Original Research Article

Maternal obesity and periodontitis cause increased gingival epithelium and connective tissue and reduced root resorption and alveolar bone loss in female rat offspring

ABSTRACT

Aims: The aim of this study was to evaluate the periodontal tissues of female offspring of obese rat mothers that had been subjected to experimental periodontitis.

Study design: Experimental research.

Methodology: Female Wistar rats (n=20) were used; half (n=10) received monosodium glutamate injections for obesity induction (MSG group), and the other half received saline injections (CTL group). At 70 days of life, ligature-induced periodontitis was induced. At 80 days, the rats were mated and produced offspring (F1). When the offspring were 70 days of age, they were subjected to ligature.

Results: The offspring of obese mothers without periodontitis had higher body weights and higher Lee index values than the offspring of control mothers. The MSG-CL-F1-CL group had greater gingival inflammation and lower alveolar bone loss than the CTL-CL-F1-CL, MSG-SL-F1-CL and CTL-CL-F1-CL groups. The female offspring of obese mothers showed increased numbers of osteocytes and osteoblasts and smaller root resorption areas than offspring of control mothers.

Conclusion: In conclusion, maternal obesity and periodontitis cause greater adiposity, increased gingival epithelium and connective tissue, increased numbers of alveolar bone cells and reduced root resorption and alveolar bone loss in female offspring.

Key words: Hypothalamic obesity; periodontal disease; fetal programming; metabolic programming; DOHaD

1. INTRODUCTION

Fetal or metabolic programming has been demonstrated in several studies, and it has been proposed that the intrauterine environment or the environment during infancy modulates physiological control and homeostasis and that this can result in increased susceptibility to chronic diseases throughout life [1,2]. In this way, maternal nutrition and lifestyle can influence the health of offspring in adulthood [3,4,5]. Epigenetic mechanisms linked to this association underlie developmental programming in the context of the Developmental Origins of Health and Disease (DOHaD), a term that has been adopted to indicate a broader perspective of the period of "origin" that goes beyond the prenatal period and encompasses the entire period of development [6,7].Research involving DOHaD aims to define the epigenetic mechanisms that are involved in the transfer of traits programmed during development for subsequent generations and to reveal the functions of various regulatory systems, organs and tissues in this programming [8].Thus, there is a strong association between maternal obesity and changes in fetal growth and development, which are reflected throughout the individual's life [9].

Epigenetic changes resulting from maternal obesity can predispose children to develop metabolic disease in adulthood and can result in transmission of the effects of this adverse environmental exposure to subsequent generations, significantly contributing to the global epidemic of metabolic diseases [10]. Among metabolic diseases, obesity has been increasing at alarming rates in adults and children around the world; its rapid growth and mortality risk make it a public health problem that increasingly engages the population's awareness [9,11]. Obesity results from the interaction of genetic and environmental factors such as sedentary lifestyle, excessive caloric intake, endocrine disruptors and the intrauterine environment [12].Approximately 10-15% of the world's adult population is obese, and this percentage has doubled since 1980, and the World Health Organization (WHO) estimates suggest that one in five adults will be obese in 2025 [12,13].

Obesity is a chronic metabolic condition and a risk factor for many diseases, and its inflammatory nature is widely recognized [14,15]. Adipose tissue affects the release of several inflammatory factors, including tumor necrosis factor alpha (TNF-α), which is found in high concentrations in the blood of obese individuals [16,17].Obesity is also a risk factor for hypertension, dyslipidemia, coronary heart disease, and stroke, in addition to being associated with periodontitis [18].

In periodontitis, an inflammatory process occurs in response to bacterial antigens present in biofilm. The host's response to these antigens is mediated mainly by neutrophils, monocytes/macrophages and T and B lymphocytes and results in the production of mediators, including cytokines, chemokines, proteolytic enzymes, interleukin-1 beta (IL-1β), interleukin-6 (IL-6) and TNF-α, that promote tissue degradation and bone resorption [19]. In individuals with advanced periodontitis, there is irreversible loss of periodontal ligament and bone and a progressive increase in pocket formation [20].

Obesity and periodontitis may be associated, as being overweight makes an individual more susceptible to periodontitis due to the presence of higher levels of inflammatory mediators [21].Adipocytes and macrophages present in adipose tissue produce inflammatory cytokines that promote the development and progression of periodontitis in a process that is related to changes in the immune response caused by obesity [22]. In addition, Suvan et al.[23] suggest that obese individuals have exacerbated local inflammation and possibly altered periodontal microflora.

Studies suggest that exposure of a fetus in the intrauterine environment to pathological conditions such as maternal obesity and periodontitis influences the health of the offspring in adulthood. Thus, the aim of this study was to investigate whether maternal obesity associated with periodontitis affects the periodontal tissue structure of female offspring.

