The Influence of Obesity Induced by Monosodium Glutamate in Periodontal Tissues of Female Wister Rats with Experimental Periodontitis

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Abstract

Aim of the study: Evaluate the effect of obesity-induced monosodium glutamate (MSG) and female sex hormones on the periodontal tissues of Wistar rats submitted to experimental periodontitis. Methods: Thirty-three newborn Wistar rats were divided into four groups CON, OB, CONLIG e OBLIG after received subcutaneous injections of hypertonic saline solution or MSG and induced a periodontal disease. Blood, perigonate and retroperitoneal fats, gingival tissue sample and the hemimandibular were then subjected analysis. Results: Analysis showed less alveolar bone loss in the OBLIG group compared to the CONLIG group ($p < 0.05$). In the gingival tissue analysis, no differences were found between the groups with experimental periodontitis. Conclusion: The results suggest that hypothalamic obesity may have a protective effect on alveolar bone loss in cases of induced periodontitis ,also may interfere negatively in the plasmatic concentrations of hormones LH and FSH, but there was no effect on gingival inflammation.

Keywords: Obesity, Periodontitis, Ovary Hormones, MSG.

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1. Introduction

Obesity is a global health problem and its prevalence has increased significantly in recent decades (Han et al., 2010). It is synonymous with chronic, multifactorial affecting adults and children and is responsible for increased risks of different systemic disorders, cardiovascular illnesses, diabetes and other life-threatening changes(Pataro et al., 2012, Pischon et al., 2007, Ritchie, 2007).The discovery that obesity by itself results in an inflammatory state in metabolic tissues, marked the beginning of a field of research that examines the inflammatory mechanisms in obesity(Gregor & Hotamisligil, 2011). The study of these mechanisms by which it induces physiological disorders may be facilitated with the use of animal models in research environment, the ratbeing a model that offers great potential for investigating molecular and cellular mechanisms underlying the development of obesity (Cox & Church, 2011). Among the neural models, hypothalamic obesity is the most known by subcutaneous administration of MSG in the first days after birth, where there is an acute hypothalamic degeneration, destroying specific locations including the ventromedial arcuate nucleus, causing disturbances in the control uptake and energy expenditure mechanisms, causing obesity, stopping growth, behavioral deficits and changes in cardiovascular control(Fernandes et al., 2012, Miranda et al.,2014,Olney, Adamo & Ratner, 1971).

Several studies suggest that obesity can be one of the possible factors which significantly influence the severity and progression of periodontal disease (Al-Zahrani, Bissada & Borawskit, 2003, Dalla Vecchia et al., 2005, Saito & Shimazaki, 2007, Verzeletti et al., 2012), and it is likely that the cytokines derived from adipose tissue have a key role in the process (Slotwinska & Slotwinski, 2015).The adipose tissue produces large amounts of cytokines and hormones collectively called adipokines, which in turn can modulate periodontitis, adversely affecting the treatment of diseases and also represent a mechanism by which periodontal infection may have an impact on systemic diseases (Deschner et al., 2014). Among adipokines which the adipose tissue secrets are TNF - α and IL-6, with concentrations proportional to the BMI. The increase in these pro inflammatory cytokines can explain the relationship between obesity and periodontal disease (Nascimento et al., 2013). Periodontal disease is a chronic infectious disease caused predominantly by bacteria (Slotwinska & Slotwinski, 2015), which can cause the destruction of the tissues and structures surrounding the teeth if not treated. This fact can result in tooth loss, which is a major negative impact on the quality of life of indivíduals (Shewale et al., 2016).Although the presence of microorganisms is necessary, it is not sufficient for the initiation of disease. Rather, it is unbalanced and persistent inflammatory reaction of the host against pathogens that results in the destruction of the periodontal tissues(Lira-Junior & Figueredo, 2016). The severity of inflammation varies between individuals, regardless of the degree of bacterial infection, suggesting that a dysregulation of host inflammatory response may contribute to its existence (Fabbri et al., 2014). As the periodontal diseases are modulated by the immune response, they can represent a risk factor for systemic diseases (Carvalho-Filho et al., 2016). Hemostasis periodontium involves a multifactorial complex relationship, which the endocrine system presents a relevant role (Mariotti, 1994).
Sex hormones have been considered an important influence in periodontal tissues, bone turnover rate, wound healing and progression of the periodontal disease (Mascarenhas et al., 2003).

