

## **Orthodontic tooth movement in obese rats: preliminary histoenzymological results**

### **Movimentação dentária ortodôntica em ratos obesos: um estudo preliminar**

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#### **Ana Beatriz Campione Colombari**

Dentist

Multiprofessional Residency in Cancer Care - Dentistry area  
Clinical Hospital, School of Medicine of Ribeirão Preto, University of São Paulo  
R. Ten. Catão Roxo, 3900 - Vila Monte Alegre, Ribeirão Preto - SP  
E-mail: abccolombari@gmail.com

#### **Karla Carpio Horta**

PhD

School of Dentistry of Ribeirão Preto, University of São Paulo  
Av. do Café, s/n-Monte Alegre, Ribeirão Preto, São Paulo, Brazil  
E-mail: hortakc@gmail.com

#### **Erika Cavano Küchler**

PhD, Professor

School of Dentistry of Ribeirão Preto, University of São Paulo  
Av. do Café, s/n-Monte Alegre, Ribeirão Preto, São Paulo, Brazil  
E-mail: erikacavano@gmail.com

#### **Fabio Lourenço Romano**

PhD, Professor Associate

School of Dentistry of Ribeirão Preto, University of São Paulo  
Av. do Café, s/n-Monte Alegre, Ribeirão Preto, São Paulo, Brazil  
E-mail: fabioromano@forp.usp.br

#### **Maria Bernadete Sasso Stuani**

PhD, Professor Associate

School of Dentistry of Ribeirão Preto, University of São Paulo  
Av. do Café, s/n-Monte Alegre, Ribeirão Preto, São Paulo, Brazil  
E-mail: bernadete@forp.usp.br

#### **Caio Luiz Bitencourt Reis**

PhD student

School of Dentistry of Ribeirão Preto, University of São Paulo  
Av. do Café, s/n-Monte Alegre, Ribeirão Preto, São Paulo, Brazil  
E-mail: caioluizreis@usp.br

**Mirian Aiko Nakane Matsumoto**

PhD, Professor Associate

School of Dentistry of Ribeirão Preto, University of São Paulo  
Av. do Café, s/nMonte Alegre, Ribeirão Preto, São Paulo, Brazil  
E-mail: manakane@forp.usp.br

**ABSTRACT**

The aim of this study is evaluated osteoclastogenesis on the periodontal ligament during induced tooth movement (ITM) in obese Wistar rats using the tartrate resistant acid phosphatase (TRAP) histoenzymatic analysis. Twelve rats weighing about 125 g were randomly divided into four groups of three animals each: Group I – healthy rats that received ITM in one side of the maxilla for seven days; Group II – obese rats that received ITM in one side of the maxilla for seven days; Group III – healthy rats that received ITM in one side of the maxilla for 14 days; Group IV – obese rats that received ITM in one side of the maxilla for 14 days. The other side of the maxilla was used as control side. Immediately after euthanasia, the samples were processed for histological examination using tartrate-resistant acid phosphatase (TRAP) staining to detect osteoclasts in the periodontal ligament under conventional microscopy. Moderate to intense osteoclast activity was found in both sides of the maxilla that received ITM regardless of ITM duration or obesity of the Wistar rats. Our results suggest that obesity did not affect the formation of osteoclasts during orthodontic ITM.

**Keywords:** Obesity, Resorption, Alveolar Bone Loss, Tooth Movement.

**RESUMO**

O objetivo deste estudo é avaliar a osteoclastogênese no ligamento periodontal durante movimentação dentária induzida (MDI) in ratos Wistar obesos através da análise da atividade da enzima fosfatase ácida resistente ao tartarato (TRAP). Vinte ratos pesando cerca de 125 gramas foram aleatoriamente divididos em 4 grupos de três animais: Grupo I – ratos saudáveis que receberam MDI em um lado da maxilla por 7 dias; Grupo II – ratos obesos que receberam MDI em um lado da maxilla por 7 dias; Grupo III ratos saudáveis que receberam MDI em um lado da maxilla por 14 dias - Group IIII – ratos obesos que receberam MDI em um lado da maxilla por 14 dias. O outro lado da maxilla foi utilizado como lado controle. Imediatamente após eutanásia, as amostras foram processadas para análise histológica usando coloração TRAP para detectar osteoclastos no ligamento periodontal sob microscopia de luz convencional. Atividade moderada a intensa foi encontrada em ambos os lados da maxilla independentemente da duração e da obesidade dos ratos. Nossos resultados fsugerem que a obesidade não afeta na proliferação de osteoclastos durante MDI.

