

# Changes in Parathyroid Hormone-Related Protein and 3-Dimensional Trabecular Bone Structure of the Mandibular Condyle Following Mandibular Distraction Osteogenesis in Growing Rats

Reiko Shibazaki, DDS, PhD,\* Koutaro Maki, DDS, PhD,† Tetsubiko Tachikawa, DDS, PhD,‡  
Yoshinobu Shibasaki, DDS, PhD,§ Robert J. Hinton, PhD,|| David S. Carlson, PhD,¶  
and Lynne A. Opperman, PhD#

**Purpose:** Distraction osteogenesis (DO) is commonly performed for mandibular reconstruction during the growth period. We tested the hypothesis that parathyroid hormone-related protein (PTHrP) in mandibular condylar cartilage and underlying trabecular bone in growing individuals undergo changes in response to distraction forces.

**Materials and Methods:** Forty-eight 6-week-old male Sprague-Dawley rats were used. Animals underwent unilateral mandibular distraction using a distractor that we devised, and unoperated animals were evaluated as controls. DO procedure was performed: 3 days' latency period, 0.4 mm/day rate, total 4.0 mm. Changes in cartilage morphology, PTHrP activity, and 3-dimensional trabecular bone structure changes measured by micro-computed tomography were examined at 0, 2, 4, and 6 weeks of consolidation.

**Results:** A marked irregularity was noted in the superior portion of the distracted side's condylar cartilage that resolved after distraction ceased. PTHrP was more strongly expressed in the hypertrophic layer of condylar cartilage on the distracted side than in controls, up to 6 weeks after the end of distraction. Subchondral trabecular bone volume, percent bone volume, and trabecular number in the superior and posterior regions of the condyle decreased significantly by 2 weeks after distraction. These parameters returned to normal in the posterior condyle, but not in the superior part of the condyle by 6 weeks following distraction.

**Conclusion:** These results suggest that unilateral mandibular distraction in growing rats causes temporary morphologic alterations of trabecular bone structure on the distracted side accompanied by increased production of PTHrP in the mandibular condyle.

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\*Part-Time Assistant, Department of Orthodontics, Showa University, Tokyo, Japan, and Postdoctoral Research Fellow, Department of Biomedical Sciences, Baylor College of Dentistry, Texas A&M University System Health Science Center, Dallas, TX.

†Professor and Chairman, Department of Orthodontics, Showa University School of Dentistry, Tokyo, Japan.

‡Professor and Chairman, Department of Oral Pathology, Showa University School of Dentistry, Tokyo, Japan.

§Emeritus Professor, Department of Orthodontics, Showa University School of Dentistry, Tokyo, Japan.

||Professor, Department of Biomedical Sciences and Center for Craniofacial Research and Diagnosis, Baylor College of Dentistry, Texas A&M University System Health Science Center, Dallas, TX.

¶Professor and Vice President of Research and Graduate Studies,

Texas A&M University System Health Science Center, College Station, TX.

#Associate Professor, Department of Biomedical Sciences and Center for Craniofacial Research and Diagnosis, Baylor College of Dentistry, Texas A&M University System Health Science Center, Dallas, TX.

Address correspondence and reprint requests to Dr Opperman: Department of Biomedical Sciences and Center for Craniofacial Research and Diagnosis, Baylor College of Dentistry, Texas A&M University System Health Science Center, 3302 Gaston Ave, Dallas, TX 75266-0677; e-mail: lopperman@bcd.tamhsc.edu

