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Tese

Efeito do ganho de peso sobre o metabolismo ósseo maxilar de ratos

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“Não é verdade que as pessoas param de perseguir os sonhos porque estão a ficar velhas, elas estão velhas porque pararam de perseguir os sonhos “

(Gabriel Garcia Márquez)

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A presente tese foi redigida segundo o Manual de Normas para trabalhos acadêmicos da UFPel, adotando o nível de descrição em capítulos não convencionais. Disponível no endereço eletrônico: <http://sisbi.ufpel.edu.br/arquivos/PDF/Manual_Normas_UFPel_trabalhos_acadêmicos.pdf> Acesso em: 09/01/2017.

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Resumo

ALMEIDA, Aline Ferreira. **Efeito do ganho de peso sobre o metabolismo ósseo maxilar de ratos.** 58f. Tese (Doutorado em Odontologia) – Programa de pós-graduação em Odontologia, Universidade Federal de Pelotas, Pelotas, 2017.

A periodontite apresenta como principal fator etiológico a resposta imunológica exacerbada do hospedeiro frente a microrganismos específicos e pode ter sua severidade aumentada quando associada a distúrbios sistêmicos como diabetes e obesidade. A obesidade é uma desordem crônica capaz de promover prejuízos à saúde do indivíduo bem como o surgimento de outras enfermidades crônicas. A exacerbação de liberação de citocinas pró-inflamatórias na corrente sanguínea parece ser o principal fator que justifique o aumento da severidade da doença periodontal nos indivíduos obesos. Sobre o tecido ósseo, a obesidade parece estar associada à baixa densidade mineral por estímulo a atividade osteoclática, pela presença das citocinas pró-inflamatórias como interleucina (IL)-1β, IL-6, fator de necrose tumoral alfa (TNF-α) que modulam o eixo do receptor ativador do fator nuclear kappa B (RANK), do seu ligante (RANKL) e do falso receptor osteoprotegerina (OPG). O objetivo deste trabalho foi avaliar por meio de análise morfompetrica o efeito do ganho de peso sobre o tecido ósseo periodontal de ratos. Animais foram divididos equitativamente em 2 grupos onde um foi alimentado com ração balanceada (controle) e outro com dieta hiperlipídica tipo cafeteria (obeso). No primeiro artigo, analisamos o osso alveolar das mandíbulas dos ratos utilizando microtomografia computadorizada a fim de relacionar o ganho de peso com possíveis mudanças na microestrutura do osso alveolar. Nos ratos que se tornaram obesos, o osso alveolar no septo interradicular foi gradualmente reduzido e o osso trabecular substituído em parte por osso mais esponjoso, observou-se ainda, uma diminuição no contato entre o osso alveolar e o dente, reforçando a perda óssea ocorrida nesta região. No segundo artigo, após 6 semanas de dieta padrão e dieta hiperlipídica, os animais foram submetidos a cirurgia para remoção do incisivo superior direito, recebendo cuidados pós-operatórios. Nos períodos pós exodontia (7, 14 e 28 dias) os animais foram sacrificados e a maxila direira conservada em formol 10% para posterior processamento histológico. Os cortes histológicos foram corados com hematoxilina-eosina para avaliação histomorfométrica e realizada a reação de imunoistoquímica para avaliar a expressão de OPG, RANKL, osteocalcina e fosfatase ácida resistente ao tartarato (TRAP). Na avaliação histomorfométrica, o grupo obeso apresentou reparo mais tardio quando comparado ao grupo controle. Houve aumento na expressão de TRAP no local da extração, provavelmente induzido pela elevação da marcação do RANKL, o que justificaria o atraso do reparo ósseo alveolar nas áreas de extração dentária. De forma geral, nossos estudos sugerem que a obesidade interfere no metabolismo do osso alveolar levando a redução do trabeculado e da área óssea, além de retardar o reparo de alvéolos dentários.

Palavras-chave: Periodontite, obesidade, peso, doença periodontal, extração dental.

Abstract

ALMEIDA, Aline Ferreira. **Effect of weight gain on the maxillary bone metabolism of rats.** 58p. Thesis PhD in Dentistry. Postgraduate Program in Dentistry. Federal University of Pelotas, Pelotas, 2017.

Periodontitis main etiological factor is the exacerbated immune response of the host to specific microorganisms and may have its severity increased when associated with systemic disorders such as diabetes and obesity. Obesity is a chronic disorder capable of inducing injury to the health of an individual as well as the onset of other chronic illnesses. Exacerbation of the release of proinflammatory cytokines into the bloodstream appears to be the main factor that justifies the increased severity of periodontal disease in obese individuals. Regarding bone tissue, obesity seems to be associated with low mineral density by stimulating osteoclast activity, by the presence of proinflammatory cytokines such as interleukin (IL)-1β, IL-6, tumor necrosis factor alpha (TNF-α), which modulates the axis of the receptor activator of nuclear factor-kappa B (RANK), its ligand (RANKL) and the false decoy osteoprotegerin (OPG). The objective of this study was to evaluate by morphometry the effect of the weight gain on the periodontal bone tissue of rats. Animals were divided equally in two groups one fed with balanced ration (control) and another with hyperlipidic diet cafeteria-type (obese). In the first manuscript, we analyzed the alveolar bone of the mandibles of the rats using computerized microtomography in order to relate the weight gain with possible changes in the microstructure of the alveolar bone. In the rats that became obese, the alveolar bone in the interradicular septum was gradually reduced and the trabecular bone partially replaced by a more cancellous bone, there was also a decrease in the contact between the alveolar bone and the tooth, reinforcing the bone loss in this region. In the second manuscript, after 6 weeks of standard diet and hyperlipidic diet, the animals underwent surgery to remove the upper right incisor, receiving postoperative care. In the post-exodontia periods (7, 14 and 28 days) the animals were sacrificed and the right maxilla preserved in 10% formalin for later histological processing. The histological sections were stained with hematoxylin-eosin for histomorphometric evaluation and the immunohistochemical reaction was performed to evaluate the expression of OPG, RANKL, osteocalcin and tartrate resistant acid phosphatase (TRAP). In the histomorphometric evaluation, the obese group presented later repair when compared to the control group. There was an increase in the expression of TRAP at the extraction site, probably induced by the elevation of the RANKL labelling, which would justify the delay of the alveolar bone repair in the areas of dental extraction. In general, our studies suggest that obesity interferes with alveolar bone metabolism leading to reduction of trabecular bone and in the bone area, as well as a delay the repair of dental alveoli.

Key words: Periodontitis, obesity, weight, periodontal disease, tooth extraction.

Sumário

1. Introdução

A crescente industrialização, urbanização e mecanização está associada com mudanças nos hábitos de vida e na dieta das pessoas. Uma dieta pobre em nutrientes e rica em gorduras e açúcares favorece o surgimento da obesidade (WHO 1998; HOWARD et al. 2003). O processo evolutivo os seres humanos desenvolveram processos altamente conservados que regulam o suprimento de combustível com as necessidades de energia para manter o peso corporal estável e uma reserva de gordura (SCHWARTZ et al. 1999). Quando o consumo calórico exagerado excede o gasto energético de forma sustentada, ocorre o acúmulo excessivo de gordura (WHO 2000). Obesidade é uma doença crônica caracterizada pelo excesso de gordura corporal, que causa prejuízos à saúde do indivíduo, podendo se constituir como fator de risco para outras enfermidades como o diabetes melito, câncer e artrite (WHO 1998; HOWARD et al. 2003; ANJOS 2006). Os fatores influenciadores da obesidade são: a susceptibilidade genética, ingestão de alimentos, atividade física e a taxa metabólica (KOPELMAN, 2000).

A epidemia da obesidade era uma realidade predominante dos países ricos e desenvolvidos (ALVES et al. 2013). Ao passo que países de baixa e de média renda começaram a se tornar mais globalizados, mudanças consideráveis no padrão nutricional e nos hábitos de vida vêm ocorrendo, acarretando, consequentemente, em uma significante elevação da prevalência desta doença. Assim, após a década de 1990, a obesidade vem assumindo taxas nunca antes observadas, e continuadamente crescentes (POPKIN, 2006).

