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Effect of occlusal hypofunction and its recovery on the three-dimensional architecture of mandibular alveolar bone in growing rats

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ABSTRACT

Backgroud: Normal occlusion is very important for physiological structure of mandible. However, the details of influences of occlusal hypofunction and its recovery on the three-dimensional architecture of mandibular alveolar bone in growing rats are still lacking.

Materials and methods: Forty-eight growing male Sprague–Dawley rats were randomly divided into normal ($n = 24$), hypofunctional ($n = 12$), and recovery ($n = 12$) groups. The hypofunction group was developed by inserting a bite-raising appliance between the maxillary and mandibular incisors of the rats. Two weeks after insertion, the appliance was removed to result in the recovery group; the experiment continued for two additional weeks. The experimental animals and control animals were killed weekly. In addition to measuring the body weight and masseter muscle weight of the rats, the histomorphology and microstructure of the mandibular alveolar bone were scanned using microcomputed tomography.

Results: A lighter masseter muscle and a higher and narrower alveolar process were observed in the hypofunction group compared with the control animals ($P < 0.05$). Mandibular remodeling also occurred in the hypofunctional group, as demonstrated by a smaller trabecular cross-sectional area, looser trabecular bone, decreased bone volume fraction, trabecular thickness, trabecular number, and increased bone surface density and trabecular separation, especially at week 2 ($P < 0.05$). After removing the anterior bite-opening appliance, the altered masseter muscle weight and architecture of the mandibular alveolar bone were gradually reversed and reached normal levels at the end of the experiment ($P > 0.05$).

Conclusions: A loss of occlusal stimuli can lead into mandibular alveolar bone remodeling, and the recovery of occlusion can restore the altered mandibular architecture in growing rats.

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1. Introduction

Occlusal function is generated when the upper and lower teeth are in contact during daily activities. This type of physical stimulation is necessary for the development and maintenance of mandibular structure [1,2]. As part of the mandible, the alveolar bone houses the teeth and periodontal tissues and directly bears the masticatory loading. This unique biomechanical configuration makes the alveolar bone an interesting site for studying the link between occlusal hypofunction and mandibular homeostasis, as investigated in different animal models in recent years.

Bresin *et al.* [3] demonstrated that feeding rats a soft diet can decrease bone mass and density in mandibular alveolar bone and thus considered the function of the masticatory muscle to be a determinant of the amount and density of cortical and trabecular bone. After inserting an upper posterior bite block, Mavropoulos *et al.* [4] also observed a significant modification of shape and structure in growing rats. Additionally, bilateral resection of the masseter muscles [5] and extraction of the molars [6] have obvious effects on the structure of alveolar bone. However, these methods could not eliminate the contact between molars, which generates most of the occlusal forces during the daily activities of animals, such as biting and chewing, whether they are feeding on a soft diet or using an appliance such as a posterior bite block. Moreover, myotomy or tooth extraction will cause injury to experimental animals to different extents.

To induce occlusal hypofunction, Suhr *et al.* [7] developed the approach of attaching an anterior bite plate and a metal cap to the maxillary and mandibular incisors of rats and observed biochemical changes in periodontal tissues and suppressed formation of mandibular cortical bone [8,9]. Nevertheless, the details regarding the influence of occlusal hypofunction on the three-dimensional (3D) architecture of mandibular alveolar bone in growing rats are yet to be determined. Whether rehabilitation can improve alveolar bone architecture also remains to be confirmed.

The high-resolution and accuracy of microcomputed tomography (micro-CT) have made this method a promising recent candidate for evaluating the microarchitecture of bone specimens [10,11], including the jaw bone under different occlusal conditions in three dimensions [3,4]. Therefore, the aim of this article was to evaluate the influence of occlusal hypofunction and its recovery on the 3D architecture of mandibular alveolar bone in growing rats using micro-CT. The first null hypothesis tested was that the loss of occlusion will lead into the 3D architectural alteration of mandibular alveolar bone. The second null hypothesis tested was that the restoration of occlusal stimuli has potential to reverse the adverse effects of occlusal hypofunction on the growing mandible.

2. Materials and methods

2.1. Animals and experimental design

A total of 48 5-wk-old male Sprague–Dawley rats (provided by the Laboratory Animal Centre of the Fourth Military Medical University, Xi'an, China) were equally divided into a control group (Con group, $n = 24$) and experimental group (Exp group, $n = 24$); the Exp group was subdivided into a hypofunction group ($n = 12$) and a recovery group ($n = 12$). The control animals, which did not receive any treatment, were sacrificed weekly ($n = 6$) to serve as a reference. Occlusal hypofunction was induced by inserting appliances modified from the design of Suhr *et al.* [7]. The appliances consisted of upper and lower resin bite plates inserted between the maxillary and mandibular incisors of the rats ($n = 24$), fixed by means of light-curing composite resin (3M ESPE Filtek P60 Posterior Restorative; 3M Co Ltd, Goettingen, MN) (Fig. 1A,B). Consequently, the contact between the molars was eliminated, and occlusal stimuli were reduced over a 2-wk period. Physically, the occlusal contact of the molars was nearly recovered at the end of this phase. In the rats in the recovery group, the appliances were removed after 2 wk of occlusal hypofunction. No malocclusion was observed during the experiment. Experimental animals were killed weekly, with six rats being sacrificed at each time point (Fig. 1C). The rats were housed in cages in a temperature-controlled room (24 ± 1°C) with a 12–12 light–dark cycle (lights on at 8:00 AM). Food and water were available *ad libitum*, and a powdered diet was used because the animals subjected to bite opening had difficulty in taking the usual pelleted diet. All protocols were performed under intraperitoneal administration of pentobarbital sodium (30 mg/kg body weight), and all efforts were made to minimize the animals' suffering. This study was approved by the Animal Research Committee of the Fourth Military Medical University, Xi'an, China.

2.2. Tissue preparation

After recording the body weights of the rats in each group weekly, all animals were killed via decapitation under deep anesthesia induced through intraperitoneal injections of pentobarbital sodium (50 mg/kg body weight). The masseter muscles of the right side were carefully dissected and weighed using an electronic scale (BSA224S-CW; Sartorius, Goettingen, Germany). The mandibles were dissected, defleshed, and separated at the symphysis into their two halves; they were then fixed with 10% neutrally buffered formalin for 1 wk for micro-CT scanning.

2.3. Micro-CT scanning

The left mandible specimens were fixed in a cylindrical sample holder in air and were scanned using a high-resolution micro-CT system (Inveon, Siemens, Germany; 80 kV, 500 mA, 800 ms integration time). The image pixel size was set to 1024 × 1024, and the slice thickness was set to 14.97 μm. An adaptive threshold was used to distinguish the trabecular bone area from the nontrabecular bone area and was kept constant for all the samples. The scanning time for each specimen was approximately 150 min. We selected the mid-coronal image through the central cusp of the lower first molar and quantified the contoured cross-sectional area of the trabecular bone in the alveolar bone (Fig. 1D) using a computer-assisted image analysis system (Leica QWin Plus;