*Original Article*

# Masseter Muscular Weakness Affects Temporomandibular Synovitis Induced by Jaw Opening in Growing Rats

**Miho Ozaki[a]; Sawa Kaneko[b]; Kunimichi Soma[c]**

**ABSTRACT**

**Objective:** To evaluate the influence of impaired masseter function during growth on the development of temporomandibular synovitis.

**Materials and Methods:** Sixteen 3-week-old male Wistar rats were classified into four groups. The first group served as control; and in the second group, jaw opening was forced for 3 hours when the rats were 9 weeks old. In the third and fourth groups, the masseter muscles were bilaterally resected at 3 weeks of age, and the rats in the fourth group were additionally forced to open their jaw at 9 weeks of age. All rats were sacrificed at 9 weeks. Temporomandibular joint (TMJ) tissue samples were processed for histology, and evaluated for cyclooxygenase-2 (COX-2) and inducible nitric oxide synthase (iNOS) expressions by immunohistochemistry to examine the inflammatory changes in the synovial membrane.

**Results:** The control group showed noninflammatory changes. In the jaw-opening group, vascular dilation and weak COX-2 immunoreactivity were induced by jaw opening in the synovium. In the masseter-resection group, the masseter-resected rats exhibited moderate synovial changes while in the resection with opening group, the masseter-resected rats revealed more significant inflammatory changes including synovial hyperplasia, dilated vasculature, fibrin deposits, and intense immunoreactivity for COX-2 and iNOS, all caused by jaw opening.

**Conclusions:** These results suggest that masseter activity in the growth period is an important factor in the induction of temporomandibular synovitis.

**KEY WORDS:** Masseter resection; Temporomandibular joint disorder (TMD); Synovitis

## INTRODUCTION

Many longitudinal studies have indicated that both subjective symptoms and clinical signs of temporomandibular joint disorder (TMD) increase during the growth period.[1] Although intensive skeletal growth and increasing muscular strength occur during puberty,[2] it is unknown whether these factors influence the development of TMD.

Some reports have suggested that mechanical stress causes the initial stage of TMD.[3,4] For example, forced jaw opening has been utilized to induce articular synovitis in the temporomandibular joint (TMJ), in animal models.[5,6] However, it has recently been suggested that the anatomical susceptibility of the temporomandibular tissues to trauma was a likely causative agent of TMD.[7]

Optimal masticatory muscular force during growth is necessary for normal mandibular growth.[8,9] Bilateral resection of the masseter muscles in growing rats resulting in a thinner chondroblastic layer of the condyle and larger mandibular plane angle[10] affects the morphology of the mandibles.[8] Moreover, a close relationship between the morphologic changes of the condyle and TMJ internal derangement[20] has been reported, and patients with temporomandibular arthritis tend to have high angles.[12] Considering these previous reports, various changes in the TMJ caused by compromised masseter function are believed to correlate with

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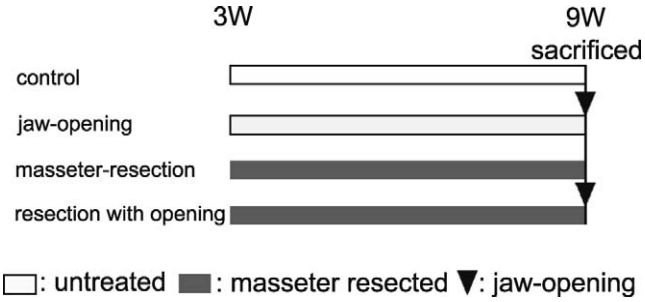


**Figure 1.** Summary of the experimental time schedules. All rats were sacrificed at 9 weeks of age, which corresponded to the end of the experimental period. Control, control group; jaw-opening, jaw-opening group; masseter-resection, masseter-resection group; resection with opening, resection with opening group.

TMD although their relationship has not been clarified yet.

