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Evaluation of the Modifications in the Inflammatory Response Promoted by Obesity in Monosodium Glutamate-induced Obese Rats

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Authors' contributions

This work was carried out in collaboration between all authors. Author DPT designed and performed the study. Authors LAMW and SESF designed the study and wrote the manuscript. Authors EHA, MAO and CABA managed the literature searches and performed the statistical analyses. Authors RKNC and ZBF designed this work and managed the analyses of the study. All authors read and approved the final manuscript.

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ABSTRACT

Aims: The purpose of this work was to investigate the inflammatory response changes caused by obesity in an experimental model of glutamate-induced obesity in rats.
Methodology: Wistar rats were injected, subcutaneously, with 4 g/kg of monosodium glutamate (MSG group) on days 2, 3, 4, 5, and 6 of life. At the age of 3 months, the male rats were tested for obesity, measuring the Lee Index, the periepididymal and retroperitoneal fat deposits, serum corticosterone, glucose, insulin tolerance, and lipid profile. The acute inflammatory response was evaluated using paw edema (induced by carrageenan or dextran), pleurisy (induced by pleural injection of carrageenan), increasing in vascular permeability (induced by intradermal injection of histamine, bradykinin, and serotonin) and leukocyte count in peripheral blood and pleural exudate.

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The chronic inflammatory response was evaluated by the cotton pellet-induced granuloma and air pouch methods.

Place and Duration of Study: Our study was carried out at Laboratory of Inflammation (State University of Maringá, Brazil) from January to December 2015.

Results: The MSG group presented an increase in both Lee Index and the fat deposits, glucose intolerance, high blood corticosterone levels, and insulin resistance, characterizing obesity. The paw edema was reduced in MSG group. The vascular permeability response induced by mediators of the inflammatory response (histamine, bradykinin, and serotonin) was not altered. The leukocyte migratory capacity to the pleural cavity was reduced in MSG group with the reduction of the circulating leukocyte number.

Conclusion: Our data provide evidence that acute inflammatory response is decreased in MSG animals, demonstrating the modification of the immune response caused by obesity. These changes in the innate immune response cannot be related to loss of responsiveness of tissues to inflammatory mediators. The decreasing in the blood leukocyte number associated with high levels of corticosterone were partially responsible for the suppression of acute inflammatory response.

Keywords: Lee index; obesity; immune response; insulin resistance; corticosterone.

1. INTRODUCTION

Obesity can be considered a chronic, complex, and etiologic multifactorial disease. In recent years, the occurrence of human obesity has increased intensively throughout the world, becoming a public health problem [1]. In recent surveys of the World Health Organization, they noted that more than one billion adults are overweight, and almost 300 million people are obese. This is a global trend and these numbers are expected to increase in the future [2].

The increase in obesity and its strong association with type-2 diabetes have aroused interest in the study of the mechanisms underlying these diseases, which are usually followed by inflammatory response disorders, increased incidence of cardiovascular diseases, and the individual incapacitation, in humans [3]. Literature data show that obesity is associated with immune dysfunction, characterized by a chronic inflammation state. It is well known that diet-induced obese rats present elevated levels of pro-inflammatory cytokines such as tumor necrosis factor (TNF-α), interleukin 6 (IL-6), interleukin 1 β (IL-1β), and lower levels of anti-inflammatory cytokines, such as interleukin 10 (IL-10) [4], promoting impact on several body functions and modifying the immune responses [5].

Furthermore, the innate inflammatory response is significantly decreased in diabetic and diet-induced obese mice [6]. A previous study demonstrated that in streptozotocin-induced diabetic rats the vascular response to permeability factors (i.e. histamine) was decreased, suggesting an insulin influence in the control of the vascular permeability and, therefore, of the inflammation [4].

