Activity and morphologic changes in the mandible after mandibular osteotomy

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Introduction: Orthognathic surgery accelerates orthodontic tooth movement, and tooth movement accelerates with demineralized bone and accelerated bone remodeling. The purpose of this study was to ascertain whether orthognathic surgery induces accelerated bone remodeling. The research design included a human model and an animal model. **Methods:** The levels of serum tartrate resistant acid phosphatase-5b (TRAP) and bone alkaline phosphatase (BALP) were measured in 15 patients after sagittal split ramus osteotomy. For the animal study, 18 rabbits were divided into 6 groups: a control group and 5 surgery groups. The rabbits in the surgery groups had osteotomies in the molar regions of the mandible. Changes in bone mass of the anterior mandibles were examined by microcomputed tomography, and changes in osteoblast and osteoclast numbers were analyzed by real-time polymerase chain reaction, hematoxylin and eosin staining, TRAP staining, and alkaline phosphatase staining. **Results:** In the 15 patients, TRAP-5b increased from 1 to 8 weeks postoperatively, and BALP increased significantly in 2 weeks postoperatively. In the rabbits, the levels of mRNA expression of TRAP were increased at 3 weeks, and matrix metalloproteinase 9 was increased at 4 and 8 weeks, whereas mRNA expression of BALP and bone morphogenetic protein 2 were increased at 4 weeks. Bone loss was detected from 1 week postoperatively and reached the maximum at 3 weeks; and bone mass and mechanical structure did not recoverer to preoperative levels until 8 weeks postoperatively. **Conclusions:** These findings show active bone remodeling induced by osteotomy. (Am J Orthod Dentofacial Orthop 2019;155:40-7)

The conventional treatment course for dentomaxillofacial deformities is generally divided into 3 phases: preoperative orthodontic preparation, orthognathic surgery, and postoperative orthodontics. It has been reported that the entire treatment lasts 21.9 to 36 months.[1-3] Preoperative orthodontics, the aim of which is to decompensate the dentition, usually takes the longest time and leads to progressive deterioration of dental function and facial esthetics. An alternative surgery-first approach approach was proposed by Nagasaka et al[4] in 2009; it proceeds without preoperative orthodontics or with minor preoperative orthodontic measures.[5] Compared with the traditional approach, the surgery-first approach leads to a significant decrease in total treatment time, usually completed within 1.5 years and on average within 1 year.[6-9] Although many patients have been reported to be successfully treated with the surgery-first approach, the underlying biologic mechanism of orthognathic surgery-accelerated orthodontic tooth movement is still unclear.

The basis of orthodontic tooth movement is known to involve alveolar bone resorption at the pressure site and bone formation at the tension site. An alteration of the metabolic rate of bone will result in a distinct rate of tooth movement.[10] Corticotomy is an efficient and commonly used surgical technique to accelerate orthodontic tooth movement. Temporary loss of bone mass over both the labial and lingual root surfaces of treated teeth has been observed in patients by cone-beam computed tomography.[11] A corticotomy model study of rats demonstrated that the bone density of alveolar bone around the surgical site was reduced at postoperative week 3 and recovered at postoperative week 8.[12] Various histologic studies have shown that corticotomy combined with accelerated tooth movement significantly increases the numbers and functions of osteoclasts on the compression side.[13-17]

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In contrast to corticotomy, with the surgical site around the alveolar bone of the target teeth, orthognathic surgical incisions are a certain distance from the orthodontic teeth. For instance, sagittal split ramus osteotomy sections are generated in the mandibular ramus and molar regions. It is unclear whether the alveolar bone responds to orthognathic surgery in the same way as corticotomy.

The alveolar bone is a relatively dynamic tissue that undergoes significant modifications throughout life. It is a metabolic process called bone remodeling or bone turnover and permits skeletal mass accumulation. Bone remodeling is the process that maintains the integrity of the skeleton through the balanced activities of its constituent cell types. These are the bone-forming osteoblasts, which produce the organic bone matrix and facilitate its mineralization, and the bone-degrading osteoclasts, which are unique exocrine cells that dissolve bone mineral and enzymatically degrade extracellular matrix proteins.

