KARLA ORFELINA CARPIO HORTA DOS REIS

EFFECT OF OBESITY OVER DENTAL, PERIODONTAL AND BONE TISSUE STRUCTURES DURING INDUCED TOOTH MOVEMENT IN RATS: MICROTOMOGRAPHIC AND HISTOLOGICAL ANALYSES

A thesis submitted to the School of Dentistry of Ribeirão Preto of the University of São Paulo, in partial fulfillment of the requirements for the degree of Doctor of Philosophy in Science

Program: Pediatric Dentistry
Concentration area: Pediatric Dentistry

Supervisor: Prof. Dr. Alberto Consolaro

Co-supervisor: Profa. Dra. Mírian Aiko Nakane Matsumoto

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Prof. Dr. _____

Judgment _____ Signature _____

CURRICULAR DATA

KARLA ORFELINA CARPIO HORTA DOS REIS

Date of birth October 19th, 1984 – Machupicchu/Cusco- Peru.

Filiation Alberto Abad Carpio Delgado (RIP)
Martha Horta Ccasa

2000-2005 Bachelor in Dentistry Catholic University of Santa Maria, UCSM, Peru
Research Project: Etiological factors of pulpal pathologies to be treated endodontically in adult patients of the Clinic of the Catholic University of Santa María
Advisor: Hair Salas Beltrán

2007-2010 Residency in Orthodontics Department of Pediatric Dentistry, Area of Orthodontics, School of Dentistry of Ribeirão Preto of the University of São Paulo, Ribeirão Preto, São Paulo, Brazil. Research Project: Molecular detection of Aggregatibacter actinomycetemcomitans on metallic brackets by the checkerboard DNADNA hybridization technique.
Advisor: Paulo Nelson Filho

2010-2012 Master of Science in Dentistry, Area of Orthodontics. Department of Orthodontics, State University of São Paulo "Júlio de Mesquita Filho", Araraquara, Sao Paulo, Brazil
Research Project: Non-radiographic assessment of the hyperdivergent class II skeletal pattern.
Advisor: João Roberto Gonçalves
Scholarship from: Coordination for the Improvement of Higher Education Personnel.

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2010-2014 Doctorate in Dental Sciences, Department of Pediatric Dentistry, School of Dentistry of Ribeirão Preto of the University of São Paulo, Ribeirão Preto, São Paulo, Brazil. Research Project: Effect of obesity over dental, periodontal and bone tissue structures during induced tooth movement in rats: microtomographic and histological analysis
Advisor: Alberto Consolaro
Co-Advisor: Mírian Aiko Nakane Matsumoto
Scholarship from : Coordination of Superior Level Staff Improvement – **CAPES**

D*EDICATION,*

This document is dedicated to my parents, ***Martha*** *and* ***Beto*** *(in memoriam), who throughout my life have given me their powerful support and unconditional love.*

To my beloved husband, ***Cristiano****, who since he came into my life has made it a wonderful adventure. I am forever grateful to you for so much love, patience, complicity and happiness.*

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ABSTRACT

To evaluate the effect of obesity over induced tooth movement in *Wistar* rats, by means of computerized micro-tomography and histological analyzes. Forty rats of approximately 125 g were randomly divided in two groups of 20 animals each, the non-obese and the non-obese groups. The left side of the maxillas of all animal received induced tooth movement (ITM = WM, with movement) for each experimental period, i.e. 7 or 14 days (OWM7, OWM14, NOWM7 and NOWM14 groups). The untreated right hemi-maxilla groups (O7, O14, NO7, NO14 groups) were also accessed. Obesity was induced by supplying a high-fat diet for eight weeks. ITM aimed the mesial movement of the left maxillary first molar using a nickel-titanium closed coil spring. After euthanasia, forty hemi-maxillas were submitted to chemical and biological processing in order to prepare the samples for microtomography examination. Also, forty hemi-maxillas were analyzed histological analyses. The number of cementoclast and osteoclasts were analyzed through staining of the enzyme tartrate-resistant acid phosphatase. Also, presence or absent of focal hyalinization (FH), frontal bone resorption (FBR) and root resorption (RR) that included active root resorption and repaired root resorption at cementum (ARRC and RRRC, respectively) and at dentin level (ARRD and RRRD, respectively), and at cervical and medial thirds of the mesial and disto-buccal roots were evaluated in sections stained with Hematoxylin-Eosin. Data was submitted to appropriate statistical analysis using the programs Graph Pad Prism 5.0 and Stata13, with a significance level of 5%. Different animal weights between groups were reported since week one ($p=0.002$ and $p\leq0.001$). Obese animals showed higher rates of ITM after 7 days than non-obese animals ($p=0.081$). This difference became more evident after 14 days ($p\leq0.001$). Bone surface density at compression site showed reduced values in obese animals submitted to ITM of 7 ($p=0.027$) and 14 days ($p=0.050$). After 7 days of ITM, obese animals presented a reduced number of

trabecula (p=0.002), increased trabecular separation (p≤0.001) and higher total porosity (p=0.027) at compression site. However, after 14 days, trabecular number, trabecular separation, and porosity, and after 7 and 14 days, bone volume, percentage of bone volume, disto-buccal root volume and angular measurements were similar between obese and non-obese animals (p>0.05). Higher quantity of osteoclast were observed after 7 days of ITM in obese animals. Obese animals also presented decreased quantity of osteoclast after 14 days of ITM when compared to 7 days. Low frequencies of FH was observed in obese and non-obese animals after 7 days of ITM. The highest frequency of FBR was found in obese animals submitted to ITM of 7 days followed by non-obese animals at the same period. This groups presented low frequencies of FBR after 14 days of ITM. Medium and low frequencies of active root resorptions at cementum and dentin respectively were observed in obese animals submitted to 7 days of ITM while low frequencies was presented by non-obese animals. High frequency of RRRD was observed in mesial root and medium frequency at disto-buccal root of obese animals submitted to 14 days of ITM, while non-obese presented low frequencies. Conclusion: Obese animals showed significantly higher rates of molar movement and different responses in morphological surrounding bone and periodontum to induced tooth movement.

Key-words: Obesity, Root resorption, Alveolar Bone Loss, Tooth movement.

DOS REIS, KOCH. **Efeito da obesidade sobre estruturas dentárias, periodontais e de tecido ósseo durante a movimentação dentária induzida em ratos: microtomographic and histological analyses.** Ribeirão Preto 2019. 91p. [Tese doutorado]. Ribeirão Preto: Faculdade de Odontologia de Ribeirão Preto da Universidade de São Paulo; 2019.

RESUMO

Avaliar o efeito da obesidade sobre a movimentação dentária induzida em ratos Wistar, por meio de micro-tomografia computadorizada e análises histológicas. Quarenta ratos de aproximadamente 125 g foram divididos aleatoriamente em dois grupos de 20 animais cada, os grupos não obesos e não obesos. O lado esquerdo das maxilas de todos os animais recebeu movimento dentário induzido (MDI = WM, com movimento) por, 7 ou 14 dias (grupos OWM7, OWM14, NOWM7 e NOWM14). Os grupos hemi-maxila direitos não tratados (grupos O7, O14, NO7, NO14) também foram avaliados. A obesidade foi induzida pelo fornecimento de uma dieta rica em gordura por oito semanas. O MDI objetivou o movimento mesial do primeiro molar superior esquerdo usando uma mola helicoidal fechada em níquel-titânio. Após a eutanásia, quarenta hemi-maxilas foram submetidas ao processamento químico e biológico, a fim de preparar as amostras para o exame microtomográfico. Além disso, quarenta hemi-maxilas foram avaliadas por análises histológicas. O número de cementoclastos e osteoclastos foi analisado através da coloração da enzima fosfatase ácida resistente ao tartarato. Além disso, presença ou ausência de hialinização focal (HF), reabsorção óssea frontal (FBR) e reabsorção radicular (RR) que incluíam reabsorção radicular ativa e reabsorção radicular reparada no cemento (ARRC e RRRC, respectivamente) e no nível da dentina (ARRD e RRRD , respectivamente) e nos terços cervical e medial das raízes mesial e disto-vestibular foram avaliados em cortes corados com Hematoxilina-Eosina. Os dados foram submetidos à análise estatística apropriada nos programas Graph Pad Prism 5.0 e Stata13, com nível de significância de 5%. Diferentes pesos de animais entre os grupos foram relatados desde a primeira semana ($p=0,002$ e $p\leq0,001$). Animais obesos apresentaram maiores taxas de MDI após 7 dias do que animais não obesos ($p=0,081$). Essa diferença ficou mais evidente após 14 dias ($p\leq0,001$). A densidade da superfície óssea do lado da compressão apresentou valores reduzidos nos animais obesos submetidos ao MDI de 7 ($p=0,027$) e 14 dias ($p=0,050$). Após 7 dias de MDI, os obesos apresentaram número reduzido de trabéculas ($p=0,002$), aumento da separação trabecular ($p\leq0,001$) e maior porosidade total ($p=0,027$) no lado de compressão. Entretanto, após 14 dias, o número trabecular, a separação trabecular e a porosidade, e após 7 e 14 dias, o volume ósseo, a porcentagem de volume ósseo, o volume radicular disto-bucal e as medidas angulares foram semelhantes entre os animais obesos e não obesos ($p>0,05$). Maior quantidade de osteoclasto foi observada após 7 dias de MDI em animais obesos e após 14 dias, houve diminuição da quantidade de osteoclastos após 14 dias de ITM quando comparados aos 7 dias. Baixas frequências de HF foram observadas em animais obesos e não obesos após 7 dias de MDI. A maior frequência de FBR foi encontrada em animais obesos submetidos

ao MDI de 7 dias, seguidos por animais não obesos no mesmo período. Esses grupos apresentaram menores frequências de FBR após 14 dias de MDI. Frequências médias e baixas de reabsorções radiculares ativas no cemento e dentina respectivamente foram observadas, em animais obesos submetidos a 7 dias de MDI, enquanto baixa frequência foi apresentada por animais não obesos. Observou-se alta frequência de RRRD na raiz mesial e média frequência na raiz disto-vestibular de obesos submetidos a 14 dias de MDI, enquanto não obesos apresentaram baixas frequências.
Conclusão: Animais obesos apresentaram taxas significativamente maiores de movimento dentário e respostas diferentes na morfologia do osso circundante e periodonto resultantes do movimento ortodôntico.

Palavras-chave: Obesidade, reabsorção radicular, perda óssea alveolar, movimento dentário.

