Mandibular condylar cartilage response to moving 2 molars in rats

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Introduction: The purpose of this study was to investigate the responses of mandibular condylar cartilage to moving 2 molars in different combinations. Methods: Rats were assigned to male and female control and experimental groups (each, n = 5). Elastic rubber bands were used to move medially the maxillary left and the mandibular right first molars in experimental group I. The same method was used to distally move the maxillary left and the mandibular right third molars, 2 mandibular third molars, and 2 maxillary third molars in experimental groups II, III, and IV, respectively. At the end of the eighth week, all condyles were examined histologically. The areas of histologic change as a percentage of total cartilage area were compared by using the Mann-Whitney U test. Results: Cartilage degenerative remodeling was observed in experimental groups II, III, and IV. The percentage areas of degenerative remodeling were higher in female experimental groups II and III than in the female control group, and in female experimental group II than in female experimental group IV and male experimental group II (all, P < 0.05). Conclusions: The mandibular condylar cartilage of female rats responded variously to different combinations of molar movement; the most obvious remodeling was observed in groups in which the maxillary left and mandibular right third molars were moved. (Am J Orthod Dentofacial Orthop 2010;137:460.e1-460.e8)

Posterior teeth play a predominant role in dietary mastication, which is attributed mainly to the biomechanical action of the temporomandibular joint (TMJ). The relationship between molar occlusion and the TMJ has long been of interest. Unilateral molar lingual crossbite and non-working side interferences, as well as the loss of molar support, have been reported to be associated with specific diagnostic groups of temporomandibular disorders (TMD) or TMJ osteoarthroses. However, 2 recent reviews suggested that there is insufficient evidence to implicate occlusal factors in the etiology of TMD. Furthermore, artificial occlusal interference has been shown to exacerbate clinical signs only in subjects with a history of TMD.

Histologic studies on animals have shown that alterations in molar occlusion lead to changes in the cartilage of the TMJ, including tissue thickening; increased tissue volume; hyperplasia and alterations in the morphology of chondrocytes; monocytic infiltration in condylar cartilage; increases in chondrogenesis, osteogenesis, and angiogenesis in the posterior condyle; and decreased proliferation of chondrocytes and the amount of extracellular matrix. However, previous animal models that investigated the relationship between abnormal molar occlusion and TMJ remodeling were all characterized by sudden changes in occlusal relationships, including the placement of an occlusal splint or metal crowns, or the unilateral removal of molars. These sudden changes in occlusion are generally different from those encountered in clinical practice.

A recent clinical study showed that a tightly locked molar occlusion, which has been suggested to be related to drifted, tilted, or supra-erupted teeth, often seen in patients who have lost posterior teeth, is associated with the incidence of TMD. A possible explanation for this observation is the altered biomechanical effect of the local contact relationship from the tightly locked occlusion on the TMJ. A supportive study on cadavers indicated that an unbalanced occlusion, including a tightly locked molar occlusion, is related to a thicker TMJ disc. Experimentally disordered occlusion in rats, achieved by moving the 2 first molars medially, was created to mimic the local contact relationship in...
tightly locked occlusion and was reported to have increased the thickness of the TMJ disc in the intermediate zone. However, no obvious changes in condyle cartilage were reported in that study.

Teeth are frequently moved in orthodontic therapy. A recently moved molar might not have a well-coordinated cusp-fossa relationship with its original occluding tooth. Biting with occlusal supports in various regions of the jaw has different biomechanical effects on the TMJ and the elevator muscles. Thus, it is of interest to investigate the responses of the mandibular condyle to various combinations of molar movement, which in turn cause alterations in precise cusp-fossa occlusal contact relationships in various regions of the dentition. However, to the best of our knowledge, the mandibular condylar response to moving different molars is still unknown.

In this study, we developed 4 types of experimentally disordered occlusion in rats by moving 2 different molars from their original positions. The aim of this study was to determine whether the mandibular condylar cartilage of rats responds differently to the 4 types of experimentally created disordered occlusion in terms of cartilage remodeling.

**MATERIAL AND METHODS**

Twenty-five male (weight, 200-210 g) and 25 female (weight, 180-190 g) 8-week-old Sprague-Dawley rats were obtained from the animal center of the Fourth Military Medical University in Xi’an, China. All surgical procedures for and care of the animals were approved by the university ethics committee and performed according to institutional guidelines. Before the experiments, the animals were maintained for 1 week to adapt to their new environment. No obvious systemic disease or disability of movement was found in any animal. They were randomly assigned to 1 control and 4 experimental groups, each group containing 5 female and 5 male rats.