Observa-se o aumento da prevalência da obesidade de forma globalizada, nas diversas faixas etárias, e em regiões onde tradicionalmente sua ocorrência era relativamente baixa (WHO, 2009). No Brasil, os dados da Pesquisa de Orçamento Familiar, realizada pelo Instituto Brasileiro de Geografia e Estatística e Ministério da Saúde, mostraram um significativo aumento de excesso de peso e obesidade em toda a população (IBGE, 2010). Dados do sistema de Vigilância de Fatores de Risco e de Proteção para Doenças Crônicas por Inquérito

Telefônico (MALTA et al. 2014) demonstram que 51% da população adulta, acima de 18 anos, estava com excesso de peso em 2012, um aumento de 8 pontos percentuais desde o último levantamento em 2006. Os dados ainda mostraram que há uma diferença na distribuição desta condição de acordo com o gênero: enquanto em mulheres o excesso de peso atinge 48%, entre os homens, este percentual chega a 54%.

As doenças periodontais são em sua maioria doenças crônicas, de origem inflamatória causadas essencialmente por microrganismos específicos (ARMITAGE, 1999). A progressão destas doenças está intimamente ligada à intensidade da resposta do sistema imune do hospedeiro frente à carga bacteriana presente nos sítios acometidos (LAINE et al., 2013). Condições sistêmicas como diabetes e obesidade têm sido apontadas como fatores de risco à progressão das doenças periodontais, uma vez que poderiam desencadear um desequilíbrio na interação bactéria-hospedeiro (GENCO; BORGNAKKE, 2013). Dentre as condições periodontais, destacam-se a gengivite induzida por biofilme bacteriano e a periodontite crônica, as formas mais prevalentes na população. A gengivite é caracterizada pela inflamação gengival causada pela presença do biofilme, com posterior crescimento do contorno gengival, coloração avermelhada acentuada e sangramento após estímulo (MARIOTTI, 1999). A periodontite crônica, por sua vez, caracteriza-se pela perda da inserção gengival e do osso alveolar, frutos da inflamação gengival, e da diminuição da resistência dos tecidos periodontais à sondagem (ANDRUKHOV et al., 2013).

Em estudos realizados em uma coorte de nascidos vivos em Pelotas houve evidencia da associação positiva entre obesidade/sobrepeso com presença de sangramento gengival e de cálculo dentário, além de maior incidência de periodontite, mostrando possível influência do excesso de peso no desenvolvimento das doenças periodontais (DICKIE DE CASTILHOS et al., 2012; NASCIMENTO, 2015). Hábitos alimentares e perfil socioeconômico podem desempenhar um papel importante na interface estado de peso e doença periodontal (Franchini et al., 2011, Thomson Et ai. 2012). Além disso, uma revisão sistemática recente mostrou associação positiva entre ganho de peso e desenvovimento de novos casos de periodontite (NASCIMENTO et al., 2015).

A obesidade provoca distúrbios no metabolismo tecidual e celular gerando alterações a nível sistêmico, podendo levar à modificação do curso da doença

periodontal. De acordo com alguns autores, sob o ponto de vista biológico, esta associação pode ser justificada pela exacerbação de citocinas pró-inflamatórias sistêmicas circulante e nos tecidos periodontais (CHAFFEE; WESTON, 2010). Sabe-se que o tecido adiposo branco é responsável pela secreção de adipocinas, substâncias responsáveis pelo estado de inflamação crônico sistêmico de baixa intensidade, assim como pela alteração na resposta imunológica (KOPELMAN, 2000).

O tecido adiposo branco (TAB), antes reconhecido órgão passivo de acúmulo de energia, é um importante órgão de função endócrina metabolicamente ativo, capaz de secretar e de expressar diversas substâncias bioativas, como as adipocinas, com ação local e sistêmica (KERSHAW; FLIER, 2004; TILG; MOSCHEN, 2006).

A resposta inflamatória clássica representa uma reação aguda frente ao desafio infeccioso ou ao dano tecidual, tendendo a evoluir para a homeostase, uma vez removido seu agente causador (MEDZHITOV, 2008). Entretanto, há uma alteração deste processo clássico diante do quadro de obesidade, já que não há manifestação dos sintomas típicos – calor, tumor, rubor, dor e perda de função – caracterizando-se por uma reação crônica de baixa intensidade (HOTAMISLIGIL, 2006).

Em indivíduos obesos a resposta inflamatória encontra-se alterada e está intimamente relacionada com a hipertrofia do tecido adiposo, uma vez que o TAB é responsável pela produção de uma gama de citocinas, em sua maioria pró-inflamatórias, como a IL (interleucina)-1β, a IL-6, o fator de necrose tumoral (TNF)-α e a proteína quimiotática para monócitos (MCP)-1, e de adipocinas, como a resistina e a leptina (CANCELLO; CLEMENT, 2006). Concomitante a isto, a obesidade também está associada à diminuição na produção de adiponectina, uma adipocina relacionada a processos anti-inflamatórios (STEFAN; STUMVOLL, 2002).

Ainda não há evidências sobre a relação causal entre a obesidade e a inflamação. Em tese, assume-se que a inflamação é um estado consequente à obesidade, embora alguns autores suportem a hipótese de a obesidade ser o resultado de uma doença inflamatória. De fato, sabe-se que obesidade e inflamação estão associadas, e apresentam condição cíclica no agravamento de ambas, estabelecendo uma relação de retroalimentação (CANCELLO;

CLEMENT, 2006).

Apesar de os estudos comprovarem o envolvimento de um quadro pró-inflamatório em condições como obesidade, há evidências de que indivíduos obesos apresentam prejuízo da resposta imune inata, inclusive da resposta inflamatória, frente a um processo infeccioso (KARLSSON; BECK, 2010; SCHMIDT et al., 2011). O reconhecimento de patógenos e a consequente secreção de mediadores inflamatórios pelas células do sistema imune inato são fatores críticos para a elaboração de uma resposta imune efetiva e consequente eliminação do agente patogênico. Sendo assim, indivíduos com sobrepeso/obesos poderiam ter risco e a gravidade de infecções aumentados (KARLSSON; BECK, 2010). Em camundongos com obesidade induzida por meio de dieta hiperlipídica, a infecção por *Porphyromonas gingivalis*, bactéria associada a quadros de periodontite, resulta em maior perda óssea alveolar e em redução da resposta inflamatória (AMAR et al., 2007), porém os mecanismos ainda não foram elucidados.

A literatura ainda tem demonstrado que a obesidade tem papel importante na cicatrização, uma vez que as citocinas pró-inflamatórias secretadas pelo tecido adiposo interferem neste processo, gerando o retardo do reparo tecidual. Segundo os autores, a processo inflamatório de baixa intensidade é responsável pelo aumento de marcadores inflamatórios sistêmicos e pelo aumento de susceptibilidade às infecções, o que gera atraso de forma significativa no processo cicatricial (PAULINO DO NASCIMENTO; MONTE-ALTO-COSTA, 2011).

No tecido ósseo, a obesidade também parece influenciar o seu metabolismo. Acreditava-se que a obesidade tinha efeitos somente benéficos sobre o metabolismo ósseo por conta do aumento de vetores de carga sobre os ossos, gerando assim aumento da proliferação e diferenciação de osteoblastos e deposição de osteócitos, estimulando a neoformação óssea. Todavia, estudos têm demonstrado que o excesso de tecido adiposo está associado com baixa densidade mineral óssea (CAO, 2011).

Baseado na literatura disponível, a obesidade parece afetar o metabolismo ósseo por meio de distintos mecanismos. Ao contrário do que se pensa, a doença pode diminuir a formação de tecido ósseo pela redução da osteoblastogênese em consequência do aumento da adipogênese, uma vez que

tanto adipócitos quanto osteoblastos são derivados da mesma célula-tronco mesenquimal (HALADE et al., 2010). Além disso, a obesidade pode aumentar a reabsorção óssea por meio da regulação positiva de citocinas pró-inflamatórias como IL-1β, IL-6, TNF-α. Estas citocinas pró-inflamatórias são capazes de estimular a atividade osteoclástica a partir da ativação da via RANKL/RANK/OPG (HALADE et al., 2010). Ainda, os adipócitos da medula óssea podem diretamente regular os progenitores osteoclásticos e as células hematopoiéticas (ADLER et al., 2014). As adipocinas, leptina e adiponectina, também parecem estar relacionadas com o processo. A ação da leptina no tecido ósseo é complexa, uma vez que tanto efeitos positivos quanto negativos têm sido reportados. Supõe-se que a produção excessiva da leptina em adultos obesos atua principalmente favorecendo a expressão de receptores para TNF-α aumentando a reabsorção óssea (VAN DIELEN et al., 2004; VAN DIELEN et al., 2001). Já a adiponectina tem efeitos anti-inflamatórios parecendo inibir a osteoclastogênese, reduzir a reabsorção óssea e aumentar a neoformação (OSHIMA et al., 2005). Como indivíduos obesos apresentam baixas concentrações desta citocina, seu efeito protetor sobre o tecido ósseo fica reduzido ou ausente (OSHIMA et al., 2005).