Summary

1. Introduction

INTRODUCTION

Overweight and obesity are defined by the World Health Organization (WHO) as a global epidemic resulting from the accumulation of abnormal or excessive fat with serious social and psychological dimensions.[1,2] According to WHO, an obese or overweight refers to a person who is too heavy for their height. This condition is considered a form of malnutrition, where more calories are consumed than necessary.[3] World obesity has more than doubled since 1980. In 2008, over 1.4 billion adults were overweight and over half a billion were obese. In this context, at least 2.8 million people died each year as a result of being overweight or obese. Statistics published by WHO got worse over the years. In 2014, more than 1.9 billion adults, aged 18 and over, were overweight. Of these, over 600 million were obese. This means that 39% of adults 18 years and older were overweight, and 13% were obese (11% men and 15% women).[1,2]

This problem is not exclusive to the adult population. Childhood obesity is one of the most serious public health challenges of the 21st century.[1] Overweight and obesity in children increases the risk of death from "non-contagious diseases" later in life. Between 2000 and 2015, the prevalence of overweight in children under the age of 5 increased worldwide and in most WHO regions. In 2010, the number of children affected was estimated at over 40 million. In 2015, globally it was estimated that 42 million children under 5 years old - 6% of all children worldly - were overweight, with the highest prevalence observed in the European Region.[1,3,4]

Considering that obesity is a difficult problem to solve, WHO stated that one of the goals for the year 2025 is to have no increase in the number of overweight cases in childhood.[5] Child overweight can be prevented through actions such as promoting

exclusive breastfeeding and adequate complementary feeding, regulating the marketing of complementary foods and non-alcoholic foods and beverages for children, and promoting physical activity from the earliest stages of life, as well as eliminating sedentary lifestyles.[5,6]

Currently considered a global pandemic, the disease has spread out in countries with different income levels. Obesity that was once associated only with high-income countries is now also prevalent in low- and middle-income countries. Among the main factors that have contributed to this global picture are the increased intake of high fat foods and the increased physical inactivity due to the increasingly sedentary nature of many forms of work, changing modes of transport and increasing urbanization. [1,2]

Mortality is such that the WHO claims that the majority of the world's population (65%) lives in countries where overweight and obesity kill more people than underweight does. Globally, there are more people who are obese than underweight - this occurs in all regions except parts of sub-Saharan Africa and Asia.[7]

Obesity mortality statistics presented by the WHO are highly worrying and can mean huge burdens on society. The high mortality rate of obesity may be justified by the fact that it is related to cardiovascular diseases (mainly heart disease and stroke), which were the leading cause of death in 2012. Diabetes, musculoskeletal disorders (especially osteoarthritis - a highly disabling degenerative joint disease) and some cancers (including endometrium, breast, ovarian, prostate, liver, gallbladder, kidney and colon) are also obesity related deasese.[7] In porcentages,[2,8,9] obesity is being described as a contributor of osteoarthritis, 44% of diabetes, 23% ischemic heart disease and 7-41% of certain cancers. In this sense, the risk of these noncommunicable diseases increases with increasing in body mass index (BMI), which

is the main parameter for determining obesity.[1] Interestingly, some authors described that the orthodontic tooth movement process could induce systemic reactions.[10,11]

In this context, due to its close relationship to chronic diseases and being considered a global pandemic, obesity has been the subject of various scientific studies. The first evidence of the connection between obesity, inflammation and diabetes was described in 1993, where Hotamisligil et al.,[12] showed an increased expression of tumor necrosis factor alpha (TNF-α) in obese rodent adipose tissue, and improved glucose tolerance after TNF-α neutralization. In obese humans, TNF-α also has greater expression in adipose tissue and muscles.[13,14] TNF-α is a proinflammatory cytokine that has the ability to activate intercellular cascades that end with insulin inhibition. This evidence contradicts the mere concept of obesity-inert lipid deposition disease.[12]

In addition to TNF-α, adipose tissue is also capable of producing numerous inflammatory mediators such as the chemotactic and monocyte protein (MCP-1) and the interleukin-6 (IL-6).[15] Studies show that adipocytes do not act alone in the local production of inflammatory mediators, and that macrophages would play a fundamental role in this process.[15,16] Macrophages accumulate in adipose tissue of obese animals and humans participating in local secretion of cytokines and chemokines, thereby generating and amplifying the local inflammatory process, and facilitating the genesis of obesity-related insulin resistance.[17,18]

Macrophages may present significant heterogeneity in their function, which depends on local factors that have different application program resources, with different parameters of inflammatory mediators, metabolic enzymes and surface markers.[19] M1 macrophages are induced by proinflammatory mediators such as

interferon-gamma (IFN-γ) and have increased production of proinflammatory cytokines such as TNF-α. M2 macrophages are induced by other cytokines such as interleukins-4 and 13 (IL-4 and IL-13), and show a profile with significant expression of anti-inflammatory mediators such as interleukin-10 (IL-10).[19] This shows that M2 macrophages participate in suppressing inflammatory responses and promoting technical repair. In adipose tissue, macrophages from non-obese mice expressed genes characteristic of M2 macrophages, such as arginase and IL-10. In obese mice, however, they presented lower expression of previous genes and higher expression of characteristic genes of M1 macrophages, such as TNF-α.[20] Based on these findings, obesity has been gaining a status of chronic inflammatory disease.[21]

Summarizing, obesity is characterized by a state of chronic low-level inflammation where the augmented adipose expansion causes adipose disfunction and increases the systemic levels of proinflammatory factors.[12] This factors are capable to release various types of adipokines, cytokines, chemokines and hormones[22,23] through which obesity influence metabolic and inflammatory responses in multiple tissues.[24] Henceforth, bone metabolism is influenced by obesity[25,26] increasing bone mineral density[27] and reducing bone remodeling.[28]

The orthodontic tooth movement involves the use of continuous, continuous interrupted or interrupted and intermittent forces.[29] As a result, these forces cause compression and tension zones in the periodontal ligament and alveolar bone, inducing morphological and microscopic reactions controlled by chemical mediators, and promoting tooth displacement by bone remodelin.[30,31] During this process periodontal ligament compression zones can lead to tissue necrosis (hyaline necrosis), the production of inflammatory mediators and the differentiation and activation of

osteoclast.[32,33] The removal of necrotic periodontal tissue by macrophages exposes the mineralized root surface to cementoclast activity resulting a orthodontically induced inflammatory root resorption,[30,33,34] which is considered a highly prevalent side effect in orthodontic treatment.[35] As related, through this process the periodontal ligament vascularity and blood flow is altered, which triggers the production and release cytokines, neurotransmitters, growth factors, colony-stimulating factors, and arachidonic acid metabolites,[36] altering bone metabolism.

That being the case, local and systemic factors affecting the rate of tooth movement, bone remodeling and root resorption during the application of orthodontic forces are one of the main challenges in orthodontic treatment. Such effects can be investigated in experimental rat models by induced movement (ITM) of the upper first molar,[37–39] and their effects could be extrapolated to humans.

The ITM of the upper first molar is evaluated at different experimental moments in order to analyze different responses over time. During the first days it is possible to assess the broad spectrum of periodontal phenomena of ITM extending from the first to 7-10 days. At 3 days of movement there is presence of hyaline areas in the periodontal ligament and slight distance to bone resorption on the pressure side. Periodontal fibers show slight stretching on the side under tension and no reabsorption areas are observed. At 5 days of movement, the observed phenomena are similar to those of the 3-day period, although more evident.[40] At 7 and 9 days of ITM, there are gaps without clastic cells and repaired by bone matrix neoformation on the pressure side. At 7 days, hyaline areas are found to be reduced and in a clear process of phagocytosis by macrophages observed in the pressure area.[40–42] Clastic cells appear in the Howship gaps of the alveolar bone cortex, on the periphery of the hyalinization

area.[33] This phenomenon characterizes a marked bone resorption at distance. At 9 days of movement, the microscopic findings show the most exuberant and well-delimited root resorption.[40]

Verna et.al.,[37] described that at the cervical level there was a steady decrease in alveolar bone fraction around the first and second molars, which became statistically significant after 7 and 14 days of ITM, and indicates greater activity of periodontal phenomena. On the other hand, the bone fraction in the apical area increased significantly around the second molar after 14 days where deposition activities replaced the previously resorbed alveolar bone. These results confirm findings of Tran Van et.al.[43] who state that around the 10th day there is a maximum peak of bone formation in osteoremodeling units (Bone Modelling Units – BMUs). Yokoya et al.,[44] analyzed tooth movement in rats under electronic and optical microscopy, and observed that the number of clasts increases until the seventh day of movement and then decreases until the 14th days.

Considering all these aspects, studies,[45–49] that interrelate systemic diseases and dentistry are arousing a growing interest. Studies relating obesity are reported in the literature, especially in the areas of periodontics,[50–52] temporomandibular disorders,[53] implantolology,[54] and cariology.[55] In the area of orthodontics, studies describe the relationship between obesity and bone maturity.[56,57] and craniofacial and dental development.[58–60]

Concerning orthodontic tooth movement, despite the literature relates that obese patients shows different craniofacial morphology[61] with a higher orthodontic treatment need in obese girls[62] very limited number of studies with conflicted results are described in the literature related to orthodontic treatment and obesity. Bremer et

al. in a pilot study reported slightly longer treatment for patients with increased body mass index.[63] Two clinical studies showed conflicting results, Jayachandran, et al.[64] reported decreased rates of tooth movement in obese patients, while, Saloom et al.[65] described higher rates. A recent study performed in mice showed that obesity attenuates the rates of orthodontic tooth movement by increased leptin levels via inhibition of osteoclastogenesis.[66] Hence, a recent systematic review concluded that the influence of body mass index over orthodontic tooth movement and related parameters remains debatable.[67] Furthermore, as related above, considering that obesity affects all age and socioeconomic groups in both developed and developing countries,[1] the quantity of patients with this condition on orthodontic practice is becoming more common. Thus, the knowledge of the impact of obesity over the orthodontic treatment is imperative.

Therefore, the purpose of this study was to analyze the impact of obesity over dental, periodontal and alveolar bone structures after induced tooth movement by means of microcomputed tomography and histological analyses.

2. Purpose

PURPOSE

The aim of the present study will be to evaluate, *in vivo*, the effect of obesity over:

- The amount of tooth movement and the morphology of the surrounding bone after the use induce tooth movement forces by means of computed microtomography;

- The number of cementoclast and osteoclasts through staining of the enzyme tartrate-resistant acid phosphatase;

- The characteristics of root and alveolar regions using hematoxylin and eosin staining and evaluated by conventional light microscopy.

3. Material and Methods

MATERIAL AND METHODS

This research was submitted and approved by the Ethics Committee for Animal Experimentation of the Campus of Ribeirão Preto of the University of São Paulo, Brazil (Process 2017.1.144.58.2). The experiment was conducted following the statements of the National Council of Animal Experimentation Control.

Animals

Forty male *Wistar rats (Rattus norvegicus, albinus*) with an average body weight of 125 g obtained from the central bioterium of the University of São Paulo, were used. The animals were housed in polypropylene cages (2 animals per cage) with a standard 12-hour light – dark cycle and a controlled temperature of 22 ± 1°C and with food and water provided *ad libitum*.

After their arrival, the animals had one week for acclimatization fed with shredded standard diet (SD) (Pragsoluções Biociências*,* Domeneghett & Corrêa LTDA., Jaú, Brazil).

Experimental design

The animals were randomly divided in two groups of 20 animals each, the non-obese (NO7 and NO14) and the non-obese (O7 and O14) groups. Following a split-mouth design, the left side of the maxillas of all animal received induced tooth movement (ITM = WM, with movement) for each experimental period, i.e. 7 or 14 days (OWM7, OWM14, NOWM7 and NOWM14 groups). The untreated right hemi-maxilla groups (O7, O14, NO7, NO14 groups) were also accessed. Forty hemi-maxillas were analyzed by computed microtomography and 40 by histological

analyses. To facilitate interpretation of the results, the hemi-maxillas were divided into subgroups:

1. NO7- hemi-maxillas of non-obese rats not submitted to ITM of 7 days;
2. O7- hemi-maxillas of obesity-induced rats not submitted to ITM of 7 days;
3. NOWM7- hemi-maxillas of non-obese rats submitted to ITM for 7 days;
4. OWM7- hemi-maxillas of obesity-induced rats submitted to ITM for 7 days;
5. NO14- hemi-maxillas of non-obese rats not submitted to ITM of 14 days;
6. O14- hemi-maxillas of obesity-induced rats not submitted to ITM of 14 days;
7. NOWM14- hemi-maxillas of non-obese rats submitted to ITM for 14 days; and
8. OWM14- hemi-maxillas of obesity-induced rats submitted to ITM for 14 days.