Bone remodeling can be assessed by evaluating serum biochemical bone turnover markers; this is only slightly invasive. Bone turnover markers include the bone isoenzyme of alkaline phosphatase (BALP) and the 5b isoenzyme of tartrate-resistant acid phosphatase (TRAP 5b). BALP plays a role as a serum bone formation marker, and TRAP 5b is an osteoclast enzyme that indicates bone resorption.[18] Bone morphogenetic protein 2 (BMP2) stimulates mesenchymal stem cells to become osteoblasts and clinical features of enhanced bone formation. Matrix metalloproteinase 9 (MMP-9) is a specific marker of osteoclasts and is prominently expressed on the surface of osteoclasts.

We hypothesized that orthognathic surgery induces regional acceleratory phenomena and enhances the rate of tooth movement by increasing bone remodeling. Thus, our aims were (1) to study postoperative changes in bone metabolism after orthognathic surgery, (2) to examine alveolar bone after mandibular osteotomy by microcomputed tomography (micro-CT), (3) to analyze changes in osteoclast and osteoblast numbers in the anterior mandible, and (4) to assess the expression of osteoclast and osteoblast marker genes.

MATERIAL AND METHODS

This prospective clinical research project was approved by the institutional review board of the Department of Oral and Maxillofacial Surgery, Hospital of Stomatology, Tongji University (number 2015-002), in Shanghai, China. All patients signed informed consent forms regarding the purposes, procedures, possible complications, and risks of this study. Fourteen patients, aged 20 to 35 years, participated; all underwent LeFort I osteotomy of the maxilla, bilateral sagittal split ramus osteotomy of the mandible, and genioplasty for their dentofacial deformities.

A 2-mL venous blood sample was collected from each patient preoperatively and at 1, 2, 3, 4, and 8 weeks postoperatively. These blood samples were repetitively sequenced at the same times as the sampling times during midmorning hours. After clotting for 30 minutes, the blood samples were centrifuged for 20 minutes at 3000 reveolutions per minute. Serum was collected and stored at −80°C until analysis of the BALP and TRAP levels. These 2 bone metabolism markers were measured using a BALP (human) ELISA kit and an ACP5 (human) ELISA kit (Abnova, Taipei, Taiwan). The absorbance was read at 405 nm using a microplate reader (Denley Dragon Well scan MK 3: Thermo, Vantaa, Finland).

One-way repeated-measures analysis of variance (ANOVA) was applied for statistical comparisons. P values less than 0.05 were considered statistically significant.

The animal ethics committee of the Department of Oral and Maxillofacial Surgery, Hospital of Stomatology, Tongji University, approved the protocol for this experiment. We used 18 female New Zealand white rabbits (mature at age 6 months; weight, 2.3 ± 0.2 kg) purchased through the Laboratory Animal Center at Tongji University. All animals were housed at the Laboratory Animal Center, where all precedures were performed.

The rabbits were randomly divided into 6 groups of 3 rabbits each. One group was the control group. The rest were surgery groups that underwent bilateral mandibular osteotomies. The rabbits were killed at 0, 1, 2, 3, 4, and 8 weeks after the operation. The surgical groups were named according to when they were killed: group 0 week, group 1 week, and so on. For example, group 0 week included the rabbits killed at week 0 after the operation.

The animal surgery protocol originated from the bilateral sagittal split ramus osteotomy, a common type of orthognathic surgery. The surgery groups had general anesthetization with an intravenous injection of 3% pentobarbital sodium (40 mg per kilogram^{-1}).[19,20] Intravenous injections of cefazolin sodium were administered 30 minutes before the surgery. After subcutaneous injection of 2% lidocaine hydrochloride (1 mL) for local anesthesia, a 3-cm long submandibular skin incision was made over the right mandible. The subcutaneous tissues were exposed by careful dissection down to the periosteum, and the bone was exposed with a periosteal incision. A rectangular cut line, piercing the cortical bone, was created on the buccal side by a high-speed rotary dental instrument and burs (8 mm diameter) under irrigation with