Finalmente, a dieta rica em gordura, uma das causas da obesidade, interfere na absorção intestinal do cálcio pela formação de sabões (*soaps*) de ácido graxo insolúveis entre a gordura e o cálcio, reduzindo a disponibilidade para deposição no tecido ósseo (LOREZEN et al., 2007; GACS; BARLTROP, 1977).

O efeito da obesidade sobre o osso alveolar também parece ser negativo. Estudos em roedores demonstraram que a obesidade foi responsável pela reabsorção espontânea de osso alveolar, causando doença periodontal (CAVAGNI et al., 2013). Entretanto, não é conhecido o mecanismo pelo qual a obesidade afeta o metabolismo ósseo. Como estudos mostram efeito negativo do excesso de tecido adiposo sobre o metabolismo ósseo, espera-se o retardo no processo de reparo e aceleração da destruição óssea em sítios expostos a periodontopatógenos. Esse fato, se confirmado, será importante para mudanças no plano de tratamento do paciente obeso, incluindo protocolos distintos de reavaliação periodontal.

2. Capítulo 1

Alveolar bone microstructural modifications induced by weight gain in rats§

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Abstract

Obesity has reached epidemic proportion with various health consequences. Few studies have addressed the relationship between obesity and microstructural changes in alveolar bone. The present study assessed the association of obesity with alveolar bone alterations in Wistar rats. Fourteen male Wistar rats were randomly divided into two groups: obese, which were fed with hyperlipidic cafeteria diet for twelve weeks in order to gain weight and control (non-obese) regularly fed rats. Body weight, Lee Index, white adipose tissue and glycaemia were recorded. After the experimental period, rats were euthanized and mandible stored in 10% buffered formalin solution. Morphometric analysis was performed using x-ray microtomography. Percentage of bone volume (P=0.023), bone density (P=0.018), trabecular thickness (P=0.008) and number (P=0.006) reduced in obese rats in comparison with control group. As expected, bone porosity increased with weight gain (P=0.048). With weight gain alveolar bone in the interradicular septum was gradually lost and trabecular bone substituted by cancellous bone. Intersection surface data showed a decrease in the contact between alveolar bone and tooth (P = 0.048). Weight gain seems to negatively influence alveolar bone microstructure resulting in a decrease in trabecular bone and increase in medullar spaces.

Keywords: Obesity, Spontaneous periodontitis, Cafeteria diet, Bone remodeling, Alveolar bone loss

1. Introduction

Obesity is a high prevalent chronic disease accompanied by morbidity and mortality [1]. Weight gain impacts on chronic health conditions, such as diabetes, hypertension, depression, among others [2-5]. Additionally, weight gain influence the establishment and progression of oral conditions, e.g. periodontitis [6, 7].

Periodontitis is a chronic inflammatory disorder that leads to the loss of the supporting structures of the teeth [8]. In most of the cases, periodontitis is induced by the response of the host immune system to the presence of a periodontopathogenic biofilm [8]. The subgingival biofilm is necessary, however not sufficient to cause tissue degradation. The host susceptibility concept encompasses the sum of unfavorable genetic, systemic and lifestyle factors [9]. This multifactorial scenario has been used to elucidate the variation in disease establishment and progression among tooth sites, individuals and populations [10].

The influence of weight gain on periodontitis progression is based on the systemic circulation of proinflammatory cytokines induced by weight gain [6, 7]. Adipose tissue expands with weight gain reducing progressively blood vessels lumen. With the reduction of supplies to adipose tissue macrophages migrate into the tissue and induce a generalized chronic low-grade inflammation. In the hyperinflammatory low-grade systemic state tumor necrosis factor-alpha (TNFα), interleukin (IL)-6 and C-reactive protein (CRP) are detected in elevated levels in the blood stream [11, 12]. These markers might indirectly induce the secretion of the receptor activator of nuclear factor-kappa B (RANK) and its ligand (RANKL) which ultimately result in osteoclastogenesis and in alveolar bone loss [13-15].

Thus, the hormones and cytokines produced in the adipose tissue might influence the homeostasis of periodontal tissues. Only one study showed an acceleration of natural alveolar bone loss in obese rats [15]. However, the study reported only visual evidence by means of photographs of maxillae stained with 1% methylene blue. The aim of this study was to evaluate the morphological changes in the alveolar bone using X-ray microtomography scan. we hypothesize that weight gain induce higher amounts of alveolar bone breakdown compared with non-obese rats.

2. Methods

2.1 Animals

This study was approved by the Animal Research Ethics Committee of the Araçatuba Dental School, UNESP, Brazil (Protocol number 2014/00293). Experiment was conducted under Good Laboratory Practice (GLP) conditions and in accordance with the guidelines of the USA National Research Council and the Canadian Council on Animal Care (CCAC).

Fourteen male Wistar rats weighting approximately 250g were used in this study. The sample size calculation considered the variability of measurements of alveolar bone loss, and acceptance as significant differences between experimental groups of 5 mm, with alpha and beta errors of 0.05 and 0.2, respectively [16]. The number of animals in each group was estimated in 6. Based on attrition rates observed in our previous studies, 7 animals were included in each group.

Animals were allocated into 2 groups according to body weight. A stratified randomization strategy comprising tertiles of body weight was used in order to

minimize possible group impairment at baseline. Control group which received a standardized rat chow (Nuvilab, Curitiba, PR, Brazil) and Weight gain group (Cafeteria diet-induced weight gain) which received a high fat and hypercaloric diet.

The hypercaloric diet, also known as CAF diet or western diet, was constituted by 55% carbohydrates, 20% lipids, 20% proteins and 5% other components (vitamins, sodium, calcium, preservatives, among others) [17]. All foods were available ad libitum both for obesity and control groups.

2.2 Blood pressure and glycaemia monitoring

The systolic arterial blood pressure measurement was performed by tail cuff plethysmography, using a plethysmography device adapted for measurement in rats. Blood glucose levels were measured by an automatic monitoring system (Accu-Check® Performa; Roche-Diagnostics Corporation, Indianapolis, IN, USA). The SBP and glycaemia were taken immediately before sacrifice.

2.3 Sample collection

Animals were euthanized after twelve weeks of standard or CAF diet in chamber saturated with halothane vapor. White adipose tissue from retroperitoneal (RWAT) and parametrial (PWAT) regions were collected and weighed to assure weight gain due to fat accumulation. Next, the lower maxilla was split in two by means of median sagittal incision following the intermaxillary suture. Alveolar bone samples were obtained through tangential cuts to the first molar mesial face and third molar distal face using surgical scissors. Samples

were immersed in 10% buffered formalin solution (pH 7.0) for 48h for tissue preservation.

2.4 X-ray Computed Microtomography (µCT) Analysis

Sample was scanned using a Skyscan 1172 µCT equipment (Skyscan, Aartselaar, Belgium). The microtomographic images were acquired using a spatial resolution of 5 micrometers (µm), in regular angular steps of 0.3°, with exposure time of 1800 ms, 2 frames and 5 random movements. The voltage of the X-ray tube was 100 kV and current of 100 µA. Three dimensional images were reconstructed using Skyscan's software (CTvol 2.3, Skyscan, Aartselaar, Belgium). In the image reconstruction process, the smoothing, ring artifacts and beam harding corrections were adjusted to improve reconstruction. Analyzes of the reconstructed samples were performed in the CTanalyzer 1.15.4.0 software (SkyScan, Aartselaar, Belgium), where the region of interest (ROI) of the reconstructed image was determined together with the binarization of the image and generation of the 3D model of each tooth of interest.

