

# Effect of obesity on alveolar bone loss in experimental periodontitis in Wistar rats

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## ABSTRACT

**O**besity has been linked to higher inflammatory status and periodontal breakdown. **Objective:** The purpose of this study was to investigate the effect of obesity on alveolar bone loss in experimental periodontitis in rats. **Material and Methods:** Twenty-four female Wistar rats were randomly divided into two groups: obese (n=13), which were fed with "cafeteria diet" (CAF diet - high amounts of sucrose and fat) for 90 days in order to gain weight, and non-obese (n=11) regularly fed rats. Ligature-induced experimental periodontitis was created in all animals. Body weight differed statistically between obese and non-obese groups (277.59 and 223.35 g, respectively) at the moment of the ligature placement. Morphometric registration of alveolar bone loss was carried out after 30 days of ligature placement to determine the effect of obesity on the progression of experimental periodontitis. **Results:** Intra-group comparisons showed significantly higher alveolar bone loss mean values in maxillary teeth with ligature ( $P<0.05$ ). Alveolar bone loss [mean (SD), mm] was not statistically different between obese and non-obese groups [0.71 (0.09) and 0.65 (0.07) mm, respectively]. However, when palatal sides are analyzed separately, obese group presented significantly higher alveolar bone loss ( $P<0.05$ ) as compared to non-obese [0.68 (0.12) and 0.53 (0.13) mm, respectively]. **Conclusions:** In spite of the weak differences, it is possible to conclude that the progression of alveolar bone loss in ligature-induced periodontitis can be potentially influenced by body weight in rats.

**Key words:** Body weight. Inflammation. Periodontal diseases.

## INTRODUCTION

The prevalence of obesity has substantially increased worldwide in the last decades<sup>8</sup>. It can cause or exacerbate different health problems, both independently and in association with other chronic diseases<sup>13</sup>. There are substantial evidences concerning the association between increased adipose tissue and high blood pressure, cardiovascular disease, diabetes mellitus and other illnesses<sup>11</sup>. Consequently, obesity has been recognized as a major public health problem.

It has been demonstrated that adipose tissue is able to secrete more than 50 bioactive molecules in the organism<sup>23</sup>. These numerous immunomodulatory factors can affect metabolic and vascular biology,

which may lead to decreased host response and increased systemic inflammation. Systematically, cross-sectional and case-control studies have found association between periodontal disease and obesity in different populations<sup>1,2,4,6,15</sup>. Nevertheless, the biological mechanisms by which obesity may affect the periodontium have not yet been determined. The existent evidence on biological plausibility is indirect.

Recently, our research group published a study assessing the progression of alveolar bone loss in rats with overweight and normal weight, using a ligature-induced periodontal disease model in Wistar rats<sup>19</sup>. The results demonstrated that overweight rats did not present higher alveolar bone loss as compared to controls. However, obesity

*per se* has not been yet established unequivocally as a possible modulating factor for periodontal destruction. Our hypothesis is that obese rats might develop higher amounts of alveolar bone loss. The aim of the present study was to compare alveolar bone loss in obese and non-obese female Wistar rats submitted to a ligature-induced experimental periodontitis protocol.

## MATERIAL AND METHODS

### Animals

Twenty-four 2-month-old female Wistar rats were used. The animals were bred and housed in standard plastic cages as described previously to ensure periodontal disease-free animals at baseline<sup>3</sup>. These conditions included wire mesh floor bedding, a finely milled diet (Supralab, Supra, São Leopoldo, RS, Brazil) and tap water *ad libitum*. A 12 h light and dark cycle was applied (light on at 08:00 h). Four to five rats were housed in each cage at a constant temperature (20°C).

### Experimental groups

The animals were randomly assigned at baseline, by means of draw stratified by body weight, into two groups as follows: Obese (n=13), which received standard feeding (Supralab, Supra, São Leopoldo, RS, Brazil) and a complementary calorie-rich diet ["cafeteria diet" (CAF diet), consisting of chocolate cookies, sugar-rich milk and fat cheese]<sup>5,19,22</sup>; Non-obese (n=11), which received standard feeding (Supralab, Supra, São Leopoldo, RS, Brazil).

### Sample size calculations

Sample size calculation was performed using data from our previous study<sup>19</sup>. Taking into consideration a mean difference in alveolar bone loss of 0.2 mm, accepting alpha and beta errors of 0.05 and 0.20, respectively, a number of nine animals *per* group was considered necessary.

