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ORIGINAL PAPER

Fat storage is partially dependent on vagal activity and insulin secretion of hypothalamic obese rat

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Abstract Hypothalamic MSG-obese rats show hyperinsulinemia and tissue insulin resistance, and they display intense parasympathetic activity. Current analysis investigates whether early subdiaphragmatic vagotomy prevents tissue insulin sensitivity impairment in adult obese MSG-rats. Hypothalamic obesity was induced by MSG (4 mg/g BW), daily, from birth up to 5 days. Control animals receiving saline solution. On the 30th day rats underwent bilateral subdiaphragmatic vagotomy or sham surgery. An intravenous glucose tolerance test (ivGTT) was performed when rats turned 90 days old. Total white fat tissue (WAT) from rat carcass was extracted and isolated; the interscapular brown fat tissue (IBAT) was weighed. Rather than blocking obesity, vagotomy reduced WAT and IBAT in MSG-obese rats when the latter were compared to sham MSG-rats. High blood fasting insulin and normal glucose levels were also observed in MSG-obese rats. Although glucose intolerance, high insulin secretion, and significant insulin resistance were recorded, vagotomy improved fasting insulinemia, glucose tolerance and insulin tissue sensitivity in MSG-obese rats. Results suggest that increased fat accumulation is caused, at least in part, by high blood insulin concentration, and enhanced parasympathetic activity on MSG-obese rats.

Keywords MSG-obesity · Parasympathetic activity · Vagotomy · Insulin resistance · Fat tissue

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Introduction

Obesity is a serious condition associated with increasing mortality and morbidity figures worldwide. However, its etiology and onset are not yet clearly understood. Obesity is usually associated with hyperinsulinemia and normo- or hyperglycemia [1–3]. Insulin is a potent stimulator of lipogenesis and fast hyperinsulinemia may be triggering the increased lipid synthesis on obesity [4]. Experimental models of obesity, such as rats with ventromedial hypothalamic (VMH) lesions and genetically obese rodents, with disturbances of their insulin secretion control and its correlation with consequent high deposits of fat in tissues, are reported [4–7]. One hypothesis (autonomic theory) refers to the derangement of the autonomic nervous system (ANS) as an important component to obesity onset [8]. It has been observed that human and obese animals have intense vagal activity and low sympathetic activity [9–12]. The pancreas is richly innervated by the sympathetic (SNS) and parasympathetic (PNS) nervous system. In fact, these nervous terminals modulate insulin secretion [13]. When activated, SNS inhibits pancreatic insulin release and PNS potentiates insulin secretion [14]. Genetically obese rodents, such as Zucker rats and *ob/ob* mice or rats with VMH bilateral injury, show hyperphagia, vagal hyperactivity, sympathetic hypoactivity, enlargement, and/or proliferation of the pancreatic islets leading to hyperinsulinemia, which may be attenuated by vagotomy

[15–17]. A less frequently explored hypothalamic obese model, which involves parasympathetic activity, deals with neonatal rodents treated with monosodium L-glutamate (MSG). Rodents develop unusual obesity regardless of food intake increase [18, 19]. Compared to non-treated animals, they are shorter and lighter [20]. While L-glutamate is an important neurotransmitter, injection of high doses in newborn rodents is excitotoxic to the brain and causes lesions especially to the hypothalamus. Major lesions are located in the arcuate nucleous (ARC) [20]. ARC neurons produce the growth hormone releasing hormone (GHRH). Indeed, MSG animals have low growth hormone (GH) levels that cause growth retardation, inhibition of muscle mass gain and short bone length [21]. However, similar to other experimental obesity models, MSG-obese rodents show massive amount of accumulated fat tissues, glucose intolerance, hyperinsulinemia, and insulin resistance [22, 23]. The fat accumulation and decrease in the tissues' insulin sensitivity in MSG-experimental model could be, at least in part, due to a high vagal activity.

Current study evaluated whether early bilateral subdiaphragmatic vagotomy prevented the development of insulin resistance in adult rats treated with MSG on birth so that the possible role of vagus nerve activity on MSG-obesity onset could be assessed.

Results

MSG-treatment effects on rat development

A batch of 30-day-old MSG-treated newborn rats had a reduction of 9.5% and 17% of body length and weight, respectively, when compared to non-treated animals; Lee index, a body mass index in rodents [24], presented a 4% increase in young MSG when compared to non-treated ones (Table 1). Perinatal MSG treatment caused a decrease of 15% and 26.8% in body length and weight, respectively, in adult rats (90-day-old), when compared to untreated ones. Lee index increased 6% in adult rats which were MSG treated during the suckling period, when compared to non-treated ones (Table 1).

