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ORIGINAL ARTICLE

Effect of body weight in the pathogenesis of ligature-induced periodontal disease in Wistar rats

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Abstract

Objective. The aim of this study was to compare ligature-induced alveolar bone loss between obese and non-obese rats. ***Material and methods.*** Thirty female Wistar rats were randomly divided into two groups: a test group comprising 14 rats fed with a "cafeteria diet" for 120 days in order to gain weight and a control group comprising 16 regularly fed rats. Ligatures were placed around the 2nd upper molars, and the contralateral teeth served as intra-group controls. After 30 days, the animals were killed and the maxillae were removed. Sodium hypochlorite was used to prepare the specimens, and the cementum-enamel junction was stained with methylene blue 1%. Morphometric analysis of alveolar bone loss was by standardized digital photographs and the distance between the cementum-enamel junction and the alveolar bone crest was measured using the software Image Tool 3.0. ***Results.*** Body weight differed statistically between test and controls (268.6 and 242.4 g, respectively). Test animals demonstrated a mean (SD) alveolar bone loss of 0.51 (0.11) mm and in the controls 0.52 (0.14) mm in teeth with ligatures. No statistically significant differences were observed (ANOVA+Tukey), except for teeth with and without ligatures in both groups. ***Conclusions.*** The establishment and progression of alveolar bone loss in rats was not influenced by body weight in the present study.

Key Words: *Alveolar bone loss, body weight, periodontitis*

Introduction

Obesity is an increasing public health problem, especially in developed and developing countries. It is a chronic condition that is associated with cardiovascular disease, hypertension, dyslipidemia, diabetes mellitus, osteoarthritis, some types of cancer and death [1,2]. It affects men and women, children, adolescents and adults [3,4].

Obesity and periodontitis are epidemiologically associated in different populations with an odds ratio varying between 1.44 and 3.4 [5–8]. When these samples are stratified, however, especially concerning gender and smoking, stronger associations are found in women and non-smokers [7,8].

Immunological and inflammatory alterations in obese individuals may partly explain the relationship between obesity and periodontitis, including the production of inflammatory mediators and cytokines by macrophages, monocytes and the adipose tissue, leptin increasing production of interleukins and interferon [9,10], a reduction in T lymphocytes and increase in TNF production [11], among others.

In a study evaluating the relationship between obesity and periodontitis, 12 normal weight Sprague-Dawley rats, 12 Sprague-Dawley rats with spontaneous hypertension and non-obese, 8 obese Zucker rats and 12 obese and spontaneously hypertense Zucker rats received induction of periodontal disease by ligatures [12], the results demonstrating that obese rats had higher bone resorption than controls.

The aims of the present study were to compare the induction of alveolar bone loss in obese and non-obese female Wistar rats and to test the hypothesis that body weight may influence alveolar bone loss.

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Material and methods

Animals

Thirty female Wistar rats, 2 months old (day 0), were used in the present study. A 12 h light and dark cycle was applied. Four to five rats were housed in each cage at a temperature of around 20°C.

Experimental groups

The animals were randomly assigned into two groups as follows: a test group given a calorie-rich diet (a "cafeteria diet" consisting of chocolate cookies and fat cheese) [13,14] for 4 months (day 0 to day 120) before being submitted to ligature-induced periodontal disease for 30 days (day 120–150), ($n=14$); and a control group given standard rat chew pellets (Nuvilab®, Curtiba, Brazil) for 4 months (days 0–120) prior to induction of periodontal disease by means of ligatures, from day 120 to day 150 ($n=16$). Water was available to both groups *ad libitum*.

During induction of the periodontal disease, the test animals continued to be fed with the "cafeteria diet" and the control animals with the standard diet.

Sample size calculations

Sample size estimates were calculated based on the data of the previous study [12]. A difference of 0.2 mm in alveolar bone loss was assumed significant in teeth with ligature. Considering an alpha error of 0.05 and a beta error of 0.20, a minimum number of 9 animals per group was calculated. For a beta error of 0.10, a minimum number of 14 animals per group was required.