2. METHODOLOGY

2.1 Sample calculation

A sample size of 20 rat mother (n=5/group) and 48 of their female offspring (n=6/group) was calculated using GPower 3.1 software [24,25] to yield an α of 5% and a power of 80% when the variables obesity and periodontal disease were considered.

2.2 Animals

Twenty female Wistar rats were used. During the first five days of life, ten of the animals received subcutaneous injections in the cervical region of 4 g/kg/day monosodium glutamate to induce the development of obesity (MSG group, n=10). The other 10 pups received injections of hyperosmotic saline solution (1.25 g/kg/day) (CTL group, n=10). The animals were adapted and maintained at the Bioterium of the Center for Biological and Health Sciences – CCBS, State University of Western Paraná, UNIOESTE, Cascavel, State of Paraná, Brazil. They were adapted and kept individually or in pairs in polyethylene cages (43x30x15) under controlled conditions of temperature (22° and 25° C), relative humidity (close to 55%) and 12-hour photoperiod (light period 7:00~19:00 h). The animals had access to chow and water ad libitum. The experimental procedures were conducted in accordance with the Ethical Principles in Animal Experimentation adopted by the Brazilian College of Animal Experimentation (COBEA) and were approved by the Ethics Committee on Animal Use (CEUA) of UNIOESTE (Protocol 0812/2017).

2.3 Induction of periodontal disease

When the animals were 70 days of age, experimental periodontal disease was induced in 5 animals from each group. For this, the animals were anesthetized by intraperitoneal injection of ketamine (75 mg/kg) and xylazine (15 mg/kg) and placed on an appropriate operating table with their mouths kept open to facilitate access to the posterior teeth. Using modified forceps and an explorer probe, a 40-cotton thread was placed around the lower right and left first molars. This ligature acted as a gingival irritant and favored the accumulation of bacterial plaque [26]. Four experimental groups (N=5/group) were established: control with ligature (CTL-CL), control without ligature (CTL-SL), MSG with ligature (MSG-CL) and MSG without ligature (MSG-SL).

Ten days after the induction of periodontal disease, the female rats were housed with nonobese control rats in the proportion of one male to two females. In the morning following mating, vaginal washings were collected, and the presence of sperm was used as an indicator of pregnancy.

2.4 Evaluation of maternal obesity and alveolar bone loss

After weaning of the offspring, the mothers were weighed and euthanized by decapitation. To confirm the presence of maternal obesity, the Lee index [the cubic root of body weight (g)/nasal-anal length (cm)] was calculated, and the animals, perigonadal and retroperitoneal fat deposits were measured.

To evaluate the success of the induction of experimental periodontitis, the left hemimandibles of the animals were removed and radiographed by a single trained examiner using a KaVo intraoral X-ray device (FOCUS, 70 kV/60 mA) with a periapical phosphor plate, an exposure time of 0.125 s and a focal length of 5 cm. The plate reading was performed by the developer of the brand KaVo – Express, and the images obtained were analyzed using Image-Pro Plus 6.0 software (Media Cybernetics, Rockville, MD, USA). A mean was taken through a linear measurement of the distance from the cementoenamel junction to the alveolar bone crest on the mesial side of the first molar [26,27].

2.5 Experiments with female offspring (first generation, F1) of CTL and MSG mothers with and without periodontal disease

At 21 days of age, the female offspring obtained from the cross (first generation, F1) were designated according to the treatment of the mothers (N=12/group): 1) CTL-CL-F1, 2) CTL-SL-F1, 3) MSG-CL-F1 and 4) MSG-SL-F1.

At 70 days of life, 6 animals from each group of female offspring were subjected to ligature-induced periodontitis for 30 days. In this way, eight experimental groups were formed (N= 6/group): (G1) CTL-CL-F1-CL, (G2) CTL-CL-F1-SL, (G3) CTL-SL-F1-CL, (G4) CTL-SL-F1-SL, (G5) MSG-CL-F1-CL, (G6) MSG-CL-F1-SL, (G7) MSG-SL-F1-CL, and (G8) MSG-SL F1-SL [26,27].

2.6 Collection of biological material

At the end of the experimental period (100 days), all animals were weighed and euthanized by decapitation. Their mandibles was removed, sectioned sagittally and separated into right and left hemimandibles. These were fixed in 10% buffered formalin for 24 hours, washed in running water for 48 hours and stored in 70% alcohol.

2.7 Radiographic evaluation of alveolar bone loss in the offspring

The left hemimandibles were radiographed by a single trained examiner on two alternate days using an intraoral X-ray device (KaVo – FOCUS, 70 kV/60 mA) with a periapical-size phosphor plate, an exposure time of 0.125 seconds and a focal length of 5 cm. The plate reading was performed by the developer of the brand KaVo – Express. Measurements of alveolar bone loss followed the same criteria as those used in the assessment of maternal alveolar bone loss [26,27].