Finally, the literature provides evidence that there is a greater activation of the hypothalamic-pituitary-adrenal axis in obese patients, particularly those with central fat distribution [7,8], resulting in more frequent release of adrenocorticotropic hormone. This effect would result in altered serum cortisol levels, which would modify the inflammatory response of the individual. However, despite the subtle findings, there are scarce evidences of clinical hypercortisolism in obese individuals. Then, it is of great importance to study the corticosteroids changes induced by obesity.

However, to the best of our knowledge, the correlation between high levels of corticosterone and decreasing the inflammatory response in obesity has been poorly investigated. Further, the changes promoted by obesity in the immune response and in the response of tissues to inflammatory mediators remain to be determined.

In this regard, the aim of this work was to investigate the modifications on the acute and chronic inflammation in obese animals. We conducted a series of experiments designed to evaluate, among other things, the involvement of histamine, bradykinin, serotonin, and corticosterone in the inflammatory response, using an *in vivo* experimental model of obesity induced by monosodium glutamate (chronic insulin resistance).

2. MATERIALS AND METHODS

2.1 Chemicals

The bradykinin triacetate, carrageenan, dextran, Evans blue, histamine hydrochloride, monosodium glutamate, and serotonin were all obtained from Sigma (St. Louis, USA); Chloral hydrate and sodium pentobarbital from were obtained from Merck (Darmstadt, Germany), and NPH insulin from Biobrás (São Paulo, Brazil).

2.2 General

Male Wistar rats were injected, subcutaneously, in the cervical region with monosodium glutamate (4 g/kg) or saline on days 2, 3, 4, 5, and 6 of life. The animals were weaned, grouped according to treatment, and housed at standard conditions (room temperature 22±0.5ºC, 12 hours light/dark cycle, 60% humidity, with food and water *ad libitum*). The rats were used in the experiments at the age of 12-14 weeks.

The metabolic alterations were characterized by blood and urine glucose levels. Further, the 24 hours urinary volume, and food and water intake were determined by keeping the animals in metabolic cage. To verify whether treatment led to obesity, the Lee Index [9] and the absolute mass of periepididymal and retroperitoneal fat were used, as previously reported [10]. The Cholesterol, HDL-cholesterol, and triglyceride levels – determined by using the commercial kits following the manufacturer guidelines (Colesterol PP®, Colesterol-HDL PP®, and Triglicérides PP®, Gold Analisa Diagnóstica Ltda, Brazil) – were also used to characterize the presence of obesity.

After characterization of obesity, the obese animals were referred as monosodium glutamate-obese group (MSG). The non-obese animals, that received only saline subcutaneously, were used for comparison with the MSG rats and statistical determination of changes in the data analyzed, and were referred as control group (Control).

2.3 Intravenous Glucose Tolerance Test

Rats were fasted for 12 hours and then anesthetized (sodium pentobarbital, 40 mg/kg body weight) intraperitoneally (i.p.). Sequential blood samples were collected from the cava vein immediately before the glucose challenge (0.5 g glucose/kg body weight), intravenously (i.v.), and 5, 10, 20, 30, and 60 minutes thereafter. After centrifugation at 4ºC, 20µL of plasma were immediately used for glucose determination using a commercial kit following the manufacturer guidelines (Glucose Liquiform®, Lab Test Diagnóstica, Brazil).

2.4 Intravenous Insulin Tolerance Test

For estimation of *in vivo* insulin sensitivity, the animals were submitted to an intravenous insulin tolerance test. Rats were fasted for 2 hours and then anesthetized (sodium pentobarbital, 40 mg/kg body weight, i.p.). Blood samples were collected from the cava vein at 0 (basal), 4, 8, 12, and 16 minutes after insulin injection (0.75 IU/kg body weight). After centrifugation at 4ºC, 20 µL of plasma were immediately used for glucose determination using a commercial kit following the manufacturer guidelines (Glucose Liquiform®, Lab Test Diagnóstica, Brazil). The plasma glucose disappearance was calculated from the linear regression of the glucose concentration values from 0 to 16 minutes of the test, as previously described [11].