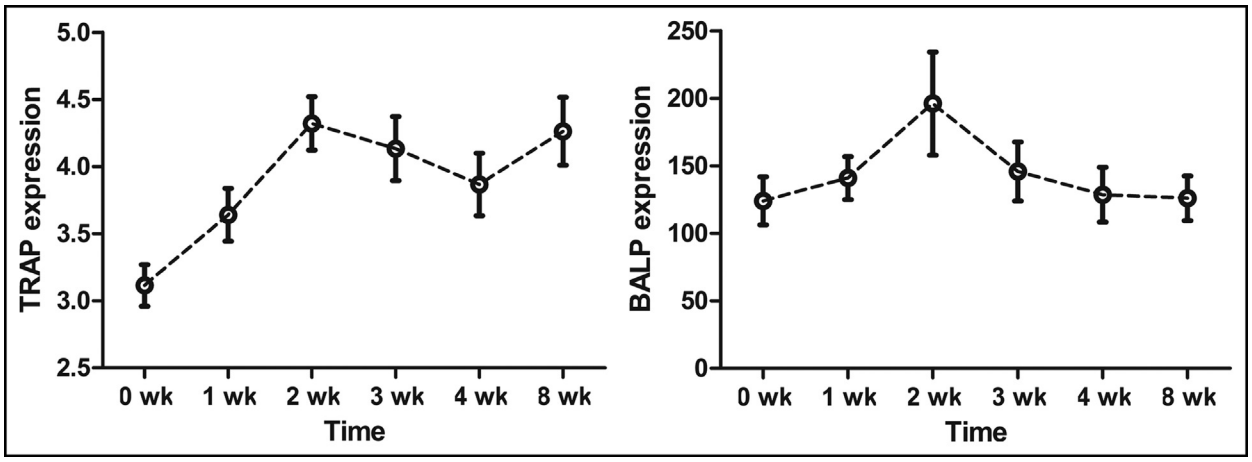

Fig 1. Accelerating bone remodeling after orthognathic surgery. Bone remodeling markers, BALP and TRAP, were measured by ELISA in patient serum. TRAP and BALP expression preoperatively, and 0, 1, 2, 3, 4, and 8 weeks postoperatively were analyzed by repeated-measures ANOVA ($P < 0.05$).

saline solution. Horizontal incisions were made 2 to 3 mm under the alveolar crest and 1 mm above the lower border of the mandible, from the third molar to the first premolar. The rectangular cortical bone was split and then replaced. The same process was applied to the left mandible.

The rabbits were killed with pentobarbital sodium at 0, 1, 2, 3, 4, and 8 weeks after surgery (3 animals at each time). They were exsanguinated and perfused through the jugular vein with 4% paraformaldehyde solution in 0.1 M of cacodylate buffer (pH 7.4). The mandibles were dissected free, separated at the symphysis into 2 halves, and fixed in the same solution for 48 hour at 4°C.

The right part of each rabbit mandible was kept in 0.5% paraformaldehyde solution until the micro-CT scan. The left part of each mandible was decalcified with 10% ethylenediaminetetraacetic acid (EDTA) at 4°C approximately 2 to 3 months before the histomorphometric analysis.

Micro-CT recreates a 3-dimensional model without destroying the original object. The mandibular process was scanned with a medical μCT50 system (Scanco Medical, Bassersdorf, Switzerland). This system excels at high resolution, providing nondestructive 3-dimensional imaging of specimens in vitro. Samples were secured in a cylindrical sample holder ($\Phi 48 \times 110$ mm) with the long axis positioned vertically. Scans were performed at a medium resolution of 24.2 mm per voxel. Three-dimensional images of each right hemimandible were acquired in all spatial directions with the software provided by the manufacturer.

The region of interest, which is the section to be measured, was drawn using a slice-based method starting from 50 slices before the middle points of the anterior mandible and moving dorsally 100 slices. Trabecular bone was carefully contoured in the first and last slices, whereas the intermediate slices were first interpolated by morphing. The region of interest was circled to examine changes in bone mass more than 10 mm from the surgical site after the mandibular osteotomy. Measurements of bone (total volume) and density were made directly from the micro-CT image data for each specimen. The following parameters were obtained by micro-CT scanning and compared between the experimental and control groups at different time points: ratio of bone volume to tissue volume (described as a percentage of the total volume that is bone), trabecular thickness, trabecular number, and trabecular separation.