Estrogen and progesterone have significant biological actions that may affect other systems including the oral cavity (Sooriyamoorthy & Gower, 1989, Pack & Thomson, 1980). Receptors of these hormones have been demonstrated in the gums, on the periosteal fibers, lamina propria fibroblasts, also dispersed in periodontal ligament fibroblasts and osteoblasts proving direct effects of sex hormones on the periodontal tissue (Jafri et al., 2015). Several studies (Pataro et al., 2012, Al-Zahrani, Bissada & Borawskit, 2003, Verzeletti et al., 2012, Nascimento et al., 2013, Saito et al., 2001, Perlstein & Bissada, 1977) show the association of obesity with the development and progression of periodontal disease, as well as the influence of hormonal changes, but there is no consensus in relation to these factors(Mariotti, 1994, Mascarenhas et al., 2003, Sooriyamoorthy & Gower, 1989, Jafri et al., 2015, Khosravisamani et al., 2014). Thus notes the importance of additional studies that describe the evidence which may occur in females with and without periodontal disease. Therefore, the aim of this study was to evaluate the effect of obesity-induced MSG and female sex hormones on the periodontal tissues of Wistar rats submitted to experimental periodontitis.

2. Materials and Methods

2.1 Animals

Pregnant rats were obtained from the central vivarium of the State University of Western Paraná, Cascavel campus, and maintained in the experimental laboratory of Endocrine Laboratory of Physiology and Metabolism under controlled temperature (21 ± 2 °) and light (12 h light cycle and 12 hours of dark - 7:00 - 19:00). At birth (considered day 0), the offspring were separated by genre, only females were kept and males were killed by decapitation. Later these animals were weaned at 21 days and received a standard diet (Nuvital, Curitiba, Brazil) and water ad libitum throughout the experimental period. 33 cycling adult Wister female rats were used in proestrus phase of the estrous cycle (body weight between 180-350g) which were kept in breeding boxes with groups of 3-5 animals per box (41 cm long x 34 cm wide x 17 cm high) and like the mother rats, they were kept in an environment with light-dark cycle and controlled temperature, with free access to water and food. All experimental protocols were approved by the Ethics Committee on Animal Use (CEUA) of the State University of Western Paraná (UNIOESTE) in accordance with the Ethical Principles for Animal Experimentation, adopted by the National Council of Animal Experimentation Control (CONCEA).

2.2 Induction of Obesity

The animals were divided into 2 groups, control (CON) and obese (OB) with 15:18 rats respectively, and then submitted to the intradermal injection in the neck during the first five days of life of 1,25 g / kg body weight saline (group CON) or MSG dose of 4g / kg (group OB).

2.3 Induction of periodontal disease

After 70 days of life the animal CON and OB groups were divided into two groups, then anesthetized by intramuscular administration of xilazinha (Virbac from Brazil Ind. Com. Ltda, São Paulo, SP, Brazil) 0,04mL / 100g body weight and ketamine (Francotar, Virbac Brazil's Indand Com. Ltda, São Paulo, SP, Brazil) in 0,08mL / 100g dose of body weight and placed on a proper operating table, which allowed the maintenance of the mouth opening of the rats facilitating access to the teeth of the posterior region. With the aid of a modified forceps and a dental explorer, all animals received a cotton ligature number 40 (Coats current Ltda., Brazil) around the first molars on both sides of the jaw in a sub marginal position to induce experimental periodontitis (Nassar et al., 2009).After this procedure 4 groups originated: CON (08 animals), CONLIG (07 animals), OB (09 animals) and OBLIG (09 animals). This ligature acted as a gingival irritant for 30 days, which favored the accumulation of bacterial plaque and the subsequent development of periodontal disease (Nassar et al., 2009).

2.4 Obesity Evaluation

At 100 days of age, the animals were weighed and naso-anal length was obtained for calculating Lee index [cubic root of body weight (g) / naso-anal length (cm)] (Bernardis & Patterson, 1968). Subsequently, after sacrifice by decapitation the perigonadal and retroperitoneal fat were removed and weighed (Aaker ®, Porto Alegre, RS, Brazil).

2.5 Collection of samples

At the end of the experimental period, the animals were decapitated. Afterwards, blood samples were collected from right brainstem, which were centrifuged at 300xG for 15 minutes and stored in a freezer at – 80^0C to perform the analysis. The perigonadal and retroperitoneal fat were removed and weighed.