2.5 Serum Corticosterone Determination

The rats were decapitated, the free-running blood collected, and the serum obtained. Corticosterone concentrations were determined by using ELISA kits (Cayman Chemical Co., USA) as reported in a previous study [12]. Briefly, samples were incubated in 96-well plates precoated with specific antibodies for 2 hours at 4ºC. Then, plates were washed and the enzyme substrate (Ellman's reagent) added for 60 minutes at 25ºC. The absorbance was determined at 412 nm in a microplate reader and the concentration of corticosterone calculated from a standard curve.

2.6 Paw Edema

Paw edema was measured as previously described by Winter et al. [13]. Carrageenan (2 mg/mL) or dextran (1 mg/mL) were injected into the plantar region of the left hind paw of the rats (0.10 mL), and the right paw received an equal volume of saline. The progress of edema was measured up to 24 hours by determining the increase in volume (µL) relative to the initial volume of the paw. The extent of edema was expressed as the difference in volume between the saline-injected paw and the paw injected with the inflammatory agent.

2.7 Vascular Permeability

Evans blue (1% aqueous solution) was administered (20 mg/kg, i.v.), followed immediately by varying doses of histamine, bradykinin, and serotonin injected subcutaneously (0.10 mL) in the dorsum of rats [14]. After 30 minutes, the rats were euthanatized with overdose of anesthetic solution (sodium pentobarbital, 200 mg/kg body weight, i.p.) and the dorsal skin removed. Portions of skin containing the stained area were cut out and the accumulated dye extracted with formamide before quantification by spectrophotometry at 620 nm. The results were expressed in µg of dye after correction for the basal values.

2.8 Carrageenan-induced Pleurisy

Prior to the pleurisy induction, aliquots of blood were obtained by caudal vein puncture of animals. To induce the pleurisy, carrageenan (50 µg) was injected as described by Vinegar et al. [15]. Four hours after, the rats were euthanatized with overdose of anesthetic solution (sodium pentobarbital, 200 mg/kg body weight, i.p.). The chest wall was opened and the inflammatory exudates collected by aspiration and the total volume determined. The total and the differential leukocyte number were determined in blood and exudates. Aliquots of blood and exudates were used to determine the total leukocyte count in a Neubauer counting chamber. For differential cell count, red blood cells were lysed by adding Turk's solution, the remaining fluid centrifuged, and cells suspended in 0.1 mL of rat plasma. Then, smears were prepared, air dried and fixed with Rosenfeld stain.

2.9 Bilateral Adrenalectomy

In anesthetized Control and MSG rats (sodium pentobarbital, 40 mg/kg body weight, i.p.) a median incision of the muscular wall was made and the adrenal glands were stripped from the surrounding adipose tissue and resected. Sham-operated Control and MSG rats were prepared by handling of abdominal viscera and closure of the incision of the abdominal wall in a similar extent as for actual adrenalectomy. These animals were also tested 8 days post-operatively.

2.10 Chronic Granulomatous Inflammation

As previously reported [16], sterile cotton (40 mg) was implanted subcutaneously in the dorsum of rats. After seven days, the animals were euthanatized and the dry weight of the cotton pellet was determined [17]. The air pouch method was also used as previously described [18]. Briefly, seven milliliters of air were subcutaneously injected into the back of each rat. After 24 hours, 4 mL of sterilized carrageenan solution (2% w/v) were injected into the air pouch. Seven days later the quantitative measurement of newly formed blood vessels was realized according to the method of Kimura et al. [19], with minimal modifications. One milliliter of Evans blue suspension was injected into the tail vein of rats. The animals were euthanatized, the entire fluid in the granuloma pouch harvested, and the capsule of granulomatous tissue removed. The volume of fluid and the wet weight of the capsule were determined. The granulomatous capsule was dissolved in extractor solution (0.5% acetone-sodium sulphate 2:1), centrifuged, and the supernatant filtered. The filtrates were measured spectrophotometrically at 620 nm against standard in digested non-injected tissue. The results were expressed in dye content (µg) per sample.

