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Obesity induction in hamster that mimics the human clinical condition

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Abstract: Although obesity is well established in hamsters, studies using diets with high levels of simple carbohydrate associated with lipids are necessary to assess the impact of this type of food in the body. In this study a high sugar and butter diet (HSB) and high temperature were employed towards this end. Obesity was successfully induced at a temperature of 30.3°C to 30.9°C after 38 days feeding the animals an HSB diet. It was shown that although diet is important for the induction of obesity, temperature is also essential because at a temperature slightly below the one required, obesity was not induced, even when the animals were fed for a longer period (150 days).The obese clinical condition was accompanied by biochemical and hematological changes, as increased cholesterol and triglyceride levels and increased leukocyte numbers, similar to alterations observed in obese humans. Furthermore, it was demonstrated that increasing the intake of simple carbohydrates associated with lipids provided evidence of inflammation in obese animals.
Key words: AIN-93, hamster, high sugar and butter diet, obesity, temperature

Introduction

Obesity is increasing gradually due to poor diet and sedentary lifestyles in the population around the world. According to the World Health Organization [54], approximately 1.9 billion adults have a body mass index (BMI) in the overweight range, and approximately 600 million are obese. This condition may be characterized as low intensity inflammation caused by the increased body weight and adipose tissue expansion, increasing the predisposition to cardiovascular diseases, dyslipidemias, cancer, chronic kidney disease, and type 2 diabetes [30].

Hypercaloric and high fat diets have been widely used for inducing obesity in animal models because there is a similarity with the pathogenesis of obesity in humans [11]. Considering the physiological similarity to humans, pigs have been used as good models for these studies

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Supplementary Figures: refer to J-STAGE: https://www.jstage.jst.go.jp/browse/expanim

Table 1. AIN-93 M diet formulated for maintenance of adult rodents and the high sugar and butter diet

Ingredient	g/kg diet	
	AIN-93M (CTRL diet)*	High sugar and butter (HSB) diet
Cornstarch	465.692	265.692
Casein (>85% protein)	140.000	140.000
Dextrinizedcornstarch	155.000	–
Sucrose	100.000	265.000
Soybean oil	40.000	40.000
Fibre (cellulose)	50.000	50.000
Mineral mix (AIN-93 M-MX)	35.000	35.000
Vitamin mix (AIN-93-VX)	10.000	10.000
L-Cystine	1.800	1.800
Choline bitartrate	2.500	2.500
Tert-butylhydroquinone	0.008	0.008
Unsalted butter	–	190.000
TOTAL	1,000	1,000
Kcal/g of diet	3.8	4.8
Carbohydrate (%)	76	45
Protein (%)	15	12
Lipid (%)	9	44

Note: Adapted from Reeves, *et al.* [39].

[47], but work with these animals in laboratories turns out to be unfeasible due to the physical structures required. Mice [52], rats [53] and hamsters have also served as models for studying obesity [50].

Some pathogens, particularly hookworms [34], *Leishmania* sp. [3], Hantavirus [42], and Ebola virus [12] are more likely or exclusively infective for particular groups of rodents, as hamsters. Furthermore, hamsters are used as a model of cardiomyopathy, diabetes, muscular dystrophy, cancer and other diseases [16]. Despite the obesity model be established in hamster, studies using diets high in simple carbohydrates and lipids, that would contribute on the understanding of some chronic diseases, are scarce in the literature, requiring further investigation as suggested by Greenwood *et al.* [20]. Morgantini *et al.* [32] shows that high glycemic carbs interfere with the absorption of lipids and contribute to the development of low intensity inflammation, as glucose has the ability to activate genes related to inflammation by epigenetic mechanisms. Hamsters are considered seasonal hibernators, a characteristic that can manifest at any time of the year depending on environmental conditions and is influenced by annual periods and shorter days and various physiological parameters [4, 22, 26]. Therefore, it is conceivable that the induction of obesity will be influenced by these parameters. Thus, the objective of this study was to induce obesity in hamsters (*Mesocricetus auratus*) in different annual periods and assessing for temperature interference in weight gain. Furthermore, we evaluated to which extension obesity induced by a diet with a high concentration of simple carbohydrates and lipids interferes with clinical and haematological parameters of animals compared to controls.