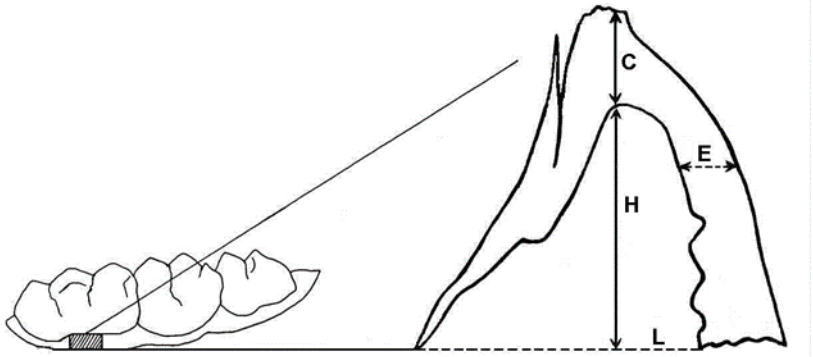
Samples of the periodontal tissue of the right hemimandibular were removed and stored for analysis. In the left hemimandibular, the gum surrounding the tooth affected by experimental periodontitis was removed and stored and then the left hemimandibular was dissected for subsequent radiographic analysis.

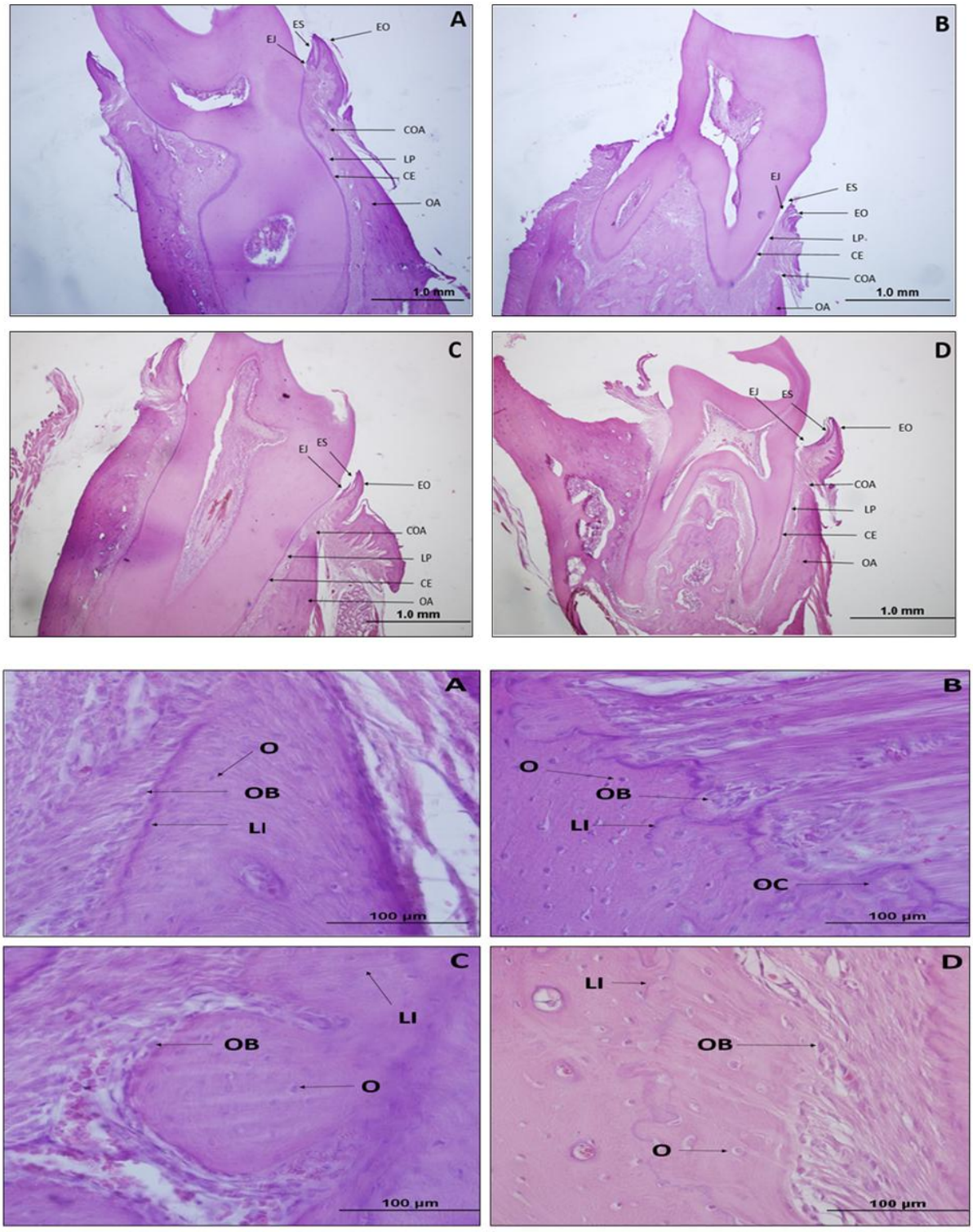
2.6 Collection of blood samples to test for hormones