Obesity induction

The non-obese group received shredded standard rat chow diet, standard diet (SD), and, the obese group received shredded high-fat diet[68] (HFD) (Pragsoluções Biociências*,* Domeneghett & Corrêa LTDA., Jaú- Brazil). Bromatological analysis (Ribersolo, Ribeirão Preto, SP, Brazil) determined that SD contained 25 g of protein, 5 g of total fat, 6 g of fiber, per approximately 100 g of diet. HFD was composed of standard rat chow plus peanuts, milk chocolate, and sweet biscuits in a proportion of 3:2:2:1,[68] and contained 22 g of protein, 23 g of total fat, 6 g of fiber per approximately 100 g of diet. All animals were fed for 9 or 10 weeks considering the duration of the applied ITM (7 or 14 days).

Metabolic measurements and body composition

Body weights were recorded weekly. Glycemia levels were recorded before and after ITM induction aided by a test strip and checked using a blood glucose monitoring system OneTouch UltraMini® (Johnson & Johnson Medical S.A., China). Variation between those two measures (Var_GLi) was also calculated. After euthanasia, the quantity of retroperitoneal (RP-fat) and epididymal (EP-fat) fat was accessed. Additionally, insulin, leptin, total cholesterol, high-density lipoprotein (HDL) cholesterol, and triglycerides levels at serum were measured using commercial kits (Merck Millipore, St. Charles, USA and Labtest Diagnóstica S.A., São Paulo, Brazil). For the determination of fed and fasted blood glucose and plasma levels, blood was collected after an 8-hours fast. Two blinded researchers conducted the analysis immediately after blood collection and serum acquisition

Induction of tooth movement (ITM)

After 8 weeks, the animals were anesthetized by intramuscular injections with a mixture of ketamine (*Ketamina*, Agener União Química Farmacêutica Nacional S/A, São Paulo, Brazil) and xylazine (*Dosaper*, Calier, Barcelona, Spain) in a ratio of 1:2 respectively and 1 mL/kg body weight). The animals were immobilized with open mouth on a designed table. Stretched orthodontic nickel-titanium coiled springs (code 35.20.064, Morelli, Sorocaba, SP, Brazil) were ligated from the left maxillary first molar to the maxillary incisors by means of stainless steel ligatures.[69,70] This procedure aimed the mesial inclination of the first molar with an active force of 50 cN in a split mouth design.

After the ITM period completed the rats were euthanized by overdose of the mixture used for anesthesia mentioned previously and a CO_2 chamber. Maxilla from each animal was dissected and sectioned in hemi-maxillas.

Microcomputed tomography (micro-CT) analyses

Before scanning, the hemi-maxillas were fixed in 10% buffered neutral formalin for 24 hours and stored at room temperature in alcohol solution. The samples were scanned by a cone-bean micro-CT system (Skyscan 1172, Bruker, Kontich, Belgium), which X-ray generator operated at a source potential of 60 kV, beam current of 165 µm, amperage of 142 µA and an exposure time of 650 ms per projection. BMP images data were reconstructed by using the NRecon software Version 1.6.10.4 with a resolution of 8 µm.

The Data Viewer Software, version 1.4.0 (Bruker, Kontich, Belgium) generated 3 dimensional models and was used to standardize the position of all samples, following a modified criteria of a published protocol.[71,72] The protocol had the first (M1) and second (M2) maxillary molars as references: a) In the sagittal plane, the occlusal surface of the M1 was horizontally positioned and the axis (x) was situated crossing the maximal diameter of the mesial and disto-buccal roots of M1 at furcation level; b) In the coronal plane, the maxillary bone was vertically orientated with the roots of the M2 pointing up and the axis (z) positioned crossing the maximal diameter of the disto-buccal root of the M1; and, c) In the transaxial plane, the M2 had its axis vertically positioned, and the roots of M1 were completely separated at furcation level (y) (Figure 1). This standardized position was saved and preserved when the sample was opened in other software.

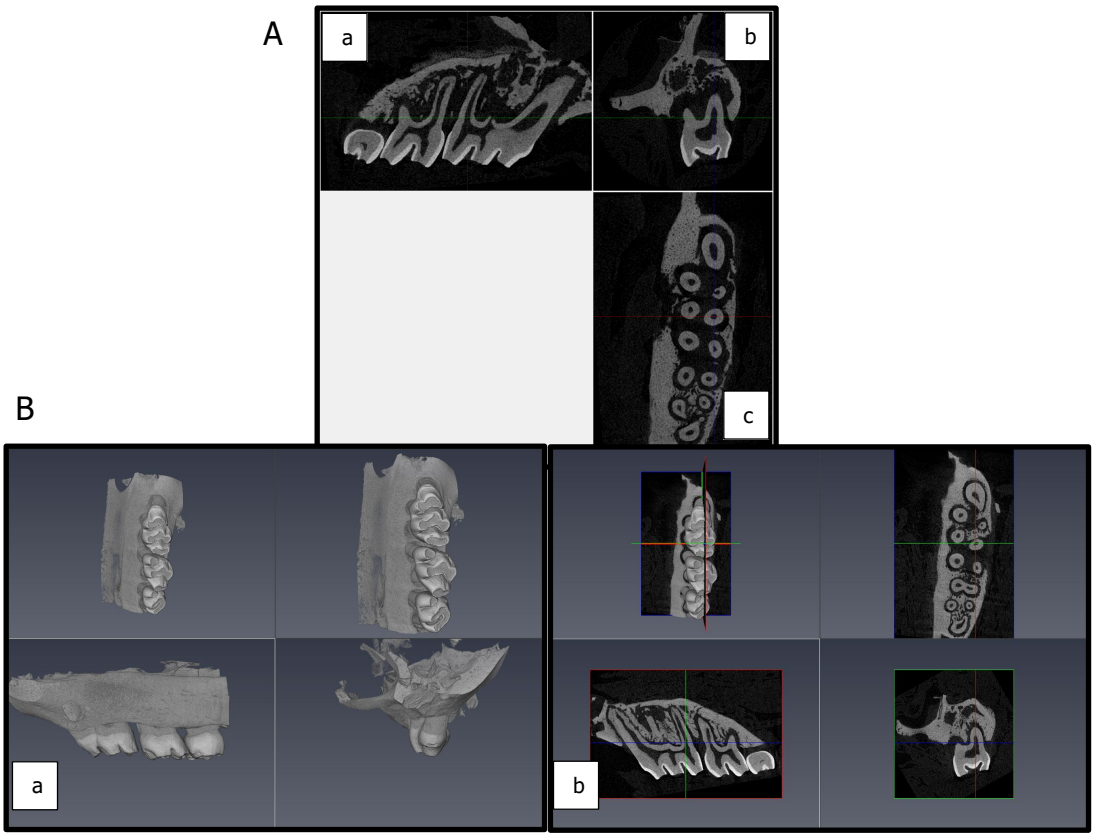

Figure 1: Standardized orientation of samples. (A) Visualization of the standardized position in Data viewer software: a) In the sagittal plane, the occlusal surface of the M1 was horizontally positioned and the axis (x) was situated crossing the maximal diameter of the mesial and disto-buccal roots of M1 at furcation level; b) In the coronal plane, the maxillary bone was vertically orientated with the roots of the M2 pointing up and the axis (z) positioned crossing the maximal diameter of the disto-buccal root of the M1; and, c) In the transaxial plane, the M2 had its axis vertically positioned, and the roots of M1 were completely separated at furcation level (y). (B) Standardized position visualized in Avizo Software a) Visualization before the Orthoviews function; b) Visualization after application the Orthoviews function.

The Avizo software (Visualization Sciences Group, Burlington, USA) reconstructed and underwent a colormap (65-255) global thresholding to extract the mineralized phase representing the 3D tooth movement and bone architecture to enable the analysis of angular and linear measurements characterizing the ITM and root volume. The final result was originated by the average of 3 measurements. CT-Analyzer software, version 1.13.5.1+ (Bruker, Kontich, Belgium) was used to analyze bone characteristics. For this analysis, the volume

of interest (VOI) was defined to include the alveolar bone of the diastema between M1 and M2, and all the area of M1, including crown and roots (Figure 2).

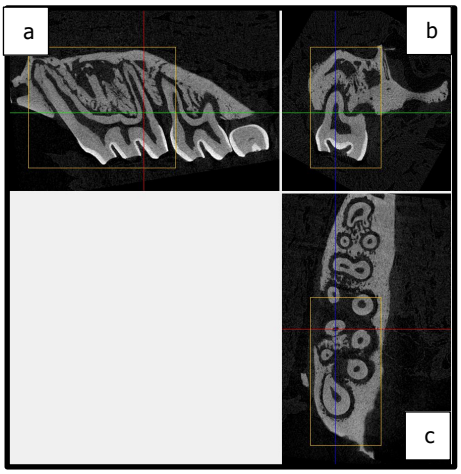

Figure 2: Selection of Volume of Interest (VOI) in 1M - CtAn software. a) Visualization of VOI in the sagittal plane; b) Visualization of VOI in the coronal plane; and, c) Visualization of VOI in the transaxial plane.

As the tension and compression sites of M1 were assessed, two cubes regions of 295x295µm and 50 slices were outlined as the region of interest (ROI) (Figure 3). To evaluate the tension site, the cube was located at the distal and medial part of the mesial root, starting at the top of the alveolar bone at furcation level. As for the compression site, the cube was localized at the mesial and median part of the disto-buccal root, also starting at the top of the alveolar bone at furcation level. The furcation level was used for being a reproducible morphological area. The VOI were constructed in all images of the coronal dataset. The images were binarized so that bone and dental structures could be distinguished according to differences in the density using a greyscale (inferior limit-65, superior limit-255; greyscale threshold 0-255). All measurements are described on figures 4, 5 and 6, and were performed by a blind, trained and calibrated researcher (ICC= 0.96).

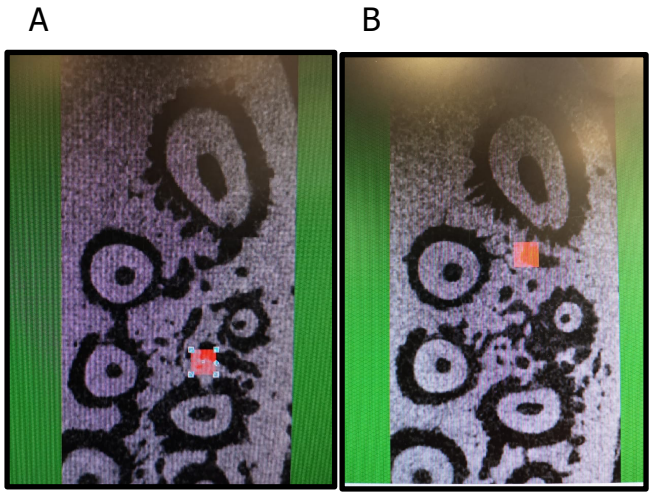

Figure 3: S Figure 11: Determination of ROI to assess alveolar bone - CtAn software. (A): compression side; (B) Tension side.