2.8 Histological processing and histomorphometric analysis

The right hemimandibles were decalcified in an acid solution (Allkimia®) for 19 hours and stored in 70° alcohol. The samples were then dehydrated in increasing concentrations of alcohol, cleared in xylene and embedded in Paraplast. For histomorphometric analysis, 5-µm-thick coronal sections were made from mesial to distal using a manual rotary microtome (Olympus 4060) equipped with a steel blade. The sections were deparaffinized with xylol, hydrated with distilled water and stained with hematoxylin-eosin (HE) for analysis. The sections were photographed at 100x magnification in an Olympus BX60 microscope equipped with an Olympus DP71 digital camera and DP Controller 3.2.1.276 software and analyzed using Image Pro-Plus 4.1.

For histological quantification of alveolar bone loss, the shortest distance between the apex of the buccal alveolar bone crest and the cementoenamel junction of the lower right first molar was measured. The measurements were repeated twice a day on three different days, and the mean of the obtained values was calculated [26,27].

Analyses of the area occupied by the gingival epithelium and underlying connective tissue were performed as described in Steffens *et al*.[28], four measurements were taken: the

height and width of the gingival epithelium and the height and width of the connective tissue. The areas were calculated by multiplying the height and width of the respective regions.

2.9 Bone cell counts

Osteoblasts, osteocytes and osteoclasts were counted in five consecutive microscopic fields of the buccal alveolar bone crest starting from the highest point of the crest. An Olympus microscope (400X magnification) was used in the counting. Two observations were made per field, and the values for each animal and each group were averaged.

2.10 Histomorphometric analysis of external root resorption

For quantitative analysis of external root resorption, photomicrographs were taken at 400X magnification and analyzed using Image-Pro Plus 6.0 (Media Cybernetics, Rockville, MD, USA); the total area of each resorption was quantified in square micrometers (μm^2).

Each measurement was performed three times, and the means of the three obtained values were calculated. When more than one area of external root resorption was present in the root, the areas were added to obtain the total resorption area per animal.

2.11 Statistical analysis

For data analysis, analysis of variance (one-way ANOVA) followed by Tukey's posttest was applied. Differences were considered statistically significant when $P<.05$. Statistical analyses and graphs were prepared using SigmaPlot 12.0 (Systat Software Inc., San Jose, CA, USA).

3. RESULTS

3.1 Evaluation of maternal obesity and alveolar bone loss

The analysis of maternal body parameters showed that body weight and snout-anal length were reduced in the mothers in the MSG-SL and MSG-CL groups compared to the mothers in the control groups. However, the MSG mothers showed increased Lee index values and increased retroperitoneal and perigonadal fat pad volume compared to animals in the control groups ($P = .05$), demonstrating the onset of obesity resulting from neonatal treatment

with monosodium glutamate. The intragroup comparison showed that the presence of periodontal disease did not affect the number of fat deposits (Table 1).

The radiographic analysis of the alveolar bone of the mothers showed that there was greater alveolar bone loss in the groups with ligature-induced periodontitis (the CTL-CL and MSG-CL groups) (*P*=.05) and that alveolar bone loss was greater in the CTL-CL mothers (*P*=.05) (Table 1).

Table 1. Body parameters and radiographic evaluation of mandibular alveolar bone loss in mothers in different experimental groups after weaning.

PARAMETERS	MOTHERS CTL-SL	MOTHERS CTL-CL	MOTHERS MSG-SL	MOTHERS MSG-CL
Body Weight (g)	247.25±5.1[a]	256.67±4.7[a]	204.62±5.1[b]	210.39±6.1[b]
Nasal-anal Length (cm)	19.3±0.2[a]	19.1±0.3[a]	17.1±0.2[b]	17.4±0.3[b]
Lee Index	325.20±3.1[a]	332.73±4.1[a]	349.5±6.2[b]	352.7±4.9[b]
Retroperitoneal Fat (g/100 g BW)	0.7±0.07[a]	0.7±0.06[a]	1.3±0.3[b]	1.2±0.7[b]
Perigonadal Fat (g/100 g BW)	1.13±0.3[a]	1.22±0.2[a]	2.2±0.3[b]	2.3±0.4[b]
Distance CEJ-ABC (pixels)	82.56±3.19[a]	155.07±8.08[b]	75.59±2.51[a]	131.73±6.28[c]

Values are expressed as the mean ± SEM. SL= without ligature. CL= with ligature. CEJ=cement-enamel junction. ABC= alveolar bone crest. N= 5 animals/group. Analysis of variance (one-way ANOVA) followed by Tukey's test.Similarly, values followed by different letters (a, b, and c) indicate significant differences among the groups (*P*< .05).