The left hemimandibles were split into 2 parts, between the anterior tooth and the premolar. The anterior mandibles were embedded in paraffin and sliced into approximately 4-μm thick sections parallel to the long axis. The sections were used for hematoxylin and eosin, TRAP, and immunohistochemistry staining.

TRAP activity was demonstrated using an acid phosphatase kit (387A; Sigma-Aldrich, St Louis, Mo) to indicate changes in osteoclast numbers. The procedure demonstrating the presence of enzyme uses 7-Bromo-3-hydroxy-2-naphthoic-o-anisidide phosphate and freshly diazotized 2-Methyl-4-([2-methylphenylazo) benzenediazonium salt. The sections were counterstained with methyl green. The numbers of TRAP-positive osteoclasts were counted in 3 randomly selected high-power (200 times) fields under light microscopy.

Alkaline phosphatase activity was detected using the BCIP/NBT alkaline phosphatase color development kit (C3206; Beyotime Biotechnology, Shanghai, China).

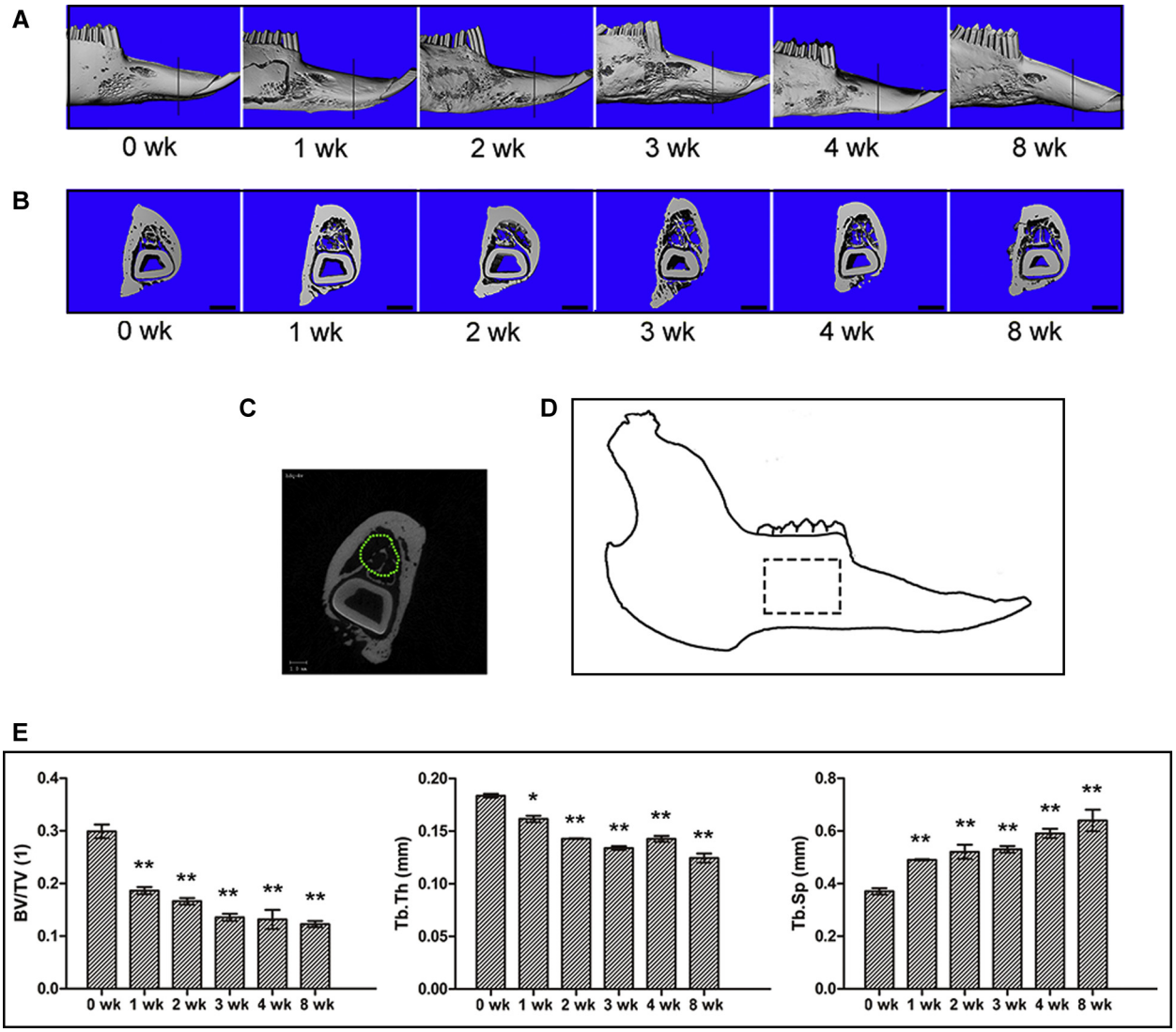

Fig 2. **A** and **B,** Bone destiny decreased in the rabbits' mandibles after mandiblar osteotomy. Micro-CT images of the rabbits' mandibles. In *A*, more holes over the buccal surface were observed postoperatively. In *B*, the cross-sectional shape of the middle of the anterior mandible is shown. The section is indicated by a *black