Intermittent posterior displacement of the rat mandible in the growth period affects the condylar cancellous bone

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ABSTRACT

Objective: To examine whether intermittent posterior condylar displacement causes changes in cancellous bone in the mandibular condyle during the growth period.

Materials and Methods: Sixteen 5-week-old male Wistar rats were divided into experimental and control groups. In the experimental group, an appliance was attached to the maxillary incisors to induce posterior displacement of the condyles in the occluded condition. Untreated rats served as the control group. Animals were sacrificed at 14 days, and the condyles were removed to analyze the three-dimensional cancellous bone structure by microcomputed tomography (micro-CT). Serial sagittal paraffin sections of the condyles were used for hematoxylin and eosin staining to investigate histomorphological changes and for tartrate-resistant acid phosphatase (TRAP) staining to identify osteoclastic cells.

Results: Micro-CT analysis showed that in the experimental group, the bone volume fraction and the degree of anisotropy were significantly decreased compared with those in the control group in the anterior region of the condyle. Moreover, the number of TRAP-positive cells was significantly greater in the same region in the experimental group than in the control group.

Conclusion: Intermittent posterior displacement of the mandible can cause region-specific changes in the profile and microarchitecture of the condylar cancellous bone. (*Angle Orthod.* 2011;81:975–982.)

KEY WORDS: Cancellous bone; Microarchitecture; Intermittent posterior displacement; Mandibular condyle

INTRODUCTION

Many studies have examined the growth of the mandible, specifically the mandibular condyles.[1–4] Condylar growth is affected by heredity, hormones, the environment, and metabolism[5] and is significant in the development of the orofacial complex. Since the condyle is subjected to forces applied to the teeth

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during mastication,[6] occlusion is important in condylar growth. Actually, malocclusion influences the condylar size,[7] cartilage thickness, and proliferation.[8] Moreover, malocclusion sometimes causes the displacement of the condylar position that normally occupies the center of the glenoid fossa, resulting in abnormal intermittent loading on the tissues in and around the temporomandibular joint (TMJ). Some authors[9–11] have especially referred to a correlation between TMJ disorder (TMD) and condylar displacement resulting from malocclusion.

Previous studies have examined the condylar cartilage under various conditions in animals. For instance, posterior displacement of the condyle reduces the number of cartilaginous cells and inhibits the proliferation of chondrocytes and the amount of extracellular matrix.[12,13] Intermittent posterior condylar displacement due to malocclusion also causes abnormal remodeling of the condylar cartilage and nerve injury.[14] During mastication, the mandibular condyle moves along the articular surface of the temporal bone and is subject to complex loading patterns. Abnormal loading in the TMJ may harmfully influence not only condylar cartilage but also the cancellous bone. However, only a few studies have examined cancellous bone of the mandibular condyle, which is located posteriorly in the glenoid fossa.

The cancellous bone structure is considered to sustain loads by aligning the shape of the trabeculae depending on the amount of stress.[15] It has been suggested[16,17] that loading causes an increase in anisotropy, which determines the shape of the trabeculae. Therefore, the posterior displacement of the condyle is likely to generate nonphysiological stress on the TMJ, and its structure may change. The present study aimed to examine whether intermittent posterior condylar displacement can cause changes in cancellous bone in the mandibular condyle.

MATERIALS AND METHODS

The experimental procedures described here were approved by the Institutions Animal Care and Use Committee (#0110103A) and were performed in accordance with the Animal Care Standards of Tokyo Medical and Dental University.

Experimental Model

Sixteen 5-week-old male Wistar rats were randomly divided into control (n = 8) and experimental (n = 8) groups. In the experimental group, we modified the guiding appliances that were used in previous studies[12,14] to induce posterior displacement of the condyle in the occluded condition. The modified guiding appliances were constructed from band material

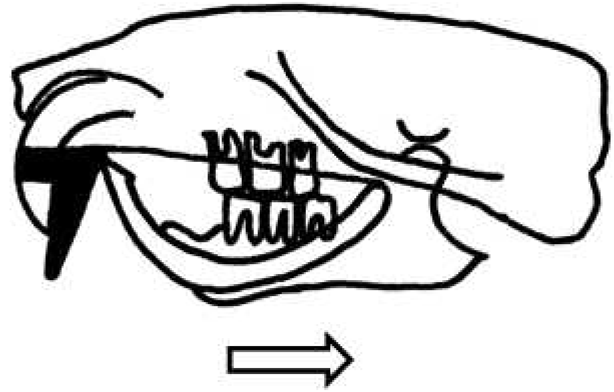

Figure 1. Experimental model. Posterior displacement of condyles (open arrow) in the occluded condition was achieved using the guiding appliance (painted in black), which was attached to the maxillary incisors.

