Original Article

Experimentally Created Nonbalanced Occlusion Effects on the Thickness of the Temporomandibular Joint Disc in Rats

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ABSTRACT

Objectives: To test the hypothesis that experimentally created physiologically nonbalanced occlusion will not affect the thickness of the temporomandibular joint (TMJ) discs in rats.

Material and Methods: Twenty-four 8-week-old Sprague-Dawley rats were equally divided into a control group that was left untreated and an experimental group where a nonbalanced occlusion was created. Elastic rubber bands, 1 mm in diameter, were inserted and 1 week later were replaced by plastic material between the first and the second molars of the left maxillary and the right mandibular dentitions to move the first molars about 0.8 mm mesially. This created and maintained a physiologically nonbalanced occlusion. The animals were euthanized 8 weeks later, and the TMJ disc thickness was measured on histologically prepared slices using an electronic meter. Two-way univariate analysis of variance was used to compare the groups (α level = .05).

Results: The intermediate zone was thicker in the experimental group than in the control group (P = .003), but no differences were found between groups regarding the anterior and posterior bands. There were no significant sex-related effects on this observation.

Conclusion: The hypothesis is rejected. The results indicate that the intermediate zone of rat TMJ disc has the ability to adapt to the alteration of the space between condyle and fossa caused by occlusion changes. Further studies on larger groups that are followed for longer times are needed. (*Angle Orthod.* 2009;79:51–53.)

KEY WORDS: Temporomandibular joint; Temporomandibular disorders; Occlusion; Rat

INTRODUCTION

Morphologic changes of the temporomandibular joint (TMJ) disc in response to etiologic tempormandibular disorder (TMD) factors are of high clinical interest.[1–11] There is a close relationship between the form and function of the disc, which in humans is thin in the intermediate zone and significantly thicker in the anterior and posterior bands. With these characteristics, the disc can be in tight contact with the articular surfaces of condyle and fossa during joint movements, which is important for maintaining normal TMJ function.[12,13] Kurita et al[14] reported that a frequently found characteristic of TMJ discs that had been surgically removed from TMD patients was that they were thicker than normal. An increased thickness may reflect a change in functional demand or may be the result of pathology.

Occlusion affects how the load is distributed in the TMJ.[15] However, in humans the occlusion has great variation, and it is difficult to compare between individuals. Therefore, there is a need for testing animal study models to investigate whether changes of occlusion would influence TMJ disc morphology. The present study was designed to simulate physiologically nonbalanced occlusion in rats, and test its effect on TMJ disc thickness.

MATERIALS AND METHODS

Rats

Twenty-four 8-week-old Sprague-Dawley rats, all raised in the same environment, were provided by the

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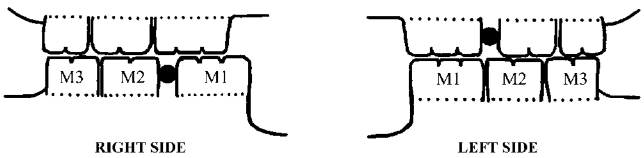

Figure 1. Illustration of the method used to create physiologically nonbalanced occlusion by producing gaps between the mandibular first and second molars on the right side and between the maxillary first and second molars on the left side. M1 is the first, M2 is the second, and M3 is the third molar. The black dots are the elastic rubber band.

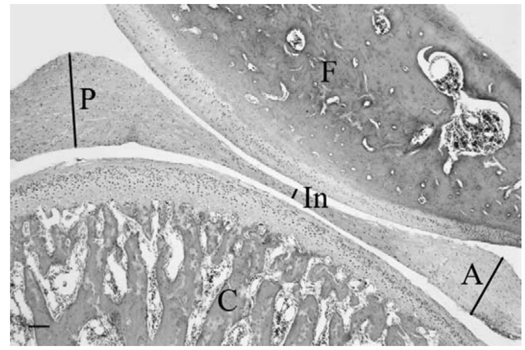

Figure 2. Illustratation showing where the TMJ disc thickness was measured in the anterior band (A), the intermediate zone (In), and the posterior band (P). C is condyle and F is fossa. Bar: 100 μm.

animal center of the Fourth Military Medical University. They were divided into one control and one experimental group with same number of animals and equal gender distribution. The study was approved by the Animal Research Committee of the Fourth Military Medical University.

Establishment of Physiologically Nonbalanced Occlusion

The rat's dental formula is I 1-1, C 0-0, P 0-0, M 3-3. There are 8 maxillary and 8 mandibular teeth with a large gap between the incisors and the first molar teeth. The rats in the experimental group were anesthetized with a muscle injection of 0.2~0.3 mg/kg 846 (The Military Veterinary Institute, University of Agriculture and Animal Sciences, Changchun, China). An elastic rubber band, 1 mm in diameter, was inserted between the first and second left maxillary molars and between the first and second right mandibular molars. After they had been inserted between the teeth, the elastic rubber bands were cut to be as short as possible to avoid disturbing the function of neighboring soft tissues. In this way the first molars were moved mesially.