Materials and Methods

Ethical statement

All animal procedures were approved by the Animal Care Ethics Committee of the Universidade Federal de Minas Gerais (UFMG, protocol number 194/2011) and were performed under the guidelines of the Brazilian Council of Animal Experimentation (CONCEA) and strictly followed Brazilian law for "Procedures for the Scientific Use of Animals" (11.794/2008).

Experimental diets

The composition of the manipulated diets in the present study was based on the AIN-93 M rodent diet as recommended by the American Institute of Nutrition (1993) with modifications of the fat components and carbohydrates as described by Maioli *et al.* [28]. The diets were offered to animals as pellets, and each diet group was maintained until the day of euthanasia. The animals were fed on diets *ad libitum.* The composition of AIN-93 M (CTRL) and the high sugar and butter diet (HSB) diet are described in Table 1.

Experimental design

This study was performed using female hamsters (*Mesocricetus auratus*) (4–6 weeks old). They were housed in the Animal Facility at the Department of Parasitology, UFMG, with a 12 h light/dark cycle. Water and manipulated diet were provided *ad libitum*. Animals at the facility are routinely treated before experiments with one 5 mg/kg dose of Ivomec ® (Ivomec Gold 1%, Merial Saúde Animal Ltda, SP, Brazil) via gavage to eliminate possible parasitic infections. The hamsters were randomly distributed into two groups each that were fed either AIN-93 M (CTRL) [39] or a high sugar and butter diet (HSB) modified from AIN-93 M [28]. The diets of each group were maintained until the day of euthanasia. Two experiments (12 hamsters/each) were conducted to evaluate the effect of temperature on hamster eating behaviour and its influence on hamster weight gain: 1) April–September (fall–spring, 150 days), and 2) December–March (summer, 68 days). A third experiment (12 hamsters) was conducted from June to November (winter–spring, 150 days) following the same methodology, and the results are not presented, but are discussed. During the first experiment, a heater was installed on the 60th day of the experiment in the animal house to increase the room temperature. The findings of the first experiment were used to standardize and optimize the weight gain of the animals for the remaining experiments. In experiment 2 and 3, the heater was maintained from the first day of the experiment. The animal house temperature was measured daily. The meteorological data for the duration of the experiments were obtained from the National Institute of Meteorology of Brazil (INMET).

Assessing obesity condition

The parameters used to considerer an animal obese were biochemical and haematological changes, increased adiposity percentage and significant weight gain compared to the control group. After animals reached a statistically significant weight gain, they were euthanized by an overdose of anaesthetic solution administered intraperitoneally (240 mg/kg ketamine plus 45 mg/kg xylazine cloridrate solution, Cetamin® and Xilazin®, Syntec, Brazil).

Measurement of hamster weight

The weight of the hamsters was measured on day 0 of the experiment and every 7 days until the end of the experiments using a semi-analytical balance.

Consumption of food and water

The diets were offered *ad libitum* to each group of animals. The consumption of diets per group was calculated by the difference in grams of food offered and remaining food found in the cages daily. Water consumption was measured twice a week and was calculated by the difference in millilitres of water offered and remaining water found in the bottle. Food and water consumption values are expressed as the mean for each group.

Blood collection and haematological parameters

The blood samples were collected on the first day of the diet (day 0) and every 30 or 38 days until the end of the experiment, based on the study of Pacanaro *et al.* [34]. The hamsters were fasted for 12 h prior to individual blood collection from the sublingual vein. Two aliquots of blood were collected: one with EDTA-potassium fluoride solution added to each sample (Doles, Goiânia, Brazil), to obtain plasma, and other, without anticoagulant, to obtain serum after centrifugation. The blood was used to evaluate red blood cell (RBC) and white blood cell (WBC) numbers. The cells were counted with a Newbauer chamber. The WBC differential count was determined with smears stained with May-Grunwald-Giemsa and examined under a light microscope (Olympus BH2, Japan).

Blood biochemical parameters

The animals were fasted for 12 h before blood collection. The biochemical parameters evaluated from plasma were total cholesterol and triglycerides and from serum, was evaluated fasting glucose. All measurements were performed using commercial kits (Doles, Goiânia, Brazil). Reference values for female hamsters were obtained from Gad [16].

