Dietary restriction inhibits bone formation in alveolar bone modeling and remodelling

Restrição alimentar inibe formação óssea em modelagem e remodelação óssea alveolar

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ABSTRACT

Objective: Methods: Balb/C mice weaned at the age of 21 days were assigned to one of the following groups: control group fed a regular hard diet ad libitum (food consumption was measured daily), and dietary restricted group (DR) received 75% of the amount of food consumed by the respective control mice the previous day. Body weight of all animals was recorded throughout. Mice were euthanized in groups of ten at 25 and 60 days of experience. Mandibles were dissected, descalcified in EDTA and embedded in paraffin. Buccolingual sections of the mesial root of the first lower molar were stained with hematoxylin-eosin and submitted to histomorphometric studies. Results: At 25 days, DR mice shows bone formation values lower than control animals for modeling and remodeling sides, coupled to an increase with bone at rest values in the modeling side. At 60 days, DR mice shows bone formation values lower than control animals in the modeling side linked with a similar increase in values corresponding to bone resorption and bone at rest. In the remodeling side, no differences in bone formation were observed between control and DR mice. However, high bone resorption and a decrease in bone at rest areas were observed. Conclusions: Dietary restriction impairs bone formation in physiologic alveolar bone modeling and remodeling.

Keywords: dietary restriction-bone, remodeling-histomorphometry.
RESUMO
Objetivo: Métodos: Os ratos Balb/C desmamados aos 21 dias de idade foram atribuídos a um dos seguintes grupos: grupo de controle alimentado com uma dieta regular e dura ad libitum (o consumo de alimentos foi medido diariamente), e grupo restrito alimentar (DR) recebeu 75% da quantidade de alimentos consumidos pelos respectivos ratos de controle no dia anterior. Registrou-se o peso corporal de todos os animais ao longo do percurso. Os ratos foram eutanasiados em grupos de dez aos 25 e 60 dias de experiência. As mandíbulas eram dissecadas, descalcificadas em EDTA e embebidas em parafina. Seções bucolíngues da raiz mesial do primeiro molar inferior foram coradas com hematoxilina-eosina e submetidas a estudos histomorfométricos. Resultados: Aos 25 dias, os ratos DR apresentam valores de formação óssea inferiores aos dos animais de controle para modelagem e remodelação dos lados, somados a um aumento com osso em repouso valores no lado da modelagem. Aos 60 dias, camundongos DR apresentam valores de formação óssea inferiores aos dos animais de controle do lado da modelagem, ligados a um aumento semelhante nos valores correspondentes à reabsorção óssea e osso em repouso. No lado da remodelação, não foram observadas diferenças na formação óssea entre ratos de controle e DR. No entanto, foi observada uma elevada reabsorção óssea e uma diminuição nas áreas de repouso ósseo. Conclusões: Restrição do cemitério impede a formação óssea em modelagem e remodelação óssea alveolar fisiológica.

Palavras-chave: remodelação de osso, restrição dietética-histomorfometria.

1 INTRODUCTION

Bone formation during growth is related to several factors such as heredity, functional and environmental (1, 2). Environmental factors include nutritional deficiencies, which may appear during growth and affect bone development causing marked variations in bone shape and size (3, 4).

There is a complex and close link between overall nutritional status and longitudinal bone growth. Numerous studies have been published about its impact on the long bones development. It was shown that malnutrition, caused by prolonged fasting or dietary restriction (DR) affects total epiphyseal growth plate height, as well as the number of chondrocytes per column in the proliferative and hypertrophic zones of the epiphyseal growth plate and the size of hypertrophic chondrocytes (4, 5, 6).

Mandibular growth and develop is caused by endochondral ossification of the mandibular condyle and it is well documented that this type of ossification is particularly sensitive to nutritional deficiencies (4, 6). Thus, DR in rats results in bone growth arrest and alterations in the size and biomechanical properties of the mandible (7, 8, 9). These data were obtained in models of active ossification, however to our knowledge, there are no available data in the literature how DR influences cellular activity during bone modeling and remodeling. This process involves bone resorption and new bone formation, which are controlled by the activity...
of osteoclasts and osteoblasts, respectively (10). Since undernutrition affects gene expression, protein synthesis, and cell differentiation (11), we hypothesized that DR could cause inhibition of osteoblasts or osteoclasts activity and influence normal bone modeling and remodeling of the mandible and others bones. Therefore, the aim of this experimental work is to evaluate, in growing mice the influence of DR in bone modeling and remodeling in alveolar bone by histomorphometric methods.

The histological bucco-lingual oriented sections of the alveolar bone allow the study of bone modeling on the lingual side and bone remodeling on the buccal side (12). Static histomorphometric studies are based on standard stereologic procedures and are employed to accurately assess bone activity (13, 14, 15). The accuracy of this methodology has been repeatedly verified (13, 14, 15, 16, 17).

2 METHODS AND MATERIALS

2.1 ANIMALS AND FEEDING PROTOCOLS

Balb/C mice weaned at the age of 21 days were supplied and maintained in CERELA-CONICET (Tucumán-Argentina).