3.2 Body parameters of the offspring

In the intragroup analysis, offspring from control mothers with periodontitis (CTL-CL-F1-CL, CTL-CL-F1-SL) showed significantly increased body weight and Lee index values compared to offspring from mothers in the CTL-SL-F1-SL group (*P* = .05); among the offspring of obese mothers, those not subjected to ligature (MSG-CL-F1-SL and MSG-SL-F1-SL) showed greater body weight gain than offspring that were subjected to ligature (MSG-SL-F1-CL) (*P* = .05). In the intergroup analysis, the offspring of obese mothers without periodontal disease (MSG-SL-F1-SL) had higher body weights and Lee index values than the offspring of control mothers without periodontal disease (CTL-SL-F1-SL) (*P* = .05) (Table 2), showing the effect of maternal obesity on the body weight of the offspring.

In the analysis of fat deposits, the CTL-CL-F1-CL, CTL-CL-F1-SL and CTL-SL-F1-CL groups showed larger retroperitoneal and perigonadal fat deposits than the group without

periodontal disease (CTL-SL-F1-SL) (*P* = .05). Comparing all groups, we found that the offspring in the CTL-CL-F1-CL, CTL-CL-F1-SL and CTL-SL-F1-CL groups had larger fat deposits than the offspring of obese mothers subjected to the same experimental conditions (MSG-CL-F1-CL, MSG-CL-F1-SL and MSG-SL-F1-CL) (*P* = .05) (Table 2).

Table 2. Biometric parameters of the female offspring in different experimental groups.

PARAMETER	G1 CTL-CL-F1-CL	G2 CTL-CL-F1-SL	G3 CTL-SL-F1-CL	G4 CTL-SL-F1-SL	G5 MSG-CL-F1-CL	G6 MSG-CL-F1-SL	G7 MSG-SL-F1-CL	G8 MSG-SL-F1-SL
Body Weight (g)	250.66 ±2.26^{a}	251.33 ±2.38^{a}	241.66 ±3.10^{ab}	234.50 ±2.31^{b}	244.33 ±2.55^{ab}	254.66 ±3.68^{ac}	236.66 ±3.86^{b}	253.83 ±3.40^{ac}
Nasal-anal Length (cm)	20.66 ±0.09^{a}	20.50 ±0.09^{a}	20.71 ±0.18^{a}	20.75 ±0.11^{a}	20.11 ±0.17^{a}	20.66 ±0.21^{a}	20.16 ±0.19^{a}	20.31 ±0.11^{a}
Lee Index	305.11 ±1.56^{a}	307.88 ±0.98^{a}	300.75 ±1.55^{ab}	295.73 ±1.90^{b}	311.03 ±2.57^{ac}	307.91 ±1.67^{a}	306.67 ±1.04^{a}	311.63 ±2.09^{ac}
Retroperitoneal Fat (g/100 g BW)	0.53 ±0.02^{a}	0.54 ±0.04^{a}	0.42 ±0.02^{b}	0.42 ±0.02^{b}	0.43 ±0.03^{b}	0.42 ±0.04^{b}	0.48 ±0.03^{a}	0.44 ±0.01^{b}
Perigonadal Fat (g/100 g BW)	0.55 ±0.04^{a}	0.52 ±0.02^{a}	0.49 ±0.02^{a}	0.35 ±0.02^{b}	0.46 ±0.02^{ac}	0.41 ±0.02^{bc}	0.37 ±0.02^{b}	0.43 ±0.01^{bc}

Values are expressed as the mean ± SEM. F1= Female offspring. N= 6 animals/group. Analysis of variance (one-way ANOVA) followed by Tukey's test.Similarly, values followed by different letters (a, b, and c) indicate significant differences among the groups (*P* = .05).

3.3 Histological analysis of the hemimandible

The histological evaluation of the offspring without periodontitis (those in the CTL-CL-F1-SL, CTL-SL-F1-SL, MSG-CL-F1-SL and MSG-SL-F1-SL groups) revealed that the oral, junctional and sulcular epithelia and the underlying connective tissue had normal morphological characteristics (Figure 1 A, B); the offspring of mothers with periodontitis had thicker gingival epithelium and joint tissues but were within normal limits. The bone crests were thick and regular in shape at the level of the cervical third of the root, and the central spongy portion of the alveolar bone presented a normal aspect (Figure 1B). In the region of the alveolar bone crest, osteoblasts formed a lining in the bone periphery, and osteocytes remained present in the central region of the bone (Figure 1C).

In the morphological analysis of the periodontium of the offspring with periodontal disease, (those in the CTL-CL-F1-CL, CTL-SL-F1-CL, MSG-CL-F1-CL and MSG-SL-F1-CL groups), inflammation and abnormal morphological characteristics of the oral, junctional and sulcular epithelia were observed (Figure 1 D, E), and inflammatory infiltrate was present in the underlying connective tissue (Figure 1F). More pronounced inflammatory conditions were present in the offspring of mothers with periodontal disease or obesity.