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Distraction osteogenesis (DO) is a treatment that involves application of prescribed physical forces to skeletal tissues that have been stimulated to undergo regenerative processes initiated by fracture repair. Because of the dynamic nature of bone healing, these controlled forces will affect the morphology of the bone through guided regeneration and growth of skeletal tissues. Clinical cases and animal experiments of DO of the craniofacial region have been reported by a number of investigators<sup>1-3</sup> since Snyder et al<sup>4</sup> first performed DO in the dog mandible in 1973. Several studies specifically focused on response of the temporomandibular joint (TMJ) as a result of DO.<sup>5-11</sup> The effects of DO on TMJ were studied by McCormick et al,<sup>6</sup> who described morphologic changes of the TMJ in a mature dog model after DO (1 mm/day rate, lengthened 10 or 20 mm), and observed flattening of condylar head, thinning of cartilage, and new bone deposition after DO. Karaharju-Suvanto et al<sup>8</sup> also performed unilateral mandibular DO in a sheep model, and they found no correlation between length of distraction and morphologic changes in TMJ. The effects of DO loading on TMJ were studied by Kruse-Losler et al<sup>9</sup> and Meyer et al,<sup>10</sup> who described the effects of various strains on the position and morphologic changes of the TMJ in an immature rabbit model after unilateral DO. They found no clinical and radiologic evidence of joint luxation, even at maximal DO rates (300,000 microstrain, 1 per day), but all cartilaginous layers were reduced in the regions of the TMJ that had been exposed to higher forces, and the fibrous layer became nearly completely destroyed after 14 days of DO. This showed a positive correlation between the degree of mechanical loading and development of degenerative alterations in the cartilage. Thurmuller et al<sup>11</sup> also unilaterally lengthened mandibles, and showed that the AP diameter of the ipsilateral condyle was decreased. The articular discs of both ipsilateral and contralateral sides showed variable thinning at the medial aspect at the end of DO. After 90 days, remodeling reduced some of those changes.

A problem with DO, which is considered to be particularly difficult in the mandible, is evaluating the optimal timing of the procedure during growth of the individual. In humans, it is assumed that the mandibular condyle undergoing mandibular distraction is compressed against the temporal component of the temporomandibular joint more strongly than that in its physiologic position.<sup>6</sup> When mandibular distraction is performed in growing individuals, the cartilage and trabecular bone of the mandibular condyle might be expected to show various responses to reaction forces during and after the procedure because they themselves are sites of growth. As a result, the effects of DO on the mandible, particularly the mandibular

condyle, in growing individuals are of considerable significance for the correction of mandibular deficiency.

Parathyroid hormone-related protein (PTHrP) has been identified as a cause of humoral hypercalcemia of malignancy associated with malignant tumors.<sup>12</sup> Recently, however, the physiologic roles of PTHrP as a regulatory factor of cell proliferation and differentiation in the fetal and postnatal periods have attracted attention.<sup>13</sup> Karaplis et al<sup>13</sup> prepared PTHrP knockout mice, and reported that homozygotes of these mice exhibited micromelic dwarfism with a small nasomaxillary region and marked mandibular hypoplasia compared with normal mice. Their report suggests that PTHrP is an important factor in jaw development, particularly mandibular growth. Yamazaki et al<sup>14</sup> and Suda et al<sup>15</sup> also examined the distribution of PTHrP and its receptor in developing mouse mandibular condylar cartilage. However, there are no observations of how PTHrP expression responds to altered mandibular positioning or forces.

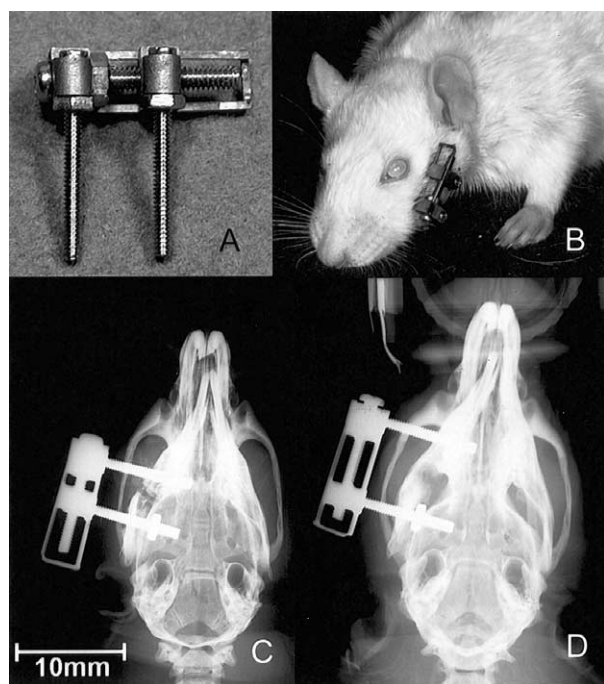
This study focuses on growth-related changes in growing rats to test the hypothesis that mandibular condylar cartilage and underlying trabecular bone in growing individuals undergo morphologic and volume changes in response to normal distraction forces. Response of both the cartilage and trabecular bone of the mandibular condyle to the distraction procedure was evaluated with regard to the localization and dynamics of PTHrP. And serial changes in 3-dimensional (3D) trabecular bone structure at the distraction site were also examined. The growth of animals was evaluated according to changes in body weight.