The blood collected after decapitation was from the right brainstem. The samples were centrifuged at 3000 rpm for 30 minutes; plasma was separated and stored in a freezer at -80°C for measurement of hormone levels.
Hormone levels were measured by radioimmunoassay. The estradiol concentration was determined using a specific kit (DSL Estradiol - 4400, Diagnostic Systems Laboratories, Texas, USA). Plasma concentrations of LH, FSH were determined using reagents obtained from the National Hormone & Peptide Program (Harbor - UCLA Medical Center, USA). The minimum detectable doses were 0,05ng / mL for H; 0.2 ng / ml for FSH. The intra-assay coefficient of variation was 4% for LH, FSH to 3%. The plasma progesterone concentration was determined using kits produced by MP Biomedicals® and error intra and inter-assay was 2.5%.

2.7 Radiographic analysis

- After euthanasia, the left hemimandibularof each animal was removed and fixed in buffered formalin (pH 7.2) for 48 hours. Radiographs were taken using a digital imaging system (Sensy-A-Ray 3:11) which uses an electronic sensor instead Rx film. The focus-film distance was set at 50 cm and the electronic sensors were exposed to 70 kV and 8 mA with a time of exposure 0.3 pulses/s. Scanned images were analyzed in 3 steps, on the Image Tools 3.0 program (The University of Texas Health Science Center, San Antonio, TX, USA) and an average taken between them by a linear measure, which ran the distance from the cement-enamel junction to the alveolar bone crest on the mesial side of the first left lower molar of the rat, with measurements in pixels (Nassar et al., 2014).
- Image 1: Schematic representation of the marginal gum of the rat, showing the reference points used for the morph metric measurements of the buccalepithelium, epithelial crest and connective tissue C: height of gingival crest epithelium, E: buccalepithelium width, H: height of connective tissue in the middleregion, L: connective tissue width in the basal region.
- Image 2: Photomicrographs of rat'stooth, longitudinal cut, hematoxylin and eosinstaining. Control group (A), control ligature (B), obese (C), obese ligature (D). Cement, CE; Alveolar bonecrest, COA; Junctional epithelium, EJ; Sulcular epithelium, ES; Periodontal ligament, LP; Alveolar bone, AO.
- Image 3: Representative photomicrographs of animals longitudinal cut, hematoxylin and eosinstaining. Control group (A), control ligature (B), obese (C), obese ligature (D). Osteocyte, O, osteoblast, OB; Osteoclast, OC; Incremental lines, LI.





2.8 Analysis of Interleukin expression 6

A portion of the gum tissue surrounding the teeth of the left side hemimandibular, subjected or not to the placement of ligature of all experimental groups were removed and used for determination by Enzyme linked immunosorbent assay (ELISA) of cytokine IL-6. For dosing of cytokine, previously sensitized plates with monoclonal antibodies (Biosource, INVITROGEN ®, California, USA) were usedaccording to the manufacturer's instructions. The plates were incubated with the supernatants of the gingival tissue or with different concentrations of recombinant IL-6 cytokine concentrations, specified by the manufacturer for 2 hours at room temperature (RT). Followed by 5 washes.

The cytokine-specific peroxidase-conjugated detection antibody was added to the plate and left at RT for 1 hour. After 1 hour of incubation, plates were washed and revealed reactivity by addition of the developing solution. The reaction was blocked after 20 minutes with stop solution and reading performed at 450 nm on a microplate reader. The concentration of cytokine in the supernatants was calculated by using linear regression curve from a standard curve performed for the respective cytokine.

2.9 Histological processing

After euthanasia of animals, the right side hemimandibular were collected, dissected and fixed in 10% formalin solution for 24 hours. After this period, they were washed in water and immersed for 1 hour in trichloroacetic acid (TCA) solution 5%. The pieces were kept in decalcification solution of TCA for 20 days and evaluated for the expected degree of decalcification, with exchange of the solution every 5 days. After decalcification tissues were immersed in 5% sodium sulfate for about 2 hours to neutralize the TCA, then the pieces were washed in running water for 1 hour, and packed in 70% ethanol at 4°C. Then the material followed the protocol for embedment in paraffin, being dehydrated in increasing alcoholic series, diaphanized in xylene and embedded in paraffin (Paraffin Purified, Vetec Quimica Fina, Rio de Janeiro, Brazil). Cuts were made in manual microtome (Olympus CUT 4055 - Charleston, South Carolina, USA) 5 mm thick. The slides were mounted and stained with hematoxylin and eosin technique (HE)(Junqueira & Junqueira, 1983).

