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Masticatory Demands Induce Region-Specific Changes in Mandibular Bone Density in Growing Rats

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ABSTRACT

This study investigates the structural adaptation of the mandibular bone when subjected to different masticatory functional and mechanical demands during growth. The effect of two experimental factors, the insertion of a bite block and the alteration of food consistency, on the bone mineral density (BMD) of the mandible was investigated in growing rats. Fifty-two male albino rats were divided into two equal groups, fed with either the standard hard diet or soft diet, at the age of four weeks. After two weeks, half the animals in both groups had their upper molars fitted with an upper posterior bite block. The remaining animals served as a control. Region-specific BMD of the mandible was subsequently measured using dual-energy X-ray absorptiometry (DXA). Soft diet and the consequent reduction of the forces applied to the mandible during mastication resulted in the reduction of BMD in all regions under study. The insertion of the bite-opening appliance (bite block) and the resulting stretching of the soft tissues led to the application of a continuous light force on the lower molars, which was associated with a significant increase of the BMD in the part of the alveolar process just below the root apices. These results raise the question of whether orthodontic treatment with similar appliances may have some, previously unsuspected, short- or long-term effects on the mandibular bone during growth and whether their effects depend on the individual soft-tissue characteristics.

KEY WORDS: Mastication, Food consistency, Bite block, Bone mineral density, Mandible, Mechanical loading.

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INTRODUCTION Return to TOC

The internal structure of bone is constantly adapting to its functional environment through processes that remove existing bone and deposit new bone. The muscles provide an important mechanical stimulus for bone formation. A number of clinical[1–3] and animal[4,5] studies suggest a relationship between masticatory muscle function and skeletal adaptation in the craniofacial region. A number of appliances used in orthodontics displace the mandible forward or downward, causing stretching of the orofacial soft tissues. An example is the bite block, which when constantly present between the upper and lower dentitions, is used to disengage the occlusion or inhibit tooth eruption.[6–8]

Masticatory muscle capacity is known to vary significantly between individuals. Both bite force[9] and thickness of the masseter muscle[10] were found to be highly variable among young individuals. On an experimental level, using a rat model, it has been proved that the functional capacity of the masticatory muscles can be altered in a reproducible way by changing the consistency of the food.[11–13] This model was used in a previous study that attempted to investigate the effect of prolonged muscular elongation induced by a posterior bite block on the length of the deep masseter in growing rats with different masticatory muscle capacities.[14] The insertion of such an appliance triggers, moreover, a dentoskeletal adaptive response of the whole craniofacial complex, as has been shown in previous studies on growing rats.[15,16] The morphological aspects of mandibular adaptation, in particular, were extensively investigated in a recent study.[17] The structural component of this adaptation remains, however, completely unknown.

The technique of dual-energy X-ray absorptiometry (DXA) allows rapid and precise measurement of bone mass or density. Specially modified for small animals, it has been applied for the analysis of rat bone, and it has been found to be highly accurate and precise.[18– 20] This cross-sectional study investigates in a rat model the region-specific changes of bone mineral density (BMD) in the mandible when subjected to different masticatory functional and mechanical demands during growth.

MATERIALS AND METHODS Return to TOC

Materials

The material of this study is based on the material of a previous morphometric investigation.[17] The experiment was carried out on 52 young male albino rats (Sprague-Dawley; B&K Universal, Södertälje, Sweden). When obtained, they were three weeks old and weighed approximately 60 g. They were kept in quarantine for one week according to the rules of the laboratory animal department. The experimental design was approved by the Ethical Committee for Animal Research in Göteborg, Sweden.

Experimental design

The animals were divided into two groups and were fed with either a hard or soft diet. The hard diet consisted of the standard food for rats in the form of pellets (R34 flour, Lactamin, Stockholm, Sweden). For the rats fed the soft diet, the same flour (R34) was mixed with water in a proportion of 1:1 to obtain a soft, porridgelike consistency. Body weight was recorded weekly to monitor the growth and health of each animal.