## Materials and Methods

### EXPERIMENTAL DESIGN

Sprague-Dawley rats ( $n = 48$ ) were used in this analysis. All rats were 6 weeks of age purchased from Saitama Exp. Animal Supply Co, Ltd (Saitama, Japan), ie, in the early growth period, with a body weight of 162.5 to 178.1 g at the start of the experiment. The rats were maintained at a room temperature of 22°C and under 12-hour light and dark cycles throughout the study. All procedures were performed in compliance with the Principles of Laboratory Animal Care established by the National Institutes of Health for the ethical treatment of animals. The animal use protocol and all experimental procedures were also reviewed and approved by the Animal Experiment Committee, Showa University (Tokyo, Japan).

The rats in the distraction group were divided into 5 subgroups of 8 animals each corresponding to 0, 2, 4, 6, and 8 weeks after the end of distraction treatment. Animals thus ranged in age from 6 to 14 weeks



**FIGURE 1.** A, The customized distraction device. B, Device attached to a rat mandible. C, Radiographic observation 1 day after the device was implanted. Note that the separated mandibular bone was fixed by the device. D, Radiographic observation 2 weeks after the end of distraction. Note the appearance of a few radiopaque image lines within the distraction gap. Scale is shown at panel C for C and D.

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at the end of the experiment. Four animals in each group were used for micro-computed tomography (CT) and 4 were used for immunohistochemistry. Two age-matched animals were used as unoperated controls for each time period.

#### MANDIBULAR DISTRACTION MODEL

After anesthesia with pentobarbital (0.08 mL/100 g body weight), a mandibular osteotomy was performed immediately posterior to the left third molar, and the small external distractor (Fig 1)<sup>16</sup> was attached to the left side of the mandible in parallel with the sagittal plane of the cranium so that the distance between the left and right heads of the mandible would not be changed by the distraction procedure.<sup>17</sup> Following a 3-day latency period, DO was performed at 0.4 mm/day over 10 days to a total distance of 4.0 mm. The animals were sacrificed 0, 2, 4, and 6 weeks after the end of the distraction procedure, and the condyles prepared for immunohistochemistry or micro-CT.

#### EVALUATION OF GROWTH ACCORDING TO CHANGES IN BODY WEIGHT

The body weight including the distractor was measured under inhaled anesthesia before surgery to at-

tach the distractor, at the beginning of distraction, at the end of distraction and 2, 4, and 6 weeks after the end of distraction.

#### IMMUNOHISTOCHEMICAL EXAMINATION OF PTHrP

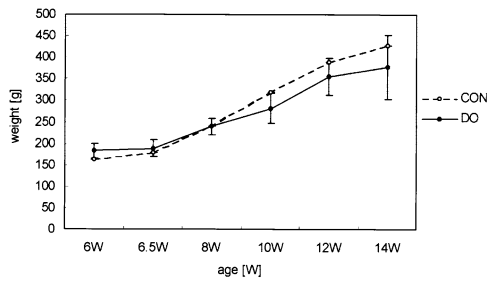
Animals were fixed by perfusion with 4% paraformaldehyde, the mandible was resected en bloc, and the mandibular condyle was separated and fixed by immersion in 4% paraformaldehyde at 4°C for 24 hours. The samples were decalcified with 10% EDTA for 7 days, dehydrated with alcohol-xylene, and embedded in paraffin. Sections 5  $\mu$ m thick were prepared and treated to quench endogenous horseradish peroxidase using 0.3% H<sub>2</sub>O<sub>2</sub> for 30 minutes. Sections were then incubated in 10% normal goat serum at room temperature for 30 minutes, followed by incubation in rabbit anti-mouse PTHrP serum (Oncogene Research Products, San Diego, CA) at a 1:100 dilution and 4°C for 24 hours. After completion of these procedures, the sections were washed with 0.01 mol phosphate-buffered saline, immersed in horseradish peroxidase-conjugated IgG in 0.05% diaminobenzidine in 0.05 mol Tris-HCl buffer, pH7.6 containing 0.02% H<sub>2</sub>O<sub>2</sub> for 1 minute at room temperature. The sections were counterstained with hematoxylin to visualize the nuclei, rinsed in tap water for 10 minutes, and cover slips mounted after confirmation of staining by light microscopy.