The National Institute of Health Guidelines for the Care and Use of Laboratory Animals (NHI publication 85-23 Rev. 1985) were observed.

All animals were housed in individual metabolic cages and acclimated to 22 °C with a 12 h light -12 h dark cycle. Mice were adapted for 2 days with a balanced complete pellet diet (BCD) providing 21% calories as protein (casein), 66% as carbohydrates (corn starch) and 13% as fat (corn oil), vitamin mixture (2.2%) (ICN, Biomedicals, Inc., Ohio, USA), salt mixture (4%) (ICN, Biomedical, Inc., Ohio, USA).

After a period of 2 days of diet acclimation, mice (40) were matched by weight and assigned to following groups:

1) control *ad libitum* group: animals were fed with *ad libitum* with BCD (the food intake was measured daily (n=20).

2) Dietary restriction group (DR): animals received 75% of the amount of food consumed by the respective *ad libitum* control mice the previous day (n=20).

Animals of control and DR groups were euthanized in groups of ten at 25 and 60 days of study period. For morphological analysis, mandibles were carefully dissected and processed for light microscopy. The samples were fixed in 10% formalin, decalcified in 10% EDTA (pH=7.2) and embedded in paraffin. Buccolinguinal sections were obtained at the level of the
mesial root of the first lower molar and stained with hematoxylin-eosin. The stained sections were observed by light microscopy and analyzed by histomorphometric studies.

2.2 BODY WEIGHT

Body weights were measured and recorded daily and expressed in grams (g) starting of the day before the experiment began until the end of the treatment period.

3 HISTOMORPHOMETRIC DETERMINATIONS

Histomorphometric measurements were performed separately on the total boundary of lingual and buccal side of the periodontal cortical alveolar bone, which correspond to modeling and remodeling activities, respectively (12). These sides were defined on digitalized microphotographs of the selected histological sections figure 3.

The following histomorphometric measurements were performed on control and experimental groups with standard stereologic methods (18): (1) Bone surface covered by osteoblasts (OblS/BS) (%): indicative of active bone formation. (2) Bone surfaces with resorption areas (with or without osteoclasts) (RS/BS) (%): indicative of bone resorption. (3) Bone surface covered by lining cells (Flat OblS/BS) (%): indicative of bone at rest.

To determine these values, zones of bone formation, bone resorption and bone rest areas observed microscopically were transferred to the corresponding zones on digitalized microphotographs of the selected histological sections. The absolute values obtained from each area were then expressed as a percentage of the total buccal and lingual periodontal cortical bone perimeter. (Figure 3).

3.1 STATISTICS

Experimental data were expressed as mean ± SD. Single comparisons between groups were assessed by Student’s t test. Differences were considered significant at p < 0.05.

4 RESULTS

Body weight results are shown in figure 1 and 2. The groups weighed the same at the time of weaning (21 days of age).

Body weight was significant different in DR mice respect to control at 25 and 60 days. (Figuras 1 y 2).

The histomorphometric analysis indicate at 25 days, DR mice shows bone formation values lower than in control animals for modelling and remodeling sides. No statistical
differences were observed in the values corresponding to bone resorption between control and DR mice in modelling and remodeling sides. Whereas surfaces corresponding to bone at rest were significantly higher in DR mice in the modeling side. Table 1.

At 60 days, DR mice shows bone formation values lower than in control animals in the modeling side linked with a similar increase in values corresponding to bone resorption and bone at rest. In the remodeling side no differences in bone formation were observed between control and DR mice, however bone resorption was statistically significant higher coupled with a decrease in bone at rest areas.

5 DISCUSSION

The results presented herein shows that dietary restriction alters bone activity, given that inhibits osteoblasts activity in physiologic alveolar bone modeling and remodeling. This effect was observed at 25 days of experience in buccal and lingual side. After 60 days of experience active bone formation was lower in DR mice only in lingual side.

The effects of undernutrition in bone activity were obtained in models of active ossification (5, 6), but no reports are available on the effect of DR in bone remodeling.

The methodology employed, using histological oriented sections of alveolar bone, allows for the study of bone activity at a given time in two periodontal cortical bones of different behavior (12), it involves the measurements of the percentage of formation, resorption and rest on the alveolar bone surface, and its analysis with statistical methods. Data on this issue can contribute to the knowledge of the effects of DR or abnormal eating behaviors in bone.

At 25 days of experience, bone formation surfaces were lower in DR mice respect to control group in both the remodeling buccal and modeling lingual side, however these areas must be considerer separately. In lingual side depression in bone formation was associated with an increase in bone rest areas, while bone resorption areas did not shows difference respect to control group. This would suggest that dietary restriction causes a lesion in the osteoblasts or their cell precursors thus leading to the inhibition of bone formation, probably due to a failure of the osteoblasts precursor cells to differentiate into active osteoblasts. It is necessary to pointed out that in normal conditions little bone resorption areas was observed on the lingual modeling side, where bone formation areas predominate (12, 17). In this work, in buccal side no bone formation areas were observed in DR mice, although in normal conditions little areas associated to osteoblasts was observed on this remodeling side, where bone resorption areas predominate (12, 17).
At 60 days of experience, bone formation areas were lower only in lingual side, while the buccal side did not show differences in bone formation between DR and control mice. The depression in bone formation in lingual modelling side was associated with an increase in bone rest and bone resorption areas, although there is not statistical difference in both latter values between DR and control mice. These results would indicate that this nutritional disorder causes a damage in cell precursors of osteoblasts and/or in osteoblasts which in turn play an important role in bone resorption. A lesion in osteoblasts would release mediators of bone resorption (19, 20) and/or lose contact with bone surface (21) allowing an increase of bone resorption areas.