Effect of vagotomy on MSG-obesity onset

Body weight and body length were not affected by the vagotomy in MSG-treated or non-treated animals and Lee index was reduced by 2.7% in operated MSG-rats. Table 1 shows that surgery did not alter Lee index in normal rats. Table 2 shows that total fat accumulation in carcass was 3.3 times higher in MSG-adult rats than in control ones; however, interscapular brown adipose tissue (IBAT) from MSG-rats increased only 2.5 times. Vagotomy reduced fat accumulation by 43% and 20%, respectively in carcass and IBAT of MSG-rats when compared to non-treated ones. While MSG rats presented fasting normoglycemia, bilateral subdiafragmatic vagotomy did not change glucose plasma levels. MSG treatment induced a huge fasting insulin plasma concentration (3.28-fold), when compared to that of normal animals; however, suppression of vagus nerve caused a drop of 66% in insulinemia.

Effect of vagotomy on glucose tolerance and insulin sensitivity

Figure 1 shows plasma glucose and insulin level changes during ivGTT. Glucose load caused a transient increase of blood glucose levels in all animal groups after 5 min; after this peak glucose levels decreased and reached concentration values which may be comparable to fasting glycemia in the 30–60 min time period. Further, MSG rats showed highest transient increased plasma glucose concentration. Figure 1, panel A, indicates that vagotomy decreased glucose levels in MSG-rats. The operation in MSG-non-treated animals did not alter the plasma glucose level oscillations when compared to those of intact rats. Figure 1, left panel C, shows total glucose concentration changes obtained by calculation of area under the ivGTT

Table 1 Development of neonatal rats treated with MSG and effects of early bilateral subdiaphragmatic vagotomy

	Age (days)	Sham-operated control	Vagotomized control	MSG sham-operated	MSG vagotomized
Body weight (g)	30	$82.94 \pm 1.06^{c,d}$	$84.57 \pm 2.33^{c,d}$	$68.75 \pm 1.73^{a,b}$	$73.00 \pm 1.54^{a,b}$
	90	$367.72 \pm 4.79^{c,d}$	$355.86 \pm 4.91^{c,d}$	$268.95 \pm 5.21^{a,b}$	$249.47 \pm 5.45^{a,b}$
Naso-anal length (cm)	30	$13.95 \pm 0.56^{c,d}$	$13.87 \pm 0.09^{c,d}$	$12.62 \pm 0.11^{a,b}$	$12.85 \pm 0.08^{a,b}$
	90	$23.04 \pm 0.14^{c,d}$	$22.89 \pm 0.15^{c,d}$	$19.50 \pm 0.13^{a,b}$	$19.58 \pm 0.18^{a,b}$
Lee index	30	$312 \pm 1^{c,d}$	$316 \pm 2^{c,d}$	$324 \pm 1^{a,b}$	$325 \pm 1^{a,b}$
	90	$311 \pm 1^{c,d}$	$310 \pm 2^{c,d}$	$330 \pm 2^{a,b,d}$	$321 \pm 3^{a,b,c}$

A batch of 30-day-old MSG and control rats were submitted to surgery. Results of 30-day-old rats represent distribution of each group before operation. Data represent mean ± SEM. Letters above numbers refer to differences ($P < 0.05$) from the following values: (a) Sham-operated control; (b) Vagotomized control; (c) MSG sham-operated; (d) MSG-vagotomized. 15–20 animals were used for each group

Table 2 Early bilateral subdiaphragmatic vagotomy effects on MSG-obesity onset

	Sham-operated control	Vagotomized control	MSG sham-operated	MSG vagotomized
Interscapular brown adipose tissue (mg/100 g BW)	73.13 ± 4.64[c,d]	77.88 ± 6.33[c,d]	182.00 ± 8.54[a,b,d]	145.00 ± 7.03[a,b,c]
Carcass fat content (g/100 g BW)	12.16 ± 1.23[c,d]	10.58 ± 0.88[c,d]	40.35 ± 2.31[a,b,d]	22.97 ± 1.06[a,b,c]
Fasting plasma insulin (μU/ml)	90.53 ± 5.05[c]	71.47 ± 10.41[c]	296.76 ± 21.54[a,b,d]	102.16 ± 7.67[c]
Fasting plasma glucose (mmol/l)	5.56 ± 0.24	5.12 ± 0.17	6.15 ± 0.20	5.93 ± 0.25

