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Cafeteria diet-induced obesity plus chronic stress alter serum leptin levels

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ABSTRACT

Obesity is a disease that has become a serious public health issue worldwide, and chronic stressors, which are a problem for modern society, cause neuroendocrine changes with alterations in food intake. Obesity and chronic stress are associated with the development of cardiovascular diseases and metabolic disorders. In this study, a rat model was used to evaluate the effects of a hypercaloric diet plus chronic restraint stress on the serum leptin and lipids levels and on the weight of specific adipose tissue (mesenteric, MAT; subcutaneous, SAT and visceral, VAT). Wistar rats were divided into the following 4 groups: standard chow (C), hypercaloric diet (HD), stress plus standard chow (S), and stress plus hypercaloric diet (SHD). The animals in the stress groups were subjected to chronic stress (placed inside a 25 cm × 7 cm plastic tube for 1 h per day, 5 days per week for 6 weeks). The following parameters were evaluated: the weight of the liver, adrenal glands and specific adipose tissue; the delta weight; the Lee index; and the serum levels of leptin, corticosterone, glucose, total cholesterol, and triglycerides. The hypercaloric diet induced obesity in rats, increasing the Lee index, weight, leptin, triglycerides, and cholesterol levels. The stress decreased weight gain even in animals fed a hypercaloric diet but did not prevent a significant increase in the Lee index. However, an interaction between the independent factors (hypercaloric diet and stress) was observed, which is demonstrated by the increased serum leptin levels in the animals exposed to both protocols.

1. Introduction

Obesity is a chronic disease that has become a serious public health issue worldwide [70]. This disease is a metabolic disorder associated with social and psychological factors, genetic predisposition, and dietary habits [8], and it affects all ages and social classes [14]. Obesity is characterized by the excessive buildup of adipose tissue, which is associated with the development of cardiovascular diseases and metabolic disorders, such as glucose intolerance, hyperinsulinemia, type 2 diabetes, dyslipidemia, and hypertension [25]. Abdominal obesity is a major risk factor for cardiovascular disease, and recent studies have demonstrated adipose tissue dysfunction, inflammation, and aberrant adipokine release in this disease [102]. Cardiovascular diseases are the major cause of morbidity and mortality worldwide, and there is considerable interest in the role of dietary constituents and supplements in the prevention and treatment of these disorders [111].

The worldwide increase in obesity is related to changes in eating patterns and the intake of hypercaloric foods [76]. Dietary behaviors that promote obesity include frequent consumption of fast food meals; frequent snacking [81]; consumption of oversized portions at home and at restaurants [53,112]; consumption of high-calorie foods, such as high-fat, low fiber foods [63]; and the intake of sweetened beverages [34]. Furthermore, compared to non-obese individuals, obese individuals tend to consume diets that have a higher energy and fat content [90].

Additionally, chronic stressors cause physiological and neuroendocrine changes [10] that are associated with increased food intake and adipogenesis [86]. Stress, combined with overeating and inactivity, can lead to overweight, and abdominal obesity is associated with a higher waist-to-hip-ratio and body mass index (BMI) [95]. In addition, studies in humans have demonstrated that disturbing the hypothalamic-pituitary-adrenal (HPA) axis function is associated with abdominal obesity [61]. Moreover, chronic stressors cause a variety of physiological and neuroendocrine changes [10] associated with changes in food intake [1], increased adipogenesis [86], decreased weight gain

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[47], and slower weight gain during chronic restraint stress [40].

Leptin secreted by adipocytes acts in the hypothalamus to regulate food intake and energy expenditure, thereby limiting adiposity [2,113]. At least two distinct neuronal groups contain leptin receptors in the arcuate nucleus, the orexigenic neurons, which produce neuropeptide Y (NPY) and agouti-related protein (AGRP), and anorexigenic neurons, which produce proopiomelanocortin (POMC) and the cocaine- and amphetamine-regulated transcript (CART) [3]. Leptin insensitivity or the lack of leptin activity results in an obese phenotype [104,106]. The reduced expression of leptin receptors may contribute to brainstem leptin insensitivity in diet-induced obesity [92]. Leptin is involved in hypothalamo-pituitary-adrenal (HPA) responses to stressful stimuli [9,22]. Restriction stress increased the leptin levels, and although the mechanism of these responses to stress is not clear, endogenous leptin may play important roles in stress responses [75] In addition, hyperleptinemia is an independent risk factor for cardiovascular disease [54] and a strong predictor of acute myocardial infarction [42].

A stressful lifestyle has been associated with changes in eating habits that result in increasing weight and obesity, and it can be related to leptin activity in the brainstem with respect to the HPA axis. Therefore, this study evaluated the effects of a hypercaloric diet plus chronic restraint stress on the serum leptin and lipids levels and the weight of specific adipose tissue fractions (mesenteric, MAT; subcutaneous, SAT and visceral, VAT) in a rat model.