Experimental procedures

The animals were weighed weekly during the obesity induction period (pre-experimental phase) and throughout alveolar bone loss induction. After having reached a difference of approximately 15% in body weight, cotton ligatures (Ethicon; Johnson & Johnson®, São Paulo, Brazil) were placed around one of the upper 2nd molars, i.e. the contralateral tooth the intra-group control [15,16]. The animals were killed 30 days after ligature placement (day 150), when a fasting period of 4 h was introduced in order to sample blood glucose in mg/dl [16]. The ethics committee of the Lutheran University of Brazil approved the study.

Laboratory procedures

Following sacrifice, the left and right segments of the maxillae were defleshed and fixed in 10% neutral buffered formalin for 24 h. The specimens were then immersed in sodium hypochlorite (Q,boa®, Osasco, Brazil) with 9% active chlorine for 5 h. The soft tissues were removed mechanically. Methylene blue 1% was used for 1 min to stain the cementum-enamel junction, followed by rinsing with water [17].

Measurements

Standard pictures of each specimen, together with a ruler, were taken with a Digital Camera and Medical Lenses (Nikon® D100; Ayuthaya, Thailand). A minimal focal distance was used and the specimen was placed with the occlusal surfaces parallel to the floor. A tripod was used to minimize error. Pictures were taken from the buccal and palatal aspects of the specimens. Measurements were computed using an image analysis program (Image Tool 3.0; UTHSCSA, San Antonio, USA). Bone levels were measured at the 2nd maxillary molar, buccally and palatally, on both sides (with or without ligatures) and between the cementum-enamel junction and the bone crest in the picture [17]. Four measurements per site were taken, i.e. the cusps and sulci between the reference points. The mean of these four measurements was considered the bone loss.

The examiner was unaware of the group distribution or of the presence or absence of the ligature. Measurements were converted into millimeters, utilizing a precision ruler as reference. In the present study, this distance was considered the alveolar bone loss [17].

Reproducibility

Prior to analysis, the examiner was trained and calibrated (double measurements of 10 specimens at 1-week intervals). Paired *t*-test statistics were run for comparison and no differences were observed in the mean values. The Pearson correlation coefficient between the two measurements revealed a very high correlation ($r=0.984$, $p<0.001$). After every 10 measurements during the experimental measurements, one specimen was re-evaluated in order to assess trans-experimental reproducibility, but no differences were detected.

Statistical analysis

The rat was considered the unit of analysis in this study. A normal distribution of the data was verified. Mean weights of animals were obtained on days 0, 120 and 150 and repeated measurements ANOVA was used to test the interaction between group-time. Mean blood glucose levels were calculated and analyzed by independent sample *t*-test.

Mean values of bone level were obtained for buccal and palatal aspects and compared by casual blocks ANOVA. The Tukey test was used if necessary as post-hoc for the ANOVAs. The level of significance was set at 5%.

Table I. Mean (SD) body weight of the animals

	Test ($n=14$)		Control ($n=16$)		
	Mean	Standard deviation	Mean	Standard deviation	p
0	186.94^{A}	8.09	178.40^{A}	8.53	NS
120	270.61^{C}	12.88	237.62^{B}	8.70	<0.001*
150	268.59^{C}	12.09	242.35^{B}	11.29	<0.001*

*Means followed by different letters are statistically different (ANOVA + Tukey, $\alpha = 0.05$).

Results

The body weight of the study animals is given in Table I. On day 0, no statistically significant difference was observed between animals in the test and control groups. Rats from both groups gained weight throughout the study period up to day 120. However, animals from the test group presented higher mean body weight on days 120 and 150 compared to controls.