After two weeks, half the animals in each group were fitted with upper posterior bite blocks, whereas the other half served as controls (Figure 1). As a result, there were four subgroups of 13 animals each, designated the “hard-diet bite block” (HB), the “soft-diet bite block” (SB), the “hard-diet control” (HC), and the “soft-diet control” (SC) experimental groups. The bite blocks were made of light-cured composite resin (Charisma; Heraeus Kulzer, Hanau, Germany), pressed into a custom-made mould and light cured (Litema Halogen Plura; Litema, Munich, Germany). The size of the appliance was 7 × 3 mm, and it was two mm thick above the first molar and one mm thick above the third molar. The appliance was cemented under anesthesia on the two maxillary molar rows with Vitrebond™ cement (3M Dental Products, St Paul, Minn) and light cured for 40 seconds.

Because the incisors could not come in contact after the immediate insertion of the appliance, initial impairment of the biting ability was unavoidable. To avoid group differences because of this, the control animals had their incisors cut with a diamond disk (under anesthesia) by approximately two mm. The incisal contact was reestablished in all groups within one week because of the continuous eruption of the incisors. None of the appliances fell off during the investigation period. The general behavior of all the animals was consistent throughout the duration of the experiment.

Methods

At the end of the experiment (four weeks after placement of the bite block), the animals were sacrificed in a CO_2 chamber. Their mandibles were dissected, defleshed, and separated at the symphysis into two halves. Each left hemimandible was subsequently thoroughly defleshed and fixed in pure alcohol (Figure 2).

They were subsequently scanned using a pencil-beam bone densitometer (Hologic QDR-1000, Waltham, Mass) to measure their BMD (mg/cm^2). As has been suggested before for small animals,[18,20] the “ultrahigh resolution” mode was used, and a smaller, custom-made collimator was placed over the original one. Each hemimandible was placed in a standardized way in a plastic box filled with three cm of normal saline. This has been shown to improve edge detection acting as soft-tissue equivalent.[21] Six geometrically defined areas were chosen to investigate regions of specific functional or anatomical interest (Figure 3).

- R1: The upper part of the incisor alveolar process, which is made up almost entirely of cortical bone.
- R2: The lower part of the molar alveolar process below the root apices.
- R3: The upper part of the molar alveolar process that contains the teeth.
- R4: A small area between the body of the mandible and the ramus.
- R5: The angular process.
- R6: The condylar process.

Statistical analysis

To investigate the BMD differences between the four experimental groups, the multivariate analysis of variance statistical procedure was used with the consistency of the food (hard vs soft) and the insertion of the bite block (control vs bite block) as independent factors. This allowed the evaluation of the effect of each experimental factor separately, as well as their interaction. Even if there was no difference of body weight between the groups (one-way analysis of variance), it was decided to use body weight as a covariate to avoid any possibility of bias due to different body sizes.

Error of the method

The whole methodological procedure was repeated in 20 of the mandibles two weeks after the initial measurements. The error of the method was calculated according to the formula of Dahlberg[22]: $Se = \sqrt{\Sigma d^2 / 2n}$, where Σd^2 is the sum of the squared differences between pairs of recordings and n is the number of duplicate measurements. It did not exceed 5.1 mg/cm^2. The coefficient of reliability[23] was also calculated using the formula $(1 - Se^2/St^2) \times 100$, where Se^2 is the variance of the error and St^2 is the total variance of the sample. Its range was found to be from 98.6% to 99.4% (Table 1).

RESULTS Return to TOC

The mean BMD values for each of the regions of interest (ROI) under study for the four groups are given in Table 2. The highest BMD values were recorded in the molar alveolar process (R2 and R3) and the lowest in the angular and condylar processes (R5 and R6). The consistency of the food had the most pronounced effect. Those animals fed the soft diet exhibited significantly lower BMD in all regions under study ($P < .001$). The placement of the upper posterior bite block had a region-specific effect on the BMD of the alveolar process. It led to less dense bone in the upper part of the alveolar process that includes the teeth (R3; $P < .001$), whereas it increased BMD in the region immediately below (R2; $P < .001$). There was an interaction of food consistency and bite block in the upper part of the alveolar process (R3). The effect of the appliance on the BMD of this region was significantly more important in the animals fed the hard diet ($P < .01$).