An adult Sprague-Dawley rat has 8 maxillary and 8 mandibular teeth, comprising 4 incisors and 12 molars. There is a large gap between the incisor and the first molar. For the rats in experimental group I (Exp I), an elastic rubber band was used to move the mandibular right first molar and the maxillary left first molar medially. The mandibular right third molar and the maxillary left third molar, the mandibular third molars, and the maxillary third molars are being moved distally in Exp II, III, and IV, respectively. M1, first molar; M2, second molar; M3, third molar. The circled E with arrows is the elastic rubber band.

**Fig 1.** Illustration of the differences in the experimentally disordered occlusions in the 4 experimental groups. In Exp I, the mandibular right first molar and the maxillary left first molar are being moved medially by an elastic rubber band. The mandibular right third molar and the maxillary left third molar, the mandibular third molars, and the maxillary third molars are being moved distally in Exp II, III, and IV, respectively.
rubber band (1/8 #, 3M Unitek, Monrovia, Calif), approximately 1 mm in diameter, was placed between the maxillary left first and second molars and between the mandibular right first and second molars. In this way, the first molars were moved approximately 0.8 mm medially by the elastic force of the rubber bands. One week later, the rubber band was replaced with self-curing resin (Zhangjiang Biomaterial, Shanghai, China) to maintain the gap until the end of the experiment.18 The same method was used to distally move the maxillary left third and the mandibular right third molars in experimental group II (Exp II), the mandibular bilateral third molars in experimental group III (Exp III), and the maxillary bilateral third molars in experimental group IV (Exp IV) (Fig 1). No procedure was performed in the control group. The experimental animals and their age-matched controls were killed at the end of the eighth week of the experiment. During the experiment, no rats had any adverse signs or disturbed mastication and nutrition. All animals received the same standardized hard pellet diet, which was mainly a composite of powdered corn, wheat flour, and beans (Fourth Military Medical University, Xi’an, China).

Under deep anesthesia induced by intraperitoneal pentobarbital sodium (50 mg per kilogram of body weight), the rats were perfused through the ascending aorta with 200 mL 0.1 mol/L of phosphate buffer (pH 7.4), followed by 400 mL of 4% paraformaldehyde (4% in phosphate-buffered saline solution, pH 7.4). Tissue blocks containing the TMJs were dissected and postfixed with the same fixative overnight at 4°C and then decalcified in Kristensen’s fluid (containing sodium formate and formic acid) for 1 week. After decalcification, the tissue around the TMJ was removed, and then the ramus was exposed as much as possible. The TMJs were then dehydrated in an ethanol series and embedded in paraffin wax with standardized orientation by adjusting the exposed ramus surface so that it was parallel to the upper surface of the embedding block. Serial sections, 5 µm thick, were cut through the TMJ in the sagittal plane with a microtome (RM 2135 rotary microtome, Leica Microsystems, Nussloch, Germany) and mounted on poly-l-lysine precoated glass slides. Serial sections of each condyle were stained with hematoxylin and eosin and toluidine blue for histologic assessment. To provide a reliable comparison between
Fig 3. Histology showing sagittal central sections of the mandibular condylar cartilage: A-C and G-I, hematoxylin and eosin staining; D-F and J-L, toluidine blue staining. Each vertical pair of images was from serial sections of 1 condyle. A and D, TMJ from the female control group. The arrows indicate normal chondrocytes. The extracellular matrix contains abundant and evenly distributed proteoglycan (D). B and E, C and F, G and J, H and K, and I and L are from female Exp IV, III, II, and IV and male Exp III, respectively. The dashed lines in B, C, and G illustrate the periphery of the degenerative remodeling region, with evidence of a locally moderate homogeneous eosinophilic mass and local proteoglycan loss. The arrows in C indicate the typical morphology of chondrocytes in the degraded region, characterized by pyknotic, homogeneous, eosinophilic nuclei, and condensed cytoplasm that failed to fill the lacunae. The degraded regions in G and J extend into the posterior disc attachment (black arrows). Yellow arrows in H, I, K, and L indicate local proliferative changes in the condylar cartilage characterized by cartilage protrusion that penetrated into the subchondral bone. Simultaneously, locally moderate homogeneous eosinophilic masses were often observed at the margins.
the areas of degraded regions in sections from different groups, the stained central sagittal sections of each condyle were selected for quantitative evaluation.21

Stained histologic sections were examined under a light microscope (DM 2500, Leica, Wetzlar, Germany). Image acquisition was performed by using a Leica DFC490 system. For each section, the total areas of the condylar cartilage and the degenerative remodeling region in the cartilage were quantified by outlining the periphery of the entire cartilage and the degenerative remodeling region in the cartilage by using a computer-assisted image analyzing system (Leica QWin Plus, Cambridge, United Kingdom). Area measurements were made twice by 2 independent observers (S.-B.Y. and X.-D.L.) over 2 months, and the averaged data were used to calculate the percentages of degenerative cartilage. The percentages were then ranked for statistical analysis as follows: 0, 0%; 1, 0%-10%; 2, 10%-20%; and 3, >20%.