#### THREE-DIMENSIONAL MORPHOLOGY OF THE MANDIBULAR CONDYLE USING MICRO-CT

Multi-scanning of the entire mandibular condyle was performed by 3D micro-CT (Nittetsu Elex Corp, Tokyo, Japan) at 36 kV, 100 mA, at a slice thickness of 14.67  $\mu$ m and reconstituted into 3D images using file software (TRI/3D-BON, RATOC, Tokyo, Japan). Volume cubes of a fixed quantity (0.057 mm<sup>3</sup>) were taken in sagittal plane from trabecular bone just below the cartilage layer in the superior part and at the posterior end of the mandibular condyle. Bone surface (BS), the total surface square measure of the trabeculae in the fixed volume cube; bone volume (BV), the total volume of the trabeculae in the fixed volume cube; trabecular number (TbN), the total number of trabeculae in the fixed volume cube; and trabecular thickness (TbTh), the mean trabecular thickness in the fixed volume cube; and trabecular separation were measured.

#### STATISTICAL ANALYSIS

The bone quality and the results of morphometry of the left mandibular condyle were compared between the distraction group and the control group. The data in the distracted group were compared with those in the control group by repeated measures ANOVA post hoc test at the  $P < .05$  level of significance.



**FIGURE 2.** Change in body weight with age.

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## Results

### BODY WEIGHT CHANGES

Body weight increased after the age of 8 weeks and stabilized at the age of 12 weeks in both the distracted and control groups (Fig 2). The body weight of control animals was greater than that of distracted animals between 8 and 14 weeks but the difference was not significant.

### HISTOMORPHOLOGY OF CONDYLE

By 8 weeks (end of the distraction period), marked differences were evident in the morphology of the superior part of the condylar cartilage between the control and DO groups (Fig 3A). In the distracted group at 8 weeks, total cartilage thickness was unchanged from controls, but a marked concavity (Fig 3B, right) was present on the superior aspect of the condylar cartilage in every animal. In addition, the condylar cartilage was characterized by almost complete disappearance of the chondroblastic and hypertrophic zones and a general disorganization of the layers of the cartilage was apparent. In the 6 weeks following treatment, the cartilage thickness in both groups gradually declined, with the thickness in the DO group less than in controls by 14 weeks. In the distraction group, the chondroblastic zone reappeared within 2 weeks following the end of DO, but hypertrophic chondrocytes were smaller and less numerous.

In the posterior part of the control mandibular condyle, fewer morphologic differences were present (Fig 3C). At the end of distraction (Fig 3C, 8w DO), the appearance of the DO cartilage was similar to control, but with a marked reduction of chondroblast lacuna size and size of the hypertrophic chondrocytes. In the first 2 weeks following treatment, the cartilage thickness in the distraction group showed a greater decline in thickness than the control group, but by 6 weeks post-treatment, the cartilages looked similar to one another.

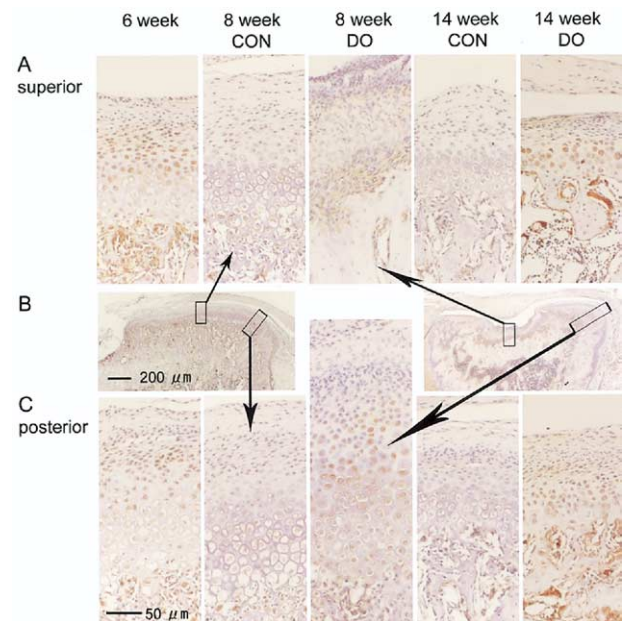
### IMMUNOHISTOCHEMICAL EXAMINATION OF PTHrP