Bone tissue was determined and the following parameters were analyzed: percent of bone volume (Tissue Volume/Bone Volume – TV/BV), intersection surface (IS), bone surface/volume ratio (Bone Surface/Bone Volume – BS/BV), bone surface density (Bone Surface/Tissue Volume – BS/TV), trabecular thickness (TT), trabecular number (TN), trabecular separation distribution (TSP), closed porosity (CP), total volume of pore space (VPS), structure model index (SMI) and trabecular pattern factor (TPF).

2.5 Statistical analysis

Normality was tested by Shapiro–Wilk test. Mean body weight and Lee Index were calculated for control and weight gain groups and compared by independent samples t-test. MicroCT data between groups were compared using the nonparametric Kruskal-Wallis test and Dunn post test, employing StataSE 14.1 software (StataSoft, College Station, TX, US). The significant level was set at 5%.

3 Results

At baseline before animals being subjected to different feeding systems no differences were observed in weight and blood pressure as observed in Table 1. Animals in the obese group increased weight by the accumulation of abdominal adipose tissue. Arterial blood pressure and glycaemia levels among obese rats were higher than in control animals (Table 1).

Table 1 – Overview of clinical measures of animals at baseline or in the end of weight gain period

	Control	Obese	P value
Arterial pressure at baseline (mmHg)	86.2 ± 11.8	91.2 ± 8.1	0.6891
Arterial pressure (mmHg)	112.3 ± 8.0	122.0 ± 10.4	0.0479*
Naso-anal measure (cm)	26.8 ± 0.67	27.8 ± 0.4	0.0919
Glycaemia (mg/dL)	77.2 ± 10.5	101.4 ± 9.8	0.0090*
Weight at baseline (g)	232.8 ± 17.6	236.2 ± 11.8	0.5779
Weight (g)	478.7 ± 43.0	636.3 ± 31.5	0.0090*
Lee index	291.3 ± 7.9	309.3 ± 3.8	0.0079*
PWAT (g)	6.9 ± 1.7	24.1 ± 4.2	0.0016*
RWAT (g)	7.9 ± 2.0	37.1 ± 5.0	0.0029*

PWAT – parametrial white adipose tissue, RWAT – retroperitoneal white adipose tissue

Bone morphometric data are presented in Table 2. Percentage of bone volume, bone density, trabecular thickness and number reduced in obese rats in comparison with control group. As expected, bone porosity increased with weight gain (Table 2). With weight gain alveolar bone in the interradicular septum is gradually lost and trabecular bone substituted by cancellous bone. Intersection surface data shows a decrease in the contact between alveolar bone and tooth (P = 0.048).

Table 2 – Comparison of bone morphometric properties as determined by μCT

	Control		Obese		P
	Mean (SD)	Median (range)	Mean (SD)	Median (range)	value
Percent bone volume (TV/BV) (%)	85.5 (2.4)	85.5 (82.0–89.0)	46.2 (34.1)	24.0 (19.1–84.0)	0.023
Intersection surface (IS) (10^{-3} pixelˆ2)	14.7 (6.8)	12.0 (11.4–28.5)	7.4 (4.3)	5.1 (2.8–12.1)	0.048
Bone surface / volume ratio (BS/BV) (1/pixel)	0.4 (0.0)	0.4 (0.4–0.4)	0.3 (0.0)	0.3 (0.3–0.4)	0.029
Bone surface density (BS/TV) (1/pixel)	0.3 (0.0)	0.3 (0.3–0.4)	0.2 (0.1)	0.1 (0.1–0.3)	0.018
Trabecular thickness (TT) (pixel)	11.7 (1.3)	11.0 (10.5–13.7)	10.0 (0.3)	10.0 (9.6–10.5)	0.008
Trabecular number (TN) (1/pixel)	0.09 (0.0)	0.09 (0.1–0.1)	0.04 (0.0)	0.02 (0.0–0.1)	0.006
Trabecular separation distribution (TSP) (pixel)	200.7 (149.2)	251.8 (0.1–353.7)	721.7 (350.9)	965.1 (318.2–994.4)	0.011
Closed porosity (CP) (%)	2.56 (6.1)	0.07 (0.1–15.0)	0.04 (0.0)	0.04 (0.0–0.1)	0.048
Total volume of pore space (VPS) (10^{-3} pixelˆ3)	10.8 (6.2)	10.8 (0.1–18.8)	241.4 (225.9)	238.3 (13.4–471.8)	0.018
Structure model index (SMI)	-0.50 (0.3)	-0.46 (-0.99– -.19)	-0.98 (0.2)	-1.01 (-1.23– -0.75)	0.018
Trabecular pattern factor (TPF)	-0.19 (0.1)	-0.18 (-0.27– -0.15)	-0.11 (0.1)	-0.10 (-0.18– -0.05)	0.045

Figure 1 illustrates the differences between the control group (A) and obese (B). An increase in periodontal ligament surface and reduction in the interradicular bone area are observed.

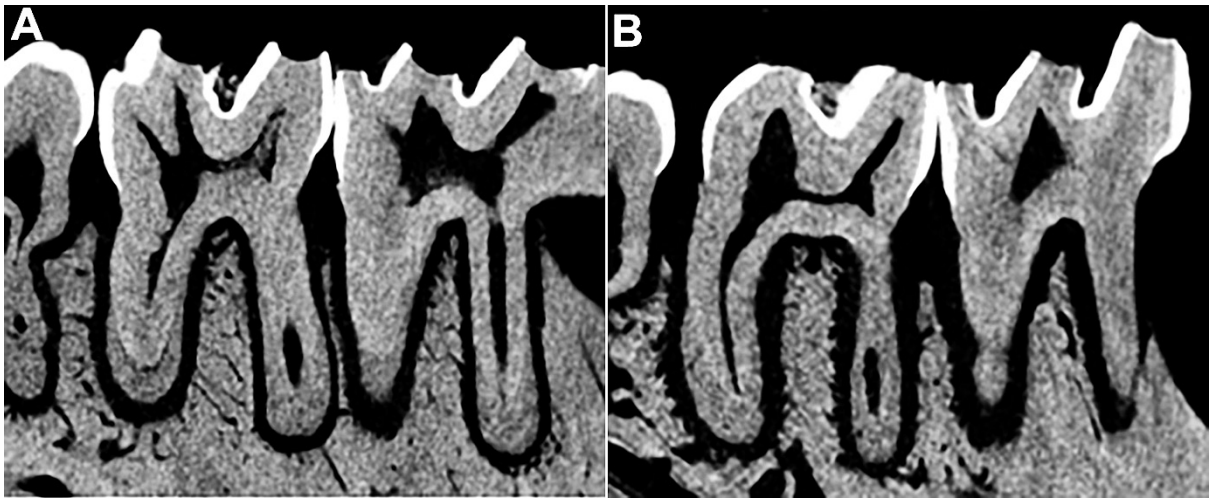

Fig. 1. Representation of the alveolar bone around the lower first molars in control group (A) and obese group (B).

Discussion

The study assessed the effect of weight gain on spontaneous alveolar bone. Diet-induced obesity was associated with alveolar bone loss and alterations in trabecular structure. The study explored for the first time the morphometrically arrangement of trabecular structure in alveolar bone in the absence of other stimuli, i.e. cotton ligatures and bacteria injections. The absence of a periodontitis-induction stimuli collaborates to analyze the effect *per se* of weight gain on periodontal tissues.

MicroCT was used to evaluate the effect of weight gain on bone structure around lower first molars in rats. The technique allows a three-dimensional analysis of microstructures and their distribution in a certain predefined area. Percentage of bone volume showed that alveolar bone was spontaneously lost

in obese rats. Also, intersection surface denotes that the periodontal ligament area is increased in obese animals. Previous studies had already demonstrated that alveolar bone could be negatively influenced by weight gain. Verzeletti et al. [18] showed an increase in the linear distance between the cemento-enamel junction (CEJ) to the bone crest using mandible photographs in obese rats with cotton ligature, posteriorly Cavagni et al. [15] using the same photograph-based methodology presented similar results in rats even without ligature. Azuma et al. [19] demonstrated that weight gain causes an increase in serum reactive oxygen species (ROS) level and gingival oxidative stress in obese rats with an increased number of neutrophils and osteoclasts in periodontal tissues.

