Abstract: This study was performed to develop a new rat model of reduced masticatory activity in order to assess the effect of this reduction on the morphology of the temporomandibular joint (TMJ) over time. Female rats were used, and ovariectomy was performed to simulate aged/postmenopausal status. Twenty-four SD rats aged 6 weeks were divided into four groups: ovariectomy/sham procedure (Ov/S); ovariectomy/reduced masticatory activity (Ov/RMA); non-Ov/S (NO/S); and non-Ov/RMA (NO/RMA). The RMA procedure involved grinding down the edges of the upper and mandibular incisors by about 3 mm and supplying the rats with a powdered diet. The bilateral TMJ was examined by micro-computed tomography at 0, 1, 2, 4, 6, and 8 weeks after the start of RMA. Condylar width was greater in the NO/S group than in the Ov/S group after the 2nd week, showing that ovariectomy reduced the width of the condyle. After the 2nd week, significant differences in condylar width were apparent between the NO/S and NO/RMA groups, and between the Ov/S and Ov/RMA groups. This RMA procedure appeared to provide a good model of reduced masticatory activity. The present findings in female rats suggest that reduction of appropriate masticatory activity in the growth period results in poor growth of the mandibular condyle and immediately induces atrophy of the mandibular condyle under conditions simulating aged/postmenopausal status.

Keywords: condylar growth, masticatory activity, micro-CT, postmenopausal change

Introduction

Mastication is the first step of food intake in mammals. Proper mastication facilitates nutrient uptake, and children are often told by their parents to chew well when eating. Sufficient mastication helps with the development of the brain, improves gustation and pronunciation, helps prevent obesity and dental caries, facilitates swallowing, and acts to regulate digestion [1,2]. These benefits of mastication are very important for the physical growth and development of children and adolescents [3,4]. On the other hand, recent studies have also demonstrated that mastication has an intrinsic relationship to dementia and cognitive function in the elderly [5,6]. Other researchers have reported that mastication may also function in adjustment to stress and pain [2]. Accordingly, maintenance of proper masticatory activity is crucial at all ages to preserve and promote quality of life.

The temporomandibular joint (TMJ) and masticatory muscles play key roles in mastication [7]. Other skeletal muscles such as the myohyoid and diaphragm muscles, and the articular disk, are also involved in mouth opening and other jaw movements. Tendons and ligaments contribute to limitation of jaw movement. Teeth can crush, grind and mix foods with saliva secreted from the salivary glands to form a bolus for swallowing, in cooperation with the tongue and cheek muscles [7,8]. Sense receptors are present in the oral cavity, while the masticatory pattern generator provides brainstem control over masticatory activity [7,9,10]. Mastication is thus achieved through harmonization of the peripheral components and the nervous system. Dental pathologies such as tooth loss, periodontitis, xerostomia, temporomandibular disorders and fractures of the jaw, along with serious diseases such as oral malignancies and cerebrovascular diseases, conditions that depress muscular function such as oral frailty and sarcopenia, post-therapeutic changes after radiotherapy and surgery near the oral cavity can all be causes of masticatory dysfunction [7]. Ideal development and growth of the TMJ, muscles and bony components correlate with masticatory activity itself.

Various animal models of reduced masticatory activity or function have been developed. Some researchers have reported that feeding experimental animals with a soft diet inhibits growth and development of the mandibular condyle by reducing masticatory activity [11-16]. Hichijo et al. [15] showed that the distances and angles between anatomical landmarks in the mandible of young rats displayed significant changes on a soft diet. Tanaka et al. [17] also demonstrated a relationship between food consistency and mandibular bone mineral density in rodents during the growth period [16]. A soft diet that reduces masticatory function can thus change the growth of the mandible in young mammals. In contrast, few investigations have examined the relationship between food consistency and the TMJ response in models involving older animals. Orajärvi et al. [18] reported that the characteristics of the TMJ condylar cartilage were correlated with loading, aging, and sex in older rats. In contrast, no studies have investigated the relationship between condylar bony change and food consistency in models involving older animals. In humans, degenerative osteoarthritic changes to the TMJ are well known to correlate with age, and are more common in postmenopausal women [19,20]. Postmenopausal status in women is associated with a wide variety of pathological conditions resulting from estrogen deficiency [21]. Osteoporotic change is one of the most prominent pathological changes in postmenopausal women. In animal experiments, ovariectomized rodents have been used to model osteoporosis or the postmenopausal female state. Ovariectomy induces loss of trabecular bone, and increases both endocortical bone resorption and cortical porosity [22]. In the TMJ region, Okuda et al. [23] have reported that ovariectomy causes alterations in the TMJ remodeling response. The authors therefore considered that masticatory activity might impact TMJ morphology in relation to postmenopausal status.