Data represent mean ± SEM. Letters above numbers refer to differences ($P < 0.05$) from the following values: (a) Sham-operated control; (b) Vagotomized control; (c) MSG sham-operated; (d) MSG-vagotomized. 15–20 animals were used for each group. Only five animals were used for fat carcass

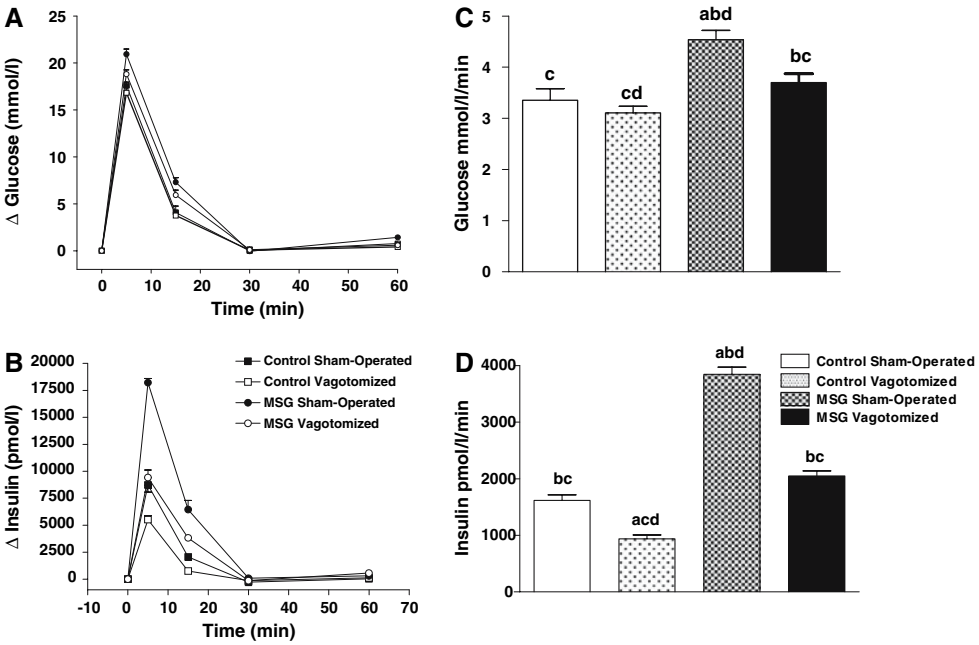

Fig. 1 *Effect of bilateral subdiaphragmatic vagotomy on intravenous glucose tolerance test of MSG-rats* Each symbol in the figures represents mean obtained from 8 to 10 rats in each group of rats. (**A** and **B**) on the left panel represent changes on plasma glucose and insulin levels during ivGTT, respectively. Total increment of plasma glucose (area under the curve) is shown on right panel (**C**) and plasma insulin on left panel (**D**). Lines over symbols or bars represent S.E.M. Letters above bars or symbols refer to differences ($P < 0.05$) from the following values: (a) sham-operated control; (b) vagotomized control; (c) MSG sham-operated; (d) MSG-vagotomized

curve. MSG treatment increased glucose levels (4.54 ± 0.18 mmol/l/min) by 35% when compared to non-treated sham-operated rats (3.36 ± 0.22 mmol/l/min); however, vagotomy induced an 18.5% decrease (3.70 ± 0.17 mmol/l/min). Glycemia from MSG-vagotomized rats failed to produce any statistical difference when compared to that of normal, intact animals. Operation did not show any changes in glucose concentration (3.11 ± 0.13 mmol/l/min) when compared to that in normal animals.

After 5 min glucose load caused an evanescent peak on insulin plasma levels, with a decrease to same levels after 30–60 min; reaching levels similar those presented in fasting. Such oscillations were reported in rats from all experimental groups, as shown in Fig. 1, panel B. However, MSG-treated rats presented a higher insulin rise than that in non-treated ones. Vagotomy caused reduction of transient insulin peak in MSG-rats, as well as in non-treated ones. Figure 1D shows that insulin AUC of MSG-rats increased 2.3 times (3,843 ± 302 pmol/l/min) when compared to that in non-treated rats (1,620 ± 92.7 pmol/l/min). Insulin AUC of MSG-treated (2,049 ± 91.1 pmol/l/min) or non-treated (939 ± 67.3 pmol/l/min) animals dropped 46.7 and 42% after vagus surgery. Figure 1D shows that insulin levels from MSG-operated rats were similar to normal and intact animals.