A series of arthroscopic[12] and histopathologic investigations[13] on TMD have revealed the occurrence of inflammation in the synovial membrane. Recent studies have identified that the activation of cyclooxygenase-2 (COX-2)[14,15] or the inducible nitric oxide synthase (iNOS)[16] pathway is involved in the pathogenesis of TMD. It has also been reported that these factors were expressed in human TMJ with synovitis,[16,17] supporting their vital roles in the pathophysiology of synovitis.[18]

In the present study, we examined the inflammatory changes in the TMJ synovium caused by jaw opening, in rats that underwent masseter resection, by investigating COX-2 and iNOS expressions to clarify whether the impaired masseter function is related to TMD development.

## MATERIALS AND METHODS

### Animal and Tissue Preparation

Animal protocols were approved by the Institutional Animal Care and Use Committee of Tokyo Medical and Dental University, and the experiments were carried out under the control of the University's Guidelines for Animal Experimentation.

Sixteen 3-week-old male Wistar rats were used for this study. They were randomly divided into four groups (n = 4 for each group) (Figure 1).

- The first group served as untreated controls.
- In the second group, mechanical stress was applied to both TMJs of the rats by forcing mouth opening for 3 hours at 9 weeks of age, according to previously described methods.[6,20] To hold the mandible in the maximal mouth-opening position (approximately 20 mm), a jaw-opening device was used. The rats were anesthetized with an intraperitoneal injection of 8% chloral hydrate (1 mL/200 g body weight) during mouth opening.
- In the third and fourth groups, to make the muscular weakness models, the masseter muscles were bilaterally resected at 3 weeks of age, using the same model reported previously by Monje[8] and Yonemitsu et al.[10] In brief, before surgery, all animals were deeply anesthetized with diethyl ether and an intraperitoneal injection of 8% chloral hydrate (1 mL/200 g body weight). After shaving the area, the rats were cut open, and the masticatory muscles were visualized. All superficial and deep portions of the masseter muscles were bilaterally cut off at the end of each muscle, and removed without damaging any major blood vessels and nerves around the muscles. Then, the opened skins were sutured. At the conclusion of the operation, amoxicillin (ICN Biomedicals Inc, Aurora, Ohio) (9 mg/60 g body weight) was injected to prevent infection.
- In addition, in the fourth group, the masseter-resected rats received forced maximal mouth-opening at 9 weeks of age as previously described in the second group.

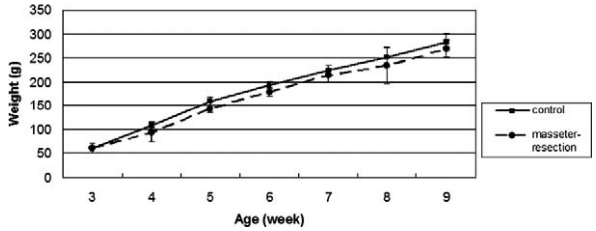


**Figure 2.** Changes in body weight during the experiment.

All rats were fed pellets, and given water ad libitum throughout the experimental period, and the body weight increased with no significant difference among each group (Figure 2). There was no difference in the pattern of jaw movement between rats that underwent masseter resection and the other rats while eating pellets.

At the end of the experimental period, ie, when the rats were 9 weeks old, all animals were deeply anesthetized and perfused transcardially with 4% paraformaldehyde in 0.1 M phosphate buffer (pH = 7.4). The rats in the jaw-opening group and resection with opening group were sacrificed immediately after the continuous jaw opening for 3 hours. The heads of the rats were immersed in the same fixative overnight, and decalcified in 10% ethylene diaminotetraacetic acid dipotassium salt dehydrate (EDTA) at 4°C for 8 weeks. All condylar areas were embedded in paraffin by conventional methods, and sliced sagittally into 6 μm-thick sections (RM 2155, Leica Co Ltd, Nussloch, Germany), and finally mounted on silane-coated slides.

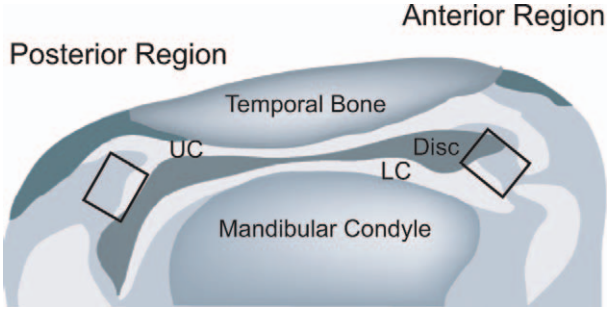


**Figure 3.** A schematic diagram of a sagittal section of the rat TMJ. The anterior and posterior boxes indicate the observed portions in each section. UC, upper joint compartment; LC, lower joint compartment.