Statistical analysis

The ranked data were processed by using SPSS software (version 11.0, SPSS, Chicago, III). Since there was no significant difference ($P = 0.847$) between the left and right condyles in the groups when compared with the nonparametric Wilcoxon test, the ranked data for the 2 sides were pooled and averaged for the analysis. The data for the female and male control and experimental groups (each group, $n = 5$) were then compared by using the nonparametric Mann-Whitney U test ($\alpha$ level = 0.05).

The size of the method error in measuring the areas of the entire cartilage and the degenerative remodeling region were calculated with the following formula,

$$\text{method error} = \sqrt{\frac{\sum d^2}{2n}},$$

where $d$ is the difference between the 2 registrations of a pair and $n$ is the number of double registrations. Ten readings were drawn randomly from the readings of each observer for method error analysis. The size of the method error for the interobserver registration was 0.018 mm$^2$. Paired $t$ tests were used to compare the interobserver registrations, and the results showed no significant difference among interregistrations ($P = 0.642$).

RESULTS

A gradual increase in body weight was recorded during the experimental period in both the control and experimental groups (data not shown). No significant difference in body weight was found among the control and experimental groups at the sampling (all, $P > 0.05$). The means and standard deviations of the rat weights at the sampling in the female control and experimental groups I, II, III, and IV were $278.0 \pm 5.7$, $273.2 \pm 17.9$, $284.6 \pm 14.6$, $266.2 \pm 33.0$, and $287.2 \pm 18.4$ g, respectively; those in the corresponding male groups were $381.2 \pm 27.1$, $364.8 \pm 26.8$, $401.8 \pm 45.6$, $389.2 \pm 32.1$, and $396.4 \pm 40.8$ g, respectively.

In the control group, there was a matched convex-to-concave occlusal relationship between the maxillary and mandibular molars (Fig 2, A). In the experimental groups, however, gaps created between the maxillary or mandibular first and second molars (Fig 2, B, C, G, and J), or between the second and third molars (Fig 2, D, E, H, and K), caused an unmatched convex-to-concave occlusal relationship. The experimentally created gaps were maintained to the end of the experiment, and thus the disordered molar occlusion relationships were operative throughout the experimental period. No observable difference was found in attrition on the occlusal surface of the experimentally moved molars compared with the corresponding molars in the control group (Fig 2, F–K).

The condylar cartilage in the control group was typically organized into 5 zones, as previously described.24 Toluidine blue staining showed that the extracellular matrix contained substantial amounts of evenly distributed proteoglycans (Fig 3, D).

In contrast, cartilage degenerative remodeling was observed in the experimental groups, typically characterized by a homogeneous eosinophilic mass and the local loss of proteoglycans (Fig 3, B, C, E, F, G, and J). These changes were most often located in the central and posterior regions of the condyle. The nuclei of the chondrocytes in these degenerative remodeling regions were typically pyknotic, homogeneous, and eosinophilic, and the cytoplasm appeared condensed and did not fill the lacunae (Fig 3, C). The magnitude of this degenerative remodeling varied appreciably. It could
be local (Fig 3, B and E) or extensive (Fig 3, C and F). Some homogeneous eosinophilic masses were so large that they extended to the posterior disc attachment (Fig 3, G and J). These regions of degenerative remodeling were observed in 7, 4, and 2 of the 10 condyles from female Exp II, III, and IV, respectively, and in 2, 1, and 1 of the 10 condyles from male Exp II, III, and IV, respectively (Table). No degenerative remodeling regions were observed in the control and Exp I animals.

Proliferative changes took the form of cartilage protrusions that penetrated into the subchondral bone in the central and posterior parts of the condylar cartilage (Fig 3, H, I, K, and L). These were observed in 2 and 5 of the 10 condyles from female Exp III and IV, and in 2, 2, 3, and 1 of the 10 condyles from male Exp I, II, III, and IV, respectively. Proliferative changes were also observed in 1 of the 10 condyles in the male control group but were not seen in female Exp I and II or the female control group. A locally moderate homogeneous eosinophilic mass and loss of proteoglycans were often concomitantly observed adjacent to the proliferative region (Fig 3, H). The surface of all condyles remained intact, and no signs of inflammatory cell infiltration were observed in any samples.

Male and female rats had different patterns in the percentages of cartilage degenerative remodeling (Table). In female rats, the percentage areas of cartilage degenerative remodeling in Exp II (P < 0.003) and Exp III (P = 0.018) were significantly higher than those of the control group, whereas those in Exp I and IV were identical to those of the control (both, P > 0.05). The percentage area of cartilage degenerative remodeling in Exp II (P = 0.012), but not in Exp III (P = 0.116), was significantly higher than that of Exp IV. There was no difference between Exp II and Exp III (P = 0.112). In male rats, the percentage areas of cartilage degenerative remodeling were not statistically different between the control and the experimental groups, or between any 2 experimental groups (all, P > 0.05).