At the start of distraction (Fig 3, 6w), PTHrP immunoreactivity was strongest in the chondroblastic layer of the condylar cartilage. Thereafter, PTHrP immunoreactivity in control condyles decreased markedly, and was limited primarily to the upper part of the hypertrophic cell layer. At the end of distraction (Fig 3, 8w DO), PTHrP immunoreactivity was considerably greater than in 8-week controls. In the disorganized superior aspect, it was evident mainly in the small cells adjacent to the cartilage-bone interface. On the posterior aspect of the condyle, immunoreactivity was localized to the chondroblastic and hypertrophic zones. This elevated PTHrP expression persisted in 14 week DO condyles.

### THREE-DIMENSIONAL TRABECULAR BONE MORPHOMETRY OF THE MANDIBULAR CONDYLE USING MICRO-CT

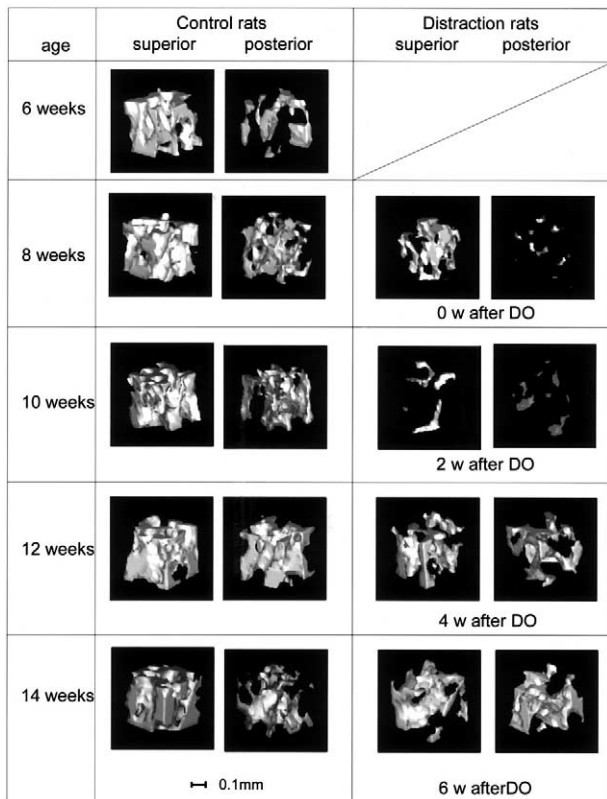
#### *Changes in Bone Volume and Bone Surface*

In the control group, the BV and BS of the superior part of the mandibular condyle increased gradually



**FIGURE 3.** Immunohistologic localization of PTHrP (brown reaction product). Micrographs showing high power (A, C) and low power (B) images through condyles. A, C, sections of the superior and posterior portions respectively of 6 week (6w) through 14 week (14w) condyles. B, location of sections shown as boxed regions in control (CON) and distracted (DO) condyles at the end of distraction. Note high levels of PTHrP in the chondroblastic layer and underlying bone at 6 weeks. PTHrP decreased in these layers by 8 weeks in control condyles, but remained elevated in the prechondroblastic and chondroblastic layers of DO tissues up to 6 weeks after distraction. Scale for big magnification: A and C, original magnification  $\times 100$ ; shown in left edged panel C; and for small magnification (B, original magnification  $\times 40$ ) is shown in left edged panel B.

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**FIGURE 4.** Radiographic images created by micro-CT. 3D structure of cancellous bone of control and distracted mandibular condyles reconstructed at 2-week intervals. Scale for all trabecular bone cube is shown in the bottom of figure.

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between 6 and 14 weeks from  $0.024 \pm 0.003$  to  $0.035 \pm 0.002$  and  $0.911 \pm 0.044$  to  $1.237 \pm 0.022$ , respectively (Figs 4, 5), along with increased body weight (from  $163.9 \pm 10.3$  to  $320.0 \pm 12.8$  g; Fig 2). In the superior region of the distracted condyle, BV and BS decreased sharply from  $0.024 \pm 0.003$  to  $0.009 \pm 0.0001$  and  $0.911 \pm 0.044$  to  $0.477 \pm 0.028$ , respectively, with both values significantly lower than control values ( $P < .009$ ).