The bone crest showed an irregular shape, and alveolar bone loss led to exposure of the cervical third of the tooth, especially in the control animals (Figure 1D). Incremental lines were present in the bone images of all groups (Figure 1 C, G). The presence of osteoclasts was noted, indicating resorption activity (Figure 1G).

All groups presented external root resorption, and there was a higher incidence of such absorption in the animals in the CTL groups with ligatures (Figure 1H).

FIGURE 1.

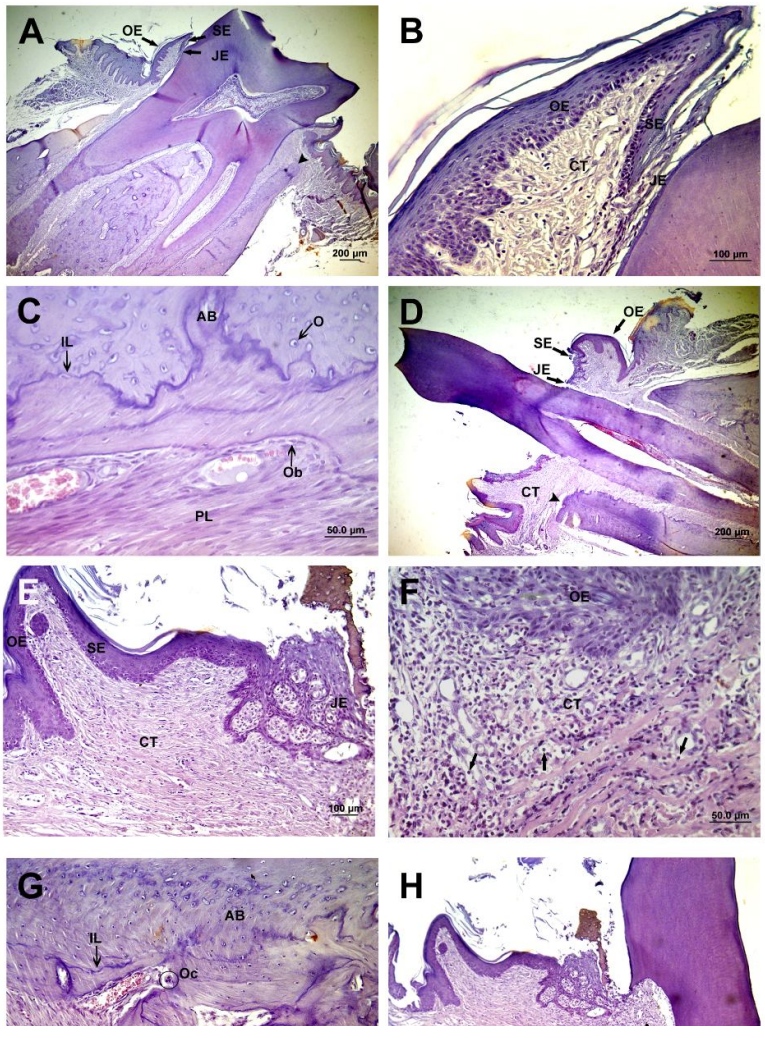

Figure 1. Photomicrograph of periodontal tissues of animals in the different experimental groups. A-B: Periodontal tissue with normal appearance. Oral epithelium (OE), sulcular epithelium (SE), junctional epithelium (JE), connective tissue (CT), alveolar bone crest (arrowhead). C: Bone cells from the alveolar bone crest under normal conditions. Osteoblasts (Ob), osteocytes (O), alveolar bone (AB), periodontal ligament (PL), incremental lines (IL). D-E: Periodontal tissues with inflammatory aspects. Oral epithelium (OE), sulcular epithelium (SE), junctional epithelium (JE), connective tissue (CT), alveolar bone crest (arrowhead). F: Underlying connective tissue with inflammatory process. Oral epithelium (OE), connective tissue (CT), presence of lymphocytes (arrows). G: Bone resorption areas. Osteoclasts (Oc), alveolar bone (AB), periodontal ligament (PL), incremental lines (IL). H: External root resorption (asterisk). Color = hematoxylin and eosin.

3.4 Histomorphometry of gingival tissue and alveolar bone loss

In the morphometric analysis of the periodontal tissues of the female offspring of the animals in the control groups, the CTL-CL-F1-CL group presented larger areas of gingival epithelium and connective tissue than did the CTL-SL-F1-CL group (P = .05), showing the effect of maternal periodontal disease on the female offspring. A similar effect was observed in the comparison between the offspring of obese mothers with and without periodontitis (MSG-CL-F1-CL and MSG-CL-F1-SL); these animals presented larger areas of gingival and connective epithelia than did the offspring of mothers without periodontitis (MSG-SL-F1-CL and MSG-SL-F1-SL) (P = .05). In addition, in comparing the MSG-CL-F1-CL group with the CTL-CL-F1-CL

group, we found that maternal obesity potentiated the inflammatory process in the female offspring's protective periodontium, resulting in a larger area of gingival epithelium and conjunctive tissue (P = .05) (Table 3).