2. Methods

2.1. Animals

Wistar rats, aged 60 days and weighing 200–250 g (60 in total), were randomized by weight and housed in polypropylene cages (49 cm × 34 cm × 16 cm). The animals were maintained on a standard 12-h light/dark cycle (lights on at 7:00 a.m. and lights off at 7:00 p.m.), in a temperature-controlled environment (22 ± 2 °C), with access to water and chow ad libitum (cafeteria diet and/or standard rat chow). The experiments and procedures were approved by the Institutional Animal Care and Use Committee (GPPG-HCPA protocol No. 09231) and were compliant with Brazilian guidelines involving the use of animals in research (Law No. 11,794). Vigorous attempts were made to minimize suffering and external sources of pain and discomfort. In addition, the minimum number of animals required to produce reliable scientific data were used.

2.2. Experimental design

The rats were acclimatized to their environment for 1 week before the start of the experiment. The animals were divided into two groups, a control group and a stress group. Each group was subdivided into two subgroups according to the chronic stress exposure and the type of diet provided (cafeteria diet or standard rat chow) as follows: standard chow (C, control and S, control plus restraint stress) and high-calorie food (HD, hypercaloric diet and SHD, hypercaloric diet plus restraint stress). The animals were weighed weekly, and the food intake was recorded daily. The experiment was performed over 6 weeks. The animals were housed in groups of four animals per cage.

2.3. Stress procedure

The animals were subjected to a chronic restraint stress model [26] using a plastic tube (25 cm × 7 cm) fixed with adhesive tape on the outside to avoid discomfort but limiting the movements of the animal; one end of the tube remained open to allow breathing [26]. The animals were exposed daily to 1 h of stress in the morning (between 9:00 and 12:00), 5 days a week for 6 weeks [26] (no stress on weekends). The animals were returned to their home cages immediately after exposure to the 1 h of stress. The control animals were maintained in their home cages throughout the experimental period. The apparatus was ventilated to avoid physical compression, hyperthermia and sweating.

Table 1
Comparison between the composition of the standard diet and cafeteria diet.

	Standard diet (%)	Cafeteria diet (%)
Carbohydrates	55	60
Protein	22	20
Lipids	4.5	15
Other constituents	18.5	5

2.4. Experimental diets

The standard rat chow (Nuvilab CR-1, NUVITAL®, Curitiba, PR, Brazil) provided an energy content of 2.93 kcal/g (information provided by the manufacturer), and the cafeteria diet totaled 4.186 kcal/g and 0.42 kcal/mL (calculated based on information provided by the manufacturer on the package label). The constituents of each diet are described in Table 1. The palatable high-calorie diet (cafeteria diet) was chosen because it mimics modern patterns of human food consumption and has been used successfully in experimental studies to induce obesity in lean animals [28,59]. This diet was adapted from a diet known as the cafeteria diet or Western diet, previously described by Estadella et al. Foods included in the cafeteria diet were crackers, wafers, sausages, chips, condensed milk and soda. Both the standard chow and the experimental diet were replaced daily with fresh food. The animals receiving the hypercaloric diet also had access to standard chow and water.

2.5. Weight parameters

The animals were weighed weekly, and the weight delta was defined as the difference between final and baseline weights. At the end of the experiment, the naso-anal length (cm) of the animals was measured to determine the Lee index. This index, which was adapted from Moura and Cols, corresponds to the ratio between the cube root of the body weight (g) and the naso-anal length (cm) of the animals multiplied by 10 [21]. The liver, adrenal glands and specific adipose tissues (mesenteric, subcutaneous and visceral) were dissected manually and were weighed using a semi-analytical balance. The data were expressed as grams of tissue per 100 g of body weight (weight tissue/bodyweight × 100). The visceral adipose tissue weight included the perigonadal and retroperitoneal fat pads.

2.6. Blood sampling and tissue collection

The animals were killed by decapitation, and the blood and tissue samples were collected 24 h after the last session of restraint stress and after a 12-h fast. A trained practitioner performed the euthanasia. The trunk blood was collected and centrifuged for 5 min at 5000 × *g* at room temperature. This method was used to facilitate the collection of large volumes of blood serum for analysis. Importantly, this model allows the determination of biochemical effects,