2.10 Microscopic observations

Microscopic analysis was performed by a single examiner by evaluating the stained histological sections. The slides were analyzed with the aid of a commonly transmitted light microscope (Leica Microsystems, Switzerland) for morphological observations of the gingival tissue, alveolar process and osteoblasts count, osteocytes and osteoclasts of animal hemimandibular.

2.10.1 Bone morphometry

- The quantification of osteoblasts, osteocytes and osteoclasts present in five consecutive fields of the buccal bone crest from the highest point of the crest was held. For this observationa 100-fold increase in the immersion microscopewas used. Two observations were made per field, and then the average value was made for each animal and for each group. Measurement of the bone crestwas performed using a microscope togetherwith a computer, enabling to capture the images through LazEz® software. A measurement of the smallest distance between the apex of the buccal bone crest and cement-enamel junction was performed. The measurements were repeated once a day on three separate days, and then the average values made.

2.10.2 Morphometrygum

- Morphometric measurements were made on the buccal marginal gums and right tongue in all groups, using an image analyzing program connected to a microscope with objective light of 10x, with intervals of 10 cuts between one count and another serialization of slices (about 70 mm). Measurements were made from predetermined points on morphological marginal gingiva, as illustrated in Figure 1. Results were expressed in nm.

2.11 Statistical Analysis

All numerical values are expressed as mean ± standard deviation. The Shapiro-Wilk test was conducted to evaluate the normality of distribution and then the ANOVA test carried out and consequently the Tukey's test at $p < 0.05$ to assess differences between the groups.

3. Results

3.1 MSG effect on the development of obesity in rats with and without induced periodontitis

MSG administration promoted changes in OB and OBLIG groups, showing through the Lee index significant difference when compared to the CON and CONLIG groups ($p < 0.05$), this difference was not seen when comparing the obese groups to each other ($p > 0.05$). The animals that received neonatal treatment with MSG showed a decrease in weight of the rats ($p < 0.05$). The weight of the retroperitoneal fat and peritoneal of rats treated with MSG showed significant difference compared to the other groups ($p < 0.05$) (Table 1).

Table 1: Effect of neonatal treatment with MSG on body parameters in CON, CONLIG, OB and OBLIG rats. Values represent mean ± standard deviation.

	CON	CONLIG	OB	OBLIG
Lee index (g/cm)	0.333±0.008A	0.329±0.012A	0.347±0.013B	0.356±0.006B
Final weight of animals (g)	226.50±16.44A	21A.42±18.90A	179.00±17.72B	189.22±9.02B
Retroperitoneal fat (mg)	1671.14±536.12A	1237.00±157.15A	2813.44±944.14B	3084.77±895.04B
Perigonadal fat(mg)	3260.00±1228.97A	2544.14±220.21A	5983.44±1356.30B	6259.11±1568.40B

Different letters mean that the data is statistically different within the same parameter with $p < 0.05$.

3.2 Serum levels of LH, FSH, Estradiol and Progesterone

By statistical analysis it can be seen that the LH concentration showed a difference between the groups CON and CONLIG with OB and OBLIG. The same thing happened when evaluating the concentration of FSH, demonstrating that animals subjected to MSG for obesity induction have a lower concentration of both LH and FSH compared to the control groups ($p < 0.05$). In relation to progesterone and estradiol, no significant differences were observed between the groups (Table 2).

Table 2: Concentration of LH, FSH, Progesterone and Estradiol of the rats of the experimental groups. Values represent mean ± standard deviation.

	CON	CONLIG	OB	OBLIG
LH (ng/mL)	0.31±0.04A	0.32±0.08A	0.26±0.05B	0.21±0.06B
FSH(ng/mL)	1.28±0.11A	1.10±0.22A	0.82±0.06B	0.86±0.02B
Progesterone(ng/mL)	56.55±22.98A	68.46±23.79A	51.48±33.02A	41.48±28.25A
Estradiol (pg/mL)	170.67±84.06	155.62±49.02A	181.63±86.44A	186.07±121.70A

Different letters mean that the data is statistically different within the same parameter with $p < 0.05$.

3.3 Radiographic analysis of the average distance from the cement-enamel junction to the alveolar crest of the first lower left molar

Radiographic analysis observed increased alveolar bone loss in animals exposed to experimental periodontitis ($p < 0.05$), demonstrating the effectiveness of induction of experimental periodontitis on the alveolar bone, but in the CONLIG group, loss was more pronounced than in the group OBLIG (Table 3).

Table 3: Average distance from the cementum-enamel junction to the alveolar bone crest of the mesial side of the first left lower molar of the rats of all groups. Values represent mean ± standard deviation and are expressed in pixels.