Imposition of a soft diet using powdery, pasty or liquid diets can be used for creating models of reduced masticatory activity in rodents. One difference between rodents and other mammals is the characteristics of the incisors; rodent incisors have open roots and continue to grow throughout life. The upper and lower incisors of adult rats grow 2.2 mm/week and 2.8 mm/week, respectively [24]. Rodents always grind their incisors to maintain an appropriate length and shape. Overgrowth of rodent incisors can cause eating dysfunction, trauma around the oral cavity, and even death (Crossley DA et al. Ferrets, rabbits and rodents 2nd ed., 370-382, Saunders, 2004). Tooth grinding is a normal activity in rodents, but abnormal in humans. For this reason, the authors considered that any reduction in masticatory activity among rodents fed a soft diet might be limited, and thus inappropriate as a model of reduced mastication.
Materials and Methods

The protocol of the present study was reviewed and approved by the institutional review board for animal experiments (approval numbers: AP14-D009 and AP15-D018).

Experimental animals

Twenty-four female Sprague-Dawley rats aged 6 weeks were randomly divided into four groups as follows:

1. Ovariectomized/sham procedure (Ov/S) group
2. Ovariectomized/reduced masticatory activity (Ov/RMA) group
3. Non-ovariectomized/sham procedure (NO/S) group
4. Non-ovariectomized/reduced masticatory activity (NO/RMA) group

Two days before starting this experiment, bilateral ovariectomy was performed by an experienced veterinarian under general and local anesthesia for the 12 rats in the Ov/S and Ov/RMA groups. Sham surgery was not performed for the 12 rats in the NO/S and NO/RMA groups. All experimental animals in the Ov/S and Ov/RMA groups were kept for acclimation in the institutional animal room for 2 days after ovariectomy until the first day of the experiment. The reduced masticatory activity (RMA) procedure was applied in the Ov/RMA and NO/RMA groups, and the sham procedure was used in the Ov/S and NO/S group for 8 weeks. Interpretation of these groups and the procedures performed in each group are shown in Table 1. Details of the RMA procedure and sham procedure are described in the next section.

RMA and sham procedure

To achieve reproducible reductions in masticatory activity in the experimental rodents, two procedures were conducted in the Ov/RMA and NO/RMA groups. First, the edges of the upper and mandibular incisors were ground down by about 3 mm with water irrigation using a dental micro motor with a dental diamond point under inhaled general anesthesia (isoflurane; Forane, Abbott Japan, Tokyo, Japan) every week. Second, rats in the Ov/RMA and NO/RMA groups were fed a powdered experimental diet (22 g/day, MF powder type; Oriental Yeast, Tokyo, Japan) every day.

In the sham procedure groups (Ov/S and NO/S), the maximum mouth-opening position was maintained for 30 s under general anesthesia every week and the rats were fed 22 g of an experimental pellet diet (MF pellet type; Oriental Yeast) each day.

All groups were housed at two animals per cage in a facility maintained at 23 ± 1°C with 50 ± 10% relative humidity on a 12 h light/12 h dark cycle for 8 weeks (until 14 weeks old).