Histological process

Hemi-maxilla's tissues were collected and fixed in 10% formalin for 24 hours, then demineralized in 23% EDTA, dehydrated, clarified and embedded in paraffin. The transverse cutting method was employed to get serial sections at a thickness of 5 µm. Selected sections were deparaffinized and stained with hematoxylin and eosin. The presence or absent of focal hyalinization (FH), frontal bone resorption (FBR) and root resorption (RR) that included active root resorption and repaired root resorption at cementum (ARRC and RRRC, respectively) and at dentin level (ARRD and RRRD, respectively), and at cervical and medial thirds of the mesial and disto-buccal roots were evaluated. Two blinded examiners performed this evaluation (kappa: 0.81). Tartrate-resistant acid phosphatase staining (TRAP) test was performed using a leukocyte acid phosphatase kit (Sigma-Aldrich Corporation, Saint Louis, EUA) according to the manufacturer's protocol, and, counterstained with hematoxylin. TRAP-positive multinucleated (> 3 nuclei) cells that attached to the alveolar bone surface and cementum surfaces of the

mesial and disto- buccal roots were counted by one blind researcher. The analyses were performed in an Axio Imager microscope (Zeiss, Oberkochen, Germany).

Statistical analysis

SPSS statistical (version 16.0, SPSS, Chicago, USA) and Stata13 (version 13, Stata CorpLP, Texas, USA) software were used to analyze the data. Shapiro-Wilk and Levene tests were employed to access data normality and dispersion of metabolic and body composition. Metabolic and body composition measurements were analyzed using t- test and one-way ANOVA followed by Bonferroni as post-test and Kruskal-Wallis test with Dunn's post-test.

The micro-CT measurements were analyzed by a mixed model for repeated measurement. For the analyses of frequencies at the histological assessment a non-parametric chi-square and Kruskal-Wallis were used. For TRAP result assessment a negative binomial regression model was used. A significance level was set at 95% (α=0.5).

4. Results

RESULTS

Only statistically significant differences are described.

The body weight was similar after one week of adaptation, i.e. at week 0 ($p=0.7$). However, a statistically significant increase was observed in obese groups since week 1 until the end of the experiment ($p<0.01$ and $p<0.001$). The variation in body weight showed statistically significant differences between the groups since week 1 until week 7 ($p<0.001$, $p<0.01$, $p<0.05$), but no differences were observed at week 8 and 9. When the sample was stratified according to ITM duration (7 and 14 days), body weight ($p=0.000$ and $p=0.002$), RP- Fat ($p=0.000$ and $p=0.044$), and EP- Fat ($p=0.001$ and $p=0.002$), showed statistically significant differences between the groups (Figure 4).

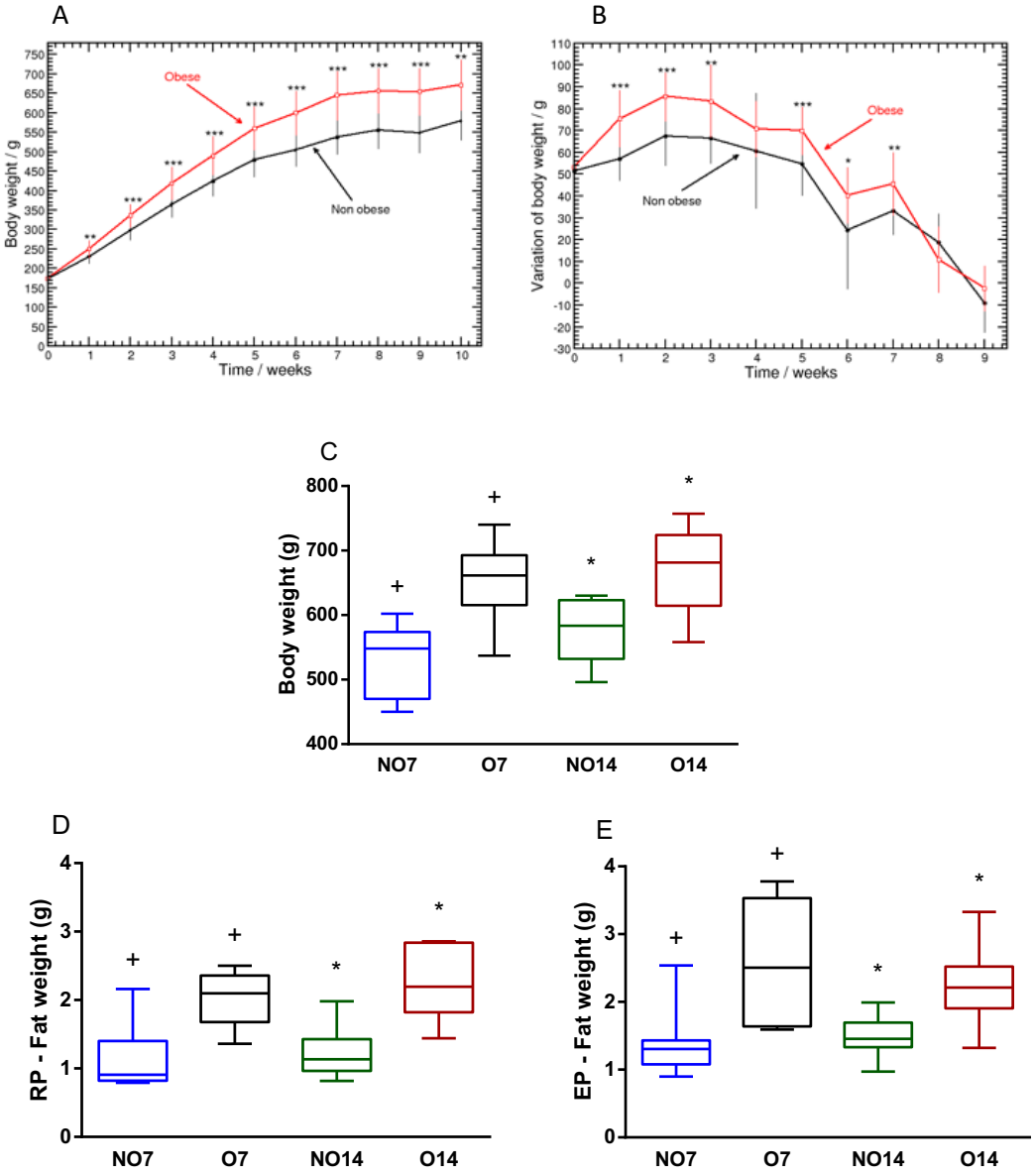

Figure 4: Metabolic measurements and body composition. (A) Body weight and (B) variation of body weight throughout the experiment analyzed for obese and non-obese animals. Data were reported as means, *p<0.05 **p<0.01 ***p<0.001. (C) Body weight, (D) RP- Fat and (E) EP- Fat, analyzed by groups according to the ITM duration (7 and 14 days). T test was used to access (A) and (B). (C) was analyzed with one-way ANOVA followed by Bonferroni. (D) and (E) were analyzed by Kruskal-Wallis test with Dunn's test for multiple comparison. Equal symbols mean statistically significant differences between the groups, p<0.05

The levels of glycemia variance and insulin were not statically different between the groups (p>0.05). However, leptin levels accessed showed statistically significant differences between the groups, (p=0.011 and p=0.025).

The lipid profile showed no differences between the groups (p>0.05) (Figure 5).

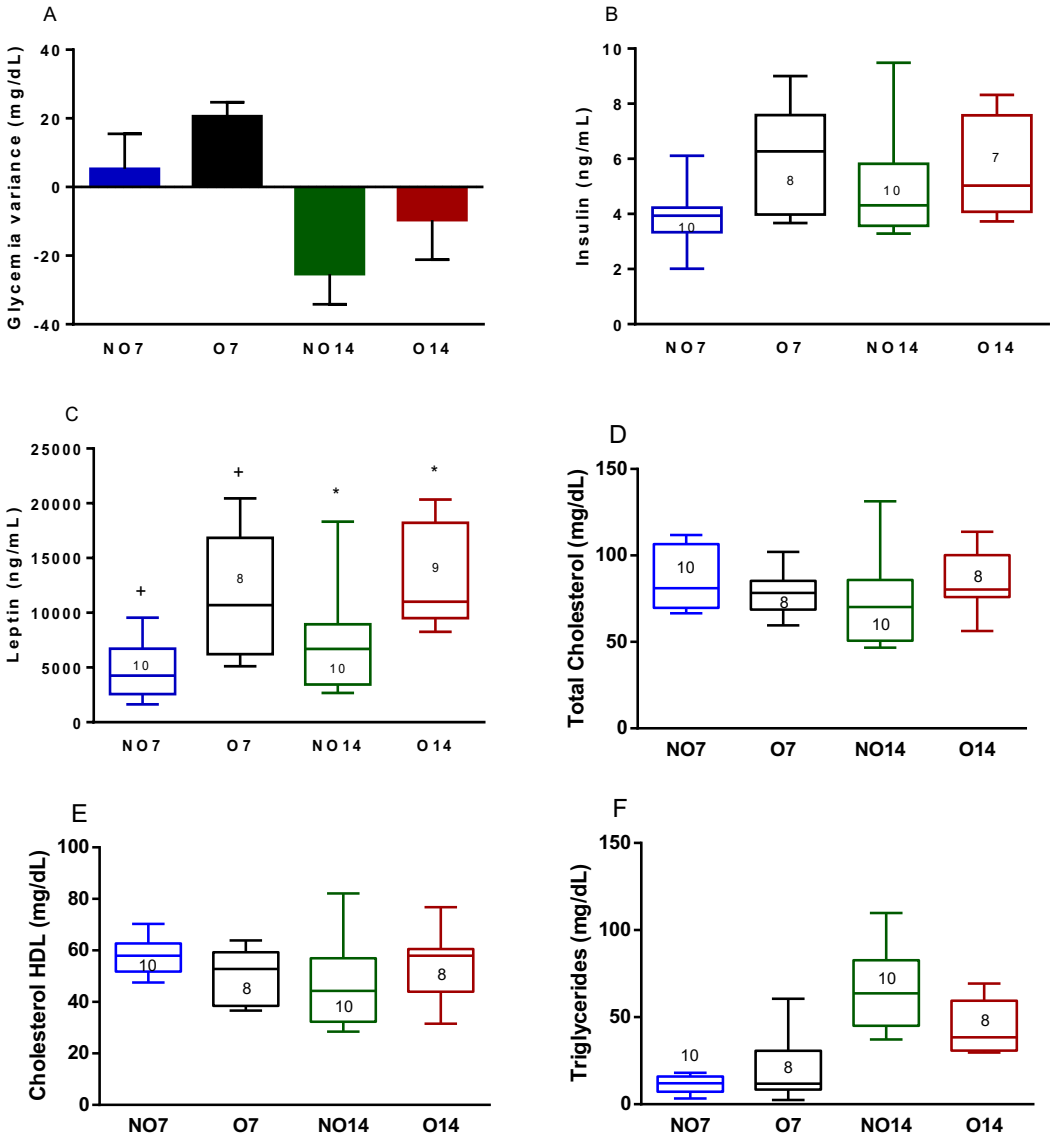

Figure 5: Metabolic measurements. (A) Glycemia variation, (B) Insulin, (C) Leptin, (D) Total Cholesterol, (E) Cholesterol HDL and (F) Triglycerides levels, accessed by groups according to the ITM duration (7 and 14 days). (A) and (F) were analyzed by Kruskal-Wallis test with Dunn's test for multiple comparison. (B), (C), (D) and (E) were analyzed with one-way ANOVA followed by Bonferroni. Equal symbols mean statistically significant differences between the groups, p<0.05. The total sample number (n) is indicated inside or above the column.