Osteoclast differentiation may be stimulated by oxidative stress in rats [20]. Indeed, even though Azuma et al. [19] showed that the degree of gingival oxidative stress induced by weight gain might induce osteoclastogenesis, the authors failed to show an influence of the oxidative stress-induced osteoclastogenesis on alveolar bone resorption. In this study, alveolar bone porosity and the separation of trabeculae was significantly increased with weight gain. These data results from the reduction in the number and thickness of trabecular bone.

Trabecular pattern factor (TPF) is an index to evaluate the connectivity of trabecular bone based on the concavity or convexity of the total bone surface. Concavity and convexity imply connectivity and isolated disconnected structures, respectively [21]. Lower TPF indicates more connected trabecular lattices and vice versa. The high prevalence of enclosed cavities and concave surfaces are represented in negative TPF values. In the obese group a significantly lower trabecular number ($P = 0.006$) with high trabecular separation distribution ($P =$

0.011) was observed resulting in a higher trabecular pattern factor (P=0.045) and higher structure model index (P=0.018).

Population-based epidemiologic studies showed association between obesity and periodontal tissues breakdown without exploring the underlying mechanisms [22-24]. Even though previous studies have mentioned increased proportion of periodontopathogenic bacteria in obese [25], the periodontal destruction seems to be more related to the inflammatory aspects [26, 27].

We hypothesize that bone resorption in obese rats was the result from enhanced osteoclastic activity. Lorincz et al. [28] showed a maintenance in serum levels of osteocalcin and an elevation in serum tartrate-resistant acid phosphatase (TRAP), markers of bone formation and resorption respectively. These data seem to correlate with the increase in the low-grade systemic state previously reported [6, 7, 23, 29]. TNFα, IL-6 and IL-1 are highly released into the blood stream with weight gain [11, 12] and through the induction of the expression of RANK and RANKL result in osteoclastogenesis and in alveolar bone loss [13-15].

In sum, weight gain seems detrimental to alveolar bone microstructure resulting in a decrease in trabecular bone and increase in medullar spaces. These facts might explain the acceleration in periodontal structures loss previously mentioned in literature.

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3. Capítulo 2

Influence of weight gain on the modulation of wound healing following tooth extraction

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§ Artigo formatado de acordo com as normas da revista Bone

Abstract

Obesity is characterized by extreme body fat accumulation related to lean body mass. Hyperlipidic diet increases the serum level of lipid peroxidation, which stimulate the differentiation of osteoclasts. This study assessed the association of obesity with alveoli repair alterations after tooth extraction. Forty-two male Wistar rats were randomly divided into two groups: obese, animals fed with hyperlipidic cafeteria diet in order to gain weight and control (non-obese) regularly fed rats. After twelve weeks, the upper right central incisor was extracted and animals were killed after 7, 14 and 28 days of tooth extraction. Slides were stained with hematoxylin and eosin or subjected to immunolocalization reaction for RANKL, OPG, Osteocalcin and TRAP. Results were quantitatively evaluated. Bone area was higher in control group all over the experiment with more TRAP-positive cells and TRAP-positive labeling in the obese group. RANKL was homogeneously expressed along the experiment with no differences among the groups, conversely OPG levels reduced in the obese groups 14 and 28 days after tooth extraction (P=0.0143 and 0.0046, respectively). Osteocalcin labeling was higher in the control group after 7 days of tooth expression (P=0.0143), with no differences in posterior evaluations. Obesity induced a delay in bone repair after tooth extraction. The increase in the number of TRAP-positive clastic cells observed in the extraction site seems to be mediated by the reduction in the expression of OPG.

Keywords: Obesity, Cafeteria diet, Bone remodeling, Osteocalcin, Tooth extraction

1. Introduction

Obesity is a chronic disease in which the body excessively accumulates fat [1], a risk factor for many systemic diseases, such as type II diabetes, cardiovascular disease and cancer [2]. It has been ranked among the most prevalent diseases in rich-income countries as well as in those of low and middle income [3].

The literature has demonstrated that obesity is associated with low-grade chronic inflammation [4]. It has been hypothesized that the white adipose tissue is responsible for secreting constantly non-specific proinflammatory cytokines, such as interleukin (IL)-6, IL-1 beta and tumor necrosis factor-alpha (TNF-alpha), so as specific adipokines (leptin and adiponectin) [5]. Additionally, as a consequence of the expansion of the adipose tissue during weight gain, macrophages infiltrate the tissue, increasing the inflammatory load [5]. IL-1, IL-6 and IL-8 can indirectly modulate the expression of the receptor activator of NF-kappa B ligand (RANKL) and osteoprotegerin (OPG) which may ultimately result in bone loss [6, 7].

Bone metabolism comprises the balance between resorption and formation, induced by osteoclasts and osteoblasts, respectively [8]. Bone turnover is mainly regulated by the RANK/RANKL/OPG axis [8]. Essentially, RANKL expressed on the osteoblast cell surface binds to RANK to stimulate osteoclast differentiation and maturation. On the other hand, osteoprotegerin (OPG) acts as a decoy receptor for RANKL, which in turn, prevents osteoclast differentiation and activation [9]. Thus, among the factors that may alter bone metabolism, chronic inflammation and increased inflammatory profile are responsible for inducing osteoclast activation, and hence, bone resorption [10].

The relationship between obesity and bone metabolism is still controversial within the literature. Initially, obesity was described as beneficial to bone tissue, since mechanical loading conferred by body weight would stimulate bone formation [11]. However, studies have also identified detrimental effects of obesity on bone metabolism [12, 13]. According to the literature, the chronic inflammatory frame combined with increased levels of proinflammatory cytokines induced by excessive body weight might nullify potential beneficial effects of obesity on bone turnover [8]. This detrimental impact of obesity on bone metabolism may be more perceived in body locations that do not experience the benefits of mechanical loading, such as the alveolar bone. Studies have demonstrated that obesity may induce alveolar bone loss in rats [14, 15], and in humans [16].

Even though studies have demonstrated the effects of excessive body weight on bone loss, there is a lack of information about the influence of obesity on the mechanisms involved in bone healing, more specifically in the alveolar bone. Given the risen prevalence of obesity, it is of relevance for clinicians to understand whether obesity would influence bone metabolism. Therefore, this study aimed to evaluate the influence of obesity on potential mechanisms related to bone healing in rats.

2. Methods

2.1. Animals

This study was approved by the Animal Research Ethics Committee of the Araçatuba Dental School, UNESP, Brazil (Protocol number 2014/00293). Experiment was conducted under Good Laboratory Practice (GLP) conditions

and in accordance with the guidelines of the USA National Research Council and the Canadian Council on Animal Care (CCAC).

Forty-two male Wistar rats weighting approximately 250g were used in this study. The sample size calculation considered the variability of measurements of alveolar bone repair, and acceptance as significant differences between experimental groups of 5 mm, with alpha and beta errors of 0.05 and 0.2, respectively [17]. The number of animals in each group was estimated in 6. Based on attrition rates observed in our previous studies, 7 animals were included in each group.

Animals were allocated into 2 groups according to body weight. A stratified randomization strategy comprising tertiles of body weight was used in order to minimize possible group impairment at baseline. Control group which received a standardized rat chow (Nuvilab, Curitiba, PR, Brazil) and Weight gain group (Cafeteria diet-induced weight gain) which received a high fat and hypercaloric diet.

The hypercaloric diet, also known as CAF diet or western diet, was constituted by 55% carbohydrates, 20% lipids, 20% proteins and 5% other components (vitamins, sodium, calcium, preservatives, among others) [18]. All foods were available ad libitum both for obesity and control groups.

2.2. Blood pressure and glycaemia monitoring

The systolic arterial blood pressure measurement was performed by tail cuff plethysmography, using a plethysmography device adapted for measurement in rats. Blood glucose levels were measured by an automatic monitoring system (Accu-Check® Performa; Roche-Diagnostics Corporation,

Indianapolis, IN, USA). The SBP and glycaemia were taken at preoperative and postoperative time periods (7, 14 and 28 days).