(Rocky Mountain Morita, Tokyo, Japan) measuring 8.5 × 8 × 3 mm and attached to the maxillary incisors with composite resin (Clearfil Liner Bond II; Kuraray Co Ltd, Okayama, Japan) for 14 days (Figure 1). All of the rats were fed powdered diet because it was difficult for the experimental group to eat pellets, and rats were given water throughout the experimental period. Animals were sacrificed at 14 days.

Microcomputed Tomography Analysis

After anesthesia was induced by the inhalation of diethyl ether and the intraperitoneal injection of chloral hydrate (400 mg/kg), the animals were perfused intracardially with 4% paraformaldehyde in 100 mM of sodium phosphate buffer (pH = 7.4). The left mandibles were immediately removed and fixed with the same fixative at 4°C for 24 hours. To determine the three-dimensional morphology and cancellous bone structure, we used microcomputed tomography (micro-CT) (SMX-100CT, Shimadzu, Kyoto, Japan). A direct, model-independent method was used to quantify various architectural parameters[18] for each specimen. These parameters included the bone volume fraction (BV/TV), trabecular number (TbN), trabecular thickness (TbTh), trabecular separation (TbSp), trabecular spacing (TbSpac), and the degree of anisotropy (DA).[19,20] A region of interest (1.0 × 1.0 × 0.2 mm) was selected at the center of the anterior and posterior halves of the condylar head (Figure 2A).

Histological Preparation

The mandibles were decalcified in 4.13% ethylenediamine tetraacetic acid solution (pH = 7.4) at 4°C for 8 weeks; next the mandibles were embedded in paraffin using conventional methods. Serial 6 μm–thick sections were cut using a microtome (RM2155; LEICA Co Ltd, Nussloch, Germany) parallel to the sagittal