After about 3 days, a gap about 0.8-mm wide, was created between the molars, and a physiologically nonbalanced occlusion was created (Figure 1). Seven days later the rubber bands were removed and replaced with plastic material to maintain the spaces until the end of the experiment. The inserted material was carefully managed to make sure that it did not contact the opposing teeth or interfere with the occlusion. During the operating procedure, which took about 5 minutes, the mouth opening was about 15 mm. No procedures were performed on the control animals.

Preparation of the Joint Specimen

Under deep anesthesia with sodium pentobarbital (60 mg/kg, intraperitoneally), the rats were perfused with 200 mL normal sodium through the ascending aorta, followed by 400 mL paraformaldehyde (4% in phosphate-buffered saline, pH 7.4). The TMJs were dissected, 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. The TMJs were then dehydrated in ethanol before being embedded in paraffin wax. The paraffin-embedded TMJs were cut into 5-μm thick slices, about 30 slices per joint. One slice was selected from the middle sagittal section of each joint for staining with hematoxylin eosin (HE). The others were saved for use in a future study.

Thickness Measurement

The slices stained with HE were observed and photographed under a microscope (Leica DM 2500, Wetzlar, Germany). The disc thickness was measured separately for the anterior and posterior bands and for the intermediate zone as reported by Hansson et al[2] and by Kurita et al[14] (Figure 2) using a computer-assisted image-analyzing system (Qwin Pro, Leica Microsystem Imaging Solutions Ltd, Cambridge, UK). One disc in the experimental group was discarded because of a technical error in the tissue preparation. In one disc from the control group the intermediate zone could not be measured because it was curled. One hundred forty measurements were obtained by measuring the three sites of each disc. All measurements were made by one of the authors without knowing which group the samples belonged to.

Statistical Analysis

The SPSS 11.0 package (SPSS Co, Chicago, Ill) was used to describe and analyze the data. First, paired sample *t*-tests were used to compare the values from the right and left side TMJ discs. No significant differences were found. Then data from the bilateral TMJ discs of each rat were averaged. For the two rats with missing data, averages were calculated using the available data. The averages were then used as the dependent variables in two-way univariate analyses of variance, which were performed separately for the anterior and posterior bands and the intermediate zone. Disc thickness was compared between the control and the experimental groups and between genders. An α level of .05 was used for all statistical tests.

Table 1. Comparison of the thickness (μm) of the temporomandibular joint disc between groups with (experimental group) and without (control) experimentally created physiologically nonbalanced occlusion[a]

	Control Group (mean ± SD)	Experimental Group (mean ± SD)	*P*
Anterior band	369.1 ± 48.17	371.0 ± 61.65	NS
Intermediate zone	56.9 ± 19.84	87.6 ± 22.26	.003
Posterior band	534.7 ± 92.14	538.8 ± 82.51	NS

[a] NS indicates not significant.

RESULTS

The effect of group was statistically significant for the intermediate zone, $F_{1,20} = 11.89$, $P = .003$, but not for the anterior and posterior bands. The intermediate zone was significantly thicker in the experimental group than in the control group (Table 1). The effect of gender was not statistically significant.

DISCUSSION

The results indicate that experimentally created, physiologically nonbalanced occlusion can be associated with increased thickness of the intermediate zone of the TMJ disc in rats. There are obvious anatomic differences in TMJ morphology and function between human and animal TMJs making it less probable that the changes necessarily occur at the same disc parts, but the important point is that the changes in occlusion were associated with changes in TMJ disc morphology in this animal study. This encourages more extensive studies on larger groups of animals followed for longer periods.

The mesial displacement of molars prevented normal contact relationship between the dental arches as illustrated in Figure 1. This probably caused a vertical displacement of the mandible with its condyles. This means the nonbalanced occlusion could have prevented the rats from closing their mouth as tightly as they used to in the previous centric occlusion and/or moved the condyles into their previous positions in the fossa. The degree of misalignment may have been small. However, it most probably had an effect by changing the space of the joint cavity and the distribution of internal joint pressure. The effect might have reduced internal joint pressure in the central area, thereby decreasing the mechanical load in the intermediate zone and causing the disc to respond by thickening in that part.

Further studies on the measurements of the spatial relations between fossa, condyle, and disc, performed in rats with experimentally created physiologically nonbalanced occlusion, are also needed to clarify details about the possible effects on jaw movement patterns.

CONCLUSIONS

- The intermediate zone of rat TMJ disc may have the ability to adapt to experimental occlusion changes by increasing thickness.
- If confirmed in extended studies with larger groups and longer follow-up time, this animal model could be a good substitute for human studies on dysfunctional occlusal changes and their effect on TMJ disc morphology.

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