2.11 Statistical Analysis

The results were expressed as the mean ± standard error of mean (SEM). Where appropriate, the data were analyzed by parametric methods: Student's t-test or one-way analysis of variance (ANOVA). Differences were considered significant at $P = .05$.

3. RESULTS

In our experiments, the obesity was characterized by increased Lee Index and accumulation of fat in periepididymal and retroperitoneal pad in MSG compared to the Control rats. Blood and urine glucose, food intake, 24 hours urine volume, and blood pressure did not differ between both groups. Only the water intake was reduced in the MSG rats (Table 1).

Although the total cholesterol concentration did not differ between both groups, the HDL-cholesterol concentrations were reduced and the triglyceride levels were elevated in MSG compared to the Control rats (Fig. 1).

Basal blood glucose levels were similar in MSG and Control rats. After glucose load, increased values of glycemia in MSG rats occurred from 5 to 30 minutes (Fig. 2 A). The area under the

curve of the glucose disappearance rate (Kitt) was significantly lower in MSG than in Control rats (Fig. 2 B).

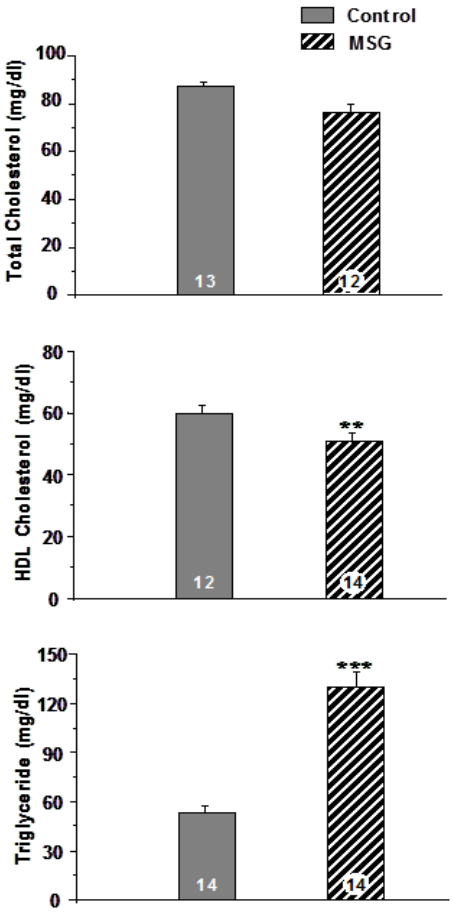

Fig. 1. Plasma lipoprotein measurements – total cholesterol, HDL cholesterol, and triglyceride – in MSG and Control rats

*Results are expressed as mean ± SEM. ** P = .01; *** P = .001, compared to the Control group (data analyzed by Student's t-test). MSG = monosodium glutamate-obese rats; n = number of rats*

The serum corticosterone levels were significant higher in MSG (4.65±0.41 µg/dL) than in Control rats (2.16±0.34 µg/dL). After adrenalectomy, serum corticosterone levels were undetectable in both groups.

The paw edema induced by carrageenan or dextran in MSG rats was markedly reduced compared to the Control rats (Fig. 3 A and B).

The increase in vascular permeability induced by histamine (1.0; 5.0; 10.0 µg), bradykinin (0.1; 0.5; 1.0 µg) and serotonin was similar in MSG and Control rats (Fig. 4 A, B, and C).

