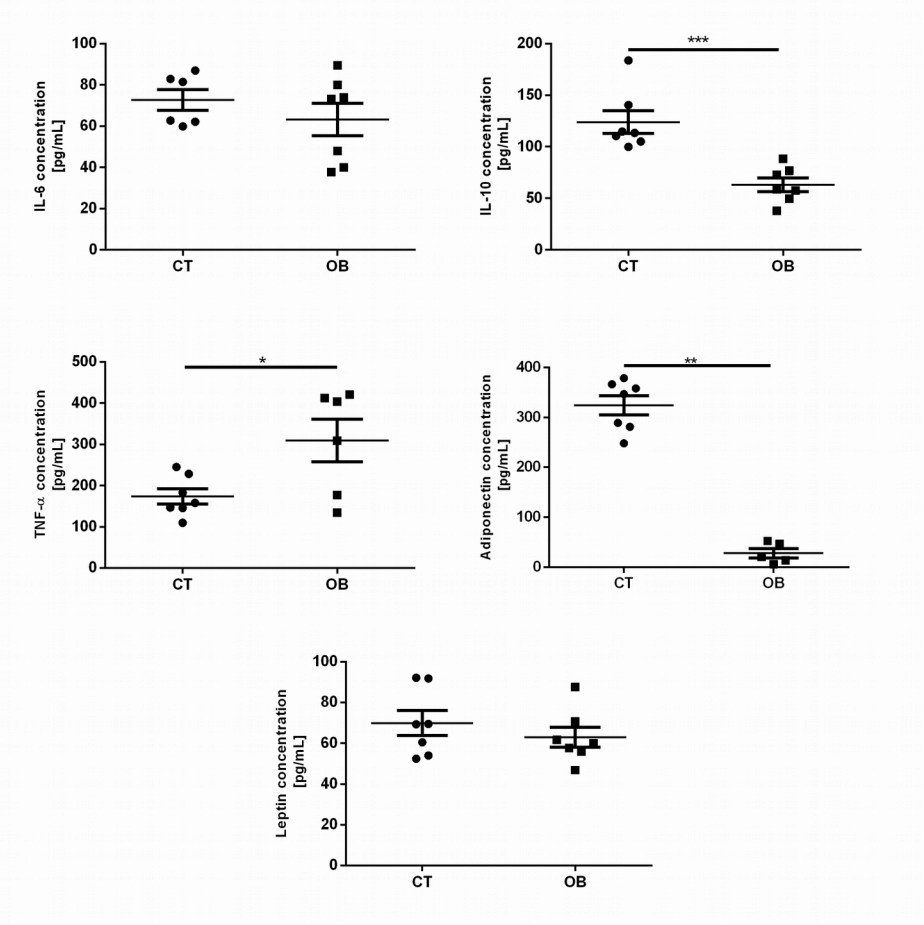


Fig 3. Morphometric analysis of liver tissue in CT or OB animals. Average ± S.E.M. n = 7 per group. *** p<0,001.

The metabolic and morphological changes were supported by significant reduction in the antioxidant capacity of the liver catalase enzymes (6.76 ± 0.40 vs. 4.65 ± 0.40 U / g of protein), p <0.01 and superoxide dismutase (1.74 ± 0.07 vs. 1.40 ± 0.11 U / g of protein), p <0.05 when compared to animals in the CT and OB groups, respectively.

Given that a number of changes in the levels of cytokines and hormones is reported in obesity, as an increase of TNF-á, IL-6 and leptin as well as reduction of IL-10 and adiponectin, it was investigated whether such changes would be found in fat animal model proposed in this study (Figure 4). Animals treated with a diet rich in fat and carbohydrates had an increased TNF-á concentration (TC = 173.8 ± 18.28 pg / ml and OB = 309.7 ± 51.71 pg / mL) and IL-reduction 10 (CT = 123.9 ± 11.08 pg / ml and OB = 63.11 ± 6.53 pg / ml) and adiponectin (CT = 324.1 ± 19.06 pg / ml and B = 27.7 ± 9.23 pg / mL) compared to animals fed control diet. IL-6 and leptin concentrations remained unchanged between groups.