DISCUSSION Return to TOC

Dual-energy X-ray absorptiometry

Although there are several other techniques available, DXA is considered a reference technique to measure BMD.[24] It uses two X-ray beams with different levels of energy. After subtraction of the soft-tissue absorption, the bone absorption of each beam is used to calculate the BMD. In pencil-beam systems, like the one used in this study, the acquisition of these radiographic images requires a scan in two directions, using a point-collimated X-ray source and a single collimated detector. This technique has been successfully used for the analysis of rat bone but only rarely used to measure BMD in specific areas of the rat mandible.[25– 27] When BMD values are very small, there are areas of the bone that are not recognized as such by the software. This is why the bone edge of regions R5 and R6 had to be manually corrected in order to improve the validity of our results. The reliability of measuring BMD in the ROI chosen for this study was found to be very high, confirming previous reports. However, the ability to position the explanted bone and define the ROI consistently using the same anatomical landmarks is of at most importance in such studies.

Animal model

The mandible can be considered a structure that maintains optimal biometrical relations between the articulations, the teeth, and the points where muscular forces are applied.[28] Each half of the rat lower jaw (hemimandible) consists of different functional units: the alveolar process housing the continuously erupting incisors, the alveolar process of the molars, and the angular, coronoid, and condylar processes, which are all sites of attachment for the major masticatory muscles.[29] The size and shape of these processes are dictated by the relative development and organization of the adductor muscles. This makes it an excellent model for studying the effect of an altered loading environment on bone growth and remodeling. The major optimal design bone-remodeling theories[30,31] stipulate that the trabecular structure of bone is optimized to offer maximum resistance to stresses and strains with a minimum amount of bone mass. The correspondence among trabecular structure, bone density, and mechanical loading has been demonstrated in several studies.[32,33]

Effect of food consistency

Soft-diet results in the reduction of the intermittent forces exerted on the mandible during mastication. Prolonged use of food with a soft consistency has been shown to reduce the functional capacity of the masticatory muscles in the rat.[11–13] In this study, BMD decreased significantly in all ROI in the animals fed the soft diet. This confirms the results of a previous study where BMD was expressed in millimeters of aluminum-equivalent thickness. In that study, a soft diet led to lower BMD values in specific areas of the rat mandible, which roughly corresponded to the areas investigated in this study.[34] In another recent study, it has been shown that BMD was significantly lower in the coronoid and the angular processes of the mandible in a powdered-diet group.[35] This is also in line with a previous histomorphometric study, where the rate of bone formation at the lateral periosteal surface of the ramus was found to be significantly lower in rats fed a soft diet.[36]

Effect of bite block

The advantage of an upper posterior bite block, compared with an appliance that would have kept the mandible in a protruded position, is that it allows free anteroposterior and jaw-opening movements. The bite block, however, may have been a mechanical disadvantage during mastication, especially in the hard-diet group. Body weight, however, did not differ between groups at any time point during the course of the experiment. Such an appliance opens the bite, stretching the masticatory muscles, which although gradually adapt in length,[14] must probably compensate for a less favorable mechanical environment.

In this study, the bite block exerted a continuous light force on the lower molars throughout the course of the experiment. This increased the compressive loads in the bone around and below the molars, which were in direct contact with the appliance during mastication. This resulted in an increase of BMD in the lower part of the alveolar process (R2) below the root apices. The exertion of a continuous light force inevitably led to tooth movement (intrusion) with areas of bone resorption and bone apposition and enlargement of the periodontal space. This is most probably the reason why there was a decrease of BMD in the upper part of the alveolar process, which included the molars. This effect was significantly more important for the animals on the hard diet where the mechanical loading was more important.

CONCLUSIONS Return to TOC

In this study, alteration of the masticatory functional and mechanical demands in a rat model led to region-specific BMD changes in the mandible during growth. The reduction of the forces exerted during mastication (soft diet) resulted in reduction of bone density in all regions under study. The insertion of a bite-opening appliance (bite block) caused the stretching of the soft tissues, resulting in application of a continuous light force on the lower molars, which increased the BMD in the part of the alveolar process just below the root apices. Although the rat mandible differs significantly both morphologically and functionally from the human mandible, it could be questioned whether orthodontic treatment with similar appliances might have some short- or long-term effects on the mandibular bone and whether these effects depend on the individual soft-tissue characteristics.