The morphometric and radiographic analysis of the alveolar bone of the female offspring showed that there was greater alveolar bone loss (P = .05) in the CTL and MSG groups subjected to ligature-induced periodontitis. However, the offspring in the MSG-CL-F1-CL group had lower alveolar bone loss than the animals in the MSG-SL-F1-CL and CTL-CL-F1-CL groups, demonstrating a protective effect of maternal obesity and/or periodontitis on the alveolar bone of the offspring (P = .05) (Table 3).

Table 3. Gingival tissue area and histomorphometric and radiographic evaluation of mandibular alveolar bone loss in the female offspring in different experimental groups.

PARAMETER	G1 CTL-CL-F1-CL	G2 CTL-CL-F1-SL	G3 CTL-SL-F1-CL	G4 CTL-SL-F1-SL	G5 MSG-CL-F1-CL	G6 MSG-CL-F1-SL	G7 MSG-SL-F1-CL	G8 MSG-SL-F1-SL
Gingival epithelium area (μm^2)	8587.64 ±368.61[a]	3379.55 ±282.02[b]	5442.38 ±120.47[c]	2823.00 ±171.46[b]	12758.29 ±1013.73[d]	4176.95 ±627.73[c]	7207.20 ±340.43[a]	2457.10 ±263.18[b]
Connective tissue area (μm^2)	466337.00 ±26939.68[a]	193445.50 ±12258.81[b]	504452.50 ±30692.65[ac]	237982.69 ±22629.00[b]	580878.96 ±36555.87[c]	262309.08 ±14539.27[b]	462904.15 ±27740.85[a]	243534.89 ±6696.94[b]
Distance CEJ-ABC (µm)	1243.90 ±42.79[a]	674.91 ±14.85[b]	1258.22 ±49.77[a]	514.94 ±13.29[b]	1064.42 ±46.62[c]	652.24 ±13.08[b]	1379.00 ±61.09[a]	611.17 ±9.14[b]
Distance CEJ-ABC (pixels)	113.93 ±9.04[a]	69.84 ±2.60[b]	125.80 ±7.74[a]	66.12 ±6.48[b]	121.24 ±8.64[a]	75.88 ±5.05[b]	130.52 ±4.08[a]	61.44 ±7.08[b]

Values are expressed as the mean ± SEM. F1= Female offspring. N= 6 animals/group. Analysis of variance (one-way ANOVA) followed by Tukey's test.Similarly, values followed by different letters (a, b, c,and d) indicate significant differences among the groups (P = .05).

3.5 Bone cell counts

The animals in the CTL-SL-F1-SL group showed significantly increased numbers of osteocytes compared to the CTL-CL-F1-CL and CTL-CL-F1-SL animals. In the quantification of osteoblasts, the CTL-CL-F1-CL group showed a significant increase compared to the other CTL groups (P = .05). In the MSG groups, offspring with periodontal disease (those in the MSG-CL-F1-CL and MSG-SL-F1-CL groups) had higher numbers of osteocytes and osteoblasts than the offspring in the MSG-CL-F1-SL and MSG-SL-F1-SL (P = .05). In the intergroup comparison, we found that the obese groups (MSG-CL-F1-CL, MSG-CL-F1-SL and MSG-SL-F1-CL) presented significantly increased numbers of osteocytes and osteoblasts compared to the CTL-CL-F1-CL,

CTL-CL-F1-SL and CTL-SL-F1-CL groups (*P* = .05) (Table 4), showing the influence of maternal obesity.

In the osteoclast analysis, the offspring with periodontitis, regardless of the groups (CTLs or MSGs), had a greater number of clastic cells than did the offspring without periodontal disease (*P* = .05) (Table 4).

Table 4. Quantification of alveolar crest bone cells in the female offspring in different experimental groups.

PARAMETER	G1 CTL-CL-F1-CL	G2 CTL-CL-F1-SL	G3 CTL-SL-F1-CL	G4 CTL-SL-F1-SL	G5 MSG-CL-F1-CL	G6 MSG-CL-F1-SL	G7 MSG-SL-F1-CL	G8 MSG-SL-F1-SL
Osteocytes (n°)	377.50 ±7.77[a]	371.50 ±9.76[a]	387.20 ±5.61[ab]	406.16 ±5.75[bc]	407.20 ±6.09[bc]	384.25 ±6.57[ab]	425.50 ±7.69[cd]	404.20 ±5.63[bc]
Osteoblasts (n°)	108.00 ±3.41a	80.40 ±3.03b	82.00 ±2.27b	76.16 ±2.53b	113.60 ±2.04ac	103.50 ±1.95ac	115.25 ±5.79ac	97.60 ±4.86acd
Osteoclasts (n°)	2.00 ±0.24[a]	0.60 ±0.13[b]	1.20 ±0.10[c]	0.33 ±0.14[b]	2.00 ±0.16[a]	0.25 ±0.13[b]	2.25 ±0.32[a]	0.60 ±0.13[b]

Values are expressed as the mean ± SEM. F1= Female offspring. N= 6 animals/group. Analysis of variance (one-way ANOVA) followed by Tukey's test.Similarly, values followed by different letters (a, b, c, and d) indicate significant differences among the groups (*P* = .05).