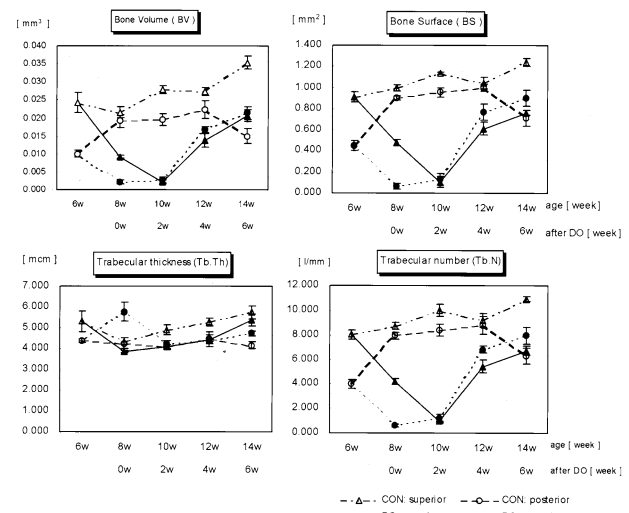
In the posterior part of the control condyle, the BV and BS remained constant from 6 to 12 weeks, but then declined from  $0.022 \pm 0.002$  and  $0.997 \pm 0.078$  to  $0.015 \pm 0.002$  and  $0.713 \pm 0.076$ , respectively, at 14 weeks. In the posterior part of the condyle on the distracted side, the decreases were even more marked than those seen in the superior portion of the condyle, with declines to values significantly ( $P < .009$ ) lower than those of controls ( $0.002 \pm 0.0001$  and  $0.070 \pm 0.017$ , respectively). While the BV and BS values in the posterior part of the distracted condyle remained unchanged by 10 weeks, these values in the superior region of the condyle continued to decline, becoming similar to the values in the posterior region

at 10 weeks ( $0.002 \pm 0.0001$  and  $0.107 \pm 0.007$ , respectively). The BV and BS values of both the superior and posterior region of the distracted condyle remained significantly lower than values for controls at 10 weeks ( $P < .009$ ).

From 10 to 14 weeks, the BV and BS values ( $0.021 \pm 0.002$  and  $0.902 \pm 0.075$ , respectively) in the posterior regions of the distracted condyle increased, with both values becoming significantly greater than those in the posterior part of the control condyle ( $P < .009$ ). Neither the BV or BS values of the superior region of the distracted condyle ( $0.021 \pm 0.002$  and  $0.763 \pm 0.038$ , respectively) approximated those in the superior region of the control condyle ( $0.035 \pm 0.002$  and  $1.237 \pm 0.022$ ;  $P < .009$ ) over this time period.

#### Changes in Trabecular Number and Trabecular Thickness

The TbN in both control and distraction groups reflected closely the BS measurements, with the TbN of the distracted tissues decreasing rapidly during the distraction period from  $8.019 \pm 0.388$  to  $4.196 \pm 0.248$  ( $P < .009$ ) in the superior region of the condyle and from  $3.99 \pm 0.393$  to  $0.613 \pm 0.147$  ( $P < .009$ ) in the posterior region (Figs 4, 5). As for BS, the TbN in the superior region of the distracted condyle continued to decrease for 2 weeks following distraction, after which it recovered, but the values did not reach control levels by 14 weeks ( $6.717 \pm 0.337$  versus  $10.885 \pm 0.193$ ;  $P < .009$ ). The TbN of the posterior part of the condyle gradually recovered to control



**FIGURE 5.** Changes over time of volume (A), surface (B), thickness (C), and number (D) of bone trabeculae beneath superior and posterior aspects of mandibular condylar cartilage. After taking micro-CT readings, reconstituted volume cubes were measured in control (CON) and distracted (DO) bones.

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levels by 14 weeks ( $7.946 \pm 0.664$  versus  $6.27 \pm 0.664$ ).