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TABLES Return to TOC

TABLE 1. The Method's Error and Reproducibility for Each of the Regions of Interest Under Study[a]

	R1	R2	R3	R4	R5	R6
Method's error (mg/cm²)	5.1	4.74	4.9	3.4	2.5	1.4
Coefficient of reliability (%)	99.4	99.3	98.9	98.6	98.7	98.7

[a] R1 indicates upper part of the incisor alveolar process; R2, lower part of the molar alveolar process below the root apices; R3, upper part of the molar alveolar process including the teeth; R4, area between the body of the mandible and the ramus; R5, angular process; R6, condylar process.

TABLE 2. Mean Values (and Standard Deviation) of Region-Specific BMD (mg/cm^2) for the Four Experimental Groups. The Effect of Each Experimental Factor and Its Interaction on the BMD Values Obtained was Investigated by Means of a MANOVA[a]

	Experimental Groups				MANOVA[b]		
	HC	HB	SC	SB	Bite Block	Soft Diet	Interaction
R1	200.8 (7.2)	196.0 (7.9)	179.1 (7.0)	177.6 (9.9)	NS	↓, ***	NS
R2	216.9 (10.7)	240.9 (13.1)	194.1 (6.9)	222.8 (12.5)	↑, ***	↓, ***	NS
R3	234.1 (4.8)	195.2 (7.1)	188.0 (7.3)	170.9 (5.8)	↓, ***	↓, ***	**
R4	130.5 (8.9)	121.1 (7.8)	110.8 (8.1)	113.6 (6.9)	NS	↓, ***	NS
R5[c]	62.3 (2.4)	60.8 (2.8)	55.0 (3.8)	57.1 (2.7)	NS	↓, ***	NS
R6[c]	64.1 (3.0)	63.3 (3.1)	60.0 (3.9)	59.5 (3.9)	NS	↓, ***	NS

[a] BMD indicates bone mineral density; MANOVA, multivariate analysis of variance; HC, hard-diet control; HB, hard-diet bite block; SC, soft-diet control; SB, soft-diet bite block; R1, upper part of the incisor alveolar process; R2, lower part of the molar alveolar process below the root apices; R3, upper part of the molar alveolar process including the teeth; R4, area between the body of the mandible and the ramus; R5, angular process; R6, condylar process.

[b] Arrows ↑ and ↓ denote the direction of the effect on the independent variables, increase and decrease of the value, respectively; ** $P < .01$; *** $P < .001$.

[c] After manual bone edge correction.

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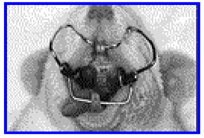
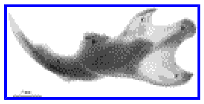


Click on thumbnail for full-sized image.

FIGURE 1. The bite block in place. A custom-made cheek retractor was used to facilitate the procedure that took place under anesthesia. (With permission from Mavropoulos et al, 2004.[17])



Click on thumbnail for full-sized image.

FIGURE 2. Left hemimandible of the rats used in the study (Sprague-Dawley). (A) Incisor alveolar process. (B) Molar alveolar process. (C) Coronoid process. (D) Condylar process. (E) Angular process

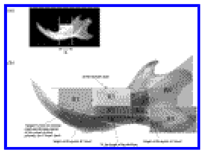


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FIGURE 3. (a) The rat mandible as it appears on screen after a densitometric scan. (b) ROI used for the bone density measurements. X, lateral length of the dentition; R1, upper part of the incisor alveolar process; R2, lower part of the molar alveolar process below the root apices; R3, upper part of the molar alveolar process including the teeth; R4, area between the body of the mandible and the ramus; R5, angular process; R6, condylar process; and "line," the minimal step defined by the software, representing in this case 0.11 mm

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