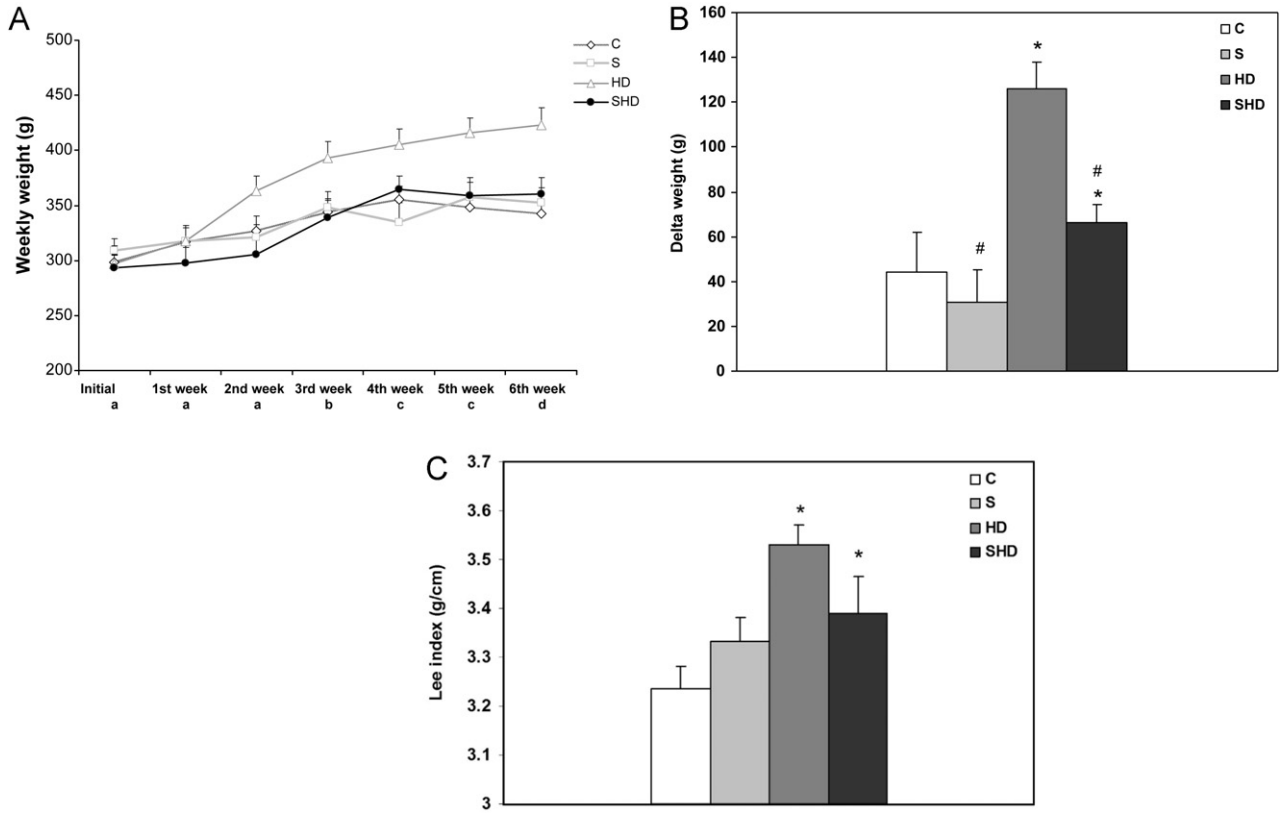

Fig. 1. Data are expressed as the mean ± SEM, $n = 8$ animals/group. C, control group (receiving standard chow alone); S, control + restraint stress; HD, hypercaloric diet; SHD, hypercaloric diet + restraint stress. (A) Weekly weight: (a) significant difference from other groups (repeated measures ANOVA, $P < 0.05$), (b) significant difference in relation to initial weight, 1st, 2nd, 5th and 6th weeks (repeated measures ANOVA, $P < 0.05$), (c) significant difference in relation to initial weight, 1st and 2nd weeks (repeated measures ANOVA, $P < 0.05$), and (d) significant difference in relation to 1st, 2nd and 3rd weeks (repeated measures ANOVA, $P < 0.05$), (B) delta weight, and (C) Lee index. *Significant effect of hypercaloric diet (two-way ANOVA, $P < 0.05$, $n = 8$). #Significant effect of chronic stress (two-way ANOVA, $P < 0.05$, $n = 8$–10).

including hormonal effects. The serum was frozen at −70 °C for subsequent analysis.

2.7. Biochemical assays

The serum corticosterone levels were measured using a commercially available ELISA kit (Catalog No. 900-097, Assay Designs, Inc., USA), and the data are expressed as ng/mL. The serum leptin levels were measured using a commercial Linco ELISA Kit (Catalog No. 00EZRL-83, Linco Research, USA), and the data are expressed as ng/mL. The concentration of glucose, total cholesterol, HDL and TAG was measured spectrophotometrically using Bioliquid kits (Laborclin, Paraná, Brazil), and the data are expressed as mg/dL. The VLDL and LDL values were calculated using the Friedewald equation (VLDL = TAG/5, LDL total cholesterol − (HDL–VLDL) [37].

2.8. Statistical analysis

The results were expressed as the mean ± standard error of the mean (S.E.M.). The baseline weight of the animals was compared between the groups using one-way ANOVA. The data and interactions were evaluated using two-way ANOVA (diet, stress, diet × stress) followed by Bonferroni correction for multiple comparisons when necessary and two-way ANOVA for repeated measures (effect of time, diet, stress, time × stress, time × diet, time × stress × diet, and diet × stress interactions) followed by Bonferroni correction when necessary. The between-group differences were considered significant at $P < 0.05$.