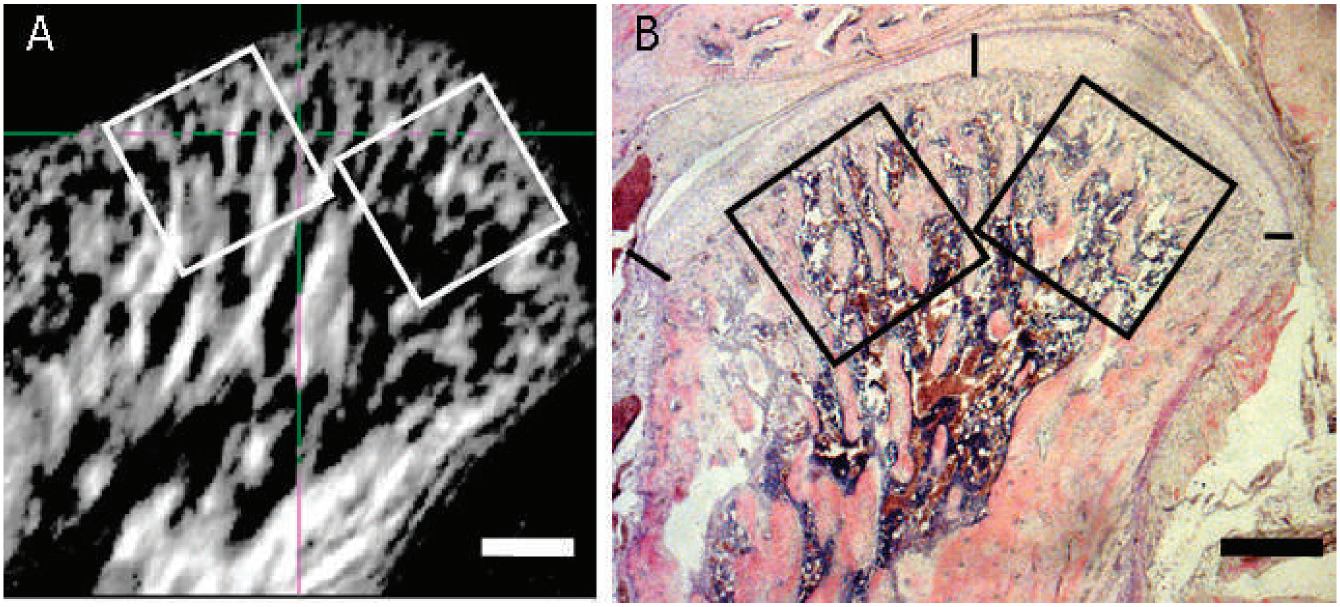

Figure 2. The selected regions of interest in the sagittal central section of the condyle. (A) Two blocks (1.0 × 1.0 × 0.2 mm) were selected for microcomputed tomography (micro-CT) analysis. (B) The surface of the condylar head was equally divided into two parts (ie, anterior and posterior); the lines are two edges and the midline of the cartilage layer. Two blocks were cut from the center of the subchondral bone of each part for histological analysis. Bars depict 500 μm.

plane of the mandibular condyle. The sections were used for hematoxylin and eosin (HE) staining for the observation of histomorphological changes and for tartrate-resistant acid phosphatase (TRAP) staining (we used 'TRACP&ALP double-stain kit,' TaKaRa, Otsu, Japan) to identify osteoclastic cells.

Histomorphometry and Quantitative Analysis

The observation area was photographed using a light microscope (Nikon Microphoto-FXA, Nikon, Tokyo, Japan) equipped with a digital camera (DXm1200, Nikon). The condyle was divided into two areas with reference to a previous study[20]; a region of interest (1.0 × 1.0 mm) was selected at the center of the anterior and posterior regions (Figure 2B). We counted the number of TRAP-positive cells in the regions of interest.

Statistical Analysis

The control and experimental groups were compared with a Student's *t*-test with a 95% significance level using statistical analysis software (Statview 5.0; SAS Institute, Cary, NC).

RESULTS

The body weight of the animals increased, with no significant difference between the control and experimental groups during the experimental period (data not shown). There were no significant differences in inspecting the three-dimensional images of the condyles in the control and experimental groups (Figure 3). However, there were significant decreases in both BV/TV and DA in the experimental group in the anterior region. On the other hand, there were no significant differences in any of the parameters between the control and experimental groups in the posterior region (Figure 4).

Compared to the control group, the amount of trabecular bone seemed to be decreased in the anterior region of the condyle in the experimental group in the histological investigation in sections with HE staining (Figure 5A), which agrees with the fact that there was a significant decrease in BV/TV in the anterior region in the experimental group (Figure 4A). TRAP-positive cells below the hypertrophic layer of the chondrocytes of 7-week-old rats were found in both groups (Figure 5B). The number of TRAP-positive cells in the anterior region of the condyle significantly increased in the experimental group (Figure 6).

DISCUSSION

In this study, we used rats as an experimental model; rats have often been used for similar studies in the literature.[1,5–8,13,14] The modified guiding appliance in our study induced malocclusion (ie, posterior displacement of the mandible) in the occluded condition. The rats in the experimental group were able to masticate in the same way as the control rats. There was no significant difference in body weight between the two