Groups	Average
CON	14.33 ± 1.08 A
CONLIG	20.14 ± 1.45 B
OB	15.18 ± 1.19 A
OBLIG	18.12 ± 1.82 C

Different letters mean that the data are statistically different with $p < 0.05$.

3.4 Quantification of IL-6 in gingival tissue

The concentration of IL-6 was measured from the supernatant of gingival tissue of the animal groups CON, CONLIG, OB and OBLIG. The results demonstrate that IL-6 concentration was higher in the CONLIG group compared to the other groups ($p < 0.05$) (Table 4).

Table 4: IL-6 concentration in the gingival samples of the rats of the experimental groups. Values represent mean ± standard deviation and are expressed in pg / mL.

Groups	Average
CON	0.39± 0.09A
CONLIG	0.73± 0.09B
OB	0.32± 0.06A
OBLIG	0.65± 0.02C

Different letters mean that the data are statistically different with $p < 0.05$.

3.5 Histological and morphological analyzes of right hemimandibular

3.5.1 Control Group

The morphology of periodontal CON group consisting of gum, periodontal ligament, cementum and alveolar bone tissue aspects presented following the normal range (Figure 2-A). In this sense, the marginal gum (smooth surface) and the insert (dotted surface), which surround the cervical portion of the teeth and involve the alveolar process, showed mucosal formed by the stratified squamous keratinized epithelium and the underlying connective tissue. This gingival epithelium appeared divided in zones: oral, sulcular and junctional. In the case of the conjunctive, the network of collagen fibers characteristically eosinophilic were displayed and organized into bundles, they were also blood vessels, nerves, fibroblasts, macrophages, lymphocytes, plasma cells present, among other defense system cells, and no inflammatory aspects were found. Macroscopically, the gums with a pink color, well adhered, covering the root of the teeth and with no signs of inflammation were observed in the animals of this group. The loose connective tissue periodontal ligament which represents the attachment tissue between the tooth and the alveolar bone, presented a large amount of fibroblasts and rich vascularization as well as the Sharpey fibers incorporated into the alveolar bone. In the cemented, of calcified connective tissue whichlines the ridiculer dentine, the insertion of the bundles of periodontal collagen fibers, and cement oblasts were verified. With respect to the alveolar bone that supports and protects the teeth, the osteoid region was displayed, identified in part by the presence of osteoblasts, organized and externally responsible for the synthesis of the bone matrix. Osteoclasts were also present in this region, organized in Howship gaps. In the mineralized bone matrix, osteocytes were still positioned in the gaps. In the bone matrix, alveolar bone, chancellors bone and compactcould be seen. The bony crests were elevated at the cervical third of the root.

3.5.2 Control Group Ligation

In the CONLIG group, morphological changes in the periodontium were observed. In oral, functional and secular gingival epithelium tissue shrinkage and disorganization of the collagen fibers were evident (Figure 3-B). The periodontal ligament was shown to decrease the fibers, disorganize cells and present inflammatory infiltration. The alveolar bone kept the morphological characteristics of the control group, except in the bony crest region which has an irregular shape with aspect of bone resorption and the presence of higher amounts of osteoclasts.
Still, in the macroscopic analysis of the gum, more reddish color, aspect softened with bleeding and the presence of areas with root exposure were found (Figures 2-B and 3-B).

3.5.3 Obese Group

After application of MSG OB group maintained the regularity of the oral gingival epithelium, junctional and sulcular. The other components of the periodontium: cementum, periodontal ligament and alveolar bone also showed normal characteristics very similar to the findings of the CON group. Macroscopically the gum showed healthy appearance, with pink color and no signs of inflammation (Figures 2-C and 3-C).

3.5.4 Obese Group Ligation

In the OBLIG group irregularities in the oral gingival epithelium, junctional and sulcular relating to tissue retraction were observed, but at a lower intensity than the one found in the CONLIG group. In the macroscopic evaluation a reddish coloration was verified with less tissue stiffness and, in some animals, areas of ridiculer exposure. Regarding the cementum and periodontal ligament, they showed changes in collagen fibers with an apparent decrease in volume. At the alveolar bone, irregularities were recorded in the bone crest with less obvious bone loss than what was found in the CONLIG group (Figures 2-D and 3-D).

Osteoblast, osteocytes and osteoclasts figures showed statistical differences in the MSG administration, when compared to the unligated groups (CON and OB) and the groups with the ligature (CONLIG and OBLIG) ($p < 0.05$) and also the difference between OBLIG and CONLIG, suggesting the hypothesis of protective effect of MSG on bone tissue (Table 5).