In the present study we employed the "cafeteria diet", i.e. with chocolate cookies for 5 days, followed by cheese for 2 days, with a daily consumption of approximately 4 g of standard diet and 4 g of the complement per rat. In the control group, the mean consumption of standard chew pellets was of 8.7 g per rat daily. No differences were observed in the amount of food consumed between tests and controls.

Mean (SD) fasting blood glucose levels on day 150 did not differ statistically in animals from the test and control groups (205.6 (51.5) and 198.4 (54.8) mg/dl, respectively).

The mean (SD) alveolar bone loss on day 150 in teeth without ligatures was 0.28 (0.07) for the test group and 0.29 (0.07) mm for the control group. No statistically significant differences were observed. Alveolar bone losses in teeth with ligatures were 0.51 (0.11) and 0.52 (0.14) mm for tests and controls, respectively. No statistically significant differences were observed between groups.

Discussion

The present study evaluated the effect of obesity in ligature-induced periodontal disease in rats. Several behavioral and systemic factors are associated with establishment and progression of the disease and obesity is one of the factors that may play a role in the pathogenesis [5–8,18].

Despite epidemiological association, causality cannot be claimed. Experimental studies are important in identifying risk factors and performing them in humans might be unethical [19]. Ligature-induced periodontal disease is an interesting model for studying periodontal disease pathogeneses isolating contributing variables [15,20–27].

In the present study, alveolar bone loss clearly occurred, since teeth with ligatures (in both groups) presented virtually double the amount of mean bone loss compared to teeth without ligatures. Split-mouth designs are subject to criticism, since an effect from the test manipulation could be observed in the control group as well. However, the above-mentioned differences indicate that if this occurred it was not to such an extent that the outcomes would not differentiate sides with and without ligature [28,29].

In order to study systemic differences experimentally, genetically modified animals may be used or induction methods may be applied in similar animals. Concerning obesity, induction can be by a fat-rich diet [30] or by use of the so-called "cafeteria diet" as complement [13,14].

In humans, obesity can be assessed by body mass index, total subcutaneous fat, waist-to-hip ratio or by abdominal measurements. In experimental rats, it is considered that a difference of approximately 15% between groups could account for obesity [13]. In the present study, the test animals presented a mean body weight 13.8% higher than controls at the moment ligatures were placed (day 120). This difference is of significance in rats and, at least, suggests overweight. The lack of association encountered in our results might be linked to this fact.

Consistency of diet might influence alveolar bone loss [31]. In the present study, both groups were exposed to the standard diet complemented by the cafeteria diet.

The age of the animals is also important where biological plausibility is concerned. Epidemiological studies suggest stronger associations between obesity and periodontitis in youngsters [5,32]. Hence, higher degrees of association are found in women [7,8]. Taking this into consideration, this study was performed in female rats and the ligatures were placed when the rats were 180 days old. The literature considers rats as old at 10 months of age [33,34].

Data on fasting glycemia in the present study did not reveal statistically significant differences between groups, and were considered in the normal range. This finding shows that the rats did not differ in this respect and that they were not diabetics, since the Wistar rat cut-off point for diabetes is 300 mg/dl [35]. Thus, this possible bias can be ruled out.

In the only animal study found in the literature about the topic [12], normal weight rats, obese rats, animals with hypertension and hypertense/obese rats

were used. The authors detected that obese rats present higher attachment loss than normal weight rats (mean (SD) 1.45 (0.36) mm and 1.25 (0.34) mm, respectively) during 7 weeks. A limitation of this study is that different species were used, leading to possible selection bias. On the other hand, our data are related to diet-induced obesity, which is not the same as naturally occurring obesity.

Only Wistar rats were used in the present study. Our values of alveolar bone loss are lower than those of Perlstein & Bissada [12] for teeth both with and without ligatures. The time of experiment (30 days versus 7 weeks) could explain the difference. Additionally, different methods of evaluation of support loss were used. The differences in body weight of test and control animals (13%) could account for the results, since they are of a relatively small magnitude. However, this difference may surely be claimed as overweight.