### Histomorphometry With Hematoxylin and Eosin Staining

Two sagittal sections of the central portion of the rat TMJ (Figure 3) were selected from each TMJ in all rats, and stained with hematoxylin and eosin. The stained sections were examined under a light microscope. Histopathologic grading of the stained sections was performed according to the methods of Muto et al[5] by two independent observers. For each section, the degree of synovitis was examined in the anterior and posterior portions, where the most typical changes were observed.

Synovial lining hyperplasia was graded on a scale from 0 to 2: grade 0, staining of 1–3 layers; grade 1, staining of 4–6 layers; and grade 2, staining of 7 or more layers.

Dilated vasculature was graded on a scale from 0 to 3: grade 0, not present; grade 1, involving less than one-third of the synovial membrane length; grade 2, involving one-third to two-thirds of the synovial membrane length; grade 3, involving more than two-thirds of the synovial membrane length.

Fibrin deposits and synovial adhesion were graded on a scale from 0 to 3 (as described for the vasculature).

### Immunostaining for COX-2 and iNOS Receptor

Typical sagittal sections were selected in the same way as described previously from each group. Immunohistochemical staining of COX-2 was performed using a Catalyzed Signal Amplification (CSA) System (DAKO, Carpinteria, Calif). The protocol was essentially that of the literature.[21] The primary antibody to rat COX-2 (Lab Vision, Fremont, Calif) was purchased commercially.

iNOS staining was performed as follows. Briefly, the deparaffinized sections were incubated with the iNOS antibody (CHEMICON, Temecula, Calif) for 30 minutes at 37°C, visualized using the biotin-streptavidin method (Histofine MAX-PO kit; Nichirei, Tokyo, Japan), and counterstained with hematoxylin.

The intensities of the immunohistochemical stainings for COX-2 and iNOS were evaluated in the anterior and posterior portions, where the most intense immunoreactivities were observed, and graded according to a previous study,[17] as follows: grade 0, negative, weak, or marginal staining less than 25% of the cells; grade 1, focally positive for more than 25% of the cells; grade 2, focally or diffusely positive for more than 50% of the cells. Two observers who were blind to the clinical and arthroscopic findings independently evaluated each section.

## RESULTS

### Histopathologic Examination Scoring

In the control group, the synovial membranes of the TMJs showed noninflammatory changes (grade 0) (Figure 4).

In the second (jaw-opening) group, the dilation of the vasculature was mainly observed with the synovial inflammatory changes resulting from jaw opening. The number of rats with synovial lining hyperplasia and synovial adhesion was insignificant (Table 1).

In the third (masseter-resection) group, which did not experience forced jaw-opening, the rats also exhibited moderate synovial changes, and dilation of the vasculature was the only apparent change.

On the other hand, in the fourth (resection with opening) group, synovitis-like changes such as synovial hyperplasia were induced by jaw opening in the TMJ. Multilayered changes (grade 1 and 2) and fibrin deposition were notably observed. The synovial membrane appeared hyperplastic associated with a considerable number of inflammatory cells and congested blood vessels. Vascular dilation was also found in the subsynovial connective tissues.

### Immunohistochemical Localizations of COX-2 and iNOS

In the control group, COX-2 and iNOS were not significantly present (Figures 5 and 6).

In the jaw-opening group, the definite reaction (grade 1) of COX-2 antibody was only seen in three of eight specimens, and iNOS staining was hardly recognized (Tables 2 and 3).

In the masseter-resection group, masseter-resected rats without forced jaw opening showed weak reactions of COX-2 and iNOS, and particularly that of iNOS was marginal.