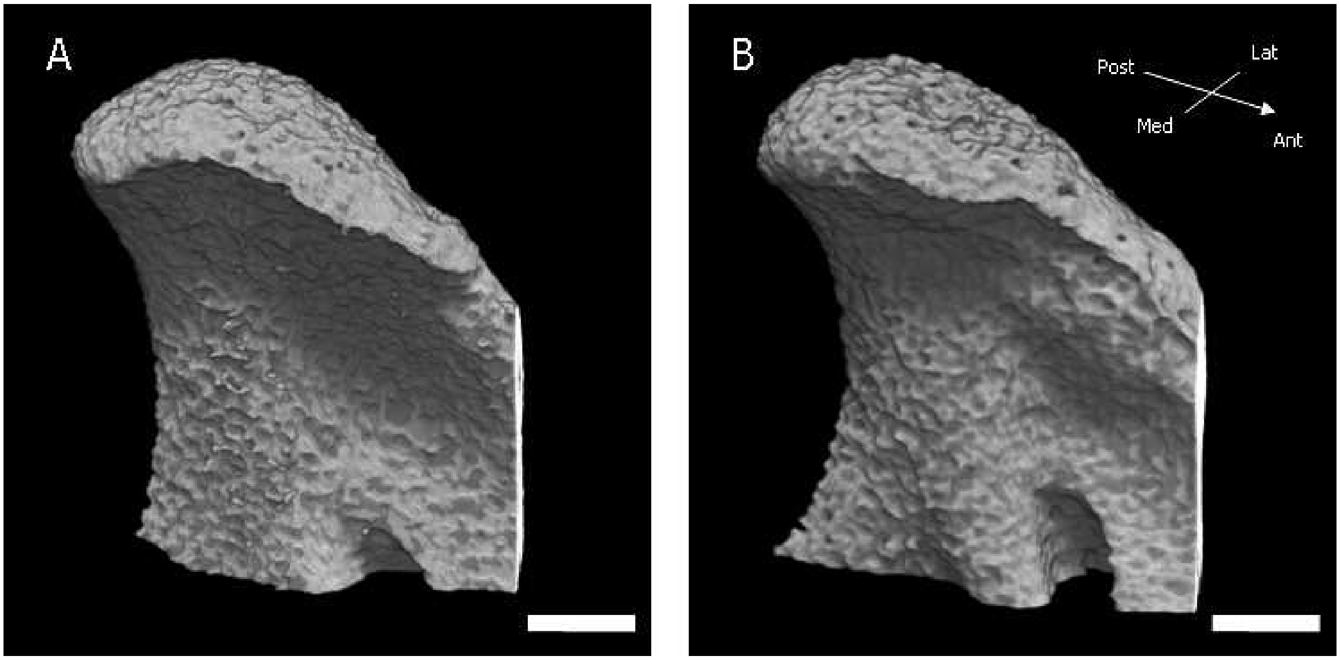

Figure 3. Three-dimensional images of the condyles. (A) Control group. (B) Experimental group. Bars depict 1 mm. Abbreviations: Post, posterior; Ant, anterior; Med, medial; Lat, lateral.

groups. Moreover, there was no anatomical difference between the right and left condyles. This indicated that the mandible displaced posteriorly without shift.

One must be cautious in interpreting the results, because all of the rats were fed powdered diet. This diet may lead to a decrease in the thickness of condylar cartilage and in the density of condylar subchondral bone.[21,22] However, one could still compare the difference between the two groups because all the rats were fed the same diet.

During mastication, complex forces are exerted on the disc and the mandibular condyle. The cartilage serves as a cushion during joint loading, whereas the cancellous bone provides structural support to the articular cartilage. Optimum mechanical loading plays an essential role in maintaining skeletal integrity. Physiological stress to the TMJ, if optimal, is very important for the development of TMJ structures during adolescence and for the remodeling of condyle in the adult.[23,24] In unloaded bone, such as that in a tail-suspension model, bone resorption and the number of the TRAP-positive cells increase.[25] A previous study[12] showed that posterior condylar displacement leads to changes in the morphology and the position of the disc that is anterior to the condylar head. In the present study, the subchondral bone structure of the anterior region changed: The bone volume fraction and the DA decreased. This indicated that joint loading was absorbed by the disc, especially in the anterior region of the TMJ.

In the present study, the micro-CT analysis showed that the bone volume fraction and the DA of the anterior region were decreased in the experimental group. Furthermore, the number of TRAP-positive cells increased based on a histomorphological analysis. It has been suggested[16,17] that loading causes an increase in anisotropy, indicating the development of a dominant direction of the trabeculae. This anisotropy applies to the mechanical properties of cancellous bone, such as stiffness and strength, while they are largely determined by its structure. Bone volume fraction and anisotropy are of special interest; 90% of the variance in mechanical properties can be explained by these parameters.[26–28] These changes indicate that the loading would be less significant in the anterior region of the TMJ in the experimental group compared to that in the control group. A previous study[12] that used the same experimental model showed that the space between the articular eminence and the mandibular condyle was wider than that observed in the control group. Thus, it is assumed that the compressive force on the anterior region of the condyle was decreased. Furthermore, the loading around the joint might be absorbed by the disc in the anterior region of the TMJ. Unfortunately, we focused on only TRAP staining to identify osteoclastic cells. The level of bone mass reflects the balance of bone-forming (osteoblasts) and bone-resorbing (osteoclasts) cells. Therefore, we should investigate not only the bone-resorbing mechanism but also the bone-forming mechanism in future studies.