Visceral adiposity

One day before the end of the experiment, all animals were weighed. After euthanasia, the visceral adipose tissue was recovered from each animal and weighed [34]. The visceral adiposity index was determined by the formula:

$$\text{Adiposity} = \frac{\text{Weight of visceral adipose tissue recovered} \times 100}{\text{Total weight of the live animal}}$$

Statistical analysis

All data were examined for normality using the Kolmogorov-Smirnov test. For analysis between two groups, Student's *t*-test was used for parametric data, and the Wilcoxon Matched Pairs or Mann-Whitney test was used for nonparametric data. The Kruskal-Wallis test was used followed by Dunn's post-hoc test for comparisons of three or more groups of nonparametric data. One-way ANOVA followed by Tukey post-hoc test was used for parametric data. The Grubbs test was used to detect outliers, which were removed from the sample. All tests were considered significant at the $P<0.05$ level. All analyses were performed using GraphPad Prism 5.0 for Windows (GraphPad Software, San Diego, CA, USA).

Results

Experiment 1: Fall-Spring

Figure 1a shows the temperature values outside and inside the animal house and the weight gain of both groups. The heater, installed on the 60th day of the experiment, increased the temperature of the animal house from 25.1°C to 30.3°C (Supplementary Fig. S1a). The weight difference between the CTRL and HSB groups began after the 90th day (30 days after installation of the heater unit in the room). Consequently, 150 days after starting the diet, the HSB group showed a significant increase in visceral fat (approximately 2×) (Fig. 1b). The external maximum temperature averaged 25.7°C in the period during which this experiment was performed.

After installing the heater, two animals of the HSB group showed aggressive behaviour and dominance in the cage. These animals interfered with the feeding performance of the remaining animals in the cage and were removed from the experiment. After the isolation of the dominant animals, the aggressive behavior persisted to the remaining animals, being detected by fights among the females and wounds in the body, but without interference in the weight gain. There was a significant difference between the feed intake of the CTRL (9.94 ± 1.78 g/day/animal) and the HSB (7.79 ± 0.19 g/day/animal) groups ($P<0.05$), whereas no difference was observed for water consumption (data not shown).

There was a significant difference in the levels of total triglycerides (Fig. 2a), cholesterol (Fig. 2b) and glucose (Fig. 2c), with increased levels of cholesterol and triglycerides to the animals from the HSB group compared to the control group. This difference was higher

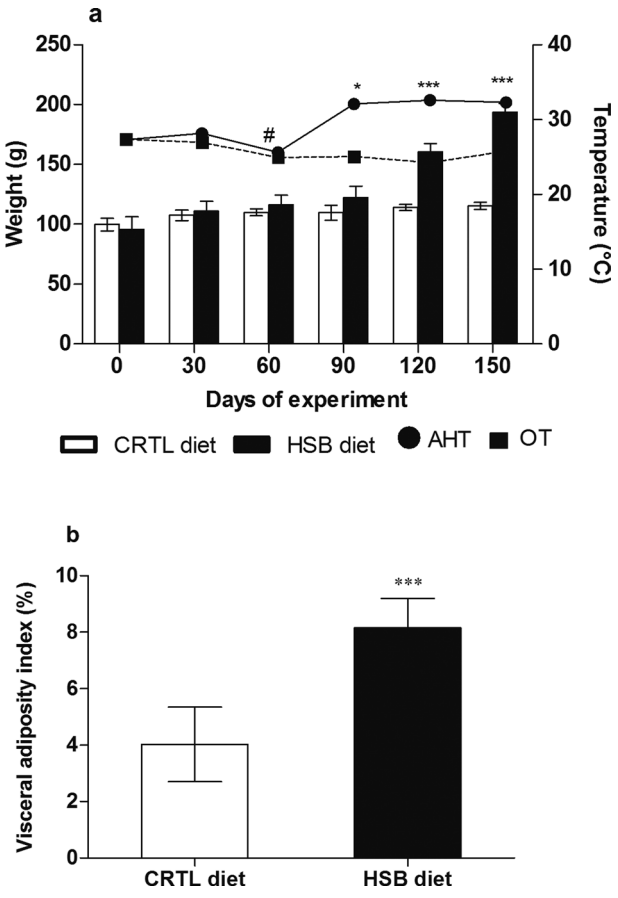

Fig. 1. (a) Hamster body weight in grams (bars) and temperature inside and outside the animal house (lines). (b) Visceral Adiposity Index at 150 days (as a percentage). Hamsters fed the AIN 93 M diet (CTRL, n=6) and hamsters fed the high sugar and butter diet (HSB, n=4). Animal house temperature (AHT) and outside temperature (OT) in degrees Celsius (°C). # Start of heater use in the animal house. * $P<0.05$ *** $P<0.001$. The data are shown as the mean ± standard deviation.

from day 90 to the end of the experiment for total triglycerides ($P<0.01$) and cholesterol ($P<0.001$), while the HSB group only showed increase in blood glucose ($P<0.001$) from day 120 to the end of the experiment.