Micro-CT analyses

The obese group showed high rates of ITM at 7 days when compared to the non-obese group (p=0.081). This difference became more evident between the obese and non-obese groups after 14 days of ITM (p=0.000). Also, higher amounts of ITM were observed in groups with 14 days of ITM when compared to the groups of 7 days of ITM in obese (p=0.000) and non-obese (p=0.000) animals. Rood distance did not show any differences between the groups (p>0.05) (Figure 5, 7 and 8).

Regarding the angular measurements, obese animals submitted to 14 days of ITM presented significantly decreased disto-buccal (p=0.000) and mesial (p=0.033) root angles with respect to the occlusal plane when compared to the side without movement. Non-obese animals submitted to 7 days of ITM presented lower values of disto-buccal root angles (p=0.031), and after 14 days, lower angles of mesial root (p=0.043), both in respect to the furcation level plane and when compared to the side without movement (Figure 5, 7 and 8).

Analyzing the disto-buccal root volume, the obese animals submitted to ITM of 14 days, presented lower values compared to the non-obese animals although with a p value of 0.076 (Figure 6, 7 and 8).

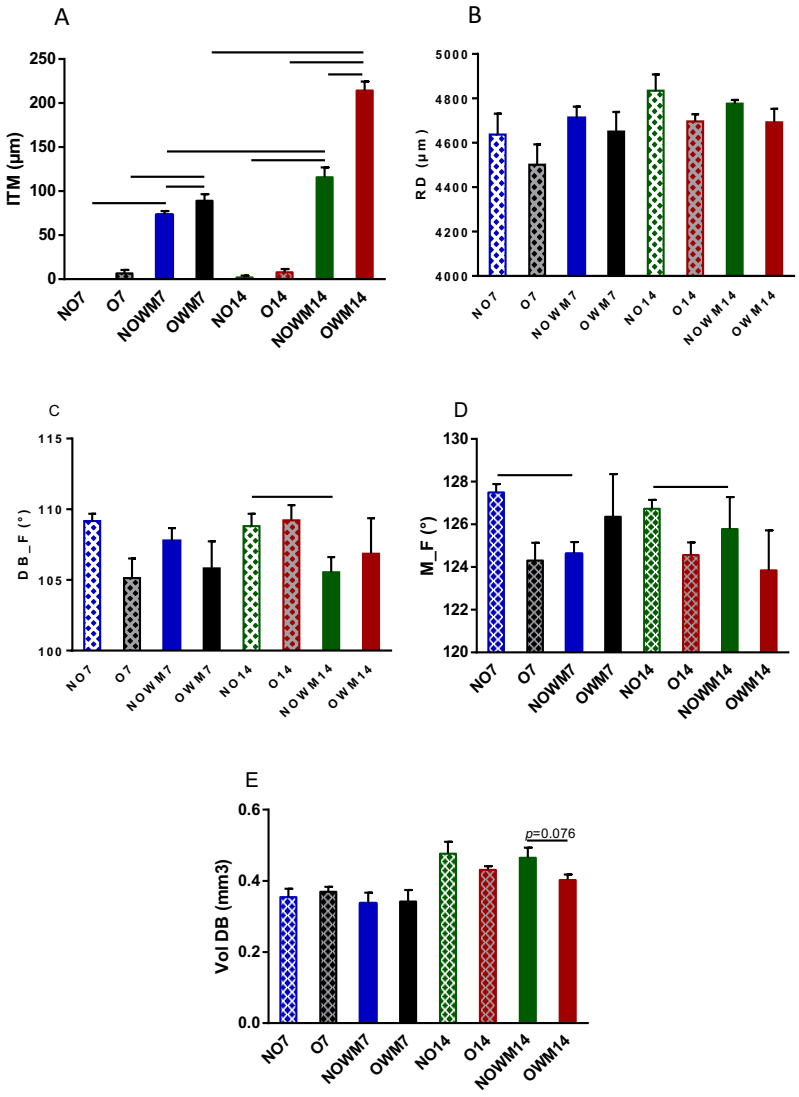

Figure 6: Linear, angular and volumetric measurements. (A) ITM (µm): distance between the nearest two landmark points of the most distal aspect of M1 and the most mesial aspect of M2 enamels; (B). RD (µm) linear distance between the apexes of the mesial roots of M1 and M2, in sagittal views; (C) DB_F (°): angle between the furcation plane (FP, plane passing through the furcation level of the M1) and the main axis of the disto-buccal root; (D) M_F (°): angle between the FP and the main axis of the mesial root; (E) Vol DB (μm^3): Volume of the disto-buccal root measured from the furcation level until the apex. This measurement was acquired using the semiautomatic thresholding. All measures were performed with Avizo software and compared with mixed model for repeated measurements. Line means statistically significant differences between the groups, $p<0.05$.

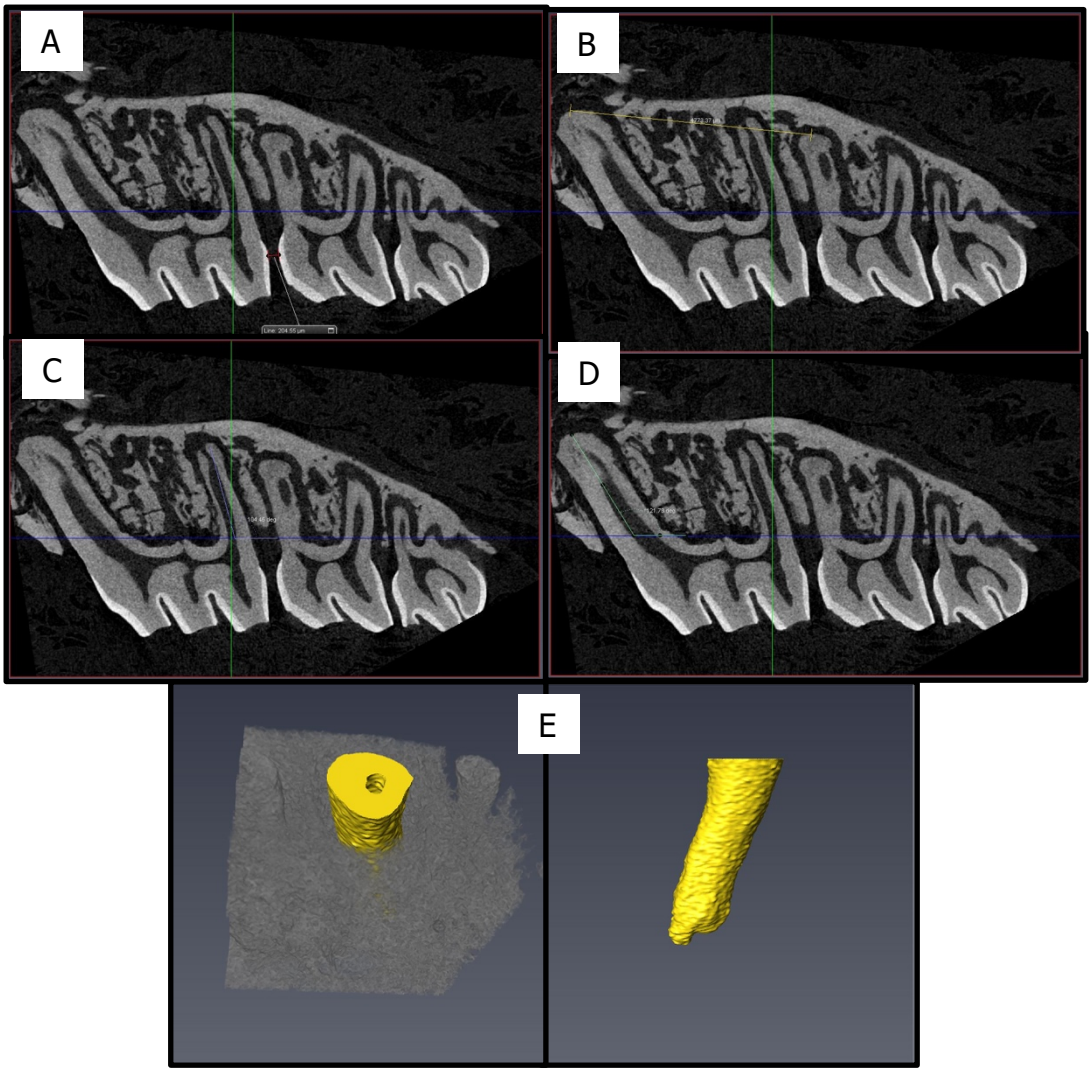

Figure 7: Linear, angular and volumetric measurements of an obese animal submitted to 14 days of ITM. (A) ITM (µm); (B). RD (µm); (C) DB_F (°); (D) M_F (°); and (E) Vol DB (μm^3).

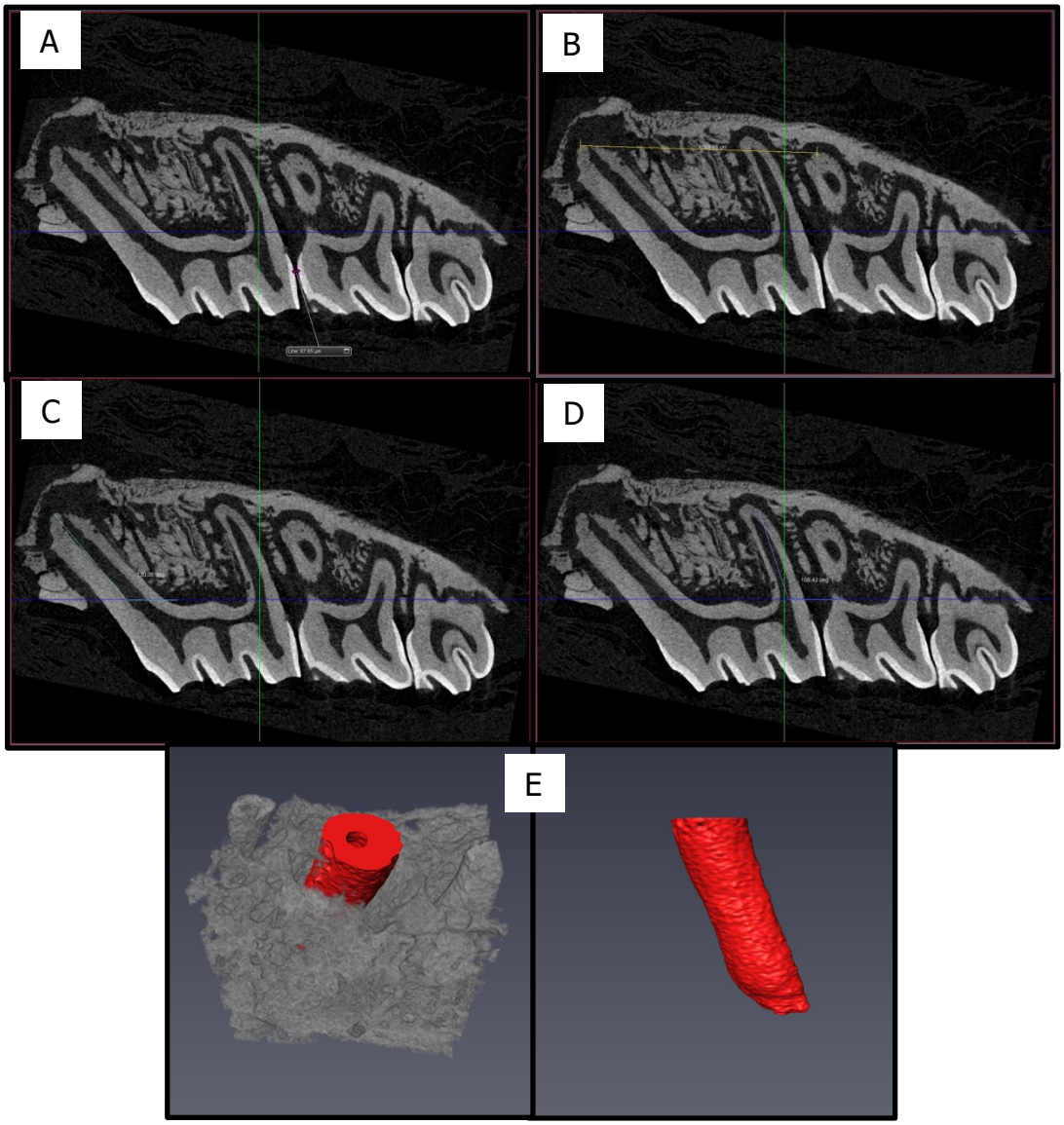

Figure 8: Linear, angular and volumetric measurements in an non- obese animal submitted to 14 days of ITM. (A) ITM (µm); (B). RD (µm); (C) DB_F (°); (D) M_F (°);(E) Vol DB (μm^3).