3. Results

3.1. Weekly weight (Fig. 1A)

The results of two-way ANOVA for repeated measures demonstrated an effect of time ($F_{(5,30)} = 77.863$, $P < 0.05$) but no effect of stress ($F_{(1,30)} = 2.947$, $P > 0.05$) or of hypercaloric diet ($F_{(1,30)} = 2.447$, $P > 0.05$) (Fig. 1, Panel A). There was no interaction between stress and diet factors ($F_{(1,30)} = 3.306$, $P > 0.05$). There was an interaction between time and stress ($F_{(5,30)} = 3.801$, $P < 0.05$) and between time and hypercaloric diet ($F_{(5,30)} = 11.137$, $P < 0.05$). In addition, there was time × stress × diet interaction ($F_{(5,30)} = 3.374$, $P < 0.05$).

3.2. Delta weight (Δ = final weight − initial weight) and Lee index (Fig. 1C)

There were no significant between-group differences for baseline weight (one-way ANOVA, $P > 0.05$, $F_{(3,30)} = 0.328$, data not shown). For the weight delta (Δ = final weight − baseline weight) (Fig. 1, Panel B), two-way ANOVA showed an effect of stress ($F_{(1,30)} = 14.599$, $P < 0.05$) and diet ($F_{(1,30)} = 23.815$, $P < 0.05$). The group means indicated that chronic stress reduced the weight delta, whereas the hypercaloric diet increased the weight delta. Regarding the Lee index (Fig. 1, Panel C), two-way ANOVA showed an effect of hypercaloric diet ($F_{(1,30)} = 10.224$, $P < 0.05$) but no effect of stress ($F_{(1,30)} = 0.184$, $P > 0.05$). Furthermore, there was an interaction between these independent factors ($F_{(1,30)} = 4.638$, $P < 0.05$).

Table 2
Relative weight of mesenteric adipose tissue, subcutaneous adipose tissue, visceral adipose tissue, adrenal glands, and liver in Wistar rats.

	C	S	HD	SHD
Mesenteric adipose	0.94 ± 0.12	0.74 ± 0.08	1.30 ± 0.06*	1.14 ± 0.12*
Subcutaneous adipose	0.74 ± 0.10	0.66 ± 0.11	1.61 ± 0.09*	1.28 ± 0.17*
Visceral adipose	3.90 ± 0.47	3.22 ± 0.38	6.28 ± 0.44*	5.44 ± 0.61*
Adrenal glands	0.0106 ± 0.0013	0.0175 ± 0.0014#	0.0120 ± 0.0013	0.0117 ± 0.0019#
Liver	3.05 ± 0.26	2.81 ± 0.18	2.55 ± 0.05	2.76 ± 0.13

The visceral adipose tissue data include the weight of the perigonadal and retroperitoneal fat pads. Data are expressed as the mean ± SEM and grams of tissue/100 g total weight. C, control group (standard chow alone); S, control + restraint stress; HD, hypercaloric diet; and SHD, hypercaloric diet + restraint stress.
* Significant effect of hypercaloric diet (two-way ANOVA, $P < 0.05$, $n = 8$).
# Significant effect of chronic stress (two-way ANOVA, $P < 0.05$, $n = 8$–10).

Table 3
Leptin, corticosterone, glucose, triglycerides, total cholesterol and high-density lipoprotein (HDL) serum levels. Very low-density lipoprotein (VLDL) and low-density lipoprotein (LDL) serum levels were calculated using the Friedewald equation.

	C	S	HD	SHD
Leptin (ng/mL)	4.93 ± 1.16	2.58 ± 0.71	9.16 ± 1.14*	12.59 ± 1.55*
Costicosterone (pg/mL)	387.38 ± 0.71	385.09 ± 1.39	386.72 ± 0.94	386.24 ± 1.16
Glucose (mg/dL)	67.92 ± 5.04	74.42 ± 4.29	71.40 ± 8.99	71.32 ± 8.37
Triglycerides (mg/dL)	49.54 ± 6.50	36.10 ± 2.06	57.83 ± 5.66*	53.17 ± 4.73*
Cholesterol (mg/dL)	41.58 ± 5.30	45.28 ± 2.23	47.50 ± 2.73*	53.77 ± 2.19*
HDL (mg/dL)	36.54 ± 1.79	35.08 ± 1.98	38.25 ± 3.76	45.07 ± 5.85
LDL (mg/dL)	9.29 ± 1.77	7.42 ± 0.66	13.27 ± 0.69*	11.90 ± 1.37*
VLDL (mg/dL)	19.59 ± 3.24	21.20 ± 2.54	23.65 ± 5.13	48.14 ± 17.02

Data are expressed as the mean ± SEM. C, control group (standard chow alone); S, control + restraint stress; HD, hypercaloric diet; and SHD, hypercaloric diet + restraint stress.
* Significant effect of hypercaloric diet (two-way ANOVA, $P < 0.05$, $n = 4$–8).