2.3. Surgery

After twelve weeks of standard or CAF diet animals were anesthetized by intramuscular administration of a solution of ketamine chloride (50 mg/kg, Vetbrands, Jacareí, SP, Brazil) and xylazine chloride (10 mg/kg, Coopers Brasil LTDA, São Paulo, SP, Brazil). Anterior maxilla antisepsis was performed using iodized polyvinylpyrrolidone and extraction of the upper right incisor was done using specially adapted tools, as previously described [19]. The margins of the wound were sutured with nylon wires (Vicryl 5-0, Ethycon, São Paulo, SP, Brazil). At the first two postoperative days, animals were fed ground rat chow to facilitate feeding, returning to standard chow after this period.

2.4. Sample collection

Animals were euthanized after 7, 14 and 28 days of tooth extraction in chamber saturated with halothane vapor. White adipose tissue from retroperitoneal (RWAT) and parametrial (PWAT) regions were collected and weighed to assure weight gain due to fat accumulation. Next, the upper maxilla was split in two by means of median sagittal incision following the intermaxillary suture. Alveolar bone samples were obtained through tangential cuts to the molar distal faces using surgical scissors. Samples were immersed in 10% buffered formalin solution (pH 7.0) for 48h for histological processing.

2.5. Histomorphometric analysis

Samples were decalcified in buffered (pH 8) 17% EDTA (Sigma Chemical Co; St Louis, MO, USA) and embedded in paraffin. Semiserial longitudinal 6μm-thick sections were stained with hematoxylin and eosin (H&E). The histomorphometric analysis of the bone area and of the middle thirds of the rat alveolus was performed in three slices from each animal. An examiner under blinded conditions performed the analysis. The analysis was performed with an optical microscope Leica Aristoplan Microsystems (Leitz, Benshein, Germany) with a magnification objective of 160 ×, coupled to an image capturing camera (Leica DFC 300FX, Leica Microsystems, Heerbrugg, Switzerland). Digitized images were analyzed in a specific software (Leica Camera Software Box, Leica Imaging Manager 50).

2.6. Immunohistochemical analysis

The next sections to the H&E-stained section were used for immunohistochemical staining to verify the expression of OPG, RANKL, Osteocalcin and tartrate-resistant acid phosphatase (TRAP) in the tissues during the healing process. After deparaffinization, the slides were washed in phosphate-buffered saline, blocked with 0.03% hydrogen peroxide (Merck, Darmstadt, Germany) and submitted to antigen recovery. Nonfat milk was used to block endogen biotin. Goat polyclonal anti-OPG, anti-RANKL, anti-Osteocalcin and anti-TRAP antibodies (Santa Cruz Biotechnology, Santa Cruz, CA, USA) were used. The secondary antibody was biotinylated anti-goat antibody (Santa Cruz Biotechnology, Santa Cruz, CA, USA).

Reactions were amplified using avidin-biotin immunoperoxidase (Dako Corp., Carpinteria, CA, USA) and developed by diaminobenzidine (Dako Corp.). Sections were counterstained with hematoxylin (Merck), dehydrated, and coverslips were mounted using Permount (Fisher Scientific Co., NJ, USA). Analysis was performed to identify the labelling characteristics of each protein.

Three slides of each specimen were selected for analysis of OPG, RANKL, Osteocalcin and TRAP expression. The area corresponding to the extraction socket was examined. The immunostaining intensity was categorized by a blinded calibrated examiner according to a scale from 1 to 4 attributed to absent/negligible, weak, moderate, and strong staining, respectively [20].

2.7. Statistical analysis

Normality was tested by Shapiro–Wilk test. Mean body weight and Lee Index were calculated for control and weight gain groups and compared by independent samples t-test. Bone formation and protein expression at the different periods were compared using the nonparametric Kruskal-Wallis test and Dunn post test, employing StataSE 14.1 software (StataSoft, College Station, TX, US). The significant level was set at 5%.

3. Results

At baseline before animals being subjected to different feeding systems no differences were observed in weight and blood pressure as observed in Table 1. Animals in the obese group increased weight by the accumulation of abdominal adipose tissue. Arterial blood pressure and glycaemia levels among obese rats were higher than in control animals (Table 1).

Table 1 – Overview of clinical measures of animals at baseline or in the 28-day period after tooth extraction

	Control group	Obese group	P value
Arterial pressure at baseline (mmHg)	83.0 ± 10.5	90.8 ± 10.9	0.3841
Arterial pressure (mmHg)	116.6 ± 4.0	129.8 ± 3.8	0.0007*
Naso-anal measure (cm)	27.1 ± 0.32	27.4 ± 0.9	0.5619
Glycaemia (mg/dL)	70.0 ± 3.5	93.8 ± 11.9	0.0026*
Weight at baseline (g)	231.4 ± 27.8	230.4 ± 17.2	0.4989
Weight (g)	509.4 ± 40.7	665.5 ± 87.7	0.0069*
Lee index	294.1 ± 4.7	318.2 ± 11.9	0.0029*
PWAT (g)	6.6 ± 0.5	26.8 ± 6.6	0.0001*
RWAT (g)	6.1 ± 0.6	39.8 ± 16.1	0.0016*

PWAT – parametrial white adipose tissue, RWAT – retroperitoneal white adipose tissue

After 7 days of tooth extraction both groups showed an initial repair of the extraction socket. The onset of alveolar bone repair was characterized by the presence, mainly in the periphery of the alveolus, of some trabeculae of osteoid material and immature bone tissue, with numerous osteocytes and irregular basophilic apposition lines, permeated by densely cellularized connective tissue still presenting hemorrhagic areas.

At 14 days, the bone tissue trabeculae started to show anastomosis and filled most of the alveolus. The connective tissue was still well densely cellularized and some smaller hemorrhagic areas could still be observed. After 28 days of tooth extration, the anastomosed bone trabeculae were thicker, although still

immature, and there was less osteoid material. The connective tissue was more scarce and mature, showing a more fibrous characteristic with less cellularity.

Small differences were observed between the specimens of the control group and the obese group, with the latter always showing a more delayed repair. The figure 1 illustrates the overall evolution of tissue repair.