On the contrary, in the resection with opening group, strong immunoreactivities for COX-2 and iNOS were observed in the synovial membrane. The immunopos-

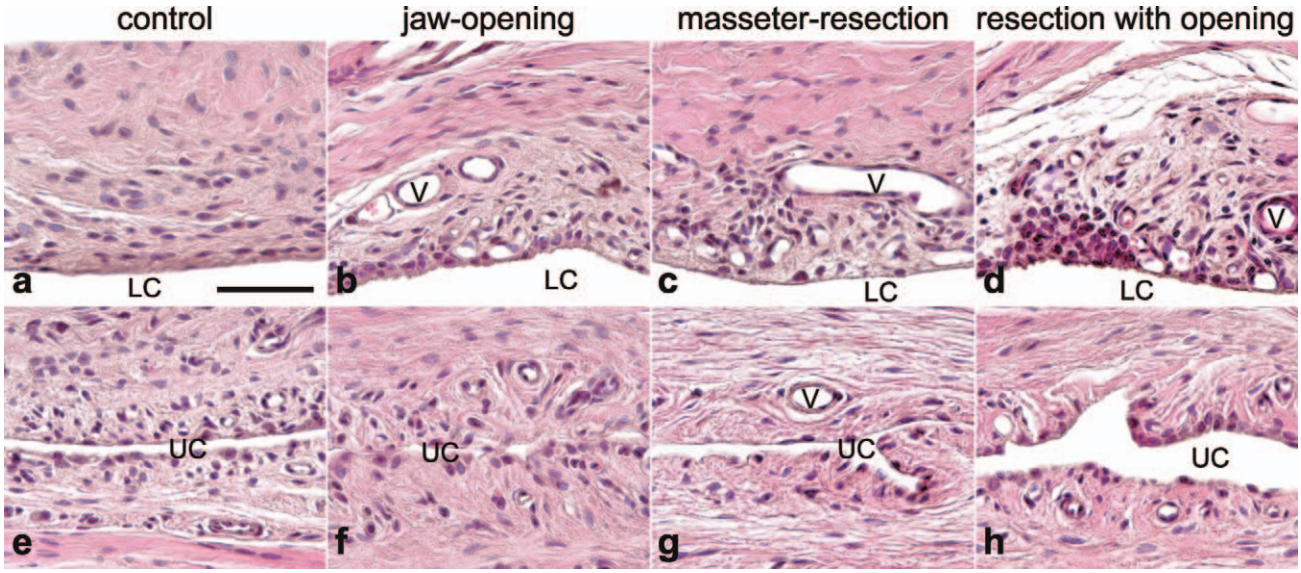


**Figure 4.** Light micrographs of 6-μm sagittal sections of the anterior (a–d), and posterior (e–h) portions in the control and experimental rat TMJs (hematoxylin and eosin staining). Panels a, e: control group. Panels b, f: jaw-opening group. Panels c, g; masseter-resection group. Panels d, h: resection with opening group. UC, upper joint compartment; LC, lower joint compartment. V, blood vessel. Bar = 50 μm.

**Table 1.** Scoring of the Thickness of the Synovial Cell Layers, Dilated Capillaries, Fibrin Deposition, and Synovial Adhesion. Each Measurement was Done from a Total of 8 Joints (4 Rats). A indicates anterior portion; P, posterior portion.

| | | Control | | Jaw-Opening | | Masseter-Resection | | Resection With Opening | |
|---|---|---|---|---|---|---|---|---|---|
| | | A | P | A | P | A | P | A | P |
| Synovial lining hyperplasia | Grade 0 | 8 | 8 | 4 | 7 | 5 | 6 | 0 | 1 |
| | Grade 1 | 0 | 0 | 4 | 1 | 3 | 2 | 5 | 6 |
| | Grade 2 | 0 | 0 | 0 | 0 | 0 | 0 | 3 | 1 |
| Dilated vasculature | Grade 0 | 8 | 8 | 0 | 0 | 0 | 0 | 0 | 0 |
| | Grade 1 | 0 | 0 | 6 | 5 | 6 | 8 | 6 | 7 |
| | Grade 2 | 0 | 0 | 2 | 3 | 2 | 0 | 2 | 1 |
| | Grade 3 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 |
| Fibrin deposits | Grade 0 | 8 | 8 | 6 | 7 | 5 | 4 | 0 | 0 |
| | Grade 1 | 0 | 0 | 2 | 1 | 3 | 4 | 7 | 8 |
| | Grade 2 | 0 | 0 | 0 | 0 | 0 | 0 | 1 | 0 |
| | Grade 3 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 |
| Synovial adhesion | Grade 0 | 8 | 8 | 7 | 4 | 8 | 7 | 7 | 3 |
| | Grade 1 | 0 | 0 | 1 | 4 | 0 | 1 | 1 | 4 |
| | Grade 2 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 1 |
| | Grade 3 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 |

