Rehabilitation of masticatory function improves the alveolar bone architecture of the mandible in adult rats

Anestis Mavropoulos a,b,⁎, Anna Ödman b, Patrick Ammann c, Stavros Kiliaridis a

a University of Geneva, School of Dentistry, Department of Orthodontics, Geneva, Switzerland
b The Sahlgrenska Academy at Göteborg University, Institution of Odontology, Department of Orthodontics, Sweden
c University Hospitals of Geneva, Department of Rehabilitation and Geriatrics, Division of Bone Diseases (WHO Collaborating Center for Osteoporosis Prevention), Geneva, Switzerland

A R T I C L E   I N F O

Article history:
Received 2 April 2010
Revised 19 June 2010
Accepted 22 June 2010
Available online 30 June 2010

Edited by: R. Baron

Keywords:
Masticatory function
Mechanical strain
Trabecular architecture
Alveolar bone
Bone mechanotransduction

A B S T R A C T

Masticatory functional changes have been shown to influence the quantity and quality of the alveolar bone during growth. This study was designed to investigate the effect of masticatory function rehabilitation on the morphology and the trabecular architecture of the mandibular alveolar bone after cessation of growth. Forty-four Sprague-Dawley male rats received soft diet in order to develop masticatory muscle hypofunction. After 21 weeks, after cessation of growth, the animals were divided into two groups: the first group continued receiving soft diet for six more weeks (hypofunction group), while the second group changed to ordinary (hard) diet with the aim to restore a normal masticatory function (rehabilitation group). A third group of 16 male rats (normal group) received ordinary (hard) diet during the whole experimental period and served as control. Micro-tomographic histomorphometry was used to evaluate the architecture of the mandibular alveolar bone (e.g. bone volume fraction, trabecular thickness, trabecular separation, etc.) at the end of the experiment (27 weeks). The height and width of the alveolar process were measured as well. The alveolar process trabecular bone volume fraction (BV/TV) was lower for the animals of the hypofunctional group as compared to those of the normal (p<0.01) and the rehabilitation (p<0.05) groups. Despite the significant improvement observed in the rehabilitation group, their BV/TV was lower in comparison to the normal group (p<0.05) at the end of this experiment. All the other micro-tomographic parameters followed the same pattern of change between groups; values of the rehabilitation group were between the values of the two other groups, differing significantly from both of them. The alveolar process was significantly shorter in the normal group in comparison to both the hypofunctional and rehabilitation groups (p<0.05). On the other hand, both the normal and rehabilitation groups were had a wider alveolar process than the hypofunctional group (p<0.05). Both alveolar height and width were significantly correlated with all micro-tomographic parameters under study. The rehabilitation of masticatory function led to a significant improvement of alveolar bone architecture in adult rats, although the negative effects of hypofunction were not totally reversed during the period under study.

© 2010 Elsevier Inc. All rights reserved.

Introduction

Mastication is the first step in the process of digestion and is meant to prepare the food for swallowing and further processing in the digestive system. All ingested solid food is transported from the front of the mouth to the occlusal surfaces of the posterior teeth. Then the food is processed by a series of masticatory cycles needed to comminute and soften it. Characteristics of the stomatognathic system, like e.g. bite force, masticatory muscle functional capacity, salivary production, will influence the masticatory function [1].

Numerous studies have shown that masticatory performance deteriorates as the number of teeth decreases [2–5]. It is known that in edentulous adults who receive implant-retained bridges or overdentures there is an improvement of both masticatory muscle strength and performance [6,7]. Serious maxillofacial discrepancies are often associated with a reduced capacity of the masticatory system [8]. Orthognathic surgery has been shown to improve masticatory muscle strength and masticatory performance in these cases [9,10]. Food hardness is sensed during mastication and affects the masticatory force [11], the jaw muscle activity [12], and the mandibular functional movements [13]. Animal experimental studies on growing rats have shown that changes of the masticatory muscle function induced by soft diet lead to a reduction of masticatory muscle strength [14]. This, in turn, results in changes of the mandibular morphology and the alveolar bone architecture [15,16]. There is sufficient evidence that similar changes occur in adult animals as well.
Masticatory hypofunction has been shown to result in changes of the lateral morphology of the mandible [17], as well as changes of the temporo-mandibular joint and the condyle in adult rats [18].

The alveolar bone receives indirectly (through the periodontal ligament) the forces exerted on the teeth during mastication. This mechanical strain of the alveolar process is superior to that of the long bones during function. For most vertebrates, long bone peak functional strains range from less than 1000 microstrain (με) during walking to between 2000 and 3200 με for more vigorous activities [19]. Finite element data suggest that alveolar bone strain during mastication can reach up to 4000–6000 με depending on food consistency [20,21]. The current investigation was designed in order to test the hypothesis that the rehabilitation of normal masticatory function in hypofunctional adult individuals may significantly alter the architecture of the trabecular bone of the alveolar process.