### Experimental procedures

A pre-experimental examination was performed to exclude animals with periodontal probing depths exceeding 0.5 mm (PCP 10-SE, Hu-Friedy, Chicago, IL, USA) to ensure that animals were periodontally disease-free before the induction of experimental periodontitis<sup>3,24</sup>. Animals were weighted weekly during the obesity induction period (pre-experimental phase, day 0-90) and throughout the experimental periodontitis (experimental phase, day 90-120).

At day 90, when a difference of approximately 20% in body weight was achieved, silk ligatures (Ethicon, Johnson & Johnson®, São Paulo, SP, Brazil) were placed around one of the upper second molars, under general anesthesia with intramuscular

5% ketamine hydrochloride (Ketamina Agener; Agener, Embu-Guaçu, SP, Brazil) and 2% xylazine hydrochloride (Calmiun; Agener) 1:1 solution (0.2 mL/100 mg). The contra-lateral maxillary molar was considered the internal control<sup>10,19,24</sup>. After 30 days of experimental periodontitis<sup>19,24</sup>, animals were killed using a carbon dioxide chamber (day 120). The experimental protocol was approved by Ethical Committee of the Lutheran University of Brazil (CEP-ULBRA 2004-027A).

### Morphometric registration of bone destruction

Following sacrifice, the left and right segments of the maxillae were dissected out manually and then immersed in sodium hypochlorite with 8.5% active chlorine (Mazzarollo, Gravataí, RS, Brazil) during 5 h to remove soft tissues<sup>10,19,24</sup>. After rinsing, the specimens were stained for 1 min in methylene blue 1% (Sigma-Aldrich, Saint Louis, MO, USA) to delineate the cemento-enamel junction.

Standardized pictures were taken of each specimen together with a ruler with a Digital Camera and Medical Lenses (Nikon D100, Ayuthaya, Thailand)<sup>10,19,24</sup>. Pictures were taken from the buccal and palatal sides of the specimens. Computerized measurements were performed by means of an image analysis program (Image Tool 3.0, UTHSCSA, San Antonio, USA). Alveolar bone loss at the second maxillary molar, buccally and palatally, on both segments of the maxillae (teeth with and without ligature), was measured. Alveolar bone loss was defined as the distance between the cemento-enamel junction and the alveolar bone crest. Five measurements *per* picture were performed and the mean of these was considered the bone loss<sup>24</sup>. All registrations were carried out blindly.

### Reproducibility

Before the analysis, the examiner was trained and calibrated by double measurements of 20 specimens with an one-week interval between them. Paired t test statistics was run and no differences were observed in the mean values for comparison. Additionally, Pearson's correlation coefficient obtained between the two measurements reflected a very high correlation ( $r=0.979$ ,  $P<0.001$ ).

### Statistical analysis

Mean values of body weight were obtained at days 0, 90 and 120. After checking for normality, mean alveolar bone loss was calculated. Intra-group comparisons (teeth with or without ligature) were performed by paired-sample t test. Inter-group comparisons were performed by independent samples t test. The animal was the unit of analysis and the alpha level was set at 0.05.

**Table 1-** Alveolar bone loss [mean (SD)] in teeth with and without ligature for obese and non-obese rats

		ABL, mm		
		Non-obese Rats	Obese Rats	P*
Without Ligature	Buccal	0.35 (0.11)	0.32 (0.09)	0.13
	Palatal	0.42 (0.11)	0.45 (0.10)	0.17
With Ligature	Buccal	0.77 (0.21)	0.75 (0.13)	0.48
	Palatal	0.53 (0.13)	0.67 (0.16)	0.00

ABL: alveolar bone loss.

\* Independent sample t test

## RESULTS

No statistically significant difference in body weight was observed between animals in obese and non-obese groups at baseline (174 and 179 g, respectively). Rats from both groups significantly gained weight throughout the study period up to day 90. However, the weight gain was higher in the obese group. At the moment of ligature placement, the mean difference in body weight between obese and non-obese groups was of approximately 20% (277.59 and 223.35 g, respectively). This statistically significant difference (t test,  $P < 0.05$ ) was maintained throughout the periodontal disease induction period.

Alveolar bone loss means at buccal and palatal sides are presented in Table 1. No statistically significant differences were observed among groups in the buccal side. However, obese rats presented higher alveolar bone loss ( $P < 0.00$ ) than non-obese rats in the palatal sides. Combining measurements from buccal and palatal sides, no differences were observed in alveolar bone loss among groups.