Table 3 shows that insulinogenic index increased to 78% in MSG-treated rats when compared to that of non-treated ones; actually surgery caused a 35% decrease in MSG-rats. The latter showed similar index values as in control intact rats. Vagotomy did not significantly change the insulinogenic index when it is compared to that in MSG-non-treated rats. Neonatal MSG treatment also induced an increase of 3.61-fold HOMA when compared to normal animals. Rate decreased 67% in MSG-vagotomized rats, although operation did not alter HOMA rate from that of normal rats.

Table 3 Early bilateral subdiaphragmatic vagotomy effects on tissue insulin sensitivity

	Sham-operated control	Vagotomized control	MSG sham-operated	MSG vagotomized
Insulinogenic index [ΔI (pmol/l/min)/ΔG(mmol/l/min)]	482 ± 39.4[c]	304 ± 24.1[c,d]	856 ± 39.0[a,b,d]	553 ± 40.0[b,c]
HOMA [fasting insulin (μU/ml) × fasting glucose (mmol/l)/22.5]	22.60 ± 1.57[c]	16.85 ± 2.62[c]	81.67 ± 6.46[a,b,d]	27.03 ± 2.32[c]

Data represent mean ± SEM of insulinogenic and HOMA indexes obtained from 8 to 10 rats in each experimental group. Letters above numbers refer to differences ($P < 0.05$) from the following values: (a) Sham-operated control; (b) Vagotomized control; (c) MSG sham-operated; (d) MSG-vagotomized

Discussion

Neonatal MSG-treatment causes obesity in rodents [19, 22, 25]. Obese animals show fasting hyperinsulinemia, tissue insulin resistance and an enormous tissue fat accretion [4, 26, 27]. However, unlike other rodent models of obesity, MSG-obese rats are neither hyperphagic nor hyperglycemic [18, 22]. Current research reports that early bilateral subdiaphragmatic vagotomy decreases tissue fat accumulation, as has been shown by the reduction of Lee index, fat contents in carcass and interscapular brown adipose tissue which contributed towards an attenuation of obesity onset.

Several studies have shown that development of obesity may be derived by an autonomic dysfunction [9, 28–33]. Hyperinsulinemia increases lipogenic activity which allows an accumulation of lipids in the tissues, as shown in MSG-treated rats [34]. Present study reports that vagotomy on MSG rats normalizes fasting insulin, although decrease in blood insulin levels did not eliminate over-fat accumulation. These data suggest that vagally mediated hyperinsulinemia induced a substantial, but not exclusive, contribution to obesity development. Moreover, SNS has terminals on the white (WAT) and on the brown adipose tissue (BAT), compounded by an important role in their metabolism [35]. Whereas SNS stimulation provokes rapidly lipolysis in WAT and BAT, low SNS activity induces lipogenesis increase [36]. A reduction of SNS activity in MSG-obese rodents has been suggested [37–39]. In addition, norepinephrine turnover is very low in WAT and BAT, as recorded in MSG-mice [40, 41]. Although direct SNS controls fat tissue accumulation, rare evidences [42] exist which show direct effect of parasympathetic nervous system (PNS) on WAT and BAT metabolism. Nevertheless, the presence of PNS terminals on fat tissues has not been demonstrated [43]. Fat accumulation, beyond SNS role, undergoes intense hormonal control. Insulin has a preponderant function on metabolism of both WAT and BAT. Lipogenesis is stimulated by insulin and there is a relationship between high insulin levels and fat-increased accumulation [11, 29, 32]. Profound decrease in fat storage of IBAT in rats has been reported when insulin antibody was added to the preparation [44]. It may be speculated that, at least partially, fat tissue accumulation is under the control of insulin level oscillations and of SNS rather than of PNS activity. Low insulin levels from MSG-vagotomized rats should be an effect to reduce fat stocks in all adipose tissue with the same magnitude; however, total WAT fat decrease, as indicated by fat carcass data, was greater than IBAT decrease. Insulin tissue sensitivity is an important component to determine fat deposits. Unlike other obese animals, insulin sensitivity of WAT does not decrease in MSG adult rats, as in muscle tissue [45]. Several authors [46, 47] and the present authors, through the insulinogenic index and HOMA also used as index of tissue insulin sensitivity have demonstrated that MSG-rats present general insulin resistance and fat tissue deposits increase on obesity development, perhaps as a consequence of maintaining WAT insulin sensitivity. It has been shown that in obese mice ob/ob insulin sensitivity decreases earlier in BAT than in WAT tissues [48]. Beyond the normalization of blood insulin levels, vagotomy also blocked the tissue insulin resistance in MSG-rats. Since low insulin level had less effect on IBAT fat accumulation when compared to WAT in MSG-vagotomized rats, this may be attributed to direct SNS effect on lipogenesis of IBAT. It has been shown that SNS denervation of normal rat IBAT provokes very significant decrease in their fat accumulation [44]. Whereas, the overall effect of vagotomy reduces total tissue fat accumulation, it may be suggested that control metabolism of WAT and BAT is different. Whereas, SNS shows a direct control over WAT and BAT, PNS controls changes in insulin secretion and tissue insulin sensitivity.