173 **Fig. 4.** Adipose tissue concentration of cytocines and hormones. Average ± S.E.M. n = 7 per
174 group. * p< 0,05; ** p<0,01 e *** p<0,001.

DISCUSSION

Diets rich in simple sugars and fats generally cause an increase of body weight, resulting in increase in the number and volume of adipocytes (Hauner 2005). Consequently, there is a deregulation of secretory activity of the adipose tissue, contributing to the development of metabolic diseases, altering the inflammatory responses and the metabolism of lipids and glucose (Lumeng & Saltiel, 2011).

The purpose of this study was to evaluate if the rodent specie *Meriones unguiculatus*, could be used in experimental studies as obesity model, given that the best model to induce a disease is the one that best imitates its pathophysiological characteristics. The present data demonstrate that this model had many typical changes in obesity as an increase in bodyweight, in addition to the relative weights of liver and adipose tissue, increase in glucose levels and circulating triglycerides, as well as TNF-α in adipose tissue and larger levels of total fat, cholesterol and triglyceride in the liver, contributing to higher intensity of hepatic steatosis. Thus, we can say that this rodent could be established as a model to study obesity.

In the early stages of adipocyte hypertrophy, a series of phenomena such as oxidative stress, hypoxia and downregulation of some chemokines initiate the inflammatory process in this tissue (Bost et al, 2005; Ito et al, 2007). During obesity, there is also the recruitment and activation of other immune cells such as macrophages that, in turn undergoes a phenotype polarization, modifying its anti-inflammatory profile of M2 macrophages or "classically activated" to the pro-inflammatory profile of M1 macrophages or "alternatively activated", increasing the secretion of various proinflammatory cytokines, such as TNF-α and IL-6 and decreasing the production of anti-inflammatory factors such as adiponectin and IL-10 (Lumeng , Bodzin & Saltiel, 2007; Gregor & Hotamisligil, 2011; Jung & Choi, 2014; van Stijn et al, 2015).

TNF-α derived from macrophages induces the release of free fatty acids (FFA) from adipocytes via lipolysis (Cawthorn & Sethi, 2008) and at the same time, these FFA released, strongly stimulate TNF-α production by macrophages (Klop, Elte & Cabezas, 2013). This paracrine interaction between adipocytes and macrophages is a "vicious cycle", further accelerating the adipose tissue inflammation and promoting inhibitory effects on energy metabolism (Clement-Postigo et al, 2011). In addition, the TNF-α produced by adipose tissue expansion, can induce the increase of synthesis and release of leptin (Paz-Filho et al, 2012), which is involved in the regulation of food intake and energy expenditure (St. -Pierre &

Tremblay, 2012). Circulating levels of leptin and its expression in adipose tissue are increased in obese individuals, probably because of leptin resistance (Myers et al, 2010).

Increased values of TNF-α were observed in our obesity model, however, we found no significant change in the amount of IL-6 or leptin in obese animals compared to the control. It is known that in humans, leptin production by adipose tissue is influenced by IL-6 production in the same tissue (Trujillo et al, 2004). Furthermore, it has been also reported that the concentration of leptin in relation to adipose mass in humans, decreases as factors associated with metabolic syndrome worsen, especially in hypertriglyceridaemia (Paz-Filho et al, 2009). Thus, we believe that in gerbils, the increase of TNF-α by itself was not able to induce increased production of leptin, and it may be necessary the additional IL-6 stimuli. Still, considering the lowest concentration of Leptin and IL-6 in relation to adipose mass, we assume that in gerbils, hypertriglyceridemia, may have them decreased as it has already been described in humans.