Although TbTh showed less dramatic changes than BV and BS over the time period studied, control TbTh decreased on the superior aspect during the distraction ( $5.322 \pm 0.502$  to  $4.353 \pm 0.178$ ) and then slowly regained the initial value by 14 weeks. By contrast, TbTh in the posterior region changed little throughout. The TbTh of the superior portion of the distracted condyle also decreased during distraction, but significantly more than that seen for the control condyle (to  $3.849 \pm 0.062$ ;  $P < .009$ ). By 14 weeks, superior TbTh values had increased but not approximated control values. During distraction, the TbTh of the posterior portion of the distracted side condyle increased sharply compared with control condyles (to  $5.78 \pm 0.469$ ;  $P < .009$ ). From 8 to 14 weeks, the TbTh of the posterior region of the distracted condyle declined, but this value remained significantly greater than that of the control condyle by 14 weeks ( $4.741 \pm 0.117$ ;  $P < .009$ ).

## Discussion

The distraction procedure used in this study was associated with pronounced morphometric changes in the subchondral trabecular bone of the mandibular condyle, as well as morphologic changes and increased PTHrP immunoreactivity in the condylar cartilage. The changes in the subchondral trabecular bone measures suggested that a progressive decrease in the overall amount of bone occurred both during the distraction process and for 2 weeks following distraction, followed by a tendency toward a partial or complete normalization to control values. This trend was characterized by a significant decrease in trabecular bone volume, trabecular bone surface, and trabecular number during the distraction and early post-distraction periods. The only bone parameter to exhibit a contrary trend was trabecular thickness, which varied relatively little and actually increased slightly during the period of active distraction. The changes in the posterior region of the condyle returned to control values completely by 6 weeks post-distraction, whereas those in the superior region did not.

Although the precise nature of condylar positioning during and following distraction is unknown, it is plausible that the DO procedure produced a change in the position of the condyle in the posterior or posterosuperior direction. The altered condylar positioning is likely caused by a combination of the osteotomy, presence of the distraction device, and the distraction process itself. Effects of altered mandibular positioning on condylar cartilage have been reported in *Macaca mulatta* monkeys,<sup>18-20</sup> rabbits,<sup>21</sup> and

rat.<sup>22-24</sup> Several reports have described changes in the superior and posterior regions of the rat condylar cartilage in response to altered mandibular positioning. Studies using appliances designed to position the mandible more posterior have shown thinner cartilage on the superior aspect of the condyle,<sup>22-24</sup> as well as decreased growth at the condyle.<sup>22,24</sup> In our study, distraction rates were similar to those used by others<sup>3,25-28</sup> in mandibular distraction; the most dramatic findings in the superior region of the distracted condyle were decreased trabecular bone volume and number.

According to Frost,<sup>28</sup> abnormally elevated compression forces applied to articular cartilage lead to inhibited growth. Therefore, it is possible that such a compressive force was responsible for the changes in cartilage appearance, and is also reflected in the decreased subchondral trabecular bone volume during distraction. Following treatment, the morphologic deformity was no longer evident, and the chondroblastic zone in the superior part of the condyle had re-appeared, but overall cartilage thickness remained narrowed compared with controls, similar to the report of Asano et al.<sup>22</sup> Subchondral trabecular bone volume also recovered, possibly related to the reappearance of the chondroblastic zone, but like condylar cartilage thickness, it did not regain control levels by 14 weeks.

In the posterior part of the condyle, subchondral trabecular bone volume decreased significantly during the distraction period, to an even greater extent than that seen in the superior region of the condyle. Interestingly, Rabie et al.<sup>29,30</sup> showed that even though overall cartilage thickness was increased during anterior positioning of the condyle, trabecular bone formation decreased for the first week after appliance placement. The differential changes in subchondral trabecular bone measures between the posterior and superior parts of the condyle are intriguing, in large part because of the different functional environments thought to characterize these regions. Copray and Liem<sup>31</sup> have shown that the superior aspect of the condylar cartilage undergoes pronounced ultrastructural changes shortly after weaning in rats while the posterior aspect remains virtually unchanged. They argued that these changes reflect an increase in articular loading on the superior surface occasioned by the changeover to hard food and that the absence of such changes in the posterior aspect suggests that this region is loaded much less or loaded under other circumstances. In fact, Hinton et al.<sup>32,33</sup> found that the superior, but not the posterior, region of the condylar cartilage showed a decrease in matrix synthesis activity in rats fed a soft diet. Mature condylar morphology is achieved by 50 days in rats. However, condylar growth, with increased trabecular

bone size and decreased marrow space is completed by 10 months.<sup>34</sup> The transformation of the condylar cartilage from adaptive to nonadaptive was considered to be complete by 220 days in rat.<sup>35</sup> After this age, the condyle was no longer considered capable of adapting in response to changing functional demands and mechanical loads.<sup>36</sup> Our 6 to 14-week-old rats (age, 42 to 98 days) were within this 50- to 220-day range, when adaptation can occur in the condyle.