Since vagotomy changes gastrointestinal motility and causes many alterations in intake and digestive behavior, it should be claimed that several effects of vagotomy, including reduction of fat accumulation, are attributed to a drastic caloric restriction. The stomach of vagotomized animals is chronically distended with food [49]. Decrease in body weight, insulin plasma levels, and gastric secretion in hypothalamic obese rats submitted to vagotomy have been interpreted as a reduction in food intake [16]; however, we have shown that early vagotomy did not alter body weight and food consumption in MSG-obese and control rats, although a large stomach distention was

observed in operated animals [18]. It should be noted that hypothalamic lesions caused by MSG injury occur mainly in the ARC area and that many neurons from ARC participate of food intake control. MSG-rodents are actually normo- or hypophagic [18, 50].

As we have shown in the present work and as other authors have reported [4, 22, 26, 27], MSG-treated rats showed a pronounced glucose intolerance and rising insulin plasma levels during ivGTT. High sensitivity to glucose-induced insulin secretion was also observed in pancreatic islets from MSG-obese rats, attributed to high parasympathetic activity on the pancreas [18, 25, 51]. Early vagotomy reduces MSG rats' glucose and insulin plasma levels during ivGTT. Result suggests that high insulin release in MSG obese rats is reduced for parasympathetic activity blocked to pancreatic islets. Indeed, pancreas or islets isolated from vagotomized Zucker obese rats [17], Wistar fatty rats [32], and MSG rats [51] showed a decrease in glucose responsivity. When submitted to vagotomy VMH-obese rats with increased acetylcholinesterase activity in pancreas [10] exhibited little or no changes in insulin secretion; however, the last datum should be received with great caution since VMH lesions have been made in 90-day-old adult rats and islets have been isolated 30–40 days after vagotomy [52, 53]. In the present study animals developed obesity early in life and underwent vagotomy when 30-day old. We may suggest that increased vagal activity targets mechanisms that contribute towards the onset of MSG obesity. Vagotomized VMH-rats treated with carbachol infusion, a cholinergic agonist, reverse the parasympathetic denervation: consequently, the animals gain weight and have high cell proliferation rate, similar to carbachol-untreated VMH-obese rats [54]. When released from PNS ends and binds itself with muscarinic receptors on pancreatic beta-cell plasma membrane, acetylcholine is responsible for insulinotropic effect of PNS activity. It has been demonstrated that, among the five subtypes (M_1–M_5), M_3 is the main muscarinic subfamily receptor involved in that signalization [55, 56]. It has been recently reported that the knockout of M_3 muscarinic receptor gene in three different mice experimental obesity models (high fat diet, goldthioglucose treatment, and ob/ob mice) improved glucose homeostasis and attenuated the obesity onset [33]. Other mechanisms are involved in obesity onset, such as the hypothalamo-pituitary-adrenal axis. Circadian control of blood corticosterone levels is disturbed in MSG-obese rats [57], as well as in other obese animals [58] and in obese human beings [59, 60]. It has been shown that adrenalectomy inhibits but does not suppress obesity onset in MSG-treated animals; however, insulin levels, tissue insulin sensitivity, and glucose tolerance were normalized. These effect were reversed by corticosterone treatment [61]. There is also evidence that infusion of glucocorticoid in the brain, third ventricle, induces hyperinsulinemia, hyperphagia, overweight, and insulin resistance. All these effects were blocked by vagotomy [62]. When these evidences are combined with our data, it may be suggested that high vagal activity must play an important role in obesity onset. Current investigation suggests that during MSG-hypothalamic obesity onset rats undergo a highly intense parasympathetic activity, which leads to increased serum insulin level and insulin resistance. Hyperinsulinemia and vagal hyperactivity, among other factors, contribute to massive fat accumulation in WAT and less intensity in BAT. Autonomic imbalance correction may be at least a potential target to prevent obesity development and its complications, such as type 2 diabetes.