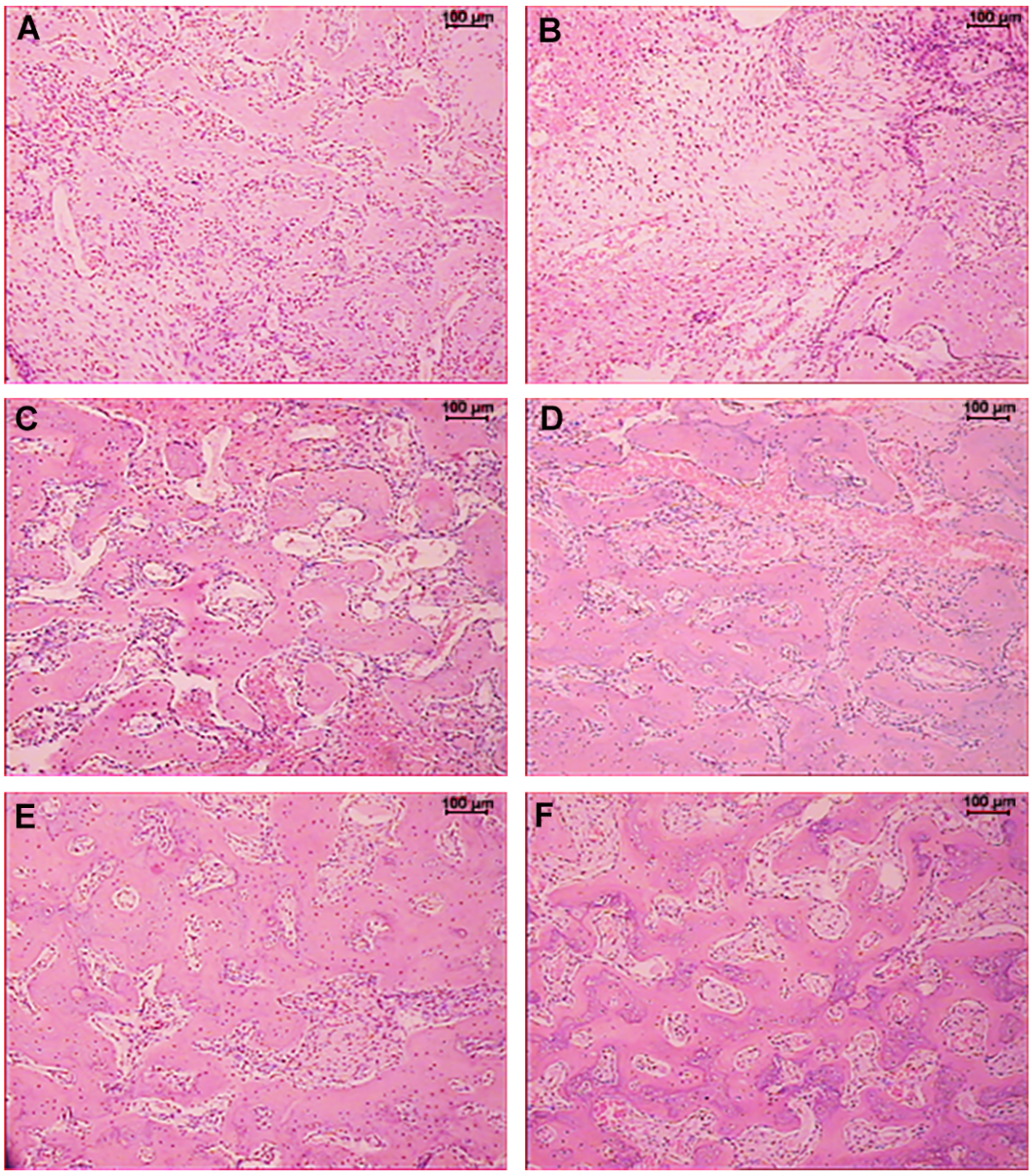

Figure 1. Photomicrographs illustrating the chronology of alveolar bone repair post-extraction. Non-obese group at 7 (A), 14 (C) and 28 (E) days post-extraction and obese group at 7 (B), 14 (D) and 28 (F) days post-extraction (HE, 100x).

Bone area was higher in control group all over the experiment with more TRAP-positive cells and TRAP-positive labeling in the obese group (Tables 2 and 3). RANKL was homogeneously expressed along the experiment with no differences among the groups (Fig. 2), conversely OPG levels reduced in the obese groups 14 and 28 days after tooth extraction (Table 3 and Fig. 3). Osteocalcin labeling was higher in the control group after 7 days of tooth expression, with no differences in posterior evaluations (Table 3 and Fig. 4).

Table 2 – Mean and standard deviation data regarding bone area formation and the number of TRAP positive cells

	Control group	Obese group	P value
Bone area 7 days (mm^2)	35.0 ± 6.6	15.8 ± 5.5	0.0023*
Bone area 14 days (mm^2)	47.1 ± 5.7	31.8 ± 8.0	0.0085*
Bone area 28 days (mm^2)	57.2 ± 6.9	40.4 ± 16.4	0.0479*
TRAP positive cells 7 days	111.2 ± 24.9	120.6 ± 53.8	0.7321
TRAP positive cells 14 days	216.4 ± 54.8	261.0 ± 115.4	0.4574
TRAP positive cells 28 days	156.4 ± 49.2	237.4 ± 70.6	0.0491*

Table 3 – Median and range data regarding immunohistochemistry scores

	Control group	Obese group	P value
OPG 7 days	2 [2-2]	2 [2-2]	1.0000
OPG 14 days	3 [3-3]	2 [2-3]	0.0143*
OPG 28 days	3 [3-3]	1 [1-2]	0.0046*
RANKL 7 days	3 [3-3]	3 [2-3]	0.1336
RANKL 14 days	3 [2-3]	3 [2-3]	0.5127
RANKL 28 days	3 [2-3]	3 [2-3]	1.0000
TRAP 7 days	1 [1-1]	1 [1-2]	0.1336
TRAP 14 days	2 [1-2]	2 [2-3]	0.0478*
TRAP 28 days	2 [1-2]	3 [3-3]	0.0046*
OC 7 days	3 [3-3]	2 [2-3]	0.0143*
OC 14 days	3 [2-3]	2 [2-3]	0.2207
OC 28 days	3 [2-3]	2 [2-3]	0.2207

OPG – osteoprotegerin, RANKL - receptor activator of NF-kappa B ligand, TRAP - tartrate-resistant acid phosphatase, OC - osteocalcin

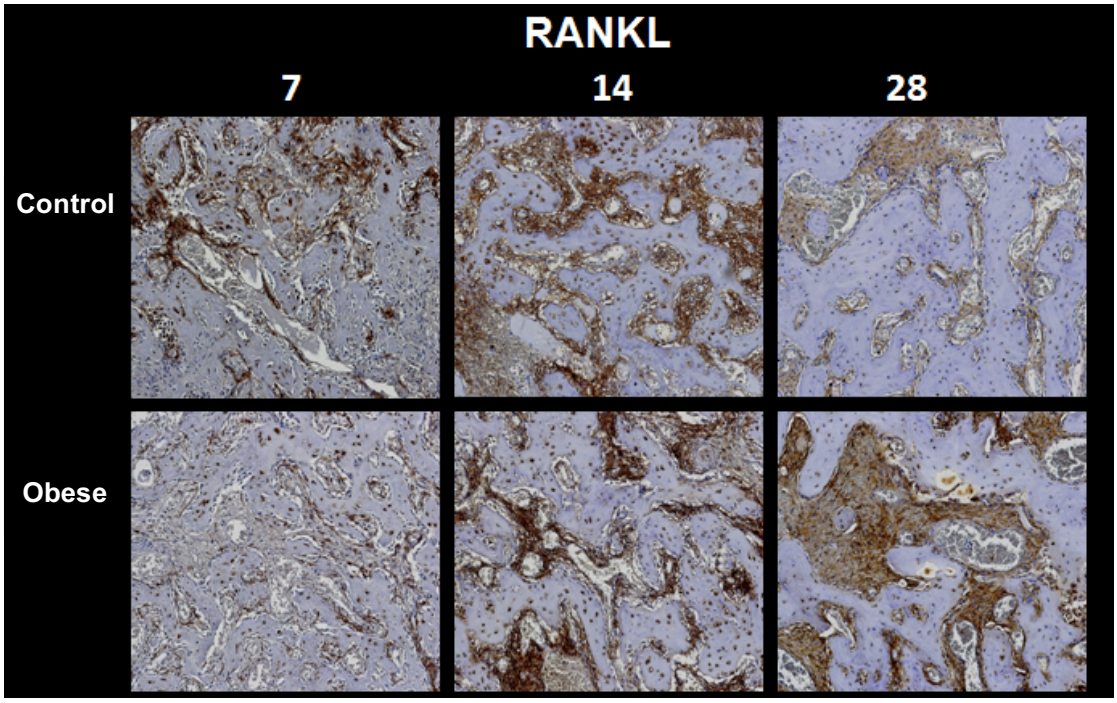

Figure 2. Illustrative images of the receptor activator of NF-kappa B ligand (RANKL) immunolabeling in both groups after 7, 14 and 28 of tooth extraction

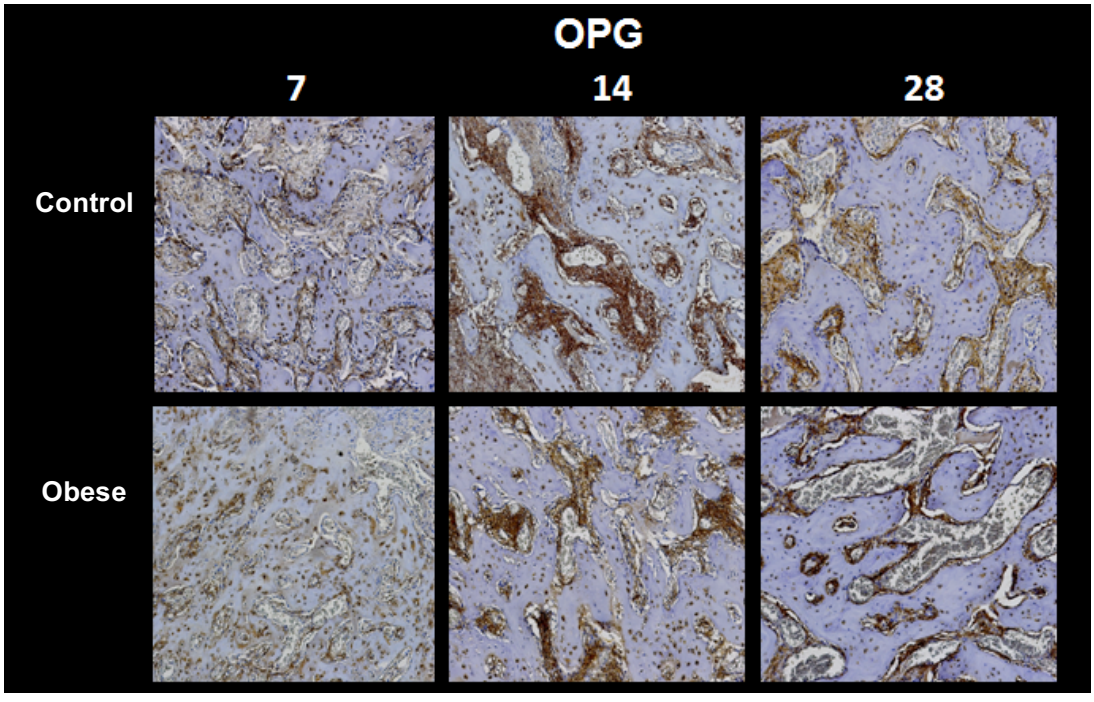

Figure 3. Illustrative images of osteoprotegerine immunolabeling in both groups after 7, 14 and 28 of tooth extraction.