itive synovial cell layers for COX-2 and iNOS in the TMJ became thicker than those of the controls. COX-2 immunoreactivity was seen in the nuclei of superficial cells, infiltrating mononuclear cells, fibroblast-like cells, and blood vessels of the hypertrophic synovium in both the anterior and posterior portions. Definite iNOS immunoreactivity was recognized in the cytoplasm of superficial cells, endothelial cells, and synovial fibroblasts in each portion of the hypertrophic synovium. As a whole, iNOS immunoreactivity was weaker than that of COX-2.

## DISCUSSION

This study indicates that the inflammatory changes of the temporomandibular synovial membrane induced by jaw opening are enhanced in rats that undergo masseter resection in the growing period.

In rats with normal masticatory muscular force, with forced jaw opening, dilation of the vasculature was the only prominent change, and subtle COX-2 staining was found in the synovial tissues. These observations seemed to result from indirect joint-loading modifica-

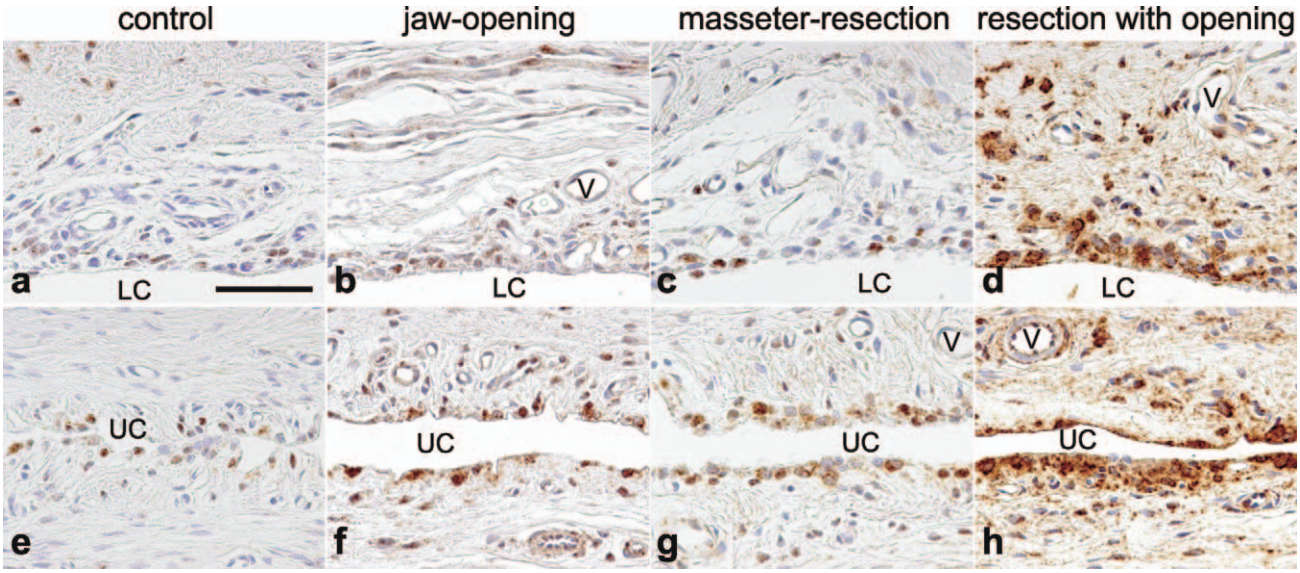


**Figure 5.** Light micrographs of sagittal sections of the anterior (a–d) and posterior (e–h) portions in the control and experimental rat TMJs immunostained for COX-2. Panels a, e: control group. Panels b, f: jaw-opening group. Panels c, g; masseter-resection group. Panels d, h: resection with opening group. UC, upper joint compartment; LC, lower joint compartment. V, blood vessel. Bar = 50 μm.