Computed tomography (CT) examination

Micro-CT (R_mCT; Rigaku, Tokyo, Japan) was used for TMJ evaluations in the rats [25,26]. The head and face region including both sides of the TMJ was scanned at 0, 1, 2, 4, 6, and 8 weeks after the first RMA or sham procedure under general anesthesia. Body weight measurement was also performed on the same day as CT scanning. Imaging conditions were as follows: tube voltage: 90 kV; tube current, 100 μA; scan time, 17 s; field of view (FOV), φ 24 mm × H 24 mm, isotropic voxel size: 50 μm. Datasets from scanned images were exported to a workstation (I-viewR; Morita, Kyoto, Japan) and reconstructed (slice thickness and pitch: 250 μm each).

At the 8th week, CT of the maxillofacial region was performed for assessment of tooth abrasion (φ 64 × 64 mm; voxel size, 133 μm).

Image evaluation

The mandibular ramus was oriented parallel to the sagittal plane on axial sectional images and the mandibular plane was set parallel to the horizontal plane on the sagittal sectional images (Fig. 1A). The section line for making cross-sectional images on the sagittal images was adjusted to a position that passed through the top of the condyle and was vertical to the mandibular plane (dotted line in Fig. 1A and 1B-1). The section line to make cross-sectional images, passing through the top of the condyle and vertical to the mandibular plane, was set parallel to the horizontal plane (white solid line). White dotted line indicates the section line which is passing through the top of the condyle and vertical to the mandibular plane as the Fig. 1A. Arrow indicates condyle of the mandible and mandibular angle, respectively. B-1: sagittal sectional micro-CT of the rat mandible. White dotted line also indicates the section line to make cross-sectional images, passing through the top of the condyle and vertical to the mandibular plane. Arrow and arrowhead indicate condyle of the mandible and mandibular ramus, respectively.

Table 1

| Group                      | n  | Ovariectomy | Food (22 g/day) | Weekly procedure                                      | Group interpretation                                      |
|----------------------------|----|-------------|-----------------|-------------------------------------------------------|----------------------------------------------------------|
| Ov/S                       | 6  | Done        | Pellet type     | Maintaining maximum mouth opening position for 30 s    | Postmenopausal woman with proper masticatory activity     |
| Ov/RMA                     | 6  | Done        | Powder type     | Grinding edge of incisors in maximum opening position | Postmenopausal woman without proper masticatory activity  |
| NO/S                       | 6  | Not done    | Pellet type     | Maintaining maximum mouth opening position for 30 s    | Adolescent girl with proper masticatory activity          |
| NO/RMA                     | 6  | Not done    | Powder type     | Grinding edge of incisors in maximum opening position | Adolescent girl without proper masticatory activity       |

The present study was conducted to develop a new rat model of reduced mastication in order to assess the temporal effect of this reduction on the morphology of the TMJ in female adolescent rats and female rats subjected to ovariectomy to simulate aged/postmenopausal status.
ference (HSD) method if significant differences were detected among all groups. All statistical analyses were performed using SPSS Statistics for Windows version 25.0 (IBM, Armonk, NY, USA). A probability level of less than 5% ($P < 0.05$) was considered to indicate statistical significance.

**Results**

Fluctuations in body weight for each group are shown in Fig. 2. No significant differences in mean body weight were seen among the four groups after ovariectomy had been performed in the Ov/S and Ov/RMA groups (data not shown). However, on the first day of the experiment (0 w; 2 days after ovariectomy), the ovariectomized groups (Ov/S and Ov/RMA) were already significantly heavier than the groups without ovariectomy (NO/S and NO/RMA). No significant differences in body weight were detected between Ov/S and Ov/RMA, or between NO/S and NO/RMA at any points during the experimental period.

Attrition of molars was more marked in the NO/S group than in the NO/RMA group (quantitative evaluation was not performed). This finding suggested that masticatory activity was reduced by the reduced masticatory activity (RMA) procedure.

![Fig. 2 Time-dependent changes in rat body weight. Ov/S and Ov/RMA groups are heavier than NO/S and NO/RMA groups at all timepoints.](image)

**Fig. 2** Time-dependent changes in rat body weight. Ov/S and Ov/RMA groups are heavier than NO/S and NO/RMA groups at all timepoints.