Bone characteristics presented various differences between the groups (Figure 9 and 10).

In the analysis of bone volume, the obese animals had low values at compression and tension (p=0.009 and p=0.002) sites after 7 days of ITM when

compared to the side without movement. Obese animals submitted to ITM showed higher values after 14 days at compression and tension sites (p=0.043 and p=0.006) than after 7 days of ITM. At tension site non-obese animals presented lower values after 7 and 14 days (p=0.013 and p=0.008) of ITM when compared to the side without movement.

The percent bone volume over total volume values were increased when comparing the obese group submitted to ITM of 7 to the ones of 14 days at compression and tension sites (p=0.048 and p=0.007). Also, reduced values were found in the obese group after 7 days of ITM at compression and tension sites (p=0.011 and p=0.002) when compared to the side without movement. At tension site, reduced values were showed in the non-obese group after being submitted to ITM of 7 and 14 days (p= 0.014 and p=0.008) in comparison to the side without movement.

Bone surface density values at compression site were reduced at the obese compared to non-obese animals submitted to ITM of 7 (p=0.027) and 14 days (p=0.050). Additionally, in the obese group after 7 days of ITM reduced values were observed at compression and tension sites (p=0.002 and p=0.004) when compared to the side without movement. Same results were presented in non-obese animals at tension site (p=0.029).

The trabecular thickness values in the obese group after 14 days of ITM at compression and tension sites (p=0.000 and p=0.010) were reduced in comparation to the side without movement, and after 7 days at compression site (p=0.001). Same reduction was found in the non-obese group with ITM of 14

days at compression and tension sites (p=0.003 and p=0.099) when compared to the side without movement. Lower values were seen when obese and non-obese animals submitted to ITM of 7 days were compared to animals with ITM of 14 days at tension and compression sites (obese, p=0.000 and p=0.001, and non-obese animals, p=0.000 and p=0.000). Hemi-maxillas of obese and non-obese animals not submitted to ITM presented higher values after 14 days at than at 7 days at compression (obese, p=0.000; non-obese, p=0.000) and tension sites (obese, p=0.000, and non-obese, p= 0.000)

The trabecular number was reduced in obese animals submitted to ITM of 7 days compared to the non-obese group at compression site (p=0.002). After 7 days of ITM obese animals presented reduced values at tension and compression sites (p=0.000 and p=0.033). in comparation with the side without movement. Non-obese animals presented this reduction at tension site (p=0.000). Obese and non-obese animals not submitted to ITM presented lower values after 14 days at compression (obese, p=0.008; non-obese, p=0.005) and tension sites (obese, p=0.000; non-obese, p= 0.001) when compared to the values of 7 days.

The trabecular separation values were increased in obese animals compared with the non-obese group after ITM of 7 days at compression site (p=0.000). Also, obese animals after 7 days of ITM presented high values at tension and compression sites (p=0.000 and p=0.002) when compared to the side without movement. This increase was observed at tension site in non-obese animals submitted to 7 (p=0.015) and 14 days of ITM (p=0.018) when comparing sides with and without movement. While a reduction of the value was observed

when compared obese animals submitted to ITM of 7 and 14 days at compression (p=0.000) and tension sites (p=0.044).

The total porosity showed high values in obese animals compared to the non-obese ones after 7 days of ITM at the compression site (p=0.027). At the same period increased values in obese animals were observed at tension and compression sites (p=0.001 and p=0.032), but only in tension side for non-obese animals (p=0.031) when comparing sides with and without movement. After 14 days of ITM, at the tension side increased values were observed for obese (p=0.037) and non-obese animals (p=0.029) when comparing sides with and without movement. The obese animals submitted to 14 days of ITM presented reduced values compared to 7 days of ITM at compression and tension sites (p=0.001 and p= 0.034). Obese animals not submitted to ITM presented lower values after 14 days at tension side (O7 vs O14, p=0.031).

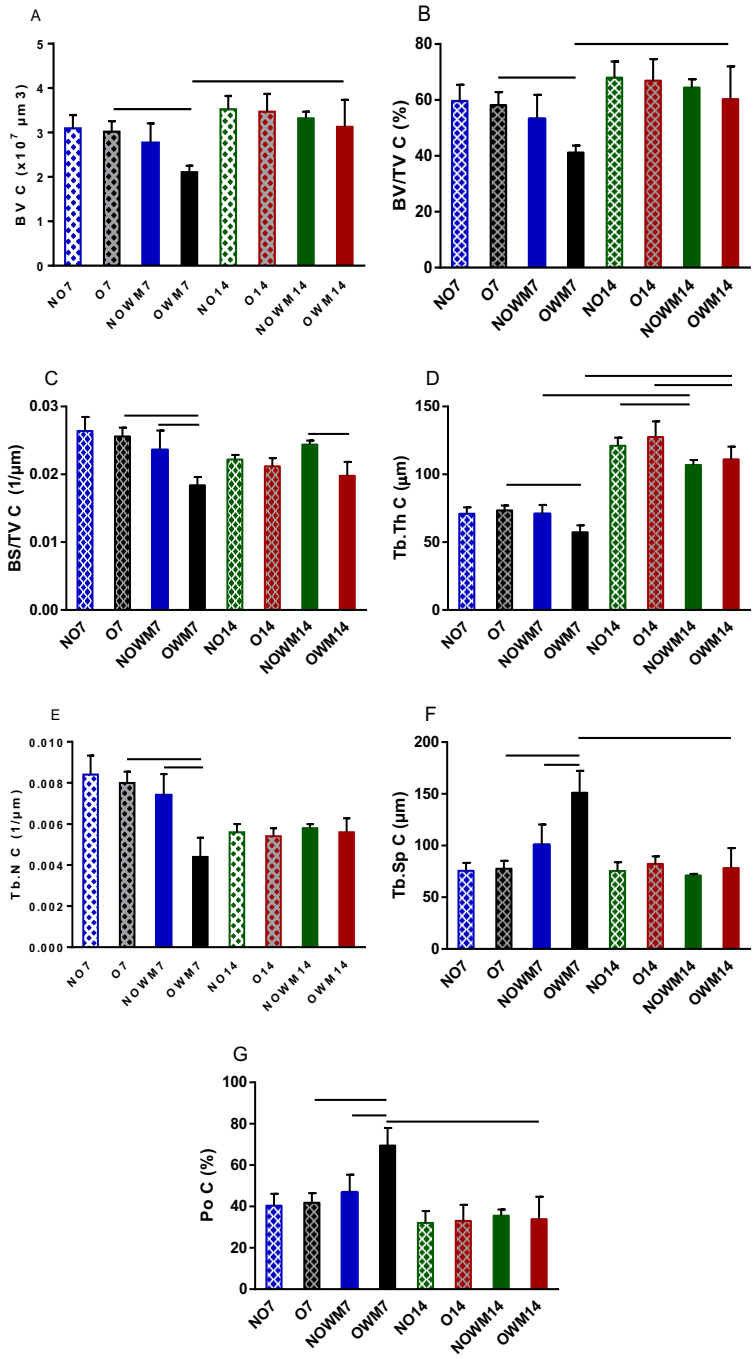

Figure 9: Bone characteristics of the compression site. (A) BV C (µm^3): Bone volume, volume of the region segmented as bone at compression side; (B) BV/TV C (%): bone volume fraction, percent of the segmented bone volume to the total volume of the region of interest at compression side; (C) BS/TV C (1/µm): Bone surface density, ratio of the segmented bone surface to the total volume of the region of interest at compression side; (D) Tb.Th C (µm): Trabecular thickness, mean thickness of trabeculae at compression side; (E) Tb.N C (1/µm): Trabecular number, average number of trabeculae per unit length at compression side; (F) Tb.Sp C (µm): Trabecular separation, mean distance between trabeculae at compression side; (G) Po C (%): Total porosity of the compression side. Measures were performed with Avizo software and compared with a mixed model for repeated measurements. Line means statistically significant differences between the groups, $p<0.05$.