Interestingly, changes in 3D trabecular bone volume, trabecular number/thickness, and PTHrP immunoreactivity in condylar cartilage in our study differed greatly between the superior and posterior regions, and the most profound changes occurred on the more loaded superior region. Although we did not measure forces transmitted to the condyle during or after distraction, it is plausible that the superior and posterior aspects of the condylar cartilage were differentially affected by alterations in the local biomechanical environment of the temporomandibular joint and condyle secondary to the altered mandibular position brought about by the distraction device.

The increased immunoreactivity for PTHrP in the mandibular condylar cartilage is remarkable because of the postulated role of this peptide in the regulation of terminal differentiation in the growth plate of limbs. The pivotal role of PTHrP as an inhibitor of chondrocyte differentiation has been shown by the targeted overexpression of PTHrP in chondrocytes using the mouse collagen type II promoter.<sup>37</sup> This targeting results in a pronounced delay in chondrocyte differentiation such that the animals are born with a cartilaginous endochondral skeleton. Long bones consist of proliferating and prehypertrophic chondrocytes in a cartilaginous matrix. The increase in PTHrP immunoreactivity seen in condyles of distracted mandibles could therefore account for the reduced hypertrophy and in some instances disappearance of the hypertrophic layer.

PTHrP expression has been little studied in the temporomandibular joint, which has a different structure and developmental history from the growth plate of the limbs. To our knowledge, 2 studies have examined PTHrP in the mandibular condylar cartilage: those of Suda et al<sup>15</sup> and Rabie et al.<sup>30</sup> In comparisons of normal mice with those with a disrupted PTHrP allele, Suda et al<sup>15</sup> found differences in expression of the PTHrP receptor between the embryonic condylar cartilage and tibial growth plate. In the tibia, the type I PTH/PTHrP receptor is found in the proliferative cell layer only, whereas in the condyle this receptor is also found in the hypertrophic chondroblasts. However, the PTHrP protein is localized to the proliferative and early hypertrophic zone of the tibial growth plate, and to the equivalent prechondroblastic and chondroblastic layers, respectively, of the condyle. Interest-

ingly, Rabie et al found that mandibular advancement triggered PTHrP expression and slowed chondrocyte hypertrophy using a bilateral mandibular advancement appliance similar to our findings with mandibular distraction.

These findings are among the first to indicate that altered biomechanical environment can effect a change in expression levels of PTHrP, perhaps with concomitant changes in growth of the affected cartilage and the underlying bone. In support of this idea, skeletal unloading has been shown to reduce cellular responsiveness to PTH, inhibiting the osteogenic response, alkaline phosphatase activity, and mineralization normally stimulated by PTH treatment.<sup>38,39</sup> However, a direct cause and effect between altered mechanical load, elevated PTHrP, and the alterations in cartilage and bone remains to be shown because biomechanical loading may regulate expression of one or several upstream regulators of PTHrP expression such as *Ihh* or *Tgf- $\beta$* .

It is significant that distraction of the mandible resulted in both morphologic and immunohistochemical changes in the condylar cartilage and volume changes in the underlying trabecular bone because the timing of DO is controversial in clinical orthodontics. One of the most important clinical dilemmas involving DO is whether the procedure can be applied to growing individuals. However, there have not been any studies of morphologic and qualitative changes in the growing mandibular condyle as a result of DO. The results of this study suggest that mandibular distraction in growing individuals may compromise the growth ability of the mandibular condyle through biomechanical factors, at least temporarily. The more profound the procedure, the more likely it is that long-term alterations in growth ability at the condyle may occur. Because the condyle is the primary site of mandibular growth, distraction procedures need to be planned carefully on the basis of understanding of the growth pattern.

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