Methods

Animals

Sixty male rats of the Sprague–Dawley strain (average age 21 days) were used. The Ethics Committee of the University of Gothenburg approved the experimental protocol.

Experimental design

Forty-four rats received ordinary food (R34, Lactamin, Södertälje, Sweden) mixed with water in standardized proportions (2:5, R34/ water). This means that the rats were fed a soft diet in order to reduce their masticatory muscle functional capacity. The rats were fed and watered ad libitum. Another 16 rats served as the control (normal group), and received ordinary food (which is hard) during the whole experimental period.

The duration of the experimental period was 27 weeks. During this time the rats were weighed every second week. After 21 weeks when the animals had nearly ceased their body growth the 44 rats of the experimental group were divided into two groups (22 rats in each), matched for weight. One group continued with soft diet until the end of the experiment (hypofunctional group). The other group received ordinary food to get a possibility to train their masticatory muscles (rehabilitation group). At the end of the experimental period the animals were killed in a CO2 chamber. Their mandibles were dissected, defleshed, and separated at the symphysis into their two halves.

Microtomographic histomorphometry (μCT)

Micro-tomographic histomorphometry of the mandibular alveolar process was performed with a high-resolution μCT system (μCT 40, Scanco Medical AG, Bassersdorf, Switzerland) as previously described [22,23]. In summary, three-dimensional images of each left hemi-mandible were acquired with a voxel size (nominal resolution) of 16 μm in all spatial directions. Samples were secured in a cylindrical sample holder in air. The resolution was set to medium (500 projections with 1024 samples each).

The volume of interest (VOI) was drawn on a slice-based method starting from the first slice containing the crown of the first molar and moving dorsally 100 slices in the area of the alveolar process between the roots of the molars and the root of the incisor. Trabecular bone was carefully contoured on the first and the last slice, while the intermediate slices were first interpolated by morphing (Fig. 1B). Each slice was subsequently visually inspected and the contour was modified where deemed necessary. Micro-computed histomorphometric indices were calculated directly from the binarized VOI. Total volume (TV) was the volume of the whole sample examined. Bone volume (BV) and surface (BS) were calculated using tetrahedrons corresponding to the enclosed volume of the triangulated surface. Mean trabecular thickness (Tb.Th.) was determined from the local thickness at each voxel representing bone. Trabecular number (Tb.N.) was calculated by taking the inverse of the mean distance between the middle axes of the structure. Furthermore, the height and the width of the alveolar process were measured perpendicularly to the height at the midlevel of the 1st molar apices.

Statistical analyses

All data are represented as mean and standard error of the mean (mean ± SEM). Analysis of variance (ANOVA) was performed in order to detect any differences between groups. The Bonferroni correction was used to perform post hoc comparisons between groups. The Pearson correlation test was applied in order to detect correlations

---

Fig. 1. Left hemi-mandible of the rats used in the study (Sprague–Dawley): (A) Photograph superimposed on a drawing of the skull (a, the molar alveolar process under study in this investigation, which is the equivalent of the alveolar process in humans; b, the incisor process; and c, the condylar process); (B) micro-CT scan (the dashed line represents the volume of interest (VOI) as it appears on one slice; (C) the same slice where the measurements of alveolar process height and width are represented by the dashed lines (the white circle shows the position of the mandibular canal).
between the morphometric and the micro-tomographic variables. All statistical analyses were performed using the SPSS statistical package (SPSS 13.0, SPSS Inc., Chicago, IL, USA). A result was considered as statistically significant at \( p \leq 0.05 \).

Results

Body weight

No statistical significant difference in body weight was found at week 21 (when the hypofunctional group was split into two groups) between the rats fed soft diet (567 ± 73 g) and the rats fed hard diet (573 ± 64 g), nor between the three groups at the end of the experimental period (week 27): the mean body weight (±SD) for the hypofunctional group was 605 g (±87 g), for the rehabilitation group was 599 ± (83 g) and for normal group was 631 g (±81 g).

Morphometric parameters of the alveolar process (Table 1)

The alveolar process was significantly shorter in the normal group in comparison to both the hypofunctional and rehabilitation groups (\( p < 0.05 \)). On the other hand, both the normal and rehabilitation groups were found to have a wider alveolar process than the hypofunctional group (\( p < 0.05 \)). In other words, the alveolar process of the functionally rehabilitated animals regained its width but did not lose its height.