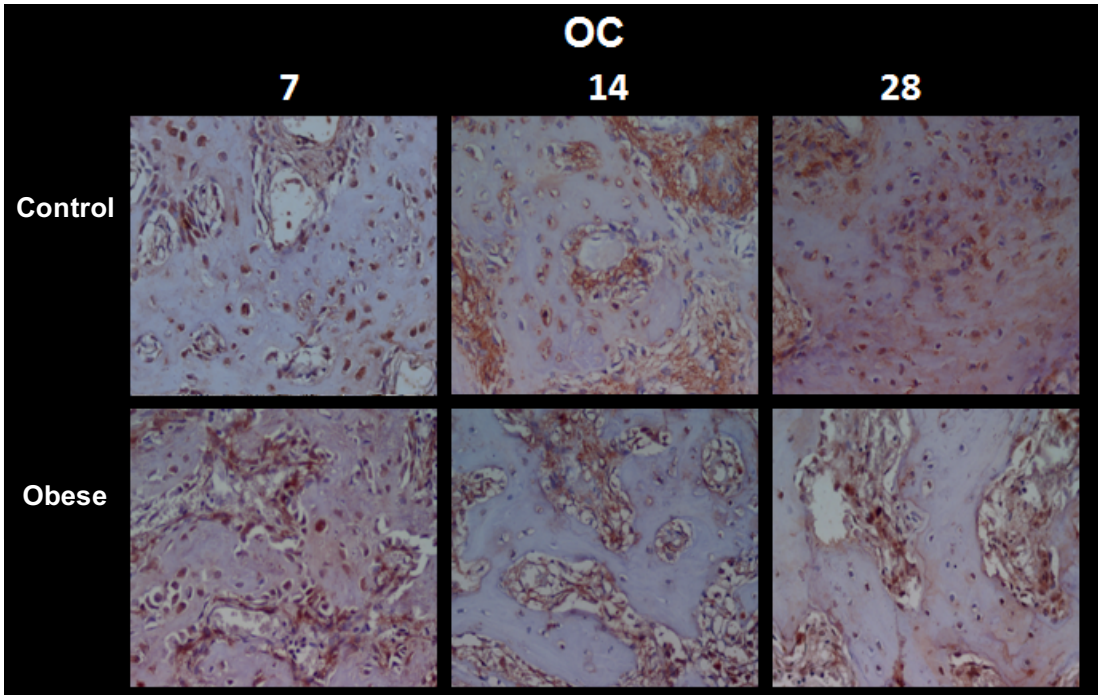

Figure 4. Illustrative images of the osteocalcin immunolabeling in both groups after 7, 14 and 28 of tooth extraction

4. Discussion

Obesity is a highly incident and prevalent disease associated with morbidity and mortality [21]. It is associated with unhealthy lifestyle behaviors, including high levels of calorie intake and/or low physical activity. Obesity is also a risk factor for systemic conditions, such as type II diabetes, cardiovascular disease, osteoporosis and cancer, generating high healthcare costs [2]. A high-fat diet might interfere with intestinal calcium absorption with fatty acids forming unabsorbable calcium soaps and therefore to low calcium absorption for tissue mineralization [22].

Obesity might affect bone metabolism by different mechanisms. Osteoblastogenesis is inversely related with adipogenesis since adipocytes and osteoblasts have a common mesenchymal stem cell origin [23]. Moreover, bone resorption increases by the upregulation of proinflammatory cytokines release,

e.g. IL-6 and TNF-α [24]. Adipocytes proliferation and growth constrains blood vessels compromising cellular nutrition and foci of necrosis are observed [25]. Macrophages are recruited to necrosis sites, exacerbating the inflammatory load in an up-regulation feedback [25].

Proinflammatory cytokines such as IL-6 and TNF-α stimulate osteoclast activity through the modulation of the RANKL/RANK/OPG pathway [6, 7]. RANKL bound to RANK determines osteoclastogenesis through the nuclear factor κB and protein kinase B-mediated signaling pathways, which promote bone resorption [6, 7]. Instead, RANKL bound to OPG inhibits osteoclastogenesis, impairing bone resorption. An imbalance between the RANKL/OPG ratio, bone metabolism disorganization probably occurs [7].

In this study the OPG/RANKL ratio was decreased and as a result, osteoclastogenesis was increased, which is consistent with the immunohistochemistry and histomorphometric data. A previous study mentioned that adipocyte-secreted cytokines in cell cultures affected the OPG/RANKL/RANK axis, causing an intensification in osteoclast differentiation [26]. Our results corroborate in part with a previous study [27] which reported a decrease in OPG secretion in a model of obesity simulation. Conversely, in other *in vitro* studies [26-28] RANKL expression increased but RANKL levels were not affected in our animal model.

Even though osteoclastogenesis and osteoblastogenesis are important for bone metabolism after tooth extraction, other proteins are important in bone formation. Osteocalcin is synthesized by osteoblasts along bone formation and it is used as a biomarker for the bone remodeling process [29]. OC is carboxylate in a posttranslational process in order to bind to hydroxyapatite and be retained

in bone tissue [29]. Thus, it is important to evaluate OC labeling in the new formed bone tissue. In this study, differences were observed between both groups. Control group presented OC precipitated all over the new bone matrix inside the extraction site as expected. Conversely, in the obese group even though labeling scores were high due to many osteoblasts labeling but the mineralized matrix was poorly impregnated. Previous studies in older persons showed that weight gain and development of metabolic syndrome are associated with a reduction in the concentration of OC in the blood stream, mediated by triglycerides and HDL cholesterol, waist circumference, and hypertension [30]. Our study showed that OC impregnation in the new tissue might be influenced by weight gain and all the consequences of this fact.

The reduced number of trabecular bone formed in the obese group might be explained also by the elevated number of TRAP-positive clastic cells observed in the new formed tissue compared to the control group. TRAP participates in the degradation of collagen intracellularly in osteoclasts [31, 32] and in the osteoclast adhesion and migration when secreted in the extracellular matrix [33].

In sum, obesity induces a delay in bone repair after tooth extraction. The increase in the number of TRAP-positive clastic cells observed in the extraction site seems to be mediated by the reduction in the expression of OPG rather than an overexpression of RANKL. Also, osteocalcin fixation seems to be reduced by the weight gain.

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3. Considerações Finais

Nossos estudos sugerem que a obesidade interfere no metabolismo do osso alveolar levando a redução do trabeculado e da área óssea, além de retardar o reparo de alvéolos dentários. Nos ratos que se tornaram obesos, o osso alveolar no septo interradicular foi gradualmente reduzido e o osso trabecular substituído em parte por osso mais esponjoso, ocorrendo uma diminuição no contato entre o osso alveolar e o dente, reforçando a perda óssea ocorrida nesta região. Na análise imunoistoquímica, houve aumento na expressão de TRAP no local da extração, provavelmente induzido pela elevação da marcação do RANKL, o que justificaria o atraso do reparo ósseo alveolar nas áreas de extração dentária. De forma geral, nossos estudos sugerem que a obesidade interfere no metabolismo do osso alveolar levando a redução do trabeculado e da área óssea, além de retardar o reparo de alvéolos dentários.

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