Table 5: Histological analysis of the right hemimandibula of the rats of the experimental groups for the quantification of osteocytes, osteoblasts and osteoclasts. Values represent mean ± standard deviation and units are expressed.

	Osteoblasts	**Osteocytes**	**Osteoclasts**
CON	27.25±1.52A	66.12±2.54A	0.15±0.01A
CONLIG	23.41±0.38B	56.56±1.21B	0.25±0.02B
OB	26.60±0.38A	64.04±1.62A	0.14±0.02A
OBLIG	24.70±1.19C	58.89±1.22C	0.2±0.02C

Different letters on the same line indicate statistically significant differences ($p < 0.05$) between the groups, within the same parameter.

3.6 Gingival histomorphometric analysis of the right hemimandibular

A statistically significant difference in all parameters studied was observed when comparing the groups with ligation (CONLIG and OBLIG) to groups without ligation (CON and OB). It is worth noting that among the groups receiving the ligature, a greater gingival inflammation was observed, but there is no statistical difference between them. This suggests that MSG had no influence on gingival inflammation (Table 6).

Table 6: Gingival histomorphometric analysis of the right hemimandibula of the rats of the experimental groups. Values represent mean ± standard deviation and are expressed in pixels.

	C	E	H	L
CON	33.35±5.56 A	33.92±1.09A	79.95±12.60 A	66.61±16.99 A
CONLIG	43.37±5.97 B	37.96±3.99B	162.92±25.84B	105.60±27.00B
OB	26.09±9.78 A	29.50±3.87C	75.18±39.79A	50.99±13.94A
OBLIG	40.02±5.88 B	35.18±1.16B	143.65±24.99B	93.27±16.10B

Different letters on the same line indicate statistically significant differences ($p < 0.05$) between the groups, within the same parameter

4. Discussion

MSG is a harmful neuroexcitatory amino acid to the central nervous system, its neonatal subcutaneous application in rats results in lesions in the arcuate nucleus and middle eminence of the hypothalamus, provoking disturbances in the mechanisms of absorption and energy expenditure, inducing obesity (Fernandes et al., 2012, Olney, Adamo & Ratner, 1971, Brandelero et al., 2012). In this study MSG administration caused increased Lee index in groups of obese rats and greater accumulation of retroperitoneal and perigonadal fat in these animals as well as shown in a recent study by Gaspar et al (2016) (Table 1). But there was no difference between groups OB, a fact that can be justified due to this treatment predisposing of various endocrine and behavioral abnormalities, such as changes in cardiovascular control, sexual dysfunction, growth disorders, obesity and hipogonadismo (Miranda et al., 2014, Cunha et al., 2010).Table 1 also shows the final weight of the animals, which demonstrates that the rats treated with MSG, showed a lower weight when compared to the rats in the control group. This difference can be attributed due to the side effects of the application of MSG, since among the described endocrine changes is the alteration of the secretion of the growth hormone (Miranda et al., 2014, Maiter et al., 1991). Since MSG neonatal administration in rats produces serious injuries in certain hypothalamic nuclei, with repercussions in different neuroendocrine axes (Camihort et al., 2005),it is possible to suggest that the results of hormone concentrations found in this study (Table 2) are based on this factor. It has been shown that both LH and FSH concentrations showed altered values in the groups that were subjected to induction of obesity. A study by Donhamet al.(1990) in female hamsters showed that MSG application did not affect daily secretion of LH and FSH, but adult animals become infertile. Lamperti and Baldwin (1982) demonstrated baseline FSH drop as well as LHRH (LH releasing hormone) in animals treated with MSG. Systemic endocrine imbalances produce significant impact on periodontal homeostasis.

The circulating levels of female sex hormones alter the host response to bacterial plaque and periodontal healing of the wound, and have an important role in bone growth and peak bone mass(Khosravisamani, 2014 & Compston, 2001). A reduced plasma concentrations of LH and FSH observed may hypothetically be negatively influencing the periodontal tissue of rats subjected to experimental periodontitis, although no observed changes in progesterone concentrations and estradiol (Nemeroff et al., 1981). When analyzing the bone tissue, we observed sharp alveolar bone loss in the groups with induced periodontitis, and this difference was more significant in the CONLIG group (Tables 3 and 5). Most of the studies that relate periodontal bone loss to obesity, present positive results in this association, suggesting that obesity may contribute to disease severity (Al-Zahrani, Bissada & Borawskit, 2003, Verzeletti et al., 2012, Saito et al., 2001, Gorman et al., 2012). However, there are several experimental models that are used to induce obesity, which can result in different responses. As the onset and progression of periodontal disease can be modified by biological, environmental and behavioral risk factors, and cytokines derived from adipose tissue may play a modulatory role in inflammatory processes(Suresh & Mahendra, 2014),hypothesis arises that the systemic inflammation present in the obese can affect the susceptibility to chronic infectious diseases, such as periodontitis. This fact was first proved in 1977, by Perlstein and Bissada (1977) and subsequently by Nascimento et al.(2013); Cavagni et al. (2013); Verzeletti et al. (2012); who carried out studies in rats using the coffee diet to induce obesity and showed a difference between the studied groups with a higher occurrence of periodontitis in obese rats.