Materials and methods

Animals and MSG treatment

Male newborn *Wistar* rats received a subcutaneous injection of MSG [4 g/Kg body weight (BW)/day], or hyperosmotic saline solution (1.25 g/Kg BW/day), during the first 5 days of life [63]. Pups were weaned on the 21st day of life and had free access to standard rodent chow (Nuvital, Curitiba PR Brazil) and water; they were housed in standard cages and maintained in a temperature-controlled room (23 ± 2°C) with lights on from 07:00 to 19:00 h.

Vagotomy operation

A batch of 30-day-old MSG and control rats were anesthetized intraperitoneally (ip) with nembutal (40 mg/Kg BW) and underwent laparotomy; the dorsal and subdiaphragmatic vagal trunks were separated from the esophagus and severed with the aid of a stereomicroscope [64]. In sham vagotomized rats the vagus nerve was separated from the esophagus, but not cut. At the end of the experimental period (60 days after surgery), stomach food retention from each group was evaluated to confirm the vagotomy operation. Stomach was weighed and stomach weight/total BW ratio was taken. Confidence interval of 99% was employed to calculate the mean ratio for controls and its upper limit was used as a rejection criterion for vagotomy effectiveness [49]. Although increased gastric fill is a functional response to vagotomy, other measures exist, such as decline in body weight, decrease water intake and gastric secretion [52, 65], which were not used in current research. Only three animals, operated were discharged by above-mentioned criteria.

Intravenous glucose tolerance test (ivGTT)

A batch of 90-day-old sham-operated control, vagotomized control, sham-operated MSG and vagotomized MSG rats were anesthetized ip with nembutal (40 mg/Kg BW) to implant a silastic cannula into the right jugular vein [66]. After overnight fasting, rats received a glucose load (1 g/Kg BW) by means of a cannula. Blood samples were collected in heparinized syringes at 0 (before glucose administration) 5, 15, 30, and 60 min for plasma glucose and insulin. After each bleeding, replacement equivalent to 0.9% saline solution was made. Plasma samples were stored at –20°C for posterior determination of glucose concentration by glucose oxidase method [67] and of insulin by radioimmunoassay [68]. Total glycemia (ΔG) and insulinemia (ΔI) increase was calculated by using the area under glycemia and insulinemia curves for the 60 min of ivGTT, subtracting fasting values (AUC). Insulinogenic index was calculated by ΔI (pmol/l/min)/ΔG (mmol/l/min) to estimate tissue insulin sensitivity.

HOMA index

Tissue insulin sensitivity was also evaluated by the previously validated [46] homeostasis model assessment (HOMA), whereby the HOMA index of insulin resistance (HOMA – IR) = fasting insulin (μU/ml) × fasting glucose (mmol/l)/22.5, described by Mattheus and coworkers [69].

Obesity evaluation

A batch of 30- and 90-day-old rats (sham or operated), MSG-treated or non-treated, were weighed and their naso-anal length was taken to calculate the Lee index [body weight$^{1/3}$ (g)/naso-anal length (cm)] × 1,000, used as a predictor of obesity in MSG-rodents [24]. Animals were anaesthetized by an ip injection of nembutal (40 mg/Kg BW) and killed by cervical dislocation. Total lipid carcass was also extracted from all rat groups. After the removal of viscera, carcass was weighed, chopped, and submitted to lipid extraction with ethanolic KOH, previously described [62]. Briefly, suspension was washed three times with petroleum ether to remove non-saponifiable lipids. The other lipid fraction was acidified with sulfuric acid and saponified lipids extracted with petroleum ether. The latter were dried and weighed [70]. Interscapular brown adipose tissue was rapidly removed in its entirety and weighed to assess fat accumulation in the tissue.

Statistical analysis

Results were given as mean ± SEM. Data were submitted to variance analysis (ANOVA). In the case of analyses with a significant F, the differences between means were evaluated by Bonferroni *t*-test. Probability values less than 0.05 were considered statistically significant. Tests were performed using GraphPad Prism version 3.02 for Windows (GraphPad Software®).

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