The unbalanced production of cytokines, with a reduced production of anti-inflammatory cytokines, such as adiponectin, is a characteristic of a diet-induced obesity. Under normal conditions, this cytokine is widely produced by adipose tissue and acts mainly promoting the activation of protein kinase activated by adenosine monophosphate (AMPK), which phosphorylates regulatory enzymes of glycolysis, gluconeogenesis and lipid oxidation in liver and skeletal muscle (van Stijn et al, 2015). Another important function of adiponectin is the induction of IL-10 expression in M2 macrophages, another counter-regulatory inflammation cytokine (van Stijn et al, 2015). In obese individuals, plasma levels of IL-10 can be re-established with weight loss (Golubović et al, 2013). Our obesity model showed decreased levels of adiponectin and IL-10 in adipose tissue, suggesting that metabolic disorders found in plasma and liver of the animals are resulting from this reduction.

Taken together, the changes arising from obesity observed in this work with the increased cytokines inducing lipolysis, increased local inflammation and decreased adipokines that regulate inflammation, we can say again that the animal model used was successful.

The increase in TNF-α production, together with lower levels of adiponectin, suggests peripheral resistance to insulin action (Yamauchi et al, 2002), which can be observed by an increase in plasma glucose in OB animals. Hypertriglyceridemia, investigated in this model may result from increased release of FFA from adipocytes via activation of the lipase hormone sensitive enzyme (Clemente-Postigo et al, 2011) and lower oxidation of FFA in adipose tissue and

less use of glucose in the liver and skeletal muscle. There is also less activation of the lipoprotein lipase (LPL) enzyme, thus causing lower uptake of very low density lipoproteins (VLDL) and increase levels of circulating triglycerides (Yamauchi et al, 2002). Once the OB animals showed decreased adiponectin concentration in adipose tissue and increased TNF-α, we believe that these metabolic pathways are certainly impaired, as it has been reported in humans (Matsubara et al, 2002; Chang et al, 2012).

Steatosis, it is often only an initial trigger for a series of diseases in the liver tissue, such as non-alcoholic steatohepatitis (NASH) and therefore cirrhosis (Tilg & Moschen, 2010). In steatosis, hepatocytes are more susceptible to the action of bacterial toxins derived from the intestine, mitochondrial dysfunction, dysregulated apoptosis, oxidative stress, the action of proinflammatory cytokines and adipokines, and activation of pro-fibrogenic factors, that lead to disease progression (Tarantino et al, 2010; Polyzos et al, 2012).

Under normal circumstances, the liver aerobic metabolism involves a stable production of pro-oxidants such as reactive oxygen species (ROS) and reactive nitrogen species (RNS), which are equilibrated by a similar rate of its consumption by antioxidants (Winterbourn, 2008). An imbalance in these rates, in favor of pro-oxidants, constitutes the phenomenon of oxidative stress, characterized by oxidation of essential biomolecules for ROS and RNS, causing the loss of their biological functions and cell viability (Görlach et al, 2015). In addition, ROS may indirectly activate transcription factors such as nuclear factor kB (NF-kB) (Tornatore et al, 2012) thus causing the production of cytotoxic mediators, proinflammatory and fibrogenic mediators by the Kupffer cells (Takeuchi-Yorimoto , 2013). However, as the hepatic disease progresses, there is further reduction of antioxidant capacity of the liver, as demonstrated by the significant reduction in activity of catalase and superoxide dismutase, reaching a systemic reduction of antioxidant capacity of the body in the later stages steatohepatitis, as demonstrated in a study of patients with NASH (Videla et al, 2004). Thus, we consider the reduction of hepatic activity of antioxidant catalase enzymes and superoxide dismutase, verified in this work, characterized the transitional state between NAFLD and NASH, and it is possible that, over time, the increased oxidative stress in the liver from animals in the OB group, unleashed the most serious manifestations of liver disease.

CONCLUSIONS

Hypercaloric diets with simple fats and carbohydrate content induce obesity in gerbils. This is the first work that uses the gerbil as an experimental model for the study of this morbid condition. It is possible to affirm that the gerbils (*Meriones unguiculatus*) can be widely adopted as an effective model for the study of obesity induced by diet.

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