**Palavras Chave:** Obesidade, Reabsorção, Perda Óssea Alveolar, Movimentação Dentária.

**1 INTRODUCTION**

Overweight and obesity are defined by the World Health Organization (WHO) as a global epidemic disease resulting from the accumulation of abnormal or excessive fat,

which can be harmful to health.<sup>1,2</sup> According to WHO, a person is classified as obese or overweight when their weight is too high for their height<sup>3</sup>. Worldwide obesity more than doubled since 1980. In 2014, over 1.9 billion adults aged eighteen years or older were classified as overweight and over 600 million as obese. This problem does not affect only adults, and child obesity is one of the most serious public health challenges of the XXI Century.<sup>1</sup>

WHO statistics about mortality due to obesity raise a matter of great concern that may represent a great burden to society. At least 2.8 million people die every year because of overweight or obesity.<sup>1</sup> High mortality rates due to obesity are explained by its association with cardiovascular diseases, particularly heart diseases and strokes, besides diabetes, musculoskeletal disorders, and some types of cancer, such as those that affect the endometrium, breast, ovary, prostate, gallbladder, kidney and colon.<sup>2</sup>

It should be expected that overweight and obese patients need of orthodontic treatment. Therefore, the anatomic phenomena that affect bone and dental structures when orthodontic forces are applied should be known, and their clinical relevance is evident. Local and systemic factors that affect the rate of induced tooth movement (ITM), bone remodeling and root resorption during the application of orthodontic forces are some of the major challenges of orthodontic treatments. Such effects may be investigated using rat models with induced movement of the maxillary first molar,<sup>4-6</sup> and results may be extrapolated to human beings.

Studies in the literature have found associations of obesity with periodontics,<sup>7-10</sup> temporomandibular joint dysfunction,<sup>11</sup> implantology,<sup>12</sup> and cariology.<sup>13</sup> In Orthodontics, studies have described the association of obesity with bone maturity,<sup>14,15</sup> and with dental and craniofacial development.<sup>16-18</sup> However, the effect of obesity on the recruitment and activity of the cells responsible for bone and root resorption (osteoclastogenesis) has not been investigated. Thus, the aim of this preliminary in vivo study was evaluating the impact of obesity on osteoclastogenesis during ITM in obese Wistar rats using histoenzymatic analyses and tartrate-resistant acid phosphatase (TRAP) staining.

## 2 MATERIAL AND METHODS

This study was approved by the Local Ethics Committee for the Use of Animals (protocol: 2017.1.144.58.2). The study sample was composed of 12 male heterogenic Wistar rats (*Rattus norvegicus, albinus*) approximately 3 weeks old, weighing about 125g, obtained from the Local Central Animal Care Facility.

The animals were kept in a room with natural humidity and temperature of  $23\pm 2^{\circ}$  C, controlled by an air conditioning and heating system. A timer was used to automatically control lighting for 12-12h light-dark cycles. The animals had free access to feed and filtered water<sup>19</sup>.

Obesity was induced using a hyperlipidemic mash diet (PragSoluçõesBiociências, Domeneghett&Corrêa Ltda., Jaú, Brazil) containing 3 components: 2 parts of peanuts, 2 of chocolate and 1 of cookies.<sup>20</sup> The diet was adapted from a cafeteria diet, or western diet, originally described by Estadella et al.<sup>21</sup> This diet has 39.7% carbohydrates, 24.5% lipids, 22.3% proteins, 7.5% gross fiber and 6% minerals and others. The rats that did not undergo obesity induction (healthy rats) were fed a normal commercial mash diet (Integral Controle PSB 22% AA, PragSoluçõesBiociências, Domeneghett&Corrêa Ltda., Jaú, Brazil). The feed, as well as water, were offered ad libitum and replaced daily, for 9 and 10 weeks. The weight of the animals was measured weekly during the whole study using an electronic scale.

The sample was divided randomly into four groups according to time of ITM and obesity. All animals underwent orthodontic procedures in one side of the maxilla. The opposite side was the control for that group.

- 1) **Group I (non-obese):** Three healthy rats that underwent ITM for 7 days.
- 2) **Group II (obese):** Three obese rats that underwent ITM for 7 days.
- 3) **Group III (non-obese):** Three healthy rats that underwent ITM for 14 days.
- 4) **Group IV (obese):** Three obese rats that underwent ITM for 14 days.