3.6 Histomorphometric analysis of root resorption

Among the animals in the CTL groups that were subjected to experimental periodontitis (CTL-CL-F1-CL and CTL-SL-F1-CL), a greater amount of root resorption was observed than was seen in the CTL groups not subjected to ligature (*P*<0.05). Among the offspring of obese mothers, larger areas of root resorption were also found in animals with periodontitis; however, comparison of the offspring of MSG mothers with those of CTL mothers, regardless of the presence of a ligature in the offspring, showed that the offspring of obese mothers had smaller areas of root resorption (*P*<0.05) (Table 5).

Table 5. Quantification of root resorption area in the female offspring in different experimental groups.

PARAMETER	G1 CTL-CL-F1-CL	G2 CTL-CL-F1-SL	G3 CTL-SL-F1-CL	G4 CTL-SL-F1-SL	G5 MSG-CL-F1-CL	G6 MSG-CL-F1-SL	G7 MSG-SL-F1-CL	G8 MSG-SL-F1-SL
Root resorption (μm^2)	18736.69 ±6582.48[a]	1777.61 ±107.93[b]	15924.27 ±1997.34[a]	2064.18 ±166.52[b]	15169.66 ±2124.58[a]	789.56 ±125.20[c]	4681.58 ±213.20[d]	392.87 ±35.22[c]

Values are expressed as the mean ± SEM. F1= Female offspring. N= 6 animals/group. Analysis of variance (one-way ANOVA) was performed followed by Tukey's test.Similarly, values followed by different letters (a, b, c, and d) indicate significant differences among the groups (P = .05).

4. DISCUSSION

Maternal obesity has become a global epidemic, and this has aroused interest in understanding the relationship between the effects of maternal obesity on fetal programming and future obesity [29]. Analysis of the maternal body parameters of the rats in our study showed that body weight and snout-anal length were reduced in mothers in the MSG-SL and MSG-CL groups compared to the mothers in the control groups. Neonatal treatment with monosodium glutamate is known to damage the hypothalamus, compromising the production of growth hormone-releasing hormone (GHRH), reducing growth hormone (GH) levels, and resulting in growth retardation and inhibition of muscle mass gain. In addition, MSG-induced obesity generates changes in the central cholinergic pathways, located mainly in the hypothalamus; these changes result in decreased food intake, reduced body mass and increased peripheral fat pad volume, as occurred in our animals, and demonstrate the role of acetylcholine (ACh) in the regulation of food intake and energy expenditure [30,31,32].

Although rats with MSG-induced obesity usually present normal or decreased food intake, it is possible to observe the presence of excess adipose tissue, and this was confirmed in our study through the analysis of MSG mothers, which were found to have increased Lee index values and increased retroperitoneal and perigonadal fat pad volumes. The hypothalamic–pituitary–adrenal (HPA) axis and the autonomic nervous system (ANS) play important roles in obesity and metabolic function. Obese rats treated with monosodium

glutamate exhibit an altered HPA axis and ANS changes, with high parasympathetic activity, hyperinsulinemia and vagal hyperactivity, resulting in visceral fat accumulation [31,32,33,34].

Regarding body parameters, the offspring of control mothers with periodontitis showed significantly increased body weights and Lee index values compared to the offspring of control mothers without periodontal disease, even when obesity was not induced in the offspring. Periodontitis is characterized by a low-grade inflammatory state, and the associated systemic inflammation can promote insulin resistance through inhibition of insulin receptor signaling. Consequently, the body attempts to compensate for this resistance by increasing insulin secretion, causing hyperinsulinemia and resulting in fat storage and obesity [35,36]. Moreover, ghrelin, a hormone found at high levels in individuals with periodontitis, is related to increased appetite [36,37].

An effect of maternal obesity on the body weight of the offspring was noted in our study: the offspring of obese mothers without periodontitis had higher body weights and Lee index values than the offspring of control mothers without periodontal disease. The central nervous system and the cardiometabolic system are the systems that appear to be most vulnerable to fetal programming during critical periods of development, and the presence of an unfavorable nutritional and/or hormonal environment can cause permanent changes in these systems. The molecular mechanisms involved in this highlight the sensitivity of the offspring's epigenome to maternal obesity. The great plasticity and ability to respond to environmental factors such as nutrients, oxygen, and hormones is a characteristic of fetal life that can alter gene expression levels through epigenetic modifications [29,38,39]. A previous study by Wentzel et al.[40] showed that metabolic disturbances in pregnant and obese rats can have profound adverse effects on the offspring, as was observed in our study.