line* in *A*. The 4-sided figure in the section is the rabbit's anterior tooth. The trabecular bone was sparse, and the cortex of the bone was thin postoperatively. In general, the lesion regions were larger at 3 weeks after the operation than those at the other times. **C,** Illustration of the method for locating the region of interest for micro-CT analysis. The regions of interest were located at the center of the anterior mandibles (starting from 50 slices before the middle points of the anterior mandible and moving dorsally 100 slices). **D,** Surgical model; *dotted line* shows the incisions. **E,** The data at 1, 2, 3, 4, and 8 weeks postoperatively were compared with the preoperative data by 1-way ANOVA. $^{*}P < 0.05$; $^{**}P < 0.01$.

The sections were incubated in the dark for approximately 4 hours and counterstained with methyl green.

For each week-8 group of rabbits, 1 mL of blood was collected from a vein in the rabbit's ear at 0, 1, 2, 3, 4, and 8 weeks postoperatively and added to an EDTA K2 tube. Sequencing of the collected blood samples was repeated at the same time as the sampling times during midmorning hours.

By pipetting from EDTA K2 tubes, 500 μL of blood was immediately added to an RNA protect Animal Blood Tube (76554; Qiagen, Calif) to maintain the stability of the RNA. The RNeasy Protect Animal Blood Kit (73224;