The trabecular bone structure in the posterior region did not change in our study. Unlike the case of

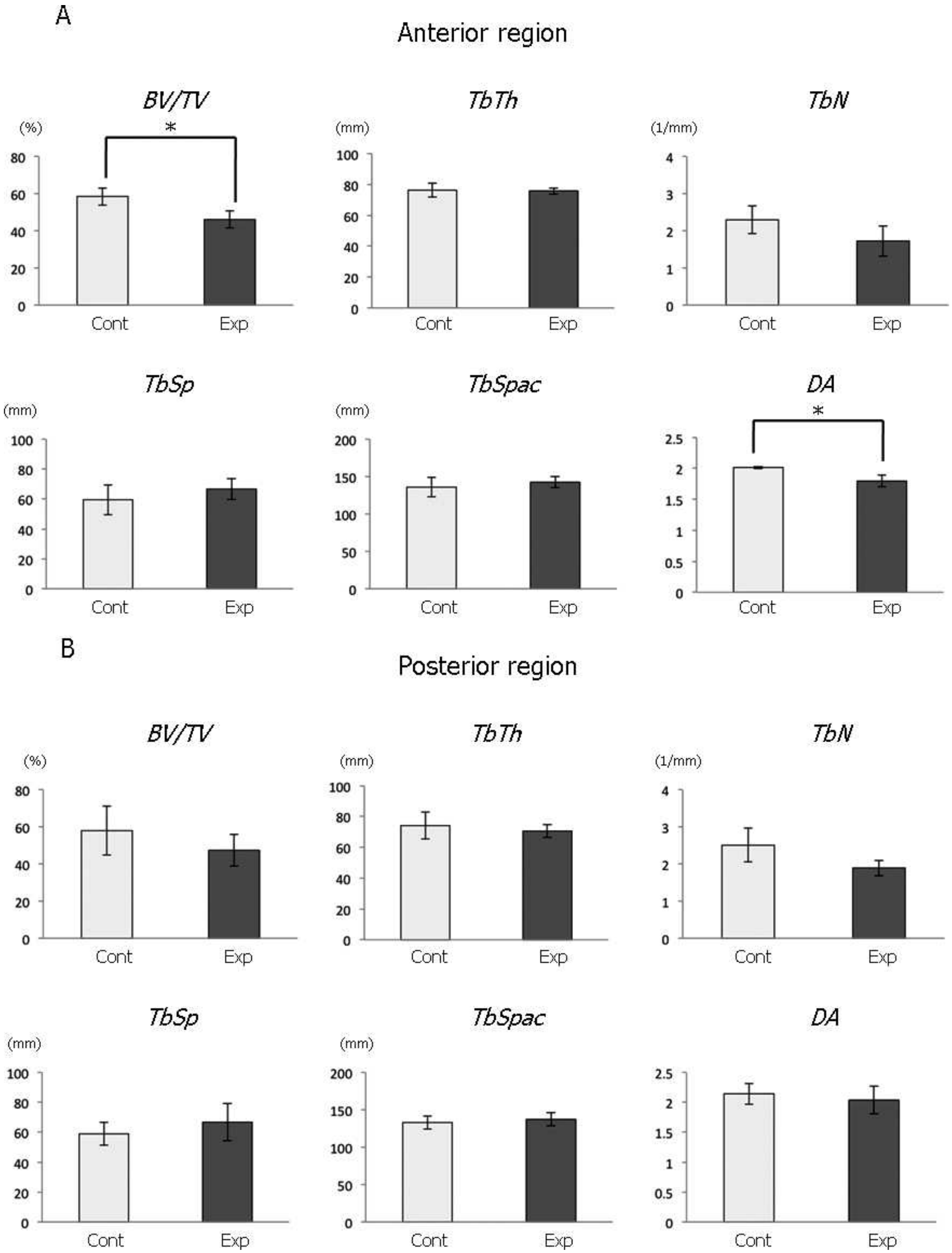

Figure 4. Comparison of the architectural parameters of the condylar subchondral bone within the selected regions of interest (ROIs). The ROIs were the anterior (A) and posterior (B) regions (n = 4). BV/TV indicates bone volume fraction; TbN, trabecular number; TbTh, trabecular thickness; TbSp, trabecular separation; TbSpac, trabecular spacing; and DA, degree of anisotropy. Value is shown as mean ± standard deviation. * $P < .05$.