Micro-tomographic histomorphometry of the alveolar process (\( \mu \)CT) (Fig. 2)

The alveolar process trabecular bone volume fraction (BV/TV) was found to be lower for the animals of the hypofunctional group as compared to those of the normal (\( p < 0.01 \)) and rehabilitation (\( p < 0.05 \)) groups. Despite the significant improvement observed in the rehabilitation group BV/TV was lower than in the normal group (\( p < 0.05 \)) at the end of this experiment. All the other micro-tomographic parameters followed the same pattern of change with values for the rehabilitation group being in the middle of the values for the two other groups, and differing significantly from both of them. The only exception was connectivity density where the rehabilitation group was not significantly superior to the hypofunctional group.

Correlation of morphometric and micro-tomographic histomorphometric parameters (Table 2)

Both alveolar height and width were significantly correlated with all micro-tomographic parameters under study in the normal and
The same superscript letter denotes a statistically significant difference (*p* < 0.05).

### Discussion

In the present study we were able to show that a prolonged period of seven months with low masticatory demands in the hypofunctional group during adolescence and early adulthood had a significant effect on the mandibular morphology and the mandibular alveolar process as compared to animals with normal masticatory function. At the end of the experiment, those animals fed a soft diet were found to have a higher and narrower alveolar process with a trabecular bone network of inferior quality and quantity. In those animals that changed their diet from soft to hard for the last 6 weeks of the experiment (rehabilitation group) a “catch-up” effect was observed. The alveolar process gained its width although it did not become shorter as in the normal group. Micro-tomographic histomorphometry showed that the trabecular bone regained significantly, but only partially, its normal qualitative and quantitative characteristics.

The teeth are an integral part of the masticatory system, which is aimed at the preparation of food for swallowing. They form the occlusal area where the food particles are physically broken down into smaller particles to facilitate swallowing and digestion [1,24]. When the food is available ad libitum, eating frequency has been estimated to be around 30 times per day in the rat [25]. The number of chewing cycles needed to prepare food for swallowing is rather constant within a subject for one type of food [26,27]. The number of processing cycles increases as foods become more difficult to chew. Adding fluid to a solid dry food has been shown to reduce the muscle activity and the number of chewing cycles until swallowing [28].

In this investigation, the change of diet from soft to hard in order to rehabilitate the masticatory muscle hypofunction took place during a period where no significant growth changes occur in the rat skeleton. The capacity of the bone to respond to functional alterations even at adult age has previously been shown in adults who start with racquet sports getting a thicker cortical wall of the humeral shaft after long-term loading [29]. In the present study the animals in the rehabilitation group had a training period of 6 weeks. Normal alveolar bone architecture was only partially restored during this period. It is possible that a longer training period would further improve the alveolar bone quantitative and qualitative characteristics.

A very interesting finding was that a “catch-up” effect was observed as far as alveolar process width is concerned. The animals that went from hypofunction to normal function (rehabilitation group) ended up with the same width as the animals in the normal group, although the animals in the hypofunction group had significantly narrower alveolar process. The alveolar process is subjected to heavy loading during mastication. The teeth transmit this stress to the alveolar bone through the periodontal ligament, which acts as a shock absorber. In this study, rehabilitation took place only after growth cessation and therefore these observations cannot be attributed to growth. When normal function was restored in the rehabilitation group, the alveolar process regained its width due to bone remodeling in order to better withstand the higher masticatory strain it was now subjected to. It seems that this led to thicker trabecular and probably cortical alveolar bone. Of note, rat molars grow and erupt normally, exactly like human molars, in contrast to rat incisors, which grow continuously to compensate for wear [30].

Teeth undergo eruption throughout life (for a review see Profitt and Frazier-Bowers [31]). Pre-emergent eruption is the tooth movement until its emergence to the oral cavity. After their emergence in the oral cavity, teeth erupt rapidly until they are in contact with their antagonists, and then they continue to erupt at a slower pace in order to compensate for the vertical growth of the lower face. To keep up with growth, human first permanent molars have to erupt about a centimeter after they first come into occlusion, so the amount of post-emergent eruptive movement is not trivial. During eruption the periodontal ligament and the alveolar bone follow the movement of the tooth, so that the alveolar process grows taller.

In the present study we found that the dentoalveolar process was taller in the hypofunctional group compared with the normal group. A plausible explanation is that the reduced mechanical loading on the molars of those animals eating soft diet allows these teeth to further erupt and the dentoalveolar process to follow their eruption. This is in line with previous findings [14,15,32] on growing rats, although the differences are more pronounced in the present study, probably due to the longer experimental period. These findings on animal experimental model are similar to those of clinical studies on patients with myotonic dystrophy [33]. In these patients, masticatory muscle hypofunction caused by the disease was associated with a vertical growth pattern and an anterior open bite due to significant posterior tooth eruption.