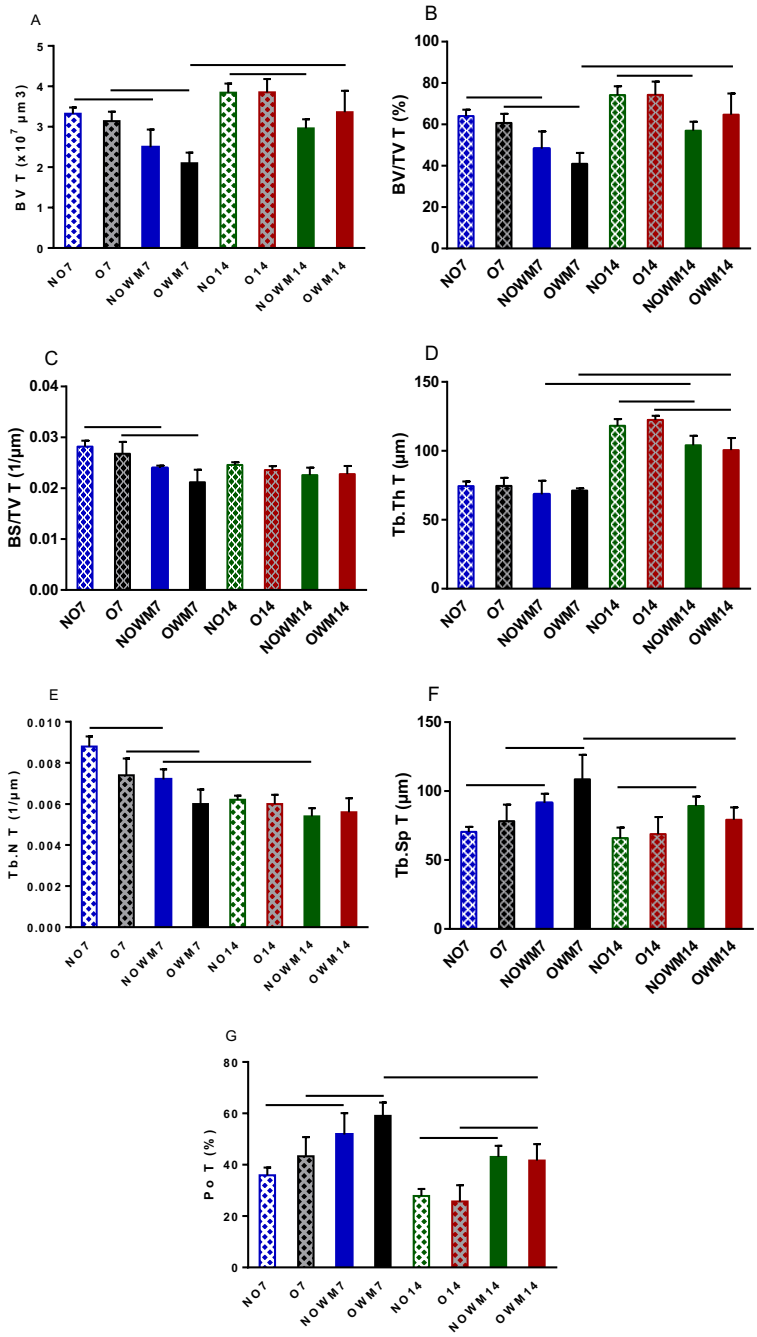

Figure 10: Bone characteristics of the tension site. (A) BV T (µm^3): Bone volume, volume of the region segmented as bone at tension side; (B) BV/TV T (%): bone volume fraction, percent of the segmented bone volume to the total volume of the region of interest at tension side; (C) BS/TV T (1/µm): Bone surface, ratio of the segmented bone surface to the total volume of the region of interest at tension side; (D) Tb.Th T (µm): Trabecular thickness, mean thickness of trabeculae at tension side; (E) Tb.N T (1/µm): Trabecular number, average number of trabeculae per unit length at tension side; (F) Tb.Sp T (µm): Trabecular separation, mean distance between trabeculae at tension side. Measures were performed with Avizo software and compared with a mixed model for repeated measurements. Line means statistically significant differences between the groups, $p<0.05$.

Histological analysis

The analysis of the sections colored with hematoxylin and eosin revealed low frequency levels of segmental hyalinization at the disto-buccal root after 7 days of ITM in obese and non-obese groups, additionally to non-obese group with no ITM.

Statistically significant differences were found when accessed frontal bone resorption. In both, mesial and disto-buccal roots, the highest frequency was found in obese animals submitted to ITM of 7 days followed by non-obese animals at the same period. Low frequency at were seem at obese and non-obese animals submitted to ITM of 14 days.

It was observed statically significant differences in the frequency of root resorption in both mesial and disto-buccal roots. Medium and low frequencies of active root resorptions at cementum and dentin respectively were observed in obese animals submitted to 7 days of ITM, whereas low frequencies were seen in non-obese animals with 7 days of ITM. Low frequency of active root resorption at cementum level (ARRC) was found in non-obese animals submitted and not submitted to ITM. At 7 and 14 days of ITM low and medium frequencies of active root resorption at dentin level (ARRD) were observed in obese animals. Low frequency level of repaired root resorption at cementum (RRRC) level was observed in obese animals not submitted to ITM at 7 days and low frequency in the non-obese group at 14 days.

High frequency of repaired root resorption at dentin level (RRRD) was observed in mesial root and medium frequency at disto-buccal root of obese animals submitted to 14 days of ITM. Non-obese animals submitted to 14 days

of ITM presented medium frequency of RRRD at the distobuccal root. In the same period animals not submitted to ITM presented low frequency of RRRD only in the mesial root, while obese animals presented this frequency in mesial and disto-buccal roots (Table 1 and Figure 11).

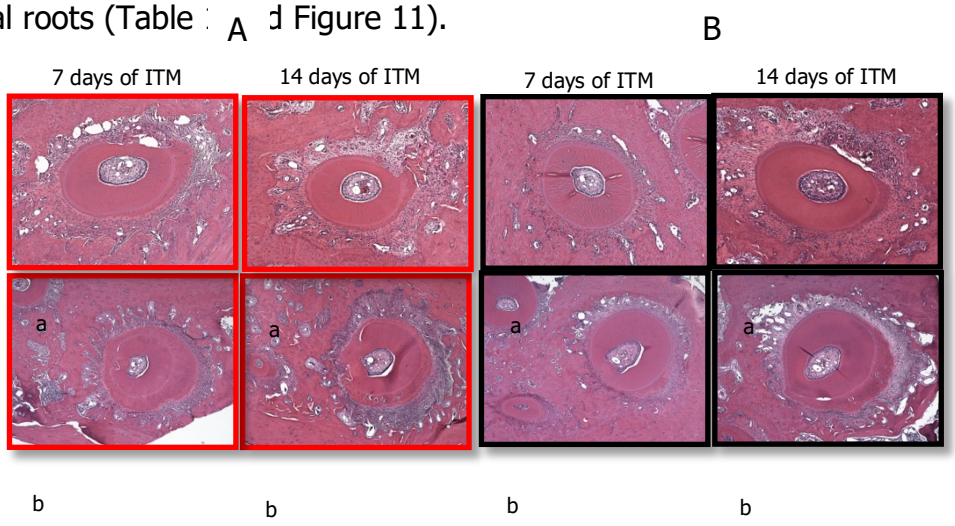

Figure 11: Histological analysis of ITM through. (A) Obese animals; (B) Non-obese animals; (a) Disto-buccal roots; and (b) Mesial roots

TRAP staining showed higher number of cementoclast in all groups submitted to ITM when compared to their corresponding group not submitted to ITM (non-obese 7 days, p=0.003; obese 7 days, p=0.001; non-obese 14 days, p= 0.002).

After 7 days of ITM obese animals presented higher number of osteoclast when compared to the non-obese animals (p=0.043). Higher number of osteoclasts were observed after 7 days of ITM in obese (p=0.022) and non-obese groups (p=0.001) when compared to the sides without movement. Obese animals submitted to ITM 14 days presented lower numbers of osteoclast compared to the ones submitted to ITM 7 days (p=0.004) (Figure 12).

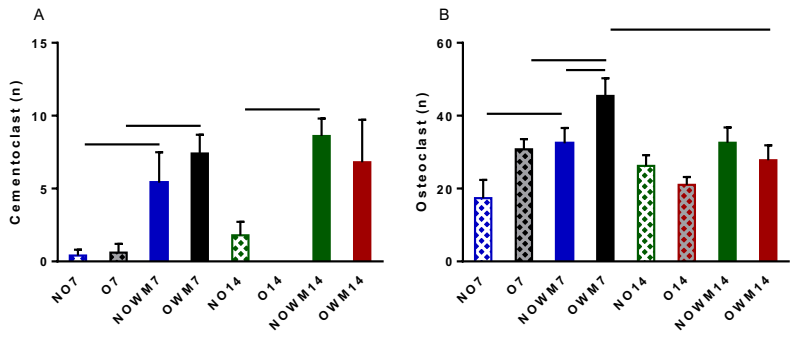

Figure 12: TRAP staining for cementoclast and osteoclast counting. Comparations were performed with a negative binomial regression model. Line means statistically significant differences between the groups, $p<0.05$

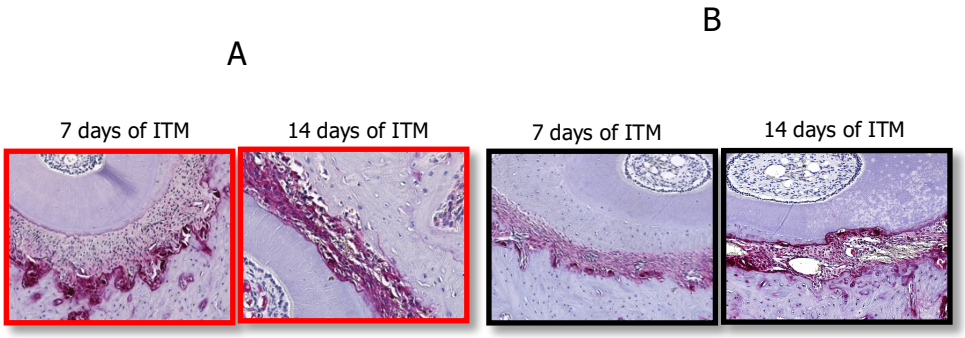

Figure 13: TRAP staining for cementoclast and osteoclast counting after ITM. (A) Obese animal (B) Non-obese animal

Table 1: Frequencies of focal hyalinization (FH), frontal bone resorption (FBR) and root resorption (RR), in mesial and disto-buccal roots (M and DB respectively) evaluated at sections stained with hematoxylin and eosin.

GROUP	FH-M	FBR-M	RR-M (%) *				FH-DB	FBR-DB	RR-DB (%) *			
	(%)	(%) *	ARRC	ARRD	RRRC	RRRD	(%)	(%) *	ARRC	ARRD	RRRC	RRRD
NO7	0	0	10	0	0	0	10	20	0	0	0	0
O7	0	0	0	0	10	0	0	0	0	0	20	0
NOWM7	0	70	10	20	0	0	20	70	40	20	0	0
OWM7	0	85	50	20	20	0	20	90	30	50	0	0
NO14	0	0	0	0	0	10	0	0	0	10	20	0
O14	0	0	10	0	0	20	0	0	0	10	0	10
NOWM14	0	20	20	0	10	0	0	20	0	20	0	40
OWM14	0	40	0	20	0	80	0	40	0	40	0	60

Data are presented as percentage (%). Fisher's exact with differences between groups same variable *$p<0.001$. FH-M: focal hyalinization at mesial root; FBR-M: frontal bone resorption at mesial root; RR-M root resorption at mesial root; RR-M root resorption at disto-buccal root; ARRC: active root resorption at cementum level; ARRD: active root resorption at cementum level; RRRC: repaired root resorption at cementum; RRRD: repaired root resorption at cementum.

4. Discussion

DISCUSSION

Obesity, a harmed global epidemic,[1,2,8,9] is characterized by a state of chronic low-level inflammation[12] that influence bone metabolism.[25,26] As establish, remodeling of the connective tissue and alveolar bone is essential to orthodontic tooth movement process.[73] However, the knowledge of the impact of the obesity over orthodontic tooth movement remains inconclusive.

Therefore, this prospective, randomized and controlled animal model study aimed the evaluation of dental, periodontal and bone responses after induced tooth movement in obese male rats. The *Wistar* rat strain is widely used in studies of induced tooth movement and metabolic disorders, and therefore considered an appropriate model with some translational potential.[74,75] Only male animals were evaluated considering that sexual hormones can influence lipid profile and glycose metabolism.[76] Furthermore, young animals were chosen in order to observe the systemic effects of the HFD throughout its growth and development phase, hence finishing the ITM when the rats have reached sexual maturity.[77]

Literature describes that HFD supplied for 12 weeks[68] or HFD with 50% of fat[78,79] are able to induce obesity with hyperlipidemia or metabolic syndrome. However, following the objective of this study the HFD used had 23g of fat and was supplied for only 8 weeks before the ITM induction, which resulted in obesity induction with no hyperlipidemia or glycose alterations.

Furthermore, the HFD[68] has been provided with shredded consistency from the first week of the experiment to ensure the amount of food intake after the ITM device was installed while also preserving the installation intact. The ITM device impacted on the animals' weight showing a decreased variation since it was installed, however this

impact was similar for obese and non-obese animals. Nevertheless, the effectiveness of obesity induction was constant from the first week until the end of the experiment. This effectiveness is reflected not only on the increased amounts of body weight but also on the significantly higher amounts of retroperitoneal and epididymal fat.