3.3. Relative tissue weights (Table 2)

The results from two-way ANOVA demonstrated the following results for the anthropometric parameters: in MAT, there was an effect of diet ($F_{(1,30)} = 14.846$, $P < 0.005$) but no effect of stress ($F_{(1,30)} = 3.256$, $P > 0.05$), and there was no interaction between these independent variables ($F_{(1,30)} = 0.041$, $P > 0.05$). In SAT, there was an effect of diet ($F_{(1,30)} = 37.479$, $P < 0.05$) but no effect of stress ($F_{(1,30)} = 2.717$, $P > 0.05$), and there was no interaction between these independent variables ($F_{(1,30)} = 1.131$, $P > 0.05$). In VAT, there was an effect of diet ($F_{(1,30)} = 22.599$, $P < 0.05$) but no effect of stress ($F_{(1,30)} = 2.414$, $P > 0.05$), and there was no interaction between these independent variables ($F_{(1,30)} = 0.027$, $P > 0.05$). The adrenal glands, as expected, showed an effect of stress ($F_{(1,30)} = 5.306$, $P < 0.05$) but no effect of diet ($F_{(1,30)} = 2.484$, $P > 0.05$), and there was an interaction between these independent variables ($F_{(1,30)} = 6.266$, $P < 0.05$). The liver demonstrated no effect of stress ($F_{(1,30)} = 0.006$, $P > 0.05$) or diet ($F_{(1,30)} = 2.553$, $P > 0.05$), and there was no interaction between these independent variables ($F_{(1,30)} = 1.698$, $P > 0.05$), demonstrating that the chronic stress and hypercaloric diet did not alter the relative liver weight.

3.4. Biochemical and hormonal parameters (Table 3)

The results of two-way ANOVA demonstrated the following for the biochemical and hormonal parameters: the leptin levels demonstrated an effect of diet ($F_{(1,27)} = 26.704$, $P < 0.05$) but not stress ($F_{(1,27)} = 0.235$, $P > 0.05$), and there was an interaction between these independent variables ($F_{(1,27)} = 5.05$, $P < 0.05$). The statistical test demonstrated that the hypercaloric diet significantly increased the serum leptin levels after 40 days of exposure. The corticosterone levels did not demonstrate an effect of hypercaloric diet ($F_{(1,26)} = 0.052$, $P > 0.05$) or chronic stress ($F_{(1,26)} = 1.643$, $P > 0.05$), and there was no interaction between these independent variables ($F_{(1,26)} = 0.695$, $P > 0.05$). Therefore, the 40-day exposure to chronic stress and/or hypercaloric diet did not alter the serum corticosterone levels. The glucose levels did not demonstrate an effect of hypercaloric diet ($F_{(1,28)} = 0.001$, $P > 0.05$) or chronic stress ($F_{(1,28)} = 0.224$, $P > 0.05$), and there was no interaction between these independent variables ($F_{(1,28)} = 0.236$, $P > 0.05$). Therefore, the 40-day exposure to chronic stress and/or hypercaloric diet was not sufficient to alter the serum glucose levels. There was an effect of diet ($F_{(1,27)} = 6.383$, $P < 0.05$) on triglyceride levels but no effect of stress ($F_{(1,27)} = 3.251$, $P > 0.05$), and there was no interaction between these independent variables ($F_{(1,27)} = 0.765$, $P > 0.05$). Therefore, the hypercaloric diet significantly increased the serum triglyceride levels. The total cholesterol levels demonstrated an effect of diet ($F_{(1,16)} = 5.014$, $P < 0.05$) but no effect of stress ($F_{(1,16)} = 2.398$, $P > 0.05$), and there was no interaction between these independent variables ($F_{(1,16)} = 0.159$, $P > 0.05$). Thus, the hypercaloric diet significantly increased the total cholesterol levels in the serum after 40 days of exposure. The HDL did not demonstrate an effect of hypercaloric diet ($F_{(1,16)} = 2.621$, $P > 0.05$) or chronic stress ($F_{(1,16)} = 0.551$, $P > 0.05$), and there was no interaction between these independent variables ($F_{(1,16)} = 1.312$, $P > 0.05$). These results showed that a 40-day exposure to chronic stress and/or hypercaloric diet for 40 days was not sufficient to alter the serum HDL levels. The LDL demonstrated an effect of diet ($F_{(1,16)} = 14.131$, $P < 0.05$) but no effect of stress ($F_{(1,16)} = 2.073$, $P > 0.05$), and there was no interaction between these independent variables ($F_{(1,16)} = 0.500$, $P > 0.05$). These results demonstrated that a hypercaloric diet significantly increased the serum LDL levels. The VLDL did not demonstrate an effect of hypercaloric diet ($F_{(1,16)} = 3.508$, $P > 0.05$) or chronic stress ($F_{(1,16)} = 2.486$, $P > 0.05$), and there was no interaction between these independent variables ($F_{(1,16)} = 1.911$, $P > 0.05$). Therefore, the exposure to chronic stress and/or the hypercaloric diet for 40 days was not sufficient to alter the serum VLDL levels.