Although obesity is considered a health risk factor, there is evidence in the literature about the protective action of obesity on bone tissue(Felson et al., 1993, Colaianni et al., 2014, Evans et al., 2015), demonstrating a positive correlation of increased body mass index with increased bone mineral density, considering the mechanical load exerted by overweight as a positive effect for bone formation (Colaianni et al., 2014, Lecka-Czernik et al., 2015 & Maggio et al., 2014). Although this mechanism is still not well established, one hypothesis would be that this increase in mechanical load would promote some stimuli on the skeleton, such as the reduction of apoptosis, increased osteoblast differentiation and bone matrix stimulation(Ehrlich & Lanyon, 2002). Another protective effect of obesity on bone tissue can be explained by insulin, since osteoblasts have an insulin receptor that stimulates osteogenic differentiation and inhibits osteoclastogenesis (Reid, 2008, Yano, Ohya & Amagasa, 1994) demonstrating the existence of a positive correlation between plasma insulin concentration and bone mineral density(Zhao et al., 2008). The beneficial effect of obesity on bone resorption was also confirmed by Brandeleroet al. (2012) which as in our experiment, used the hypothalamic obesity model and observed a protective effect on alveolar bone loss in rats.

Among the biologically active molecules secreted by the adipose tissue, leptin and adiponectin are the most abundant. Leptin levels have a positive correlation with the amount of adipose tissue and are therefore increased in obesity (Abooshahab et al., 2016 & Uddin et al., 2010).There have been reports pointing to leptin as being able to stimulate differentiation of bone marrow cells into osteoblasts, leading to an increase in extracellular matrix mineralization (Prouteau, Benhamou & Courteix, 2006).Turner et al. (2013) demonstrated in mice that the rate of bone formation increased substantially in animals after the subcutaneous application of leptin, indicating that it acts mainly in peripheral pathways increasing the activity and number of osteoblasts. Analyzing the cell count, (Table 5) it is known that under normal conditions osteocytes are the most abundant cells of bone tissue (Katayama, 2016) (Figure 3), and bone maturity can be verified by a larger amount of this (Kurikchy et al., 2013).Formation and bone resorption are in equilibrium at physiological normality so that the activity of the osteoclasts is followed immediately by the activity of the osteoblastos (Silva & Branco, 2011). We verified in our study that the quantity of osteocytes and osteoblasts was lower in the CONLIG group when compared to the others groups and, conversely, the number of osteoclasts was higher in this group, suggesting the occurrence of bone resorption, since osteoclasts play a central role in bone destruction(Kagiva, 2016). Thus, it is possible to state that the animals in this group were the target of greater bone destruction compared to the other groups studied. Evaluating the histometric analysis of the gingival tissue, we observed in our study the increase of periodontal inflammation in the groups that were submitted to ligature placement (CONLIG and OBLIG) as previously demonstrated (Verzeletti et al., 2012, Nascimento et al., 2013, Nassar et al., 2009, Nassar et al., 2014 & Brandelero et al., 2012) (Table 6). However, this procedure results in damage to tissues and periodontal structures, thus inducing experimental periodontitis (Nascimento et al., 2013).

It is also possible to verify that obesity induced by MSG did not cause changes in soft tissues because the groups that were submitted to experimental periodontitis presented statistically equal results. In a study by Mizutaniet al. (2014) with gingival samples from obese and lean Zucker rats demonstrated that obesity associated with insulin resistance can lead to endothelial dysfunction and gingival inflammation. When this relationship was studied in school-age youth, no relationship was found between gingivitis and obesity (Nascimento et al., 2013), as in our study.

5. Conclusion

Within the limits of animal experimentation, the results of this study suggest that hypothalamic obesity may exert a protective effect on alveolar bone loss when associated with experimental periodontitis. In addition, it may adversely affect the plasma concentrations of some female sex hormones. However, additional research is needed to provide more information to aid in elucidating the mechanisms by which periodontitis can influence obesity and the concentration of these hormones.

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