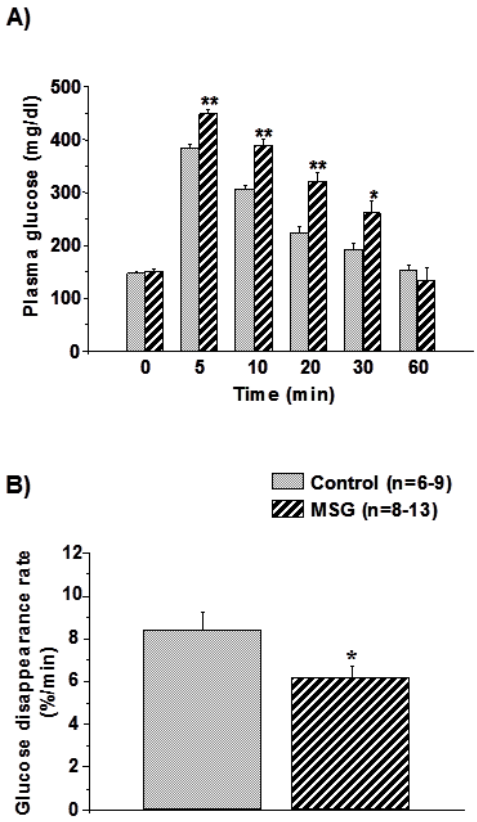

Fig. 2. Plasma glucose levels during intravenous glucose tolerance test (A) and glucose disappearance rate (Kitt) during the intravenous insulin tolerance test (B) in MSG and Control rats

*Results are expressed as mean ± SEM. *P = .05; **P = .01, compared to the Control group (data analyzed by one-way ANOVA [A] and Student's t-test [B]). MSG = monosodium glutamate-obese rats; n = number of rats*

As shown in the Fig. 5, a significant decreasing in the blood leukocyte number was found in MSG (n = 9) compared to the Control rats (n = 9) prior to the pleurisy induction. Furthermore, it was determined that both polymorphonuclear and mononuclear blood leukocytes were reduced in MSG rats before the inflammatory stimuli.

As shown in the Table 2, after carrageenan stimulus the volume of pleural exudate was similar in MSG and Control rats, however, the total number of cells migrating into the pleural cavity was markedly reduced in MSG rats. Similar increases in pleural exudates were observed in adrenalectomized MSG and Control rats. Adrenalectomy did not modify the migration of leukocytes to the pleural cavity in Control rats,

however, it restored the reduced migration found in MSG rats. In this circumstance the number of leukocytes in the pleural exsudate did not differ from those found in sham-operated control rats.

Table 1. General characteristics of the animals after 90 days of experiment

	Control	MSG
Lee index (x100)	29.97±0.20 (n = 17)	30.63±0.10[a] (n = 16)
Retroperitoneal fat (absolute mass)	1.27±0.08 (n = 8)	1.94±0.15 [a] (n = 10)
Glycemia (mg/dL)	147.8±3.6 (n = 8)	151.4±5.4 (n = 13)
Urine glucose (mg/24h)	0.76±0.20 (n = 9)	0.46 ± 0.10 (n = 8)
Food intake (g/100g body weight/24h)	8.30±0.50 (n = 10)	8.40±0.50 (n = 8)
Water intake (mL/100g body weight/24h)	15.20±1.40 (n = 8)	11.00± 1.00[a] (n = 9)
Urine volume (mL/24h)	8.50±2.20 (n = 8)	7.90±0.60 (n = 7)
Blood pressure (mmHg)	125.70±3.40 (n = 8)	118.00±2.20 (n = 7)

Results are expressed as mean ± SEM. [a] P = .05, compared to the Control group (data analyzed by Student's t-test). MSG = monosodium glutamate-obese rats; n = number of rats

The dry weight of the cotton pellet in MSG rats (0.46±0.05 g) was significantly higher than in Control rats (0.33±0.02 g). In the air pouch, the capsule weight (g/100g body weight) of newly formed granulomatous tissue and the volume of the fluid (mL/100 g body weight) in granulomatous capsule was similar in MSG and Control rats (Fig. 6 A and B). The Evans blue content in granulomatous capsule vasculature was significantly higher in MSG compared to the Control rats (Fig. 6 C).