**Table 2.** Scoring of COX-2 Expression in the Synovial Cell Layers of the Control and Experimental Rat TMJs. A indicates anterior portion; P, posterior portion.

| | | Control | | Jaw-Opening | | Masseter-Resection | | Resection With Opening | |
|---|---|---|---|---|---|---|---|---|---|
| | | A | P | A | P | A | P | A | P |
| COX-2 score | Grade 0 | 8 | 8 | 5 | 5 | 5 | 6 | 1 | 2 |
| | Grade 1 | 0 | 0 | 3 | 3 | 3 | 2 | 7 | 5 |
| | Grade 2 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 1 |

tions. Rats have similar condylar translatory movements to those of the human condyle.[22] Translatory movement of the condyle at maximal mouth opening refers to the range of motion of the condyle from the anterior slope of the glenoid fossa to a point slightly anterior to the summit of the articular eminence. Any condition that causes the condyle to move over a greater range is considered to lead to traumatic injury of the TMJ. In the TMD patient with synovial inflammation, it has been reported that increased vascularity indicates mild or early synovitis, while capillary hyperemia, fibrosis, and synovial hyperplasia indicate more pronounced synovitis.[13] Thus, in our rats, considering that hyperplasia of the synovial membrane and fibrin deposits were scarcely observed, the synovial inflammatory changes caused by jaw opening were moderate.

On the contrary, in the rats with impaired masseter function, when jaw opening was forced, more significant inflammatory changes involving synovial cell proliferation, increased vascularity and gradual fibrous changes of the subsynovial tissues were observed. Our results were essentially similar to those in previous studies showing the basic inflammatory response induced by injury of the synovial membrane.[23] Furthermore, COX-2 is normally undetectable in most tissues, but it can be expressed at high levels in macrophages and other cell types after induction with a variety of substances including inflammatory mediators.[24] The overproduction of NO generated by iNOS also causes tissue damage during inflammation.[17] Recent studies suggested that the activation of COX-2 or iNOS contributes to the pathogenesis of inflammatory arthritis including rheumatoid arthritis[15] and adjuvant-induced arthritis.[25] In the light of those reports, the intense COX-2 and iNOS expressions induced by jaw opening in the weak masseter muscular model indicate that the inflammatory changes were caused and developed in the TMJ.

It is also extremely interesting that in our study, COX-2 immunoreactivity was observed under vascular dilation and was more detectable than iNOS reaction. Based on reports that COX-2 expression in colon cancer cells stimulated angiogenesis of co-cultured endothelial cells,[26] and the upregulation of COX-2 enhanced NO production in a rat intestinal epithelial cell