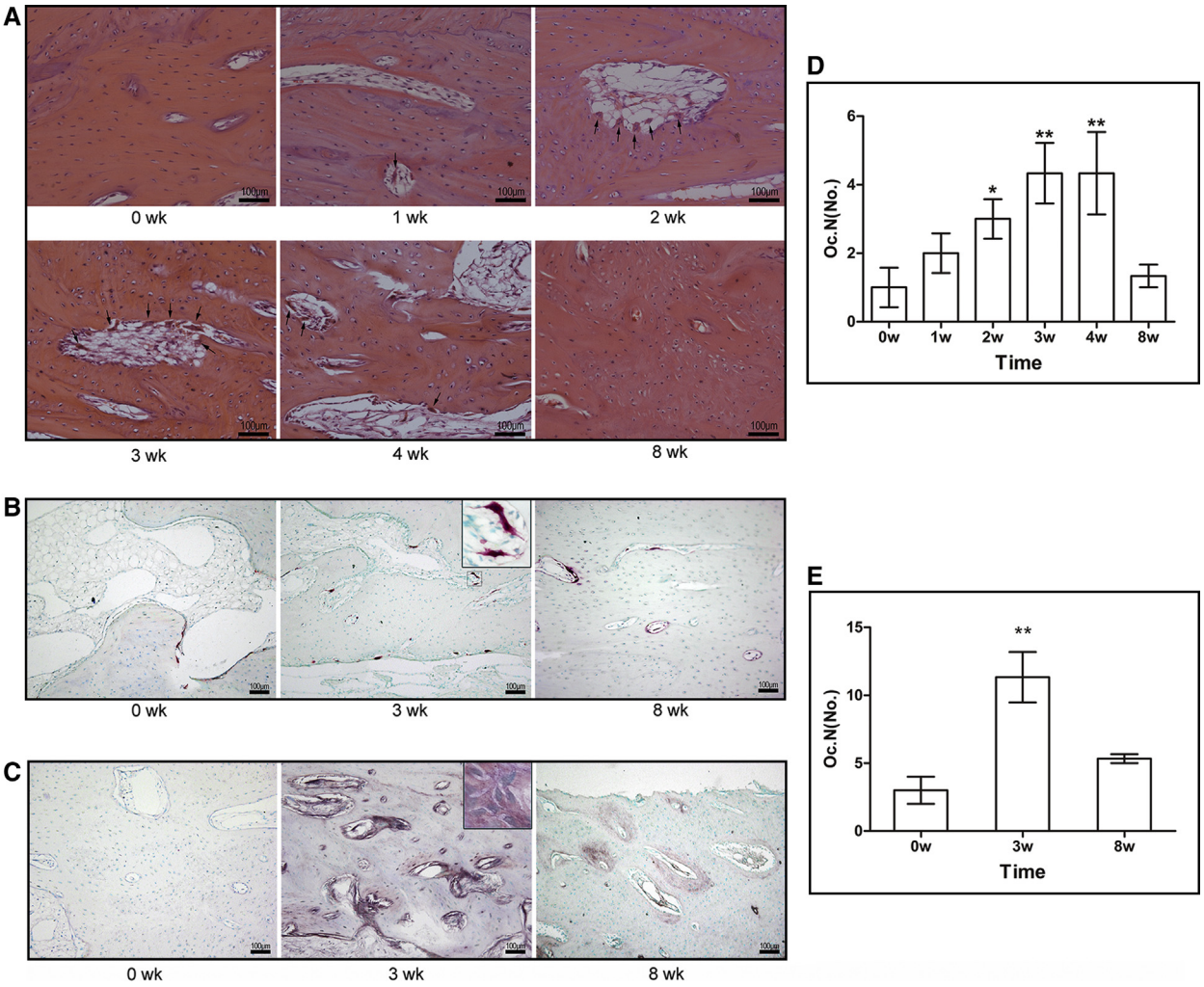

Fig 3. Osteoclasts and osteoblasts increased in the rabbits' mandibles after mandibular osteotomy. The middle points of the anterior mandibles were stained and observed in light microphotographs. **A,** Hematoxylin and eosin staining; *arrowheads* show osteoclasts. **B,** TRAP staining. TRAP-positive staining showed the osteoclasts as aubergine cells. **C,** ALP staining. Alkaline phosphatase-positive showed the osteoblasts as brown cells. **D** and **E,** More osteoclasts were observed postoperatively than preoperatively. The data at 1, 2, 3, 4, and 8 weeks postoperatively were compared with the preoperative data by 1-way ANOVA, Each error bar represents the mean ± the standard error of the mean; n = 3; **P* <0.05; ***P* <0.01.

Qiagen) was used to purify the RNA as described by the manufacturer. RNA from each sample was reverse-transcribed to cDNA using the Transcription First Strand cDNA Synthesis Kit (Roche, Basel, Switzerland).

Quantitative real-time polymerase chain reaction analysis was performed using a 3-step, SYBR Green assay with the FastStart Essential DNA Green Master Kit (Roche). Glyceraldehyde-3-phosphate dehydrogenase was used as the housekeeping gene. Samples were quantified using the LightCycler 96 System (Roche, Basel, Switzerland). The 3-step conditions were as follows: 45 cycles at 95°C for 10 seconds, 55°C for 10 seconds, and 72°C for 20 seconds. Each cDNA sample was assessed by real-time polymerase chain reaction analysis, and the analyses were repeated 3 times. The relative gene mRNA expression levels were calculated and normalized to the level of glyceraldehyde-3-phosphate dehydrogenase.