4. Discussion

In this study, we determined that the obesity induced by the cafeteria diet increased the serum leptin levels, the mesenteric, subcutaneous, and visceral adipose tissue weight, the weight delta, the Lee index, and the serum triglycerides and total cholesterol levels. The results demonstrate that exposure to the hypercaloric diet for 6 weeks induced obesity in the rats. Conversely, the exposure to the chronic restraint stress reduced the weight delta and increased

the relative weight of the adrenal glands. Additionally, we observed an interaction between these independent factors for the serum leptin levels, the Lee index, and the adrenal gland weight.

A number of studies using obesity models have demonstrated a relationship between increased adiposity and increased TG levels [66,110], and this study corroborates these findings. Different types of fat depots exhibit different properties, and their anatomic location is an important risk factor for cardiovascular diseases, metabolic disorders, and other conditions [91]. The current evidence demonstrates biological and genetic differences between adipose tissues depending on their anatomic location. Specifically, the upper body/visceral fat distribution in obesity is closely associated with metabolic complications [87]. Intra-abdominal tissues are metabolically and functionally different from subcutaneous adipose tissue (SAT) and exhibit a higher capillary density, sympathetic innervation and adrenergic receptor expression [55]. Intra-abdominal tissues release more free fatty acids, glycerol and endocrine hormones into the portal venous system and have direct access to the liver, whereas those derived from SAT are secreted into the systemic circulation [55,91].

In our study, the circulating levels of HDL and VLDL were not significantly altered by the hypercaloric diet and/or chronic stress. The animals subjected to the hypercaloric diet model demonstrated an increase in LDL cholesterol and total cholesterol, similar to the findings in earlier studies using the cafeteria diet [8,51]. Studies in humans and animals subjected to chronic stress have been linked to increased levels of serum cholesterol [29,85], and the results of our six-week restraint stress protocol confirms the association between stress and cholesterol. The high leptin levels found with the exposure to the high-calorie diet may be related to an increase in fatty tissues, especially visceral fat accumulation, because leptin is synthesized mainly in these tissues [19]. Adipose tissue secretes signaling molecules that play a central role in weight regulation and metabolic function [108]. Leptin is an adipocyte hormone that signals the status of energy stores in the peripheral tissues to the brain [33], affecting feeding behavior and metabolism [50]. This peptide plays an important role in the regulation of food intake, energy consumption, glucose metabolism, the cardiovascular system, the immune system, and the secretion of insulin and the pituitary hormone [2]. In addition, growing evidence suggests that leptin may contribute to the development of cardiac dysfunction, and chronic hyperleptinemia may increase the risk of cardiac disorders [54]. The circulating leptin levels are proportional to the total amount of the adipose tissue mass, and leptin binds to receptors within specific hypothalamic nuclei to regulate energy balance by reducing appetite [114]. Leptin acts in association with other neuropeptides, such as NPY, which increases food consumption and decreases energy expenditure [3]. NPY neurons located in the ARC are controlled by multiple neural and peripheral signals [23,24] and play an important role in several physiological functions, including cardiovascular homeostasis and the regulation of the sympathetic nervous system (SNS) activity [48,64]. Moreover, NPY receptors are highly expressed in human adipocytes, and they inhibit lipolysis [56] and participate in leptin regulation pathways [78,72]. High levels of leptin are associated with obesity but do not adequately suppress food intake, suggesting the attenuation of leptin activity caused by leptin resistance [74]. When released under conditions of stress, glucocorticoids stimulate leptin gene expression in human and mouse adipocytes [71,109]. Conversely, β-adrenergic agonists inhibit leptin gene expression in adipocytes and lower circulating leptin levels [109], leading to the loss of the regulatory mechanism of leptin [114].

Interestingly, we observed a positive interaction between the hypercaloric diet and stress exposure, which is corroborated by a number of studies in which leptin secretion is increased by sympathetic nerve stimulation, food intake, glucocorticoids, tumor necrosis factor-α, interleukin-1, and insulin and is decreased by starvation [79,99]; furthermore, restraint stress may alter leptin levels [75]. Studies on leptin-deficient ob/ob mice revealed that leptin is necessary for the normal expression of several hypothalamic genes that regulate food intake and metabolism [98].