4. DISCUSSION

Obesity is one of the most common and detrimental metabolic diseases. Characteristics of human obesity can be found in animals in which obesity is induced experimentally, as observed after the monosodium glutamate administration in neonatal rats. In fact, in our experiments higher Lee index and weight of periepididymal and retroperitoneal fat pad were found in MSG than in Control rats. We also observed an altered lipid metabolism, insulin resistance, and glucose intolerance in MSG animals. In a previous study, alterations in the lipid metabolism (triglyceride level) were observed in MSG animals only after inflammatory stress, deferring from our results in this point [20]. However, these metabolic changes were not sufficient to induce polyphagia, glycosuria, or polyuria. These data together characterize the obese state and demonstrate that the experimental model carried out is consistent with those reported in many studies [21,22,23], validating the results obtained.

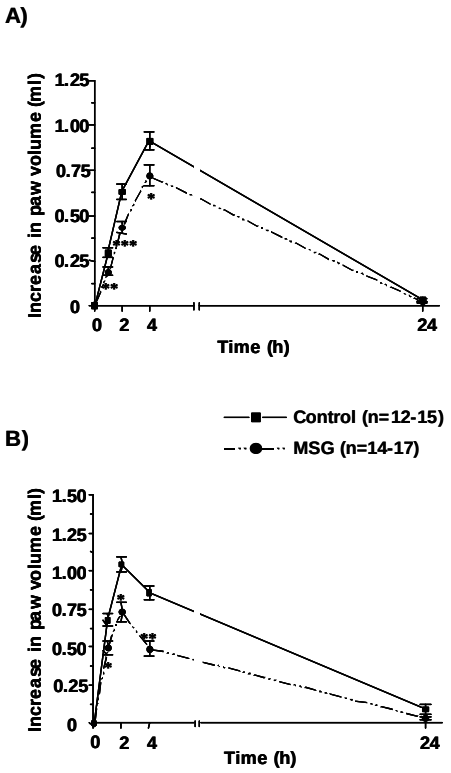

Fig. 3. Time course of the carrageenan-induced paw edema (A) and dextran-induced paw edema (B) in MSG and Control rats

*Results were obtained 90 days after injection of monosodium glutamate and are expressed as mean ± SEM. *P = .05; **P = .01; ***P = .001, compared to the Control group (data analyzed by Student's t-test). MSG = monosodium glutamate-obese rats; n = number of rats*

Even, our findings suggest that, despite of similar basal glycemia, MSG animals present glucose intolerance with insulin resistance. Data from the literature suggest that insulin resistance in obesity is related to the smaller capacity of the insulin in stimulating glucose utilization, due to a reduction in the expression of glucose transporters [21,24]. However, to elucidate the correlation between obesity and insulin resistance in this experimental model is not the

primary aim of this work. Future studies will be done with this purpose.

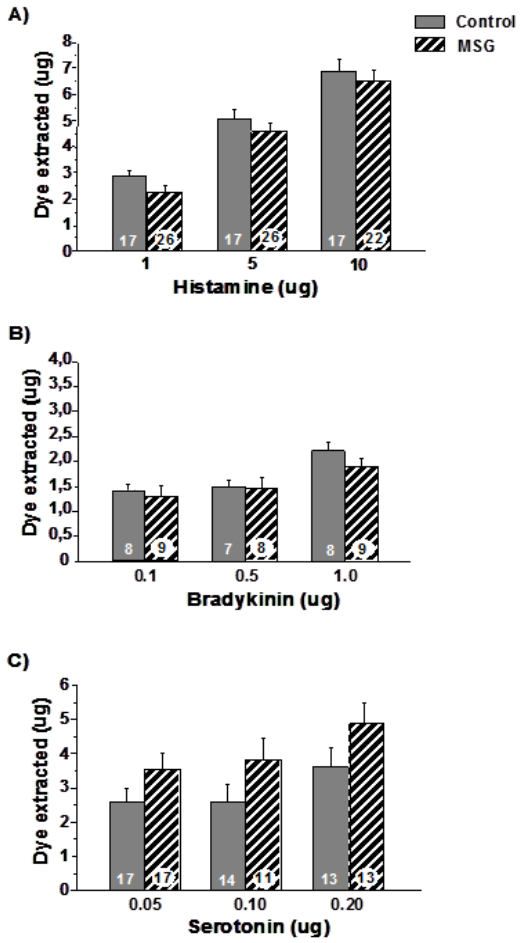

Fig. 4. Dye leakage after injection of histamine (A), bradykinin (B), or serotonin (C) obtained in MSG and Control rats after treatment with Evans blue