The importance of this study is strongly related to the elevated prevalence of obesity and periodontitis in developed and developing countries. The absence of differences observed in our results sheds some light on our understanding of the problem, in that the assumed biological interactions between obesity and periodontitis are not so significant in ligature-induced periodontal disease in rats. However, this knowledge is not suffice for us to imply that obesity does not affect the periodontal immunological response; it cannot be ruled out. All lifestyle characteristics implicated in obesity in periodontitis have to be stressed. Both obesity and periodontitis share risk factors and, in this sense, further studies should be performed aimed especially at a more integral dental approach.

The findings of the present study, taking into consideration its methodological characteristics and limitations, may lead to the conclusion that the establishment and progression of ligature-induced alveolar bone loss in rats is not influenced by body weight.

References

[1] Must A, Spadano J, Coakley EH, Field AE, Colditz G, Dietz WH. The disease burden associated with overweight and obesity. J Am Med Assoc 1999;282:1523–9.

[2] Gregg EW, Cheng YJ, Cadwell BL, Imperatore G, Williams DE, Flegal KM, et al. Secular trends in cardiovascular disease risk factors according to body mass index in US adults. J Am Med Assoc 2005;293:1868–74.

[3] Vastag B. Obesity is now on everyone's plate. J Am Med Assoc 2004;291:1186–7.

[4] Vignerova J, Blaha P, Osancova K, Roth Z. Social inequality and obesity in Czech school children. Econ Hum Biol 2004; 2:107–18.

[5] Al-Zahrani MS, Bissada NF, Borawskit EA. Obesity and periodontal disease in young, middle-aged and older adults. J Periodontol 2003;74:610–5.

[6] Wood N, Johnson RB, Streckfus CF. Comparison of body composition and periodontal disease using nutritional assessment techniques: Third National Health and Nutrition Examination Survey (NHANES III). J Clin Periodontol 2003;30:321–7.

[7] Dalla Vecchia CF, Susin C, Rosing CK, Oppermann RV, Albandar JM. Overweight and obesity as risk indicators for periodontitis in adults. J Periodontol 2005;76:1721–8.

[8] Alabdulkarim M, Bissada N, Al-Zahrani M, Ficara A, Siegel B. Alveolar bone loss in obese subjects. J Int Acad Periodontol 2005;7:34–8.

[9] Marti A, Marcos A, Martinez JA. Obesity and immune function relationships. Obes Rev 2001;2:131–40.

[10] Lamas O, Marti A, Martinez JA. Obesity and immunocompetence. Eur J Clin Nutr 2002;56:S42–5.

[11] Tanaka S, Isoda F, Ishihara Y, Kimura M, Yamakawa T. T lymphopenia in relation to body mass index and TNF-α in human obesity: adequate weight reduction can be corrective. Clin Endocrinol 2001;54:347–54.

[12] Perlstein MI, Bissada NF. Influence of obesity and hypertension on the severity of periodontitis in rats. Oral Surg Oral Med Oral Pathol 1977;43:707–19.

[13] Svensson AM, Hellerström C, Jansson L. Diet-induced obesity and pancreatic islet blood flow in the rat: a preferential increase in islet blood perfusion persists after withdrawal of the diet and normalization of body weight. J Endocrinol 1996;151:507–11.

[14] Christoffolete MA, Moriscot AS. Hypercaloric cafeteria-like diet induced UCP3 gene expression in skeletal muscle is impaired by hypothyroidism. Braz J Med Biol Res 2004;37: 923–7.

[15] Galvão MP, Chapper A, Rösing CK, Ferreira MB, de Souza MA. Methodological considerations on descriptive studies of induced periodontal diseases in rats. Braz Oral Res 2003;17: 56–62.