After 60 days of the experiment (installation of heater), hamsters fed with the high sugar and butter diet had an increase in the number of leukocytes (Fig. 3a). After the 120th day of the experiment, we observed an increase in the number of neutrophils (Fig. 3b) and lymphocytes (Fig. 3c). The monocytes were elevated from the 60th day until the end of the experiment (Fig. 3d).

Experiment 2: Summer

After it has been demonstrated the need of a heater to

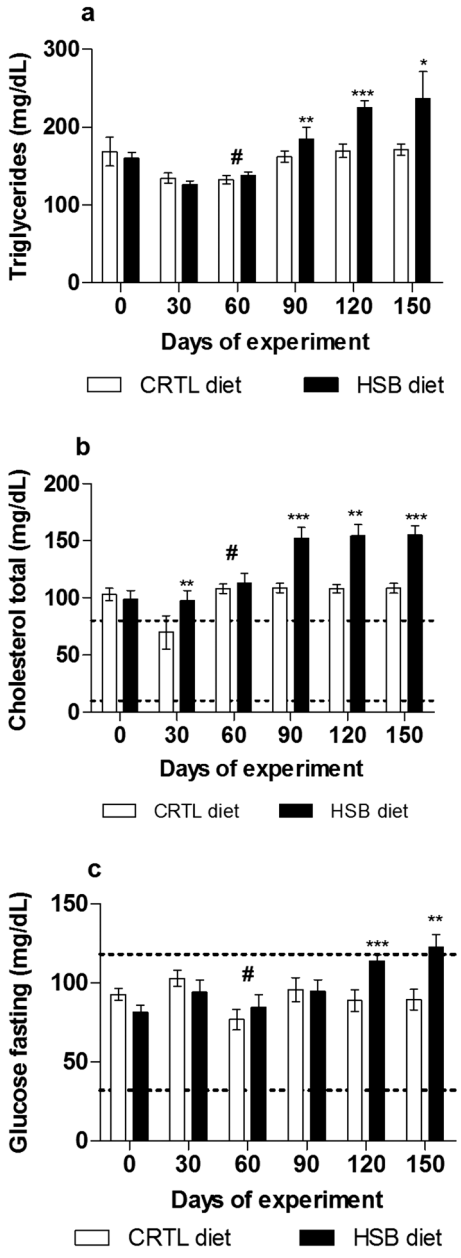

Fig. 2. (a) Triglycerides in milligrams per decilitre. (b) Cholesterol in milligrams per decilitre. (c) Glucose fasting in milligrams per decilitre. Hamsters fed the AIN-93 M diet (CTRL, n=6) and hamsters fed the high sugar and butter diet (HSB, n=6 or 4). # beginning of use of the heater in the animal house.* $P<0.05$ ** $P<0.01$ *** $P<0.001$. The data are shown as the mean ± standard deviation.

maintain the animal house temperature higher than the outside temperature, new experiments were performed to analyse other parameters, as visceral adiposity (experiments 2 and 3, data not shown). For these experiments, the animals were fed the same diet offered to animals in the first experiment.

The external temperature average for experiment 2 was 29.6°C, and the average temperature of the animal house was 30.9°C (Supplementary Fig. S1c). As in experiment 1, there was a significant difference between the feed intake of the CTRL group (8.97 ± 1.97 g/day/animal) and the HSB group (7.49 ± 0.23 g/day/animal) ($P<0.05$). No difference was observed in water consumption (data not shown). In this experiment, the HSB group had significantly different weights at the 38th day ($P<0.01$) of the experiment, and the experiment was extended for 30 days more, to analyze possible biochemical and haematological alterations, and the weights difference between the two groups remained significantly different ($P<0.01$).