## DISCUSSION

In the present study, the effect of obesity on pathogenesis of alveolar bone loss in experimental periodontitis was evaluated. Our results showed a potential interference of obesity.

Although a recent study published by our group and dealing with the same topic did not demonstrate differences in alveolar bone loss<sup>19</sup>, the biologic plausibility and the current epidemiologic data encouraged us to keep considering the hypothesis of this relationship. Additionally, the difference in the body weight of the rats in the first study could only be considered overweight and not obesity.

Obesity has been linked to a wide variety of health problems, like hypertension, cardiovascular diseases, diabetes mellitus, inflammation disorders and cancer<sup>12,13</sup>. The immunologic activity of adipose tissue may play an important role in the development of periodontal disease<sup>9,17</sup>. Studies suggest that obesity is associated with

immunocompetence alterations such as lower lymphocyte counts, lower natural killer counts and altered cytokines production<sup>14,16</sup>. Notwithstanding, clear connections with immune dysfunction<sup>20</sup> and inflammatory conditions<sup>7</sup> have been elucidated. The present study only demonstrates the ultimate outcome: alveolar bone loss. However, it should be noted that the observed effects could be related to any of the components of the causal chain. We suspect that the inflammatory profile linked to obesity accounts at least for an important part of the outcome.

Recently, epidemiologic studies have demonstrated association between obesity and different periodontal parameters<sup>2,6</sup>. A study with a representative sample in Southern Brazil conducted by our group has demonstrated an increased risk for periodontal destruction in overweight and obese women<sup>6</sup>. However, the biological mechanisms by which obesity may affect the periodontium have not yet been determined<sup>16,17,18</sup>. A recent publication reported that obese individuals had increased proportion of red-complex bacteria<sup>21</sup>, but the meaning of this finding is still unknown. The possible higher susceptibility of obese individuals seems to be more related to the inflammatory aspects. The CAF diet could have an influence on either bacterial plaque in quantitative or qualitative ways. However, to the best of our knowledge, there are no studies that have assessed this topic.

The results obtained in the present study demonstrated that in palatal sides of the maxillary segments, obese animals presented higher amounts of alveolar bone loss than non-obese ones. On the other hand, when buccal sites are considered alone or together with palatal sites, the significance is not evident. This finding should be interpreted with caution, especially due to the length of the experimental period (30 days). The subjacent studied biological plausibility should not be forgotten and the finding in palatal sites could also be found in buccal sites with longer periods of experiment.

In humans, there are different forms to assess obesity, but the Body Mass Index is currently

recommended by the World Health Organization. In studies with experimental rats, it is considered that a body weight difference of approximately 15% between groups could account for obesity<sup>22</sup>. In the present study, the test animals presented a mean body weight 19.8% higher than controls at the moment ligatures were placed (day 90). This difference is of significance in rats and is sufficient for obesity.

The consistency of the diet could influence alveolar bone loss<sup>3</sup>. However, in the present study, CAF diet was a complement and both groups were exposed to the standard diet as well. The consistency of the diet could influence alveolar bone loss<sup>3</sup>. However, in the present study, CAF diet was a complement and both groups were exposed to the standard diet as well. Another possible limitation is about the consequences of CAF diet intake. There is biological plausibility that high sucrose diet might induce glucose and insulin intolerance in rats. This pathway could result in more severe alveolar bone loss<sup>17</sup>. However, our recent study with similar methodology demonstrated no effect of diet and/or body weight gain on levels of glucose after 16 weeks of CAF diet<sup>19</sup>. Methodological aspects related to breeding and housing of the rats were observed<sup>3</sup>, contributing to improve the reliability of the results. Additionally, blinding of the examiner, randomization, use of sufficient number of animals and use of comparative groups were principles followed by this study in order to generate better evidence. Our sample size calculation indicated a minimum of 9 animals in each group. Thus, we consider the sample of this study adequate in terms of quantity.

In spite of the discrete results, obese animals seem to be affected by their condition and showed higher amounts of alveolar bone loss, especially when palatal sides were evaluated alone. The research topic is still open and, due to the importance of obesity and periodontal disease as problems affecting numerous people around the world, further studies are warranted.

## CONCLUSION

The findings from this study, within the limits of an animal investigation, lead to the conclusion that obesity potentially influences the pathogenesis of experimental periodontitis, leading to higher alveolar bone loss in female Wistar rats.

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