Although the animals in the normal group had a shorter alveolar process in comparison to the hypofunctional group, the rehabilitation of masticatory function did not alter the alveolar process height in the same way that width was affected, as mentioned earlier. There was no “catch-up” effect on alveolar process height. This is due to the fact that rehabilitation of function occurred after the cessation of growth. The vertical growth of the alveolar process depends almost entirely to tooth eruption. There is recent evidence that demonstrate that periodontal ligament tissue contains osteoprogenitor cells that have the ability to generate alveolar bone [34]. In the animals that received soft diet during their growth (hypofunctional and rehabilitation groups) molar teeth were allowed to erupt more than normally, due to the lower masticatory loading [35]. After the end of growth, rehabilitation of normal function led to higher masticatory loading, but since there is no reverse mechanism for tooth eruption the alveolar process retained its height.

In an effort to better understand the effect of normal (hard) and soft diet on the rat alveolar process we performed a correlation of all the morphometric and histomorphometric parameters under study. Both alveolar height and width were significantly correlated with all

### Table 1

<table>
<thead>
<tr>
<th></th>
<th>Normal</th>
<th>Hypofunctional</th>
<th>Rehabilitation</th>
</tr>
</thead>
<tbody>
<tr>
<td>Height (mm)</td>
<td>2.73 ± 0.21ab</td>
<td>3.12 ± 0.19ab</td>
<td>3.06 ± 0.22ab</td>
</tr>
<tr>
<td>Width (mm)</td>
<td>2.41 ± 1.10a</td>
<td>2.28 ± 0.09ab</td>
<td>2.38 ± 0.08b</td>
</tr>
</tbody>
</table>

*The same superscript letter denotes a statistically significant difference (*p* < 0.05).

### Table 2

<table>
<thead>
<tr>
<th></th>
<th>Alveolar width</th>
<th>BV/TV</th>
<th>BS/BV</th>
<th>Connectivity Density</th>
<th>Trabecular thickness</th>
<th>Trabecular number</th>
<th>Trabecular separation</th>
</tr>
</thead>
<tbody>
<tr>
<td>Alveolar height</td>
<td>r = 0.33 (p = 0.045)</td>
<td>r = 0.49 (p = 0.002)</td>
<td>r = 0.38 (0.001)</td>
<td>r = 0.54 (p = 0.001)</td>
<td>r = 0.38 (p = 0.001)</td>
<td>r = 0.46 (p = 0.004)</td>
<td>r = 0.45 (p = 0.004)</td>
</tr>
<tr>
<td>Alveolar width</td>
<td>r = 0.48 (p = 0.002)</td>
<td>r = 0.38 (p = 0.019)</td>
<td>r = 0.46 (p = 0.004)</td>
<td>r = 0.37 (p = 0.22)</td>
<td>r = 0.48 (p = 0.002)</td>
<td>r = 0.51 (p = 0.001)</td>
<td></td>
</tr>
</tbody>
</table>
the histomorphometric parameters (Table 2). This probably reflects the parallel effects of masticatory function on both the morphology and the trabecular architecture of the alveolar process. Soft diet and the resulting reduction of masticatory forces lead to more tooth eruption and a higher alveolar process during growth. Normal masticatory function (hard diet) has an anabolic effect on both trabecular and cortical bone of the alveolar process. A wider alveolar process with an enhanced trabecular bone may better withstand the important mechanical strain during mastication. This is in accordance with recent evidence of a positive correlation between maximum bite force and alveolar process width in humans [36,37].

The stomatognathic system presents a unique biomechanical configuration, which is entirely different from any other skeletal site of the body. In the rat, the forces developed during mastication have been estimated to range from 2 to 25 N (but mainly between 4 and 6 N) depending on the hardness of the food [32,38]. During normal function the mechanical loading of the long bones is inferior to that of the alveolar bone during mastication. For most vertebrates, long bone peak functional strains range from less than 1000 microstrain (με) during walking to between 2000 and 3200 με for more vigorous activities [19]. Finite element data suggest that alveolar bone strain during mastication can reach up to 4000–6000 με depending on food consistency [20,21].

There is strong evidence that bone formation is influenced by strain rate, frequency, amplitude, duration, and interopulation of rest periods [19]. It is known, for example, that bone cells respond better to a mechanical environment dominated by high strains changing at different periods [19]. Finite element data suggest that alveolar bone strain during mastication can reach up to 4000–6000 με depending on food consistency [20,21].

The results of the present study seem to confirm the hypothesis of an important effect of masticatory function rehabilitation on the alveolar bone. Significant shape adaptation of the alveolar process to a modified masticatory functional environment took place even after growth cessation. Alveolar trabecular bone architecture did improve in adult rats after functional rehabilitation although the negative effects of hypofunction were not completely reversed during the period under study. This, extrapolated to humans, could mean that after masticatory functional rehabilitation (orthognathic surgery, implant prosthetic restoration) an improvement of alveolar bone quantity and quality is to be expected. This in turn may have a positive impact on the risk or the progression of periodontal disease as has been shown in recent investigations [44].

References