Two animals from the HSB group died during the experimentation period. Therefore, the weight and visceral adiposity values for this group were measured on the 68th day for four animals. Nevertheless, the obesity had been established with significant differences between the HSB and CRTL groups as demonstrated on both parameters, final weight (Supplementary Fig. S2a) and visceral adiposity (Supplementary Fig. S2b).

In experiment 2, the HSB group had elevated levels of triglycerides ($P<0.001$; Supplementary Fig. S3a) and cholesterol ($P<0.01$; Supplementary Fig. S3b) within 38 days, which remained the case until the end of the experiment. Blood glucose levels were significantly lower in the CRTL group at the 38th day ($P<0.01$), but this did not continue through the end of the experiment. The glucose levels remained within the reference values for hamsters, and no difference was observed at the 68th day (Supplementary Fig. S3c).

The HSB group of experiment 2 had increased WBC cells from the 38th day until the end of the experiment in animals fed the high sugar and butter diet ($P<0.01$; Supplementary Fig. S4a). On the 38th day, we observed an increase in neutrophils and monocytes ($P<0.01$; Supplementary Fig. S4b and $P<0.001$; Supplementary Fig. S4d, respectively). However, on the 68th day, we observed a decline of these cells and a predominance of lymphocytes ($P<0.01$) in the blood of animals from the HSB group (Supplementary Fig. S4c).

As previously mentioned, experiment 2 had two deaths from the HSB group at 50 and 59 days, and it was observed that these animals had an excessive increase in leukocyte numbers, with values of 24,800 and 23,560 leukocyte/mm^3 for each animal.