Obesity is almost always associated with leptin resistance [12], which in animal models of obesity, may be related to several associated factors, such as impaired transporter, receptor, post-receptor, and downstream neuronal circuitry functions [6]. Leptin is transported across the blood–brain barrier (BBB) by a saturable transport mechanism, which is affected by a number of circulating substances, such as triglycerides [6]. In our study, we found high levels of serum triglycerides and leptin in response to the cafeteria diet-induced obesity. According to Banks et al., serum triglyceride levels interfere with the ability of the BBB to transport leptin and are likely a major cause of the leptin resistance observed both in starvation and obesity [6,84].

For the weight delta, an interaction was not observed between stress and exposure to the cafeteria diet; however, this interaction was observed for the Lee index. Our study corroborates several studies demonstrating that chronic stress results in weight loss in rats [72]. In rodents, chronic stress regimens, such as social subordination [101] or variable stress [72,96], reduces food intake, body weight gain, and adiposity [96]. On the other hand, other studies suggest that social and non-social stressors also increase body and lipid mass leading to metabolic disorders and obesity [60,96]. In addition, experimental studies combining the intake of a hypercaloric diet and stress exposure have produced contradictory results [7,60,65]. In this study, we demonstrated that the administration of a cafeteria diet for six weeks produced obesity-like conditions in rats, with an increase in body weight and adipose tissue weight. Notably, exposure of the animals the two procedures (the hypercaloric diet and chronic stress) produced lower weights than exposure of the animals to the hypercaloric diet alone. Therefore, we propose that the effect of the cafeteria diet on the establishment of obesity was higher than the weight loss imposed by stress. In addition, previous studies using the same stress model demonstrated an increase in sweet food intake [26,94], and this effect was associated with the increased body weight observed in the animals exposed to the two protocols (the hypercaloric diet and chronic stress). In our study the stressed rats that were fed a high-calorie diet exhibited a higher Lee index, which represents obesity. In this study, we observed significantly increased adipose tissue depots (MAT, SAT and VAT) in the animals exposed to the high-calorie diet. Several studies have reported that in animals subjected to approximately 1 h or less of restraint stress daily, hypercaloric diets cause increased abdominal adipose tissue deposition [8,28,82,45,97]. Increased adipose tissue mass is the primary characteristic of obesity and is associated with the consumption of high-calorie foods [69]. In this study, the animals fed the cafeteria diet became obese; therefore we propose that the effect of the cafeteria diet on establishing obesity [28,59,89] was higher than the weight loss imposed by the stress.

Palatable food that is rich in fat and carbohydrates ("comfort food") decreases the stress response in chronically stressed rats [80]. Sweet, fatty foods that are low in protein may also provide alleviation from stress in vulnerable people via the enhanced function of the serotonergic system [39]. We used a hypercaloric diet exhibiting features that influence the choice of foods. Eating a small amount of sweet food immediately and selectively improves an experimentally induced negative mood state, and the effect of the sweet food, e.g., chocolate, is because of its palatability. It has been hypothesized that the immediate mood effects of palatable foods contribute to the habit of eating to cope with stress [68]. It has been demonstrated that even if they are not hungry, humans [1,41,107] and animals [20] increase their food intake following stress or

a negative emotion [4,67]. Furthermore, the type of food eaten tends to be high in sugar or fat, or both [27,43,80]. On the other hand, in terms of protective functions, studies have shown that women categorized as viscerally obese exhibited habituation to repeated stressors, whereas their lean counterparts did not exhibit this behavior. Similar findings have been reported in rats [65]. Therefore, the available evidence from human studies supports the validity of the animal model and the working hypothesis in terms of both the drive-inducing effects of stress and the stress-reducing effects of eating.

The control of feeding is altered by different factors, such as biological status, available nutrients, and stress [93]. Feeding behavior involves complex mechanisms that include the caloric demands of the body and hedonic and cognitive aspects [1,32,52,58]. Moreover, the behavior can be changed by a number of factors, such as nutrient availability and stress [26]. The hormones released in response to stress may affect the appetite in different ways. Norepinephrine and corticotropin-releasing hormone (CRH) are appetite suppressants produced in response to stress [44], whereas cortisol stimulates the appetite during recovery from stress [100]. The CRH acts via CRH receptors in or near the PVN to inhibit food intake [57], although the mechanism is not understood completely. On the other hand, it has been suggested that leptin also influences CNS activity through the regulation of hypothalamic neuropeptides, such as NPY [5,17,73]. Another possible modulator of stress-eating is leptin [18,36,104], because this peptide exerts effects within the hypothalamus that regulate homeostatic food intake [49,74,88] and in the ventral tegmental area that reduces dopamine neurotransmission and extinguishes the reward value of food [71]. Tomiyama et al. suggested that leptin acts as a modulator of stress-eating. When an individual has an adaptable, flexible allostatic stress response that is sensitive enough to upregulate leptin secretion in response to stress, the individual may not fall prey to the urge to consume comfort foods. However, comfort food eating may be triggered more easily when the system does not respond, i.e., the leptin reactivity is low or absent. In summary, this study implicates the circulating leptin reactivity the potential dampening of the known shift in food preference to high fat, sweet foods following exposure to stress. Furthermore, the data point toward leptin as a potential independent modulator of stress-eating. Leptin responses to acute stress demonstrate a complex pattern, and the exact nature, cause and underlying mechanisms of the phenomenon remains to be determined [103].