The percentage area of cartilage degenerative remodeling in female rats was significantly higher than that in male rats in Exp II (P = 0.012) but was identical in all other experimental groups (P > 0.05).

**DISCUSSION**

Moving molars is often done in orthodontic practice. In our study, 2 molars in different combinations of locations were moved away from their original positions, and the responses of the condylar cartilage were observed histologically. Varying degrees of remodeling change were observed in the mandibular condylar cartilage. The obvious indications of the cartilage degenerative remodeling—a homogeneous eosinophilic mass and local loss of proteoglycans—were predominately observed in female Exp II and Exp III, and occasionally in female Exp IV and male Exp II, Exp III, and Exp IV. The degenerative remodeling of cartilage in female Exp II was the most significant in terms of both the frequency of occurrence and the size of the percentage area.

The TMJ cartilage remodels throughout life in response to the repeated mechanical loading from dental occlusion. In our study, the combination of moving a maxillary third molar on 1 side and a mandibular third molar on the other side (Exp II) induced the most obvious degenerative remodeling in the condylar cartilage. The second most effective combination for inducing degenerative remodeling involved moving 2 mandibular third molars distally (Exp III), followed by moving 2 maxillary third molars (Exp IV). The least effective combination involved moving a maxillary first molar on 1 side and a mandibular first molar on the other side (Exp I). Furthermore, the area of degenerative remodeling was predominantly in the posterior region of the condyle, with little change in the anterior area.

### Table

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The percentages were ranked as follows: 0, 0%; 1, 0%-10%; 2, 10%-20%; 3, >20%.

*P* <0.05 vs female control and Exp I groups; † *P* <0.01 vs female control and Exp I groups; ‡ *P* <0.05 vs female Exp II group.

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These differences in remodeling response among the 4 experimental groups were assumed to be due to different biomechanical outcomes of the jaw lever system. Further investigations of TMJ loading changes in response to different combinations of molar movement by using methods such as finite element analysis are expected.

Excessive mouth opening has been reported to induce osteoarthritis-like lesions in the TMJs of rabbits and rats. However, the degenerative remodeling of condylar cartilage that we observed in this study is unlikely to have been caused by trauma from mouth opening during the experimental procedures, since all rats in the experimental groups had a similar mouth-opening procedure but showed obvious differences in the condylar cartilage between groups.

Interestingly, there were no statistically significant differences in condyle cartilage changes between sides, not only in the symmetrical molar-movement groups (Exp III and IV) but also in the asymmetrical molar-movement groups (Exp I and II). The mesiodistal distances were approximately 2.0 mm for the maxillary and mandibular third molars and 3.0 mm for the maxillary and mandibular first molars. The gaps created by moving molars away from their original positions, approximately 0.8 mm in the mesiodistal distance, were thus less than half of the mesiodistal distance of the first and third molars. This means that the moved first or third molars continued to have contact in occlusion with their opposing molars but were obviously no longer maximally intercuspated. This suggested that an unmatched occlusal relationship was created in the first or third molar area. The abnormal loads from this unmatched occlusion appeared to be attributed more to the condylar cartilage degenerative remodeling than those from possible mandibular deviation caused by the asymmetrical molar movements. One observation in support of this assumption was that the asymmetrical first molar movement in Exp I did not induce degenerative changes in the condylar cartilage. Furthermore, previous studies on rats showed that a lateral functional shift of the mandible led to an increase in mandibular condylar cartilage thickness and proliferative ability, but not to degenerative remodeling of cartilage. Further investigations with finite element analysis are expected to provide more information on the underlying mechanisms.

The sex differences in the cartilage degenerative remodeling responses we observed might indicate that female animals are more susceptible to the occlusal alterations performed in this study. Because degenerative remodeling changes in joint cartilage are highly likely to cause impairment of TMJ function, it will be interesting to determine whether our results are related to the greater susceptibility of women to TMD. Furthermore, it will be of interest to investigate the effect of estrogens in this process. There are, nevertheless, anatomic differences in the morphology and function of the molars and TMJs between humans and rats, and these make it difficult to extrapolate our findings to the clinical situation. Thus, in the future, clinical research in humans might provide more relevant information.

CONCLUSIONS

Our results demonstrate that the mandibular condylar cartilage of female rats responds variously to different combinations of molar movement in terms of cartilage degenerative remodeling. Moving 2 contralateral third molars—1 from the mandible and 1 from the maxilla—leads to the most obvious degenerative remodeling changes in the condyles of female rats.

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REFERENCES