*Results were obtained 90 days after injection of monosodium glutamate and are expressed as mean ± SEM. *P = .05, compared to the Control group (data analyzed by Student's t-test). MSG = monosodium glutamate-obese rats; n = number of rats*

The reduced responses to carrageenan (injected in the paw or pleural cavity) or dextran (in the paw) occurred in MSG rats cannot be attributed to altered responses to the inflammatory mediators released by these irritants, such as histamine, bradykynin, and serotonin, since the vascular responses to these mediators were not impaired in MSG group. Similar results were obtained by Limãos and collaborators using the same experimental model [25]. Although, Castrogiovanni and collaborators demonstrated that the responses to pro-inflammatory and anti-inflammatory cytokines were normal in MSG animals [20].

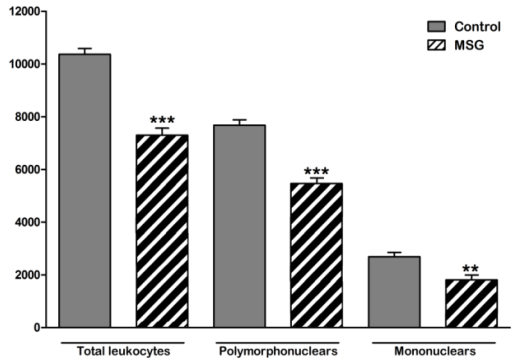

Fig. 5. Total and differential blood leukocytes count in MSG and Control rats.

*Results were obtained 90 days after injection of monosodium glutamate, just prior to the pleurisy induction, and are expressed as mean ± SEM. **P = .01, ***P = .001, compared to Control group (data analyzed by Student's t-test). MSG = monosodium glutamate-obese rats*

Although similar pleural exudate volumes, the reduced leukocyte migration demonstrated after pleurisy in MSG rats may be related to the significant decreasing in the blood leukocyte number. In obese individuals, reduced leukocyte number (mainly the polymorphonuclears) and reduced responsiveness to antigen have been observed [26]. It seems to be related to hematopoiesis dysfunctions caused by obesity. These important immune defects can downregulate the acute inflammatory response and even make the individual susceptible to infections [6]. It also partially explains the reduced paw edema development determined after carrageenan and dextran injection in MSG group.

Furthermore, higher levels of corticosterone in MSG rats may be responsible for the decreased inflammatory response and partially elucidate the reduced paw edema and leukocyte migration in MSG rats. Giving support to this hypothesis, adrenalectomy rendered MSG animals as responsive to inflammatory irritants as Control animals. Then, our data suggest that the high levels of endogenous corticosteroids, arising from the activation of the hypothalamic-pituitary-adrenal axis in MSG obesity, could determine an impaired acute inflammatory response in MSG group.

Table 2. Exudate volume and leukocytes count after carrageenan-induced pleurisy

Group	Exudate volume (mL)	Leukocyte count (cells / mm^3)	Cells x 10^3	
			MN	PMN
Control	0.55±0.50 (n = 9)	80028±3892 (n = 9)	16±2 (n = 9)	64±3 (n = 9)
MSG	0.60±0.04 (n = 9)	51361±3464* (n = 9)	9±1* (n = 9)	42±3* (n = 9)
Sham-control	0.53±0.03 (n = 7)	70100±2542 (n = 7)	17±4 (n = 7)	53±3 (n = 7)
Sham-MSG	0.47±0.05 (n = 12)	49880±3722 (n = 12)	12±2 (n = 12)	37±3 (n = 12)
Control-ADX	1.04±0.10** (n = 8)	87000±3402*** (n = 8)	26±6*** (n = 8)	61±8 (n = 8)
MSG-ADX	1.22±0.14*** (n = 12)	69320±5740** (n = 12)	12±2 (n = 12)	57±6** (n = 12)