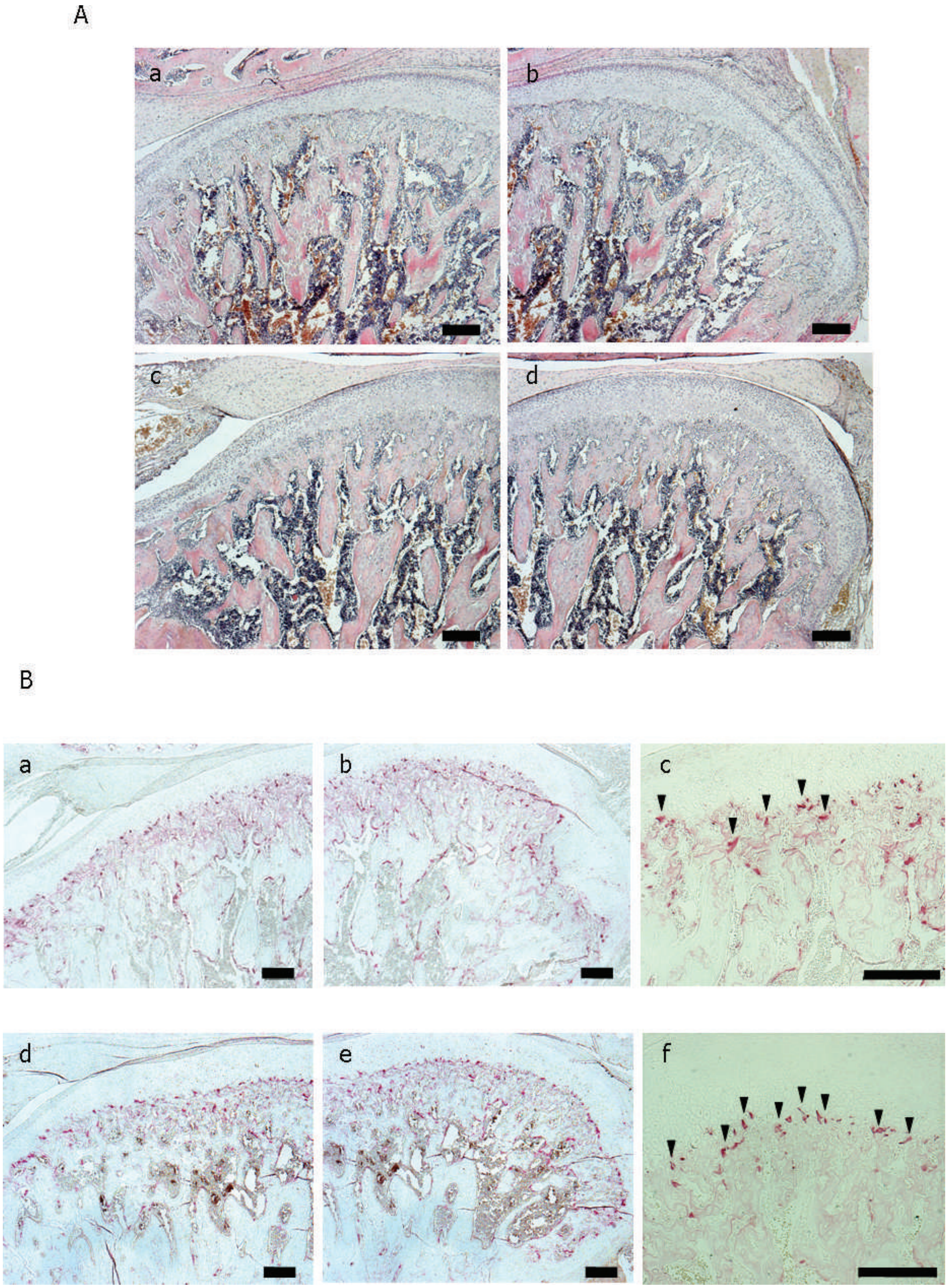

Figure 5. Light micrographs of the sagittal sections of the condyle. (A) Hematoxylin and eosin (HE) staining. a indicates anterior region in the control group; b, posterior region in the control group; c, anterior region in the experimental group. (B) tartrate-resistant acid phosphatase (TRAP) staining. a indicates anterior region in the control group; b, posterior region in the control group; c, larger magnifications of anterior region in the control group; d, anterior region in the experimental group; e, posterior region in the experimental group; and f, larger magnifications of anterior region in the experimental group. Bars depict 200 μm. Arrowheads indicate TRAP-positive cells. Original magnification 40× (a, b, d, and e) and 100× (c and f).

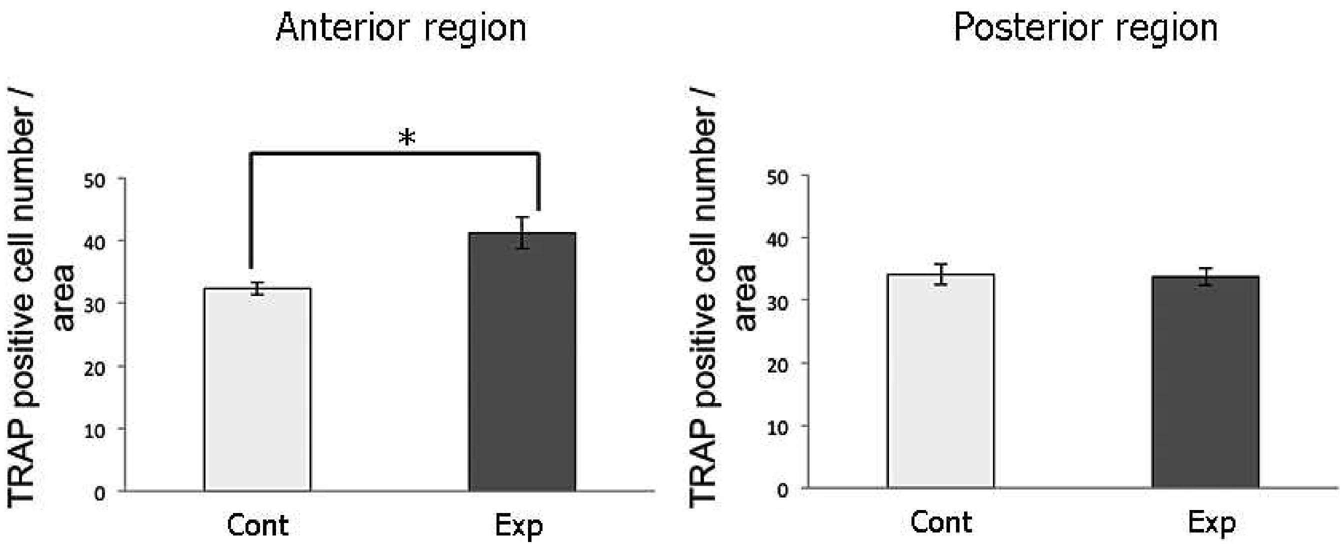

Figure 6. Number of tartrate-resistant acid phosphatase (TRAP)–positive cells in the condyle within the selected regions of interest (ROIs). The ROIs were the anterior and posterior regions (n = 4). Values are shown as mean ± standard deviation. * $P < .05$.

humans, there is no limit in movement to the posterior direction in the TMJ of the rat. In contrast, the nonphysiological stress due to posterior condylar displacement in humans must be greater because humans have a postglenoid process.

The present study revealed that posterior condylar displacement resulted in changes in the trabecular bone structure in the mandibular condyle. Since the trabecular bone structure is believed to be suitable for sustaining load,[15] this alteration indicates that the load on the TMJ was changed. This may explain why some authors[9–11] have indicated a close correlation between TMD and posterior condylar displacement resulting from malocclusion.

CONCLUSION

- Intermittent posterior displacement of the mandibular condyle can cause region-specific changes in the profile and microarchitecture of the condylar cancellous bone.

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