Using the same restraint chronic stress model used in this study, previous studies have demonstrated an increase in sweet food intake [26,106] that was reversed by diazepam or midazolam [26]. On the other hand, variable chronic stress produced a decrease in sweet food intake that was reversed by fluoxetine [38], suggesting that the restraint chronic stress and variable chronic stress protocols represent anxiety and depression animal models, respectively. The restraint chronic stress protocol produced decreased serotonin levels in the hippocampus accompanied by an increased turnover of this neurotransmitter [106]. It has been proposed that cortisol and insulin stimulate the ingestion of energy-dense "comfort foods", which protects the HPA axis from stress-induced dysfunction and the associated depression and anxiety [20]. The synthesis of the neurotransmitter serotonin (or 5-hydroxytryptamine; 5-HT) depends on the dietary availability of the precursor essential amino acid, tryptophan [15]. High-sugar, low-protein foods might influence the mood via increased synthesis of 5-HT [30,31].

In addition, the chronic stress induced a significant increase in the relative weight of the adrenal glands, regardless of the presence of the hypercaloric diet. This observation reflects the continuous stimulation of the adrenal glands by ACTH, leading to glandular hypertrophy [11,29], and confirms that the chronic animal stress model was effective. However, the exposure to repeated stress did not induce an increase in the corticosterone levels after 6 weeks, suggesting the habituation of the HPA axis. This observation corroborates the findings of previous studies suggesting that the compensatory and adaptive mechanisms of this hormone act as a protective factor for the maintenance of homeostasis. Previous studies using different repeated stress protocols for 6 weeks demonstrated the habituation to stress and corticosterone levels similar to those in the control animals [16,92,105].

In this study, significant between-group differences were not observed for the glucose levels. Regarding the chronic stress exposure, this finding corroborates a previous study demonstrating that increased glucose levels were maintained for up to 2 h after the last stress session [105]. This effect may be mediated by an adaptive process resulting from the repeated exposure to stress (habituation or metabolic tolerance) [26]. The high-calorie diet did not affect the blood glucose levels even though the animals developed obesity-defining parameters. Previous studies have shown that obese animals do not exhibit increased glucose levels because an increase in insulin release makes up for its reduced activity to maintain normoglycemia [35,82]. This type of compensatory mechanism also occurs in obese, insulin-resistant humans and involves the plasticity of pancreatic beta cells, which respond by increasing insulin secretion [46,83]. The normoglycemic state observed in our groups of animals exposed to the hypercaloric diet may be because the animals were not exposed to the diet for a sufficient length of time to produce changes in the blood glucose levels; previous studies using hyperglycemia models used longer treatment periods [13,89].

Future studies using the same experimental conditions will increase our understanding of the effects of chronic stress plus a hypercaloric diet and will facilitate the translation of the findings to humans. More specifically, in future studies, we will investigate the neuropeptide Y and the food preferences of animals subjected to chronic stress plus a hypercaloric diet. However, it is important to emphasize the limitations of extrapolating animal studies to other species. For example, the experiments were performed in male rats, which complicate the translation of the results to both genders in humans, particularly because the effects of chronic stress and food intake are affected by modulations in hormone levels [62,77]. In addition, rats demonstrate intrinsic preferences for different types of high-energy foods. Violating their preferences may have consequences on their ingestion and metabolism. However, these interpretations are not supported in this study because the animals were free to choose any combination of fat, sucrose, or chow, and the groups ate approximately equal calories from sucrose and fat. In humans, many intriguing associations have been proposed between stress, obesity, and eating. However, interpreting the associations between stress and eating is difficult because of the potential for ex post facto errors (nonrandom assignment to obesity conditions), ethical constraints on stressor severity or duration, performance issues under unusual experimental circumstances, and the confounded issues of feeling better through feeding and body-image dissatisfaction.

5. Conclusion

Exposure to a hypercaloric diet for 6 weeks induced obesity in rats, as demonstrated by the increased Lee index and weight delta, and was associated with the establishment of hyperleptinemia, hypertriglyceridemia, and hypercholesterolemia. Our results confirm that the cafeteria diet is an effective animal model for studying obesity and its consequences. In addition, the stress protocol successfully inhibited weight gain independent of the type of diet the animals were fed; however, the protocol did not prevent a significant increase in the Lee index and serum leptin levels, which signifies obesity, in animals subjected to both protocols concur-

rently.

Conflict of interest

The authors report no conflicts of interest. The authors alone are responsible for the content and writing of the paper.

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