It has been observed in this study at 60 days of experience, an increase in bone resorption surfaces in the lingual modeling side and in the bucal remodelling side. When the nutritional deficit persists for long periods, a multi-deficiency syndrome is generated which is characterized by, among others, hypocalcemia secondary to a decrease in calcium carriers blood proteins. In this context was previously found that this hypocalcaemia contributes to a decrease bone apposition and increase bone resorption due to an increase in parathyroid activity (22, 23). It has been revealed in recent years that leptin, the hormone secreted by adipocytes, has several roles in the control of bone metabolism, such as the control of osteogenesis by the central nervous system, and direct action on osteoblasts and osteoclasts (24, 25, 26, 27). Since there is a direct relationship between the amount of body fat and levels of leptin circulating, it is likely that in this study, the DR animals present low levels of this hormone in the blood, that also could have an effect on changes in bone modeling and remodeling observed. The results found in this study allow us to conclude that the effect of DR in physiologic bone remodeling is a decrease in bone-forming activity, which in turn cause reduction of bone apposition. This situation must be taken into account from a clinical point of view because these disorders of the bone activity of malnourished individuals from various causes, can lead to different types of osteopenic disorders.
REFERENCES


LIST OF TABLES AND FIGURES

1 LEGENDS FIGURE

Figure 1: Effects of dietary restriction on body weight in mice fed during 25 days. Body weight was recorded daily. After acclimation period (2 days), animals were fed with *ad libitum* balanced conventional diet (BCD) (control group) or calorie restriction diet during 25 days (DR group). *: significant difference compared to control groups at the end of study period. \( p < 0.05 \). Source: authors.

![Figure 1: Effects of dietary restriction on body weight in mice fed during 25 days.](image1)

Figure 2: Effects of dietary restriction on body weight in mice fed during 60 days. Body weight was recorded daily. After acclimation period (2 days), animals were fed with *ad libitum* balanced conventional diet (BCD) (control group) or calorie restriction diet during 60 days (DR group). *: significant difference compared to control groups at the end of study period. \( p < 0.05 \). Student’s \( t \) test: Source: authors.

![Figure 2: Effects of dietary restriction on body weight in mice fed during 60 days.](image2)
Figure 3. Bone activity was calculated in vestibular side between points A and C, and in lingual side between points B and D. In order to determine these points a line \( a \) was draw between points A and B corresponding to the uppermost point of the buccal and vestibular alveolar crest. Between the midpoint of the segment AB and the apex of the tooth a line \( b \) was drawn. Perpendicular to line \( b \) and tangential to the apex of the tooth a line \( c \) was drawn, which determined points C and D at its intersection with the buccal and lingual periodontal cortical bone respectively.

![Diagram](image)

Table 1. Histomorphometric determinations in buccal and lingual sides in dietary restricted mice fed during 25 days experience.

<table>
<thead>
<tr>
<th></th>
<th>Buccal remodeling side</th>
<th>Lingual modeling side</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Control%</td>
<td>DR%</td>
</tr>
<tr>
<td>OblS/BS (%)</td>
<td>17±11</td>
<td>0*</td>
</tr>
<tr>
<td>RS/BS (%)</td>
<td>30±21</td>
<td>26±10</td>
</tr>
<tr>
<td>Flat OblsS/BS (%)</td>
<td>53±13</td>
<td>64±16</td>
</tr>
</tbody>
</table>

OblS/BS%: Bone surface covered by osteoblast, indicating active bone formation.
RS/BS %: Bone surfaces with resorption areas (with or without osteoclasts) Indicating bone resorption.
Flat OblsS/BS%: Bone surface covered by lining cells, indicating bone at rest.

Values are mean ± standard deviation (\( n = 10 \) for groups).
* \( p < 0.05 \) respect controls. Student t test.

Source: authors.

Table 2. Histomorphometric determinations in buccal and lingual sides in dietary restricted mice fed during 60 days.

<table>
<thead>
<tr>
<th></th>
<th>Buccal remodeling side</th>
<th>Lingual modeling side</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Control%</td>
<td>DR%</td>
</tr>
<tr>
<td>OblS/BS (%)</td>
<td>1±3</td>
<td>7±6</td>
</tr>
<tr>
<td>RS/BS (%)</td>
<td>48±13</td>
<td>68±11*</td>
</tr>
<tr>
<td>Flat OblsS/BS (%)</td>
<td>52±14</td>
<td>25±13*</td>
</tr>
</tbody>
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