## 2.1 INDUCED TOOTH MOVEMENT

Before placement of orthodontic appliances, the animals were anesthetized with a single IM dose of a sedative, muscle relaxant anesthetics (2% xylazine, Dosaper, Calier, Barcelona, Spain) mixed with a general injectable anesthetics (10% ketamine, Ketamina Agener, Agener União Química Farmacêutica Nacional S/A, São Paulo, Brazil), in a ratio of 1:2 respectively and 1 mL/kg body weight.

The objective of ITM was to move the maxillary first molar mesially using orthodontic devices built according to the study conducted by Heller and Nanda,<sup>22</sup> modified by Martins-Ortiz.<sup>23</sup> Orthodontic forces were applied during daylight because the daylight rhythms of bone metabolism have an important role in orthodontic treatments.<sup>24</sup>

Orthodontic movement was examined at 7 and 14 days to evaluate the immediate and delayed response of the structures under study.<sup>25</sup> The appliance was activated with a

50g force only once at the time of placement. It was controlled daily to check its integrity and the correct functioning, which was clinically confirmed by the presence of space between the first and second molar.

After the study, the animals were killed with an overdose of a sedative, muscle relaxant anesthetics (2% xylazine, 30mg/kg, Dosaper, Calier, Barcelona, Spain) mixed with a general injectable anesthetics (10% ketamine, 300mg/kg, Ketamina Agener, Agener União Química Farmacêutica Nacional S/A, São Paulo, Brazil) and a CO<sub>2</sub> chamber, as recommended in the euthanasia guidelines issued by the National Council for the control of Animal Experimentation (Concea) in Brazil. Immediately after euthanasia, the study side of the maxilla was removed using sterilized surgical scissors and processed for histological analysis using tartrate-resistant acid phosphatase staining.

## 2.2 DETECTION OF CLAST FORMATION USING HISTOENZYMOLOGICAL ANALYSIS OF THE ACTIVITY OF TARTRATE-RESISTANT ACID PHOSPHATASE (TRAP)

Clast formation was examined at 7 and 14 days of ITM to determine the immediate and delayed response of the structures under study and TRAP activity. The slides were incubated in a solution of 8 mg of naphthol AS-MX phosphate disodium salt (Sigma-Aldrich) in 500 µL of N,N-diethylformamide, followed by the addition of 50 mL of a 0.2-mol/L sodium acetate buffer (pH 5.0) containing 70 mg of Fast Red ITR (Sigma-Aldrich). Dehydrated sodium tartrate (50 mmol/L) was then added to the solution, and the slides were incubated at 37° C for two hours. After that, the slides were rinsed in distilled water and counterstained with hematoxylin. The TRAP-positive clasts were counted under conventional microscopy: cells with three nuclei, in resorption gaps, in direct contact with alveolar bone and around the root area. The examinations were conducted by a single calibrated observer unaware of what group was under analysis. A potential marker for bone resorption is tartrate-resistant acid phosphatase activity (TRAP). This enzyme is secreted by osteoclasts during bone resorption, which enables the identification of active osteoclasts<sup>26,27</sup> The number of TRAP positive cells was evaluated at the tension and compression sides.

Three histological sections were used for each animal, and the criteria for TRAP positive cells were: **score 0**, complete absence of immunolabeling (total absence of immunoreactivity [IR] in the cells); **score 1**, low immunolabeling (IR in about 1/4 of the immunoreactive cells per area); **score 2**, moderate immunolabeling (IR in about 1/2 of the

immunoreactive cells per area); and **score 3**, high immunolabeling (IR in about 3/4 of the immunoreactive cells per area).<sup>28,29</sup>

### 2.3 STATICAL ANALYSIS

Weight (mass) was expressed in grams. Groups were compared with each other using a *t* student test ( $\alpha = 5\%$ ) in GraphPad Prism 7.0 (San Diego, California USA)

### 3 RESULTS

Animals were weighed all through the study. Mean rat weight was not statistically different between the groups of obese and non-obese rats in week 0 (day of group randomization). However, from week 4 to 9 mean weight of obese rats was greater than the mean weight of non-obese rats. (Figure 1).