The influence of maternal periodontitis on the offspring was demonstrated in our comparison of the CTL-CL-F1-CL group with the CTL-SL-F1-CL group and the comparison of the MSG-CL-F1-CL and MSG-CL-F1-SL groups with the MSG-SL-F1-CL and MSG-SL-F1-SL groups; the offspring of mothers with periodontitis had larger areas of gingival epithelium and connective tissue. Variations in the clinical expression of periodontitis are known to be associated with genetic susceptibility factors [41,42,43]. The clinical expression of gingival inflammation in response to bacterial biofilm can be substantially modified by systemic factors,

whether inherent to the host or related to environmental influences. Epigenetic changes in the gingiva arising from the interaction of the bacterium *Porphyromonasgingivalis* with epithelial cells alter Toll-like receptors (TLRs), resulting in deficiencies in expression of and signaling by these receptors and reducing the resistance of the host to pathogens, leading to bacterial persistence and inflammation [44,45].

Furthermore, comparison of the MSG-CL-F1-CL group with the CTL-CL-F1-CL group showed that maternal obesity potentiated the inflammatory process in the protective periodontium of the female offspring, resulting in larger areas of gingival epithelium and connective tissue. These results again demonstrate fetal programming and explain the finding that systemic inflammation associated with obesity increases periodontal inflammation and the destructive processes caused by oral microorganisms. Adipose tissue releases pro-inflammatory cytokines and hormones known as adipocytokines that induce inflammatory processes and oxidative stress disorders, generating similar pathophysiology in the two diseases. Obesity can paralyze the innate immune response of the periodontium by attenuating macrophage infiltration and activation and thus aggravate periodontitis [36,46].

Our analysis of bone loss showed that there was less alveolar bone loss in the offspring in the MSG-CL-F1-CL group than in the offspring in the MSG-SL-F1-CL and CTL-CL-F1-CL groups, providing evidence for a factor that protects the offspring's alveolar bone against the effects of obesity and/or maternal periodontitis. The bones of obese patients are subjected to greater mechanical load; this can stimulate the skeletal system and result in reduced apoptosis, increased osteoblast differentiation and stimulation of the bone matrix. Furthermore, higher bone mineral density (BMD) in obese individuals is directly related to increased circulating insulin concentrations, since osteoblasts possess an insulin receptor that stimulates osteogenic differentiation and inhibits osteoclastogenesis, resulting in less alveolar bone loss. Leptin, a hormone secreted mainly by adipose tissue, also plays a protective role in bone tissue by increasing osteogenic activity and the levels of physiological antiresorptive factors [47,48,49].

The female offspring in the MSG-CL-F1-CL, MSG-CL-F1-SL and MSG-SL-F1-CL groups showed a significant increase in the numbers of osteocytes and osteoblasts compared to the offspring in the CTL-CL-F1-CL, CTL-CL-F1-SL and CTL-SL-F1-CL groups, showing the influence of maternal obesity. Among the biologically active molecules secreted by adipose

tissue, leptin and adiponectin are the most abundant, and it has been reported that leptin stimulates the differentiation of bone marrow cells into osteoblasts [49].

Comparison of the offspring of MSG mothers to those of CTL mothers showed that the offspring of obese mothers had smaller areas of root resorption, regardless of the presence of a ligature in the offspring. Odontoclasts are the cells responsible for the resorption of dental hard tissues and are morphologically and functionally similar to osteoclasts. The receptor activator of nuclear factor-kappa β ligand (RANKL), known to be a member of the tumor necrosis factor family, stimulates osteoclast activation, and RANKL expression has also been detected in odontoclasts located in root dentin undergoing resorption, suggesting a common regulatory mechanism for the cellular reabsorption of mineralized tissues such as bones and teeth. Based on this, it is possible to attribute a similar protective effect of MSG obesity on bone mass in the case of root resorption, since high plasma levels of insulin in obese individuals are associated with inhibition of osteoclastogenesis, and leptin secreted by adipose tissue is a physiological antiresorptive factor [47,50,51].

5. CONCLUSION

In conclusion, maternal obesity and periodontitis cause greater adiposity, increase the amount of gingival epithelium and connective tissue and the number of alveolar bone cells and reduce root resorption and alveolar bone loss in female offspring.

CONSENT

It is not applicable.

ETHICAL APPROVAL

This study was in accordance with the Ethical Principles in Animal Experimentation adopted by the Brazilian College of Animal Experimentation (COBEA) and were approved by the Committee on Ethics in the Use of Animals (CEUA) of UNIOESTE.

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