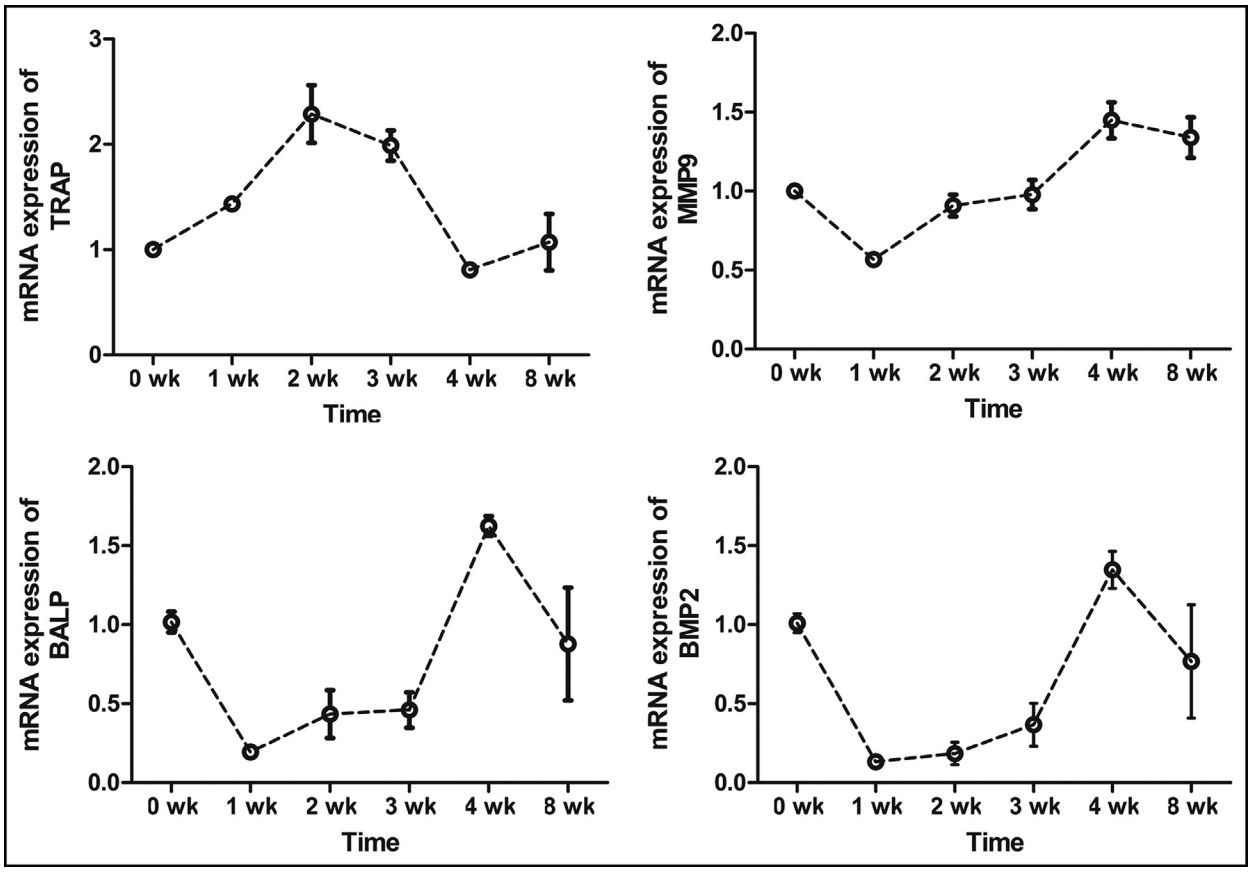

Fig 4. Gene expression of rabbits' bone remodeling markers. The preoperative data and the data at 1, 2, 3, 4, and 8 weeks postoperatively were analyzed by repeated-measures ANOVA ($P < 0.05$).

RESULTS

TRAP expression was significantly increased from the first week postoperatively. The highest level was observed in the second week after the orthognathic surgery, followed by a gradual decline that did not reach the preoperative level at week 8 postoperatively. BALP expression gradually increased in the first 2 weeks after orthognathic surgery and then gradually decreased; the highest level of BALP was observed during the 2 postoperative weeks (Fig 1).