At 7 days of ITM, there was moderate to intense TRAP-positive clast activity in both groups (Figure 2). At 14 days, the side of the maxilla that underwent tooth movement had moderate TRAP-positive clast activity, and reparative phase was observed, with a

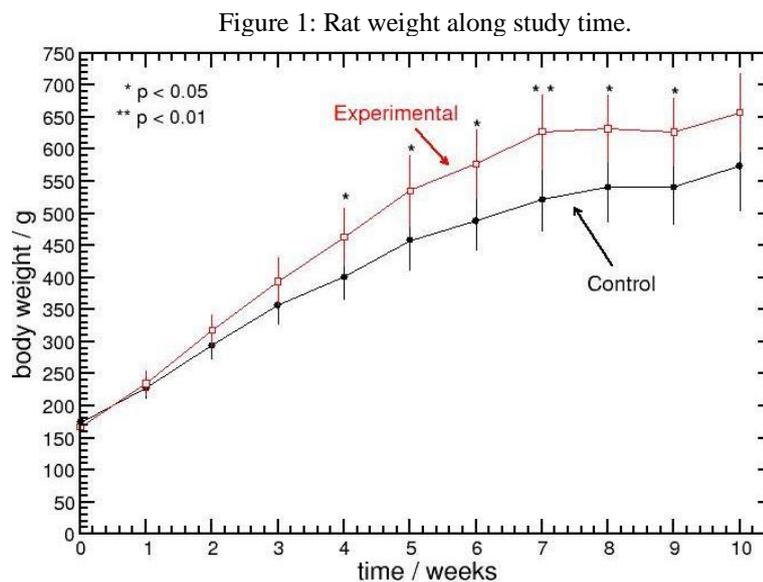
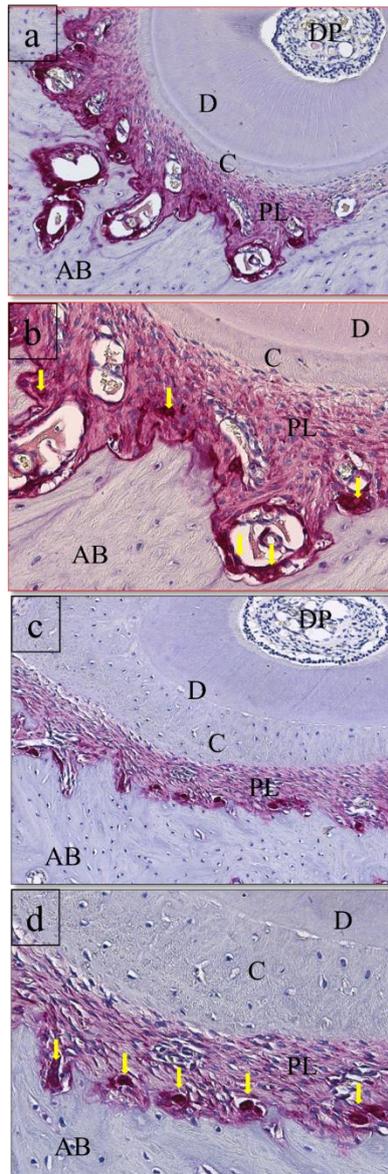
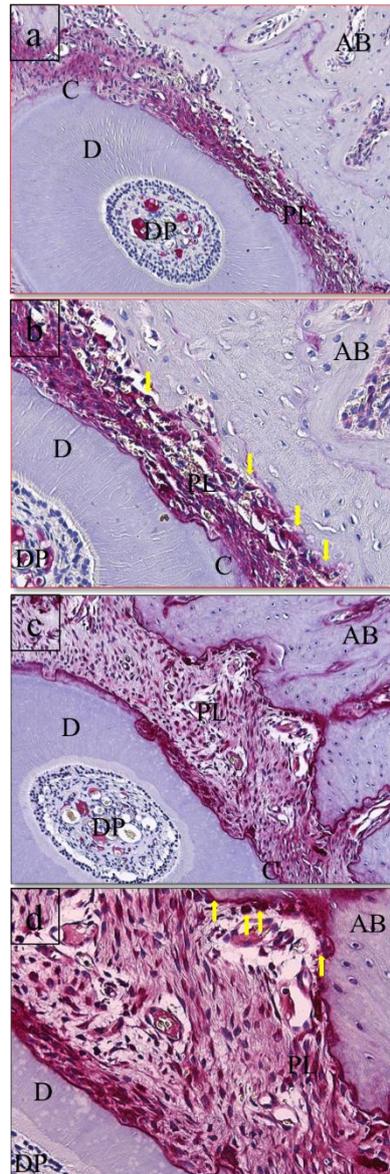


Figure 2: TRAP staining of the mesial root of maxillary first molar in obese and non-obese groups at 7 days of ITM. A and b: Obese animals, c and d: Non-obese animals; AB: alveolar bone, PL: periodontal ligament, C: cementum, D: dentin, DP: dental pulp, Yellow arrows indicate osteoclast (TRAP, a and c: 20X; b and d: 40X)



Reduced number of osteoclasts in both groups (Figure 3). However, no differences were found between obese and non-obese rats at any study time point.