*Results were obtained 90 days after injection of monosodium glutamate and are expressed as mean ± SEM. *P = .01, compared to the Control group. **P = .05, compared to Sham-control group. ***P = .05, compared to Sham-MSG group (all data analyzed by Student's t-test). MSG = monosodium glutamate-obese rats, ADX = adrenalectomized, MN = mononuclear leukocytes; PMN = polymorphonuclear leukocytes; n = number of rats.*

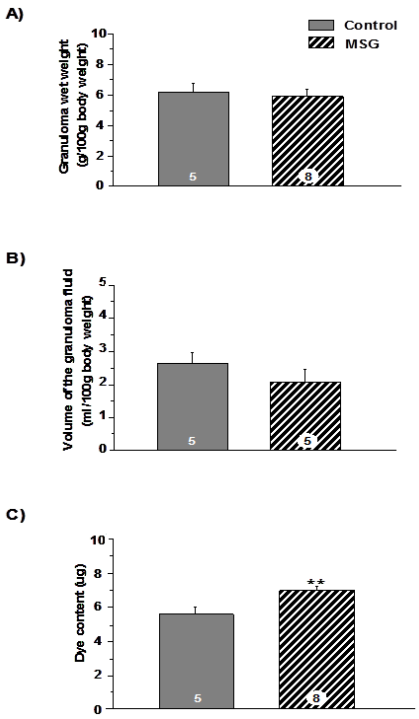

Fig. 6. Granuloma wet weight induced by carrageenan 2% (A), volume of the fluid in granulomatous capsule (B), and Evans blue content in granulomatous capsule (C) in MSG and Control rats

*Results were obtained 90 days after injection of monosodium glutamate and are expressed as mean ± SEM. *P = .05, compared to the Control group (data analyzed by Student's t-test). MSG = monosodium glutamate-obese rats; n = number of rats*

Finally, the two methods used to study the chronic inflammation allow us to evaluate the proliferative component (by the cotton pellet-induced granuloma test) and the angiogenic component (by the air pouch method) of the chronic inflammatory response. The high weight of the granuloma found in MSG animals indicates an increase in the proliferative response to injurious stimuli in obesity. The increased vascular permeability found in the air pouch indicates an increased angiogenesis in response to inflammatory stimuli. Our findings confirm the hypothesis that obesity is related to an improvement in chronic inflammatory responses [4]. However, the mechanisms involved here are not clearly explained by our findings and remain to be determined by further studies.

5. CONCLUSION

In conclusion, our data provide evidence that MSG animals show a significant reduction in the acute inflammatory response, demonstrating the modification of the immune response caused by obesity. It is important to note that these changes in the innate immune response are not related to loss of responsiveness of tissues to inflammatory mediators. According to our results, the decreasing in the blood leukocyte number associated with high levels of corticosterone are probably the main responsible for the suppression of acute inflammatory response. Finally, it was observed an enhancement of cell proliferation and angiogenesis in chronic inflammatory response in MSG animals, however, the pathways involved were not clearly determined by our findings. Further investigations may elucidate the mechanism by which obesity produces the observed changes in the hematopoietic system and offer additional

information on how the chronic inflammatory response is stimulated in the obese state.

CONSENT

It is not applicable.

ETHICAL APPROVAL

All authors hereby declare that "Principles of laboratory animal care" (NIH publication No. 85-23, revised 1985) were followed, as well as specific national laws where applicable. The experimental protocol was approved by the Ethical Committee in Animal Experimentation of the State University of Maringá (CEEA/UEM n° 017/2013).

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COMPETING INTERESTS

Authors have declared that no competing interests exist.

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