There were more surface hollows of the mandibles in the surgery groups than in the control group. Larger spaces in bone trabecula were observed postoperatively and peaked in group 3 week (Fig 2, *A* and *B*).

Micro-CT analysis showed significantly lower values for the ratio of bone volume to tissue volume and for trabecular thickness, but higher values for trabecular separation were initially observed in group 1 week. These changes persisted until the end of the experiment (Fig 2, *E*). Maximal bone loss seemed to occur at 8 weeks postoperatively.

At 0 weeks (baseline), few TRAP-positive cells were found in the cancellous bone. More osteoclasts (TRAP-positive) were observed at 3 weeks after surgery than preoperatively ($P < 0.05$). There were no significant differences between the preoperative assessment and that at 8 weeks postoperatively (Fig 3). TRAP gene expression was higher at 1 week, 2 weeks, and 3 weeks postoperatively compared with the preoperative assessment. The highest gene expression of TRAP was detected at 2 weeks postoperatively; it was 2-fold higher than the preoperative value. The mRNA expression of matrix metalloproteinase 9, a marker of mature osteoclasts, increased to the greatest extent at 4 weeks postoperatively ($P < 0.05$) (Fig 4).

Alkaline phosphatase-positive cells were mainly located on the trabecular bone surface, and more alkaline phosphatase-positive cells were observed in group 3 week and group 8 week and stained more intensely than those in the control group (Fig 3, *C*). Compared with the preoperative assessment, the mRNA expression of BALP, a marker of early-stage

osteoblasts, and bone morphogenetic protein 2, acting as a potent stimulator of ectopic bone formation, were lower at 1 week, 2 weeks, and 3 weeks postoperatively and were significantly higher at 4 weeks postoperatively($P < 0.05$) (Fig 4).

DISCUSSION

A decrease in regional bone density and a high level of bone turnover during corticotomy have been proposed as a sort of regional acceleratory phenomenon (RAP). RAP, a complex reaction characterized by the acceleration of regional soft and hard tissue vital processes, seems to be evoked by any regional stimulus of sufficient magnitude.[21] RAP in dentistry and orthopedics may result from local trauma, such as mucoperiosteal flap surgery, orthodontic force, infection, and systemic changes in bone due to menopause and parathyroid hormone treatment.[12] Osteotomy, a common orthognathic surgery, is a type of trauma. The manifestation of RAP after osteotomy and the underlying mechanism require further study.

In this study, acceleration of bone remodeling after orthognathic surgery was observed in clinical samples at 1 to 8 weeks postoperatively. Both osteoblasts and osteoclasts were active. During the animal experiment, mandibular orthognathic surgery was found to impact the metabolism throughout the whole mandible, instead of only in places adjacent to the operation area in the alveolar bone. We suppose that the term "regional" varies directly with the magnitude of the stimulus. In rat models of a whole defect in the left tibia, a decrease in bone mass was observed in sites adjacent to the defect as well as in tibiae without defects and the fourth lumbar region.[22]

This study indicated that TRAP, a marker of osteoclasts, began to be active from the first week after the osteotomy. BALP, a marker of osteoblasts, and bone morphogenetic protein 2, a morphogenic bone formation, were increased at 4 weeks postoperatively. In the next phase of our experiment, we found that the expressions of IL-1β, IL-6, and TNF-α were significantly increased 3 to 7 days after the orthognathic surgery. These inflammatory cytokines enhance osteoclastogenesis.[23] It has been reported that a RAP response is associated with an increase in systemic inflammatory markers.[24] We considered that the inflammation led to the osteoclast and osteoblast cell population shifts in numbers after the mandibular osteotomy.

This study confirmed the occurrence of RAP after mandibular osteotomy. Mandibular osteotomy could affect bone metabolism throughout the bone tissue of the whole mandible. The postoperative changes improve our understanding of the clinical orthodontic and orthognathic surgery start time and guidance for assessments of accelerated bone metabolism and the rate of orthodontic tooth movement, providing a theoretical basis for clinical understanding and how to accelerate orthodontic tooth movement.

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