Figure 3: TRAP staining of mesial root of maxillary first molar in obese and non-obese groups at 14 days of ITM. A and b: Obese animals, c and d: Non-obese animals; AB: alveolar bone, PL: periodontal ligament, C: cementum, D: dentin, DP: dental pulp, Yellow arrows indicate osteoclast (TRAP, a and c: 20X; b and d: 40X)



#### 4 DISCUSSION

This study was conducted in the context of an increase in obesity rate in world, which has become a public health problem, and its possible effects on bones and periodontal structures of teeth moved orthodontically. Studies suggest that a high BMI is a risk factor for systemic diseases, such as cardiovascular disease, diabetes, some types of cancer and several bone and muscle disorders.<sup>2</sup> Michelogiannakis et al.<sup>30</sup>, reported that screening and educational health community programs, particularly those for children and adolescents, are highly recommended to educate the public about the deleterious effects of obesity in general and in oral health, including several possible complications during

orthodontic treatments. Further studies should be conducted to evaluate the association between orthodontic treatments and BMI and to evaluate the efficacy of intervention protocols in the prevention of child obesity during the treatment.<sup>30</sup>

This study was conducted with molar teeth of male Wistar rats (*Rattus norvegicus*). This lineage was selected because it is an adequate model and has some potential for the extrapolation of results to humans.<sup>19,20,25,31</sup> Male rats were used to try to avoid the effect of sex hormones that might have affected results. Age of Wistar rats was determined according to their sexual maturity. Rats reach sexual maturity in about 6 weeks.<sup>32</sup> To simulate the systemic effects of the study diet during all the growth time and body development, rats weighing 125 g were chosen so that ITM was completed when rats had already reached sexual maturity.

To induce obesity in the animals, the hyperlipidemic diet described above was chosen, as it simulates human meals, known as fast food, in studies described in the literature.<sup>20,33-35</sup> However, Damanaki et al.<sup>36</sup> found that this model of animal research induced by diet has limitations that should be taken into considerations when attempting to extrapolate data to human beings, because the levels of expression and the anti-inflammatory adipocin and adiponectin were not reduced, which is in contrast to chronic obesity in human beings.<sup>36</sup>

In a study of the effect of orthodontic movement on the root apex, Zhao et al. found that the application of force led, in the long term, to changes in the root apex, as it affected the shape of Hertwig's epithelial root sheath and the behavior of apical cells.<sup>37</sup>

Several authors have investigated the effect of different factors on orthodontic movements. According to Chisari et al.<sup>38</sup>, age affects movements, which may be partly associated with the decrease in biological response. Other variables that may significantly affect ITM are root length, bone level and density, and bone quality.<sup>38</sup>

Yan et al.<sup>39</sup> found that there was less formation of TRAP-positive osteoclasts in ITM in the group of obese rats. Their findings indicate that obesity may attenuate ITM by inhibiting osteoclastogenesis in rats,<sup>39</sup> which was not confirmed in this study. They also found that the elevated level of leptin in obese rats inhibited osteoclastogenesis and decreased ITM.<sup>39</sup> In this study, however, different results were found for the sites that underwent ITM when compared with those without ITM, regardless of the presence of obesity in rats.

The clinical relevance of this study lies in the fact that many obese patients seek orthodontic treatment, and the effect of obesity on the amount of movement and bone and

root resorption has not been described yet. Despite the growing number of obese and overweight patients found in orthodontic clinics, there are few studies in the literature about the effect of BMI on orthodontic movement, and its mechanisms remain to be elucidated.<sup>39</sup> Due the proximity of the genomic and physiology between rats and humans, the animal is a good model to induce obesity and to externalize the effects to humans<sup>40</sup>.

This preliminary study may provide a basis for future studies about obesity and its effects on ITM. These mechanisms should be elucidated to optimize treatments, including the determination of the variables that affect them positive or negatively. Our results suggest that obesity does not affect ITM, but our sample was small, and larger samples should be used to reach conclusive results.

## **5 CONCLUSION**

There was moderate to severe positive osteoclast activity using tartrate-resistant acid phosphatase in the sides of the maxillary that underwent ITM, regardless of time of movement and presence of obesity in Wistar rats. Obesity did not affect the formation of osteoclasts during orthodontic ITM.

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