[16] Fernandes MI, Gaio EJ, Oppermann RV, Rados PV, Rösing CK. Comparison of histometric and morphometric analyses of bone height in ligature-induced periodontitis in rats. Braz Oral Res 2007;21:216–21.

[17] Verzeletti GN, Gaio EJ, Rösing CK. Effect of methotrexate on alveolar bone loss in experimental periodontitis in Wistar rats. Acta Odontol Scand 2007;65:348–51.

[18] Albandar JM. Periodontal disease in North America. Periodontology 2000 2002;29:31–69.

[19] Beck JD. Risk revisited. Community Dent Oral Epidemiol 1998;26:220–5.

[20] Fischman SL, Greene GW Jr. The role of a local irritant in experimental periodontal pathology. Oral Surg Oral Med Oral Pathol 1964;18:637–45.

[21] Carranza FA Jr, Simes RJ, Mayo J, Cabrini RL. Histometric evaluation of periodontal bone loss in rats: the effect of marginal irritation, systemic irradiation and trauma from occlusion. J Periodontal Res 1971;6:65–72.

[22] Johnson IH. Effects of local irritation and dextran sulphate administration on the periodontium of the rat. J Periodontal Res 1975;10:332–45.

[23] Sanavi F, Listgarten MA, Boyd F, Sallay K, Nowotny A. The colonization and establishment of invading bacteria in periodontium of ligature-treated immunosuppressed rats. J Periodontol 1985;56:273–80.

[24] Klausen B. Microbiological and immunological aspects of experimental periodontal disease in rats: a review article. J Periodontol 1991;62:59–73.

[25] Yoshinari N, Kameyama Y, Aoyama Y, Nishiyama H, Noguchi T. Effect of long-term methotrexate-induced neutropenia on experimental periodontal lesion in rats. J Periodontal Res 1994;29:393–400.

[26] Koide M, Suda S, Saitoh S, Ofuji Y, Suzuki T, Yoshie H, et al. In vivo administration of IL-1 beta accelerates silk

ligature-induced alveolar bone resorption in rats. J Oral Pathol Med 1995;24:420–34.
[27] Keles GG, Acikgoz G, Ayas B, Sakallioglu E, Firatli E. Determination of systemically and locally induced periodontal defects in rats. Indian J Med Res 2005;121:176–84.
[28] Hujoel PP, Loesche WJ. Efficiency of split-mouth designs. J Clin Periodontol 1990;17:722–8.
[29] Hujoel PP, DeRouen TA. Validity issues in split-mouth trials. J Clin Periodontol 1992;19:625–7.
[30] Buison A, Pellizzon M, Ordiz F Jr, Jen KL. Augmenting leptin circadian rhythm following a weight reduction in diet-induced obese rats: short- and long-term effects. Metabolism 2004;53:782–9.
[31] Björnsson MJ, Velschow S, Stoltze K, Havemose-Poulsen A, Schou S, Holmstrup P. The influence of diet consistence, drinking water and bedding on periodontal disease in Sprague-Dawley rats. J Periodontal Res 2003;38:543–50.
[32] Reeves AF, Rees JM, Schiff M, Hujoel P. Total body weight and waist circumference associated with chronic periodontitis among adolescents in the United States. Arch Pediatr Adolesc Med 2006;160:894–9.
[33] Zhao C, Li WW, Franklin RJ. Differences in the early inflammatory responses to toxin-induced demyelination are associated with the age-related decline in CNS remyelination. Neurobiol Aging 2006;27:1298–307.
[34] Sakima A, Averill DB, Gallagher PE, et al. Impaired heart rate baroreflex in older rats: role of endogenous angiotensin-(1–7) at the nucleus tractus solitarii. Hypertension 2005;46:333–40.
[35] Dong H, Altomonte J, Morral N, Meseck M, Thung SN, Woo SL. Basal insulin gene expression significantly improves conventional insulin therapy in type 1 diabetic rats. Diabetes 2002;51:130–8.