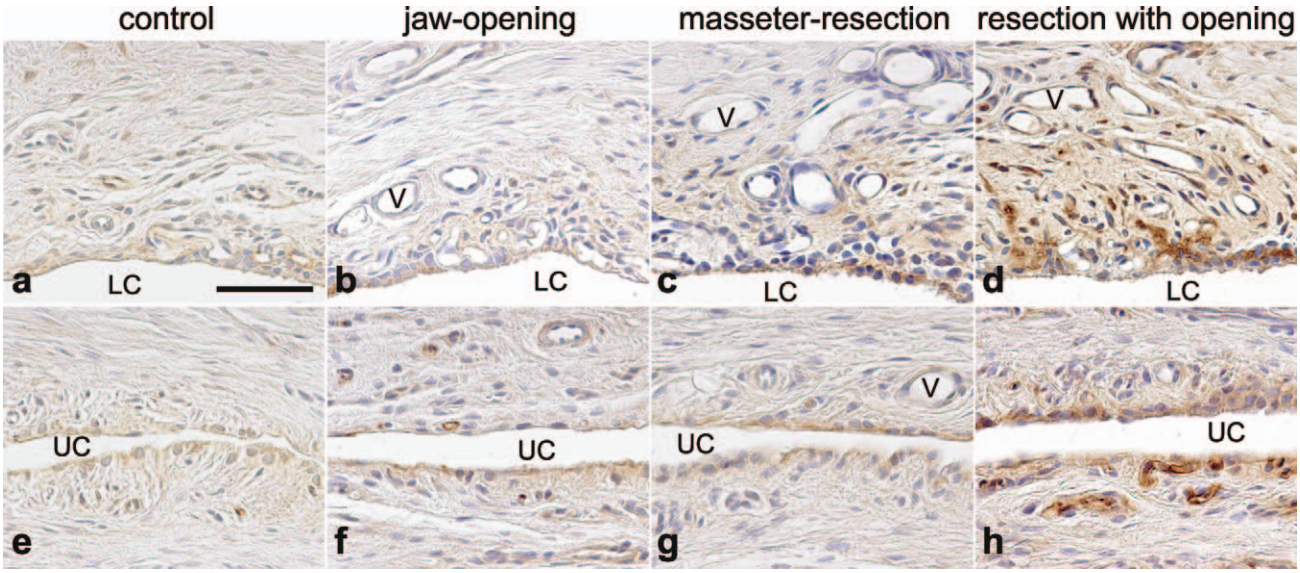


**Figure 6.** Light micrographs of sagittal sections of the anterior (a–d) and posterior (e–h) portions in the control and experimental rats TMJs immunostained for iNOS. Panels a, e: control group. Panels b, f: jaw-opening group. Panels c, g: masseter-resection group. Panels d, h: resection with opening group. UC, upper joint compartment; LC, lower joint compartment. V, blood vessel. Bar = 50 μm.

**Table 3.** Scoring of iNOS Expression in the Synovial Cell Layers of the Control and Experimental Rat TMJs. A indicates anterior portion; P, posterior portion.

| | | Control | | Jaw-Opening | | Masseter-Resection | | Resection With Opening | |
|---|---|---|---|---|---|---|---|---|---|
| | | A | P | A | P | A | P | A | P |
| iNOS score | Grade 0 | 8 | 8 | 8 | 7 | 6 | 7 | 1 | 4 |
| | Grade 1 | 0 | 0 | 0 | 1 | 2 | 1 | 6 | 4 |
| | Grade 2 | 0 | 0 | 0 | 0 | 0 | 0 | 1 | 0 |

line,[15] our findings may indicate the more swiftly COX-2 reaction is concerned with angiogenesis in response to injury than iNOS. Further studies are needed to clarify the interactions among COX-2, iNOS, and the production of angiogenic factors.

One clinical study found that the thickness of the masseter muscle alone correlates with bite force.[27] In the other clinical study, patients with myotonic dystrophy had three times less electromyographic activity in their masticatory muscles than healthy individuals[28] and had a high prevalence of malocclusions such as anterior open bite. In the morphologic analysis, bilateral resection of the masseter muscles decreased the condylar head length and shortened ramal height.[10] Accordingly, we assumed that resecting the masticatory muscle fibers would diminish the masticatory muscular force and reduce the loading on the condyle. Structural joint abnormalities induced TMJ synovitis by external trauma and hypermobility of the joint tissues.[7] In our study, the differences in synovial reactions between the rats with normal and weak masseter function confirm this idea. In addition, inflammatory changes induced by masseter resection without forced jaw opening were not significant in our study, and no significant change would occur because of the scar tissues, as suggested by other reports.[8,10] Therefore, we believe that bilateral masseter resection itself is not immediately associated with TMJ synovitis, but structurally fragile TMJ tissues resulting from impaired masseter muscular force may lead to inflammatory changes when the TMJ receives impulses from something.

To further analyze the mechanisms of TMD as a chronic pathology, biochemical long-term studies are required in addition to immunohistochemistry. This study demonstrates that the impaired masseter muscle function in the growing period may be a factor promoting the inflammatory reaction of the TMJ in response to excessive mechanical stress. Therefore, optimal growth of the masseter muscle is important to prevent the development of TMD.

## CONCLUSIONS

- The inflammatory changes of the synovium induced by jaw opening were more significant in growing rats with weak muscle function.

- These results indicate that masseter activity in the growth period is an important factor in the development of TMD.

## ACKNOWLEDGMENTS

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