The ITM design, i.e. mesial maxillary first molar induced tooth movement (50cN force), was chosen as it is the most common and widely used.[80,81] All orthodontic forces were applied during the whole-day period with the ITM device installed early in the morning considering that diurnal rhythms in bone metabolism have important implications over orthodontic treatment.[38] Studies[38,82,83] demonstrated that forces applied in molars of *Wistar* rats during the whole-day and light-period (7 am to 7 pm) showed increased rates of tooth movement, new bone formation at tension site, more osteoclast activity at compression site and more area of root resorption when compared to forces applied during the dark-period (7 pm to 7 am).

Obese animals showed higher rates of tooth movement than the non-obese animals after 7 days of ITM. This difference became more evident at 14 days of ITM. Our findings are consistent with another research[65] performed in obese patients. The research mentioned was performed comparing the response to orthodontic treatment of obese and normal-weight patients. The outcomes revealed that there were no differences in time taken to achieve the competition of tooth alignment. However, after one week of orthodontic treatment, tooth displacement increased in obese patients, furthermore, after cofounders adjustment, obese patients had significantly higher rates of tooth movement throughout the alignment period. In addition, a proinflammatory state in the gingival tissues was observed prior treatment, which was associated with

faster initial tooth movement, suggesting that obesity may significantly affect oral tissues and the response to orthodontic treatment.

On the other hand, our results in terms of tooth movement conflicts with two other studies that reported lower tooth movement in obese groups.[64,66]

Jayachandran et al.[64] tested the correlation between salivary levels of leptin and orthodontic tooth movement in over-weight and normal-weight female patients. Leptin levels were evaluated immediately after force application, 1 hour, and at 1 month. After one hour, the leptin levels were significantly higher, but after one month the leptin levels decreased lower than baseline values. The mean rate of tooth movement measured after 3 months of orthodontic treatment was lower in over-weight. The authors found a positive correlation between leptin levels and the rate of tooth movement application, where low values of leptin levels correlate with low levels of tooth movement.

Some important differences on the experimental design are seen between Jayachandran's et al.[64] research and the present study. First, we performed the experiment in male rats which despite the translational potential of our results to humans, they may be different from a patient assessment. The authors referred hyperleptinemia in female patients but we assessed only male animals. Perhaps this difference could be explained by different responses to hyperleptinemia seen in woman and men, where strong positive correlation between hyperleptinemia and bone structure are showed by women, but it seems to have a weaker effect in men.[84] Furthermore, trabecular bone is described as being regulated by estrogen, therefore, different responses may also be seen.[85] Additionally, we assessed the induce tooth movement after 7 and 14 days, while they assessed the orthodontic tooth movement

after 3 months, then different periods of time evaluated could explain different responses. However, we agree with the authors finding that described positive correlation between leptin levels and the rate of tooth movement application, because our animals had high levels of leptin and high levels of rates of tooth movement.

Yan et.al.[11] performed a research in obese-induce mice where the left maxillary first molar more moved mesially with an orthodontic force of 30 g for 3, 5 and 7 days. The amounts of tooth movement was decreased at 3 and 7 days of force application measured in micro-CT and photographs. The TRAP staining showed lower amount of osteoclast in the obese group after 3 and 7 days. No differences were observed at 5 days. In a second essay testing the effect of leptin in osteoclastogenesis, the authors found that leptin could directly inhibit osteoclasts generation and function. Our findings conflicting with the study of Yan et. al.,[66] this differences may be explained by differences on the type of animal used and different forces applied, since the authors used mice and applied lower forces than the forces we used.

The expected low angular measurements of the disto-buccal and mesial roots after ITM in both groups show the efficacy of the ITM model used. However, these angular measurements and root distance differences could not be observed between obese and non-obese groups. Perhaps if the sample size were increased we could see this difference because the graphics shows a tendency to lower angles in the obese groups.

ITM applied for 7 days had an impact on bone morphology of obese animals at the compression and tension sites. These animals presented lower values of bone volume, percentage of bone volume and reduction bone surface density. The reduced bone volume values are consistent with lower trabecular number and thickness and

high trabecular separation values. All these findings are accompanied by higher levels of total porosity. Similar results seemed in non-obese groups, however only at the tension site. When comparing the results of obese and non-obese animals, significant low values of bone surface density, trabecular number and high values of trabecular separation and total porosity at the compression site were found. These findings show that ITM applied for 7 days had more impact on bone morphology of obese animals evaluated at the compression site. The results were expected because as already described this is a predominantly resorptive period.[86]

Comparing the results between 7 and 14 days of ITM, at 14 days a repair phase can be observed in obese animals with higher amounts of bone volume, percentage of bone volume, trabecular thickness, and reduced trabecular separation and total porosity mainly at the compression site. While the non-obese group presented only increased trabecular thickness. These results show that, although the bone morphology of the obese group was more compromised at 7 days of ITM, the metabolism of these animals made them able to reach a repair phase, where their bone morphology was similar to the bone morphology of non-obese animals at 14 days. TRAP staining confirms this finding, showing a significantly fewer osteoclast in obese animals after 14 days of ITM compared to the group of 7 days of ITM.

The BV, BV/TV (%), Tb.Th and Tb.Sp, increased in the groups of obese animals with ITM of 14 days compared to obese animals with 7 days of ITM, indicates that the forces induced to bone formation with a consequent reduction of the total porosity. This bone formation was also described by other authors assessing the animals without any systemic condition.[87]

As there is no research evaluating data in obese animals using micro-CT for bone morphology evaluation, we will compare our results with researches in non-obese animals. Similar to our results, other authors[88,89] described that in animals submitted to 7 days of ITM showed no differences in bone volume fraction, trabecular number, thickness and separation. Animals submitted to ITM for 14 days showed at compression sites slight decreased bone volume fractions,[88,89] while others showed increased bone fractions with decreased number of trabecular separation.[87,90] Our results did not show any difference in bone volume fraction at 14 days. These differences may be due to the different locations of the ROI evaluated by the micro-CT analyses, additionally to the differences in on the sample's strain, age, and sex related bone metabolism.

Hematoxylin-eosin stained sections of obese and non-obese animals after being submitted to 7 days of ITM presented low frequency of segmental hyalinization, high frequency of frontal bone resorption, medium and low frequencies of active root resorption. However, consistent with the results of the micro-CT and TRAP staining, at 14 days, obese animals presented cementum and dentin repaired with high and medium frequencies, while the non-obese animals had medium frequencies.

When assessing the volume of the disto-buccal roots, no difference was observed between obese and non-obese animals after 7 days of ITM. However, on day 14, the obese animals showed a tendency to reduced volume of disto-buccal roots. Perhaps if the sample size would be increased a significant difference between the groups might be seem. This similarity was confirmed by TRAP staining, which showed that although the number of cementoclast increased in both groups after undergoing ITM, this amount was similar when compared the obese and non-obese animals in

both periods. Another research performed the evaluation of the volume of the root, finding that root resorption volume of the mesial root increased significantly after 7 days of orthodontic tooth movemet.[87] This difference with our results may be explained by the different root assessed additionally to different strain and age of the animals. Ru et. al.[87] used Sprague Dawley rats of 10-week-old with 10 g of orthodontic force applied to the mesial root which may receive higher amounts of force than the disto-buccal roots that we assess, then difference responses are expected.

Therefore, the present study showed by means of microtomography that obese presented higher rates of tooth movement in the early periods of induced tooth movement confirmed by the higher number of active osteoclasts observed by TRAP and higher frequency of frontal bone resorption by hismorphometric evaluation.

The results presented highlight potential implications of the obesity condition over bone morphology and tooth movement after orthodontic treatment, area that need to be more explore with different laboratorial assessment and clinical trials.

5. Conclusion

CONCLUSION

The obesity showed effect on dental, periodontal and bone structures, reflected on:

- High rates of tooth movement and different morphological bone responses;
- Higher number of osteoclasts while non-effect on the number of cementoclast;
- Different histological responses at periodontum, roots and bone structures.

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Annex

ANNEX A

Approval of the Project by the Animal Use Ethics Committe

UNIVERSIDADE DE SÃO PAULO
FACULDADE DE ODONTOLOGIA DE RIBEIRÃO PRETO
Comissão de Ética no Uso de Animais

Of. CEUA 106/2017

Ribeirão Preto, 13 de julho de 2017.

Ref. processo nº 2017.1.144.58.2

Senhora Pesquisadora,

Informamos que o projeto "Efeito da obesidade sobre o tecido ósseo e estruturas periodontais, durante movimentação ortodôntica: análise microtomográfica, imonohistoquímica e histológica" foi **aprovado *ad referendum*** da Comissão de Ética no uso de Animais da FORP, em 13/07/2017, emitindo o certificado anexo.

Informamos, também, que deverá ser entregue na Secretaria da CEUA, **até 12/02/2019** o **Relatório Final** contendo os resultados e/ou resumo do trabalho publicado.

Atenciosamente,

Prof. Dr. Michel Reis Messora
Vice-Coordenador no exercício da Coordenação
da Comissão de Ética no Uso de Animais

Ilma. Sra.
Profa. Dra. Mirian Aiko Nakane Matsumoto
Departamento de Clínica Infantil
desta Faculdade

./aafn

AVENIDA DO CAFÉ S/Nº - TEL: (16) 3315-0251/4123- FAX: (16) 3315-4102
14040-904 - RIBEIRÃO PRETO - SP- BRASIL

ANNEX B

Approval of the Project by the Animal Use Ethics Committee

UNIVERSIDADE DE SÃO PAULO
FACULDADE DE ODONTOLOGIA DE RIBEIRÃO PRETO
CEUA
COMISSÃO DE ÉTICA NO USO DE ANIMAIS

UNIVERSIDADE DE SÃO PAULO
FACULDADE DE ODONTOLOGIA DE RIBEIRÃO PRETO
COMISSÃO DE ÉTICA NO USO DE ANIMAIS

CERTIFICADO CEUA – FORP/USP

Certificamos que a proposta intitulada **"Efeito da obesidade sobre o tecido ósseo e estruturas periodontais, durante movimentação ortodôntica: análise microtomográfica, imonohistoquímica e histológica"**, registrada com o nº 2017.1.144.58.2, sob a responsabilidade da Profa. Dra. Mirian Aiko Nakane Matsumoto – que envolve a produção, manutenção ou utilização de animais pertencentes ao filo Chordata, subfilo Vertebrata (exceto humanos), para fins de pesquisa científica (ou ensino) – encontra-se de acordo com os preceitos da Lei nº 11.794, de 08 de outubro de 2008, do Decreto nº 6.899, de 15 de julho de 2009, e com as normas editadas pelo Conselho Nacional de Controle de Experimentação Animal (CONCEA), e foi APROVADO "ad referendum" da Comissão de Ética no Uso de Animais da Faculdade de Odontologia de Ribeirão Preto (CEUA/FORP) em 13/07/2017.

Finalidade	( ) Ensino (X) Pesquisa Científica
Vigência da Autorização	13/07/2017 a 12/01/2019
Espécie/Linhagem/Raça	Rato isogênico / Wistar
Nº de animais	40
Peso/Idade	45 - 50 g / 21 dias
Sexo	Masculino
Origem	Biotério Central do *Campus* USP – Ribeirão Preto

Ribeirão Preto, 13 de julho de 2017.

Prof. Dr. Michel Reis Messora
Vice-Coordenador no exercício da Coordenação
da Comissão de Ética no Uso de Animais

CEUA - FORP/USP