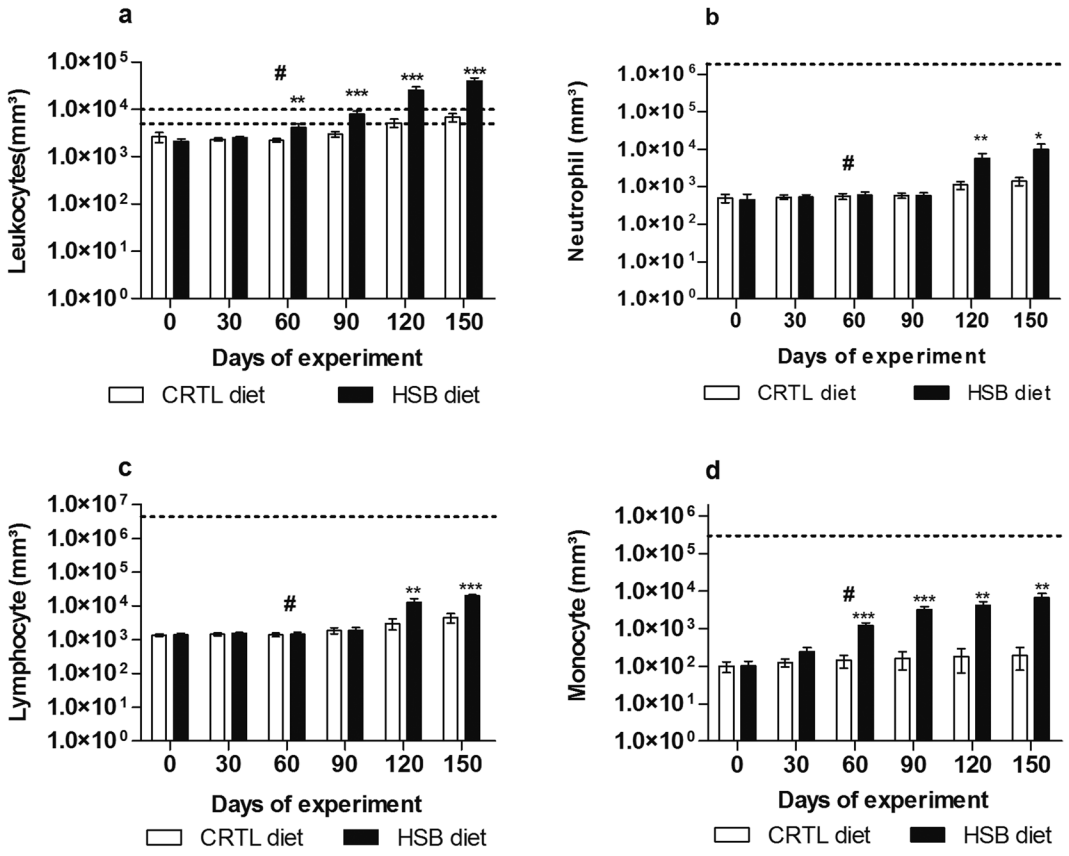

Fig. 3. Total and differential leukocyte counts. (a) Leukocytes per mm^3 of blood. (b) Neutrophils per mm^3 of blood. (c) Lymphocytes per mm^3 of blood. (d) Monocyte per mm^3 of blood. Hamsters fed the AIN 93 M diet (CTRL, n=6) and hamsters fed the high sugar and butter diet (HSB, n=6 or 4). # beginning of heater use in the animal house. * $P<0.05$ ** $P<0.01$ *** $P<0.001$. The data are shown as the mean ± standard deviation.

Discussion

The main genesis of obesity in humans is due to the ingestion of a high fat and high sugar diet. Considering that obesity represents a serious global problem, the establishment of experimental models that can be extrapolated to human's physiological conditions is crucial. Different strategies have been used to establish animal's models to study obesity, as an example by lesion of the medial hypothalamus region that is responsible for satiety [40]. However, in most of the situations the induced obesity does not results in an ideal model of study, because the parameters that are altered by high fat and high sugar diet could not be achieved. In this work, a clinical condition of obesity was established in a hamster model using a high sugar and butter diet and high temperatures.

The influence of temperature observed in experiment 1 demonstrates that temperature needs to be under close surveillance when inducing obesity in hamsters. According to Wade and Bartness [50], it is harder to induce obesity in hamsters in seasons other than summer, corroborating our data. The difficulty on the development of obesity induction protocols is because these animals have a distinct metabolism: they can hibernate at any time of year according to the environmental conditions, which may influence weight gain [4, 22, 27]. It was also observed a behavior of dominance and stress, to all groups. This behavior is common in models maintained in group. However, the animals from the HSB group exhibited an extremely aggressive behavior and cage dominance, preventing weight gain for the other animals. The stress and aggressiveness observed in obese animals can lead to an increase in cortisol levels, causing various physiological changes in the body. Recent studies have demonstrated the involvement of the 11β-HSD1 enzyme (11β-hydroxysteroid dehydrogenase type 1) in the activation of cortisol in adipose tissue and its relation to the development of obesity and insulin resistance, as corti-

sol is an insulin-antagonistic [21, 35].

Experiment 1 covered three seasons (fall, winter and spring), with a natural temperature average of 25.7°C throughout the period. Unlike other studies described in the literature [5, 24], that reported the induction of obesity in hamsters at temperatures below 25°C, hamsters in this study only reached obesity after the introduction of a heater into the animal house that increased the temperature by 5.2°C (25.1°C to 30.3°C). These results were replicated in experiment 2 (supplementary data). These data corroborate the results of Zhao [56], which indicated that temperature was an essential factor in the accumulation of fat in hamsters. Cannon and Nedergaard [8] observe that the ideal temperature for housing rodents would be 30°C because, this is a zone of thermal neutrality to these animals, with results that can be compared to the humans thermal neutrality zone. The work done by Zhang *et al.* [57] has shown that the activity of the mitochondria at a temperature of 30°C is lower in hamsters, thus reducing thermogenesis. The consumption of a hyperlipid diet, leads to an increase in oxidative stress, resulting in the elevation of free fatty acids, hyperglycemia and postprandial hypertriglyceremia [14]. These factors are responsible for the elevation of reactive oxygen species, formation of superoxide anion and production of proinflammatory cytokines [10]. Apparently, increased oxidative stress decreases the activity of the uncoupling protein 1 (UCP1), present in brown adipose tissue besides being involved in mechanisms that aggravate morbidity in obese individuals [57]. Although brown adipose tissue is present mainly in newborn humans, it is known to be of some importance for adults and its metabolism is conditioned by changes in temperature, mainly when it drop off, and that is associated with weight loss. However, the interruption of the cold exposure due to the increase of the temperature on internal environments, leads to the weight gain and thermogenesis decreases [29].

In experiment 3 (data not shown), performed during winter and spring, even using the heater, the temperature was not increased above 28.8°C and, even after 150 days, we were unable to induce obesity in the animals. Coherently with the results found on the two previous experiments, where obesity was accompanied by biochemical and haematological changes, no such alterations were detected in this experiment to any of the evaluated parameters. This can be explained by the fact that with higher temperature, animals required less energy to keep their body temperature, resulting in a positive energy balance [49]. Kodama and Pace [25] showed that the decrease in temperature decreased the accumulation of body fat in hamsters, as observed in this study, and the narrow temperature variation range (30.3°C and 30.9°C in experiments 1 and 2, respectively, to 28.8°C, in experiment 3, Supplementary Fig. S1e) may be indicative that the temperature for Syrian hamsters is really a determining factor for the induction of obesity. Although the present study did not induce obesity in a temperature equal or lower than 28.8°C, other studies have demonstrated induction of obesity in experimental models at a temperature lower than the one established in this work [8, 49]. However, these studies did not characterize the season in which the experiments were conducted. Meisel *et al.* [31] showed that obesity induction is influenced by the way animals are housed, showing that female hamsters housed in groups exhibited greater adiposity than individually housed animals. The grouping of animals leads to an increase in temperature, reducing the energy expenditure necessary to maintain body temperature, which results in a positive energy balance. Moreover, the grouping may cause stress to the animals because hamsters live alone under natural conditions [6, 18]. Foster *et al.* [15] found that hamsters subjected to stress showed statistically increased food intake, weight gain and adiposity. Given that animals in this study were housed in groups, this may have favoured the weight gain for both the HSB group and the CTRL group. In our experiments, the animals from the HSB group ingested a smaller amount of food when compared to animals from the CTRL group, which demonstrated that the gain in weight and visceral fat were not caused by hyperphagia but by the high calorie modified diet. These data are similar to those found by Ramirez [36]. On the other hand, the volume of water was not different for the CTRL and HSB groups in any of the experiments, contrary to the findings, of Townsend *et al.* [48] in an experiment using mice.

Carbohydrates are a major source of energy for the functioning of the body. However, excessive consumption of carbohydrates, causes the expansion of adipose tissue, hyperglycemia and increased cholesterol [7, 9, 43, 55], whereas the exaggerated consumption of fat also produces these changes and promotes non-alcoholic fatty liver disease [17]. Furthermore, it has been demonstrated that the type of ingested carbohydrate correlates to the degree of inflammation intensity and with

the outcome of cardiovascular diseases [17]. This fact is related to the speed that the body absorb each type of carbohydrate influencing the ability to raise the glycemic index. Although the relationship between inflammation and the intake of carbohydrates is still obscure [20], evidence indicates a possible change of pro-inflammatory cytokines [19]. The high fat diet is a more natural way to induce obesity and is able to cause alterations of the biochemical parameters in experimental models [9, 23, 33].

Studies have shown the association of hypertriglyceridemia and hypercholesterolemia with obesity, indicating that the increased levels of these molecules is related to predisposition to heart disease [2, 37, 41, 44]. The consumption of a diet high in fat and simple carbohydrates interferes with fat absorption because sugar presents the capacity of increases lipid storage on the enterocytes and of stimulate the secretion of chylomicrons [32]. As a hormonaly active tissue, visceral adipose tissue releases different bioactive molecules and hormones that are able to interact with the immune system of the individual, interfering in many pathologic processes.

Many authors have reported an increase in white blood cells, triglycerides and body mass index in obese individuals, relating these results to the inflammation seen in obesity. Whereas the inflammatory response is associated with an increase in macrophages, other cell types are involved in the inflammation mechanism of the adipose tissue, such as neutrophils, mast cells, B lymphocytes and T lymphocytes [13, 38, 45, 51]. These results corroborate the results found in the present study in which leucocytosis was induced with an increase in lymphocytes, monocytes, and neutrophils. Neutrophils play an important role in the inflammatory response and in the recruitment of macrophages [46]. They also have the ability to respond to inflammatory stimuli and are the first cells to arrive at the site of inflammation [1].

In conclusion, this study showed that the temperature and the season influence the obesity establishment in Syrian-hamsters using a diet with high simple carbohydrates and lipids. The temperature above 30°C provides the ideal conditions for establishment of obesity in this model. Furthermore, the change in the type of food caused haematological and biochemical changes, similar to what is observed in human obesity, and provided evidence of inflammation in hamsters fed with HSB diet (obese animals).

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