![Fig. 3 Sagittal sectional images of the molar region at 8 weeks. Attrition of the molar was more marked in the NO/S group than in the NO/RMA group (quantitative evaluation was not performed). This finding suggested that masticatory activity was reduced by the reduced masticatory activity (RMA) procedure.](image)

**Fig. 3** Sagittal sectional images of the molar region at 8 weeks. Attrition of the molar was more marked in the NO/S group than in the NO/RMA group (quantitative evaluation was not performed). This finding suggested that masticatory activity was reduced by the reduced masticatory activity (RMA) procedure.

![Fig. 4 A: time-dependent changes in width of the mandibular condyle among the 4 groups. The Ov/S group showed significantly decreased condylar width after the 4th week compared with the baseline (0 w) measurement. In the Ov/RMA group, condylar width decreased significantly after the 1st week. In the NO/RMA group, no notable change in the condylar width was observed over the entire study period. In the NO/S group, condylar width was significantly increased after the 1st week and reached a plateau at the 6th week.](image)

**Fig. 4 A** A: time-dependent changes in width of the mandibular condyle among the 4 groups. The Ov/S group showed significantly decreased condylar width after the 4th week compared with the baseline (0 w) measurement. In the Ov/RMA group, condylar width decreased significantly after the 1st week. In the NO/RMA group, no notable change in the condylar width was observed over the entire study period. In the NO/S group, condylar width was significantly increased after the 1st week and reached a plateau at the 6th week. Asterisks show significant differences in condylar width between Ov/S and NO/S groups. B: time-dependent changes in width of the mandibular condyle between Ov/S and NO/S. Condylar width was greater in the NO/S group than in the Ov/S group after the 2nd week. Asterisks show significant differences in condylar width between Ov/S and NO/S groups. C: time-dependent changes in width of the mandibular condyle among the 4 groups. Significant differences between Ov/S and Ov/RMA groups were evident in condylar width after the 2nd week. Asterisks show significant differences in condylar width between NO/S and NO/RMA groups. D: time-dependent changes in width of the mandibular condyle between NO/S and NO/RMA. There are significant differences between NO/S and NO/RMA groups in condylar width after the 2nd week. Asterisks show significant differences in condylar width between NO/S and NO/RMA groups.
Condylar width was greater in the NO/S group than in the Ov/S group after the 2nd week, showing that ovariectomy reduced the condylar width (Fig. 4B). Comparisons between NO/S and NO/RMA, and between Ov/S and Ov/RMA, demonstrated significant differences in condylar width after the 2nd week, confirming that the RMA procedure reduced the width of the condyle (Fig. 4C, D).

Discussion
This study using a female rat model clearly demonstrated that reducing masticatory activity during the growth period resulted in poor growth of the mandibular condyle, suggesting the importance of proper mastication for growth of the mandibular condyle in adolescence. Proper masticatory activity also preserved the width of the mandibular condyle in rats that had been subjected to ovariectomy in order to simulate an aged/postmenopausal status. These findings suggested that appropriate masticatory activity is necessary in order to maintain mandibular condylar function irrespective of age or hormonal status.

Ovariectomy reduced the condylar width of rats in comparison with the Ov/S and NO/S groups. Few previous studies have evaluated the condylar morphology of the TMJ using ovariectomized rodents [23,27], and none have employed in vivo micro-CT to evaluate the same animals over time. Ovariectomy induces a state of estrogen deficiency that can evoke changes in skeletal metabolism such as increased bone turnover with resorption exceeding formation, loss of cancellous bone over cortical bone, and decreased intestinal absorption of calcium [26]. For these reasons, ovariec- tomized rodents are widely used as a model for studies of bone metabolism in postmenopausal or elderly women [21,22,28]. In the present study, the Ov/S group was considered to represent proper masticatory activity in the postmenopausal state. Condylar width in the Ov/S group decreased slowly over time from the 4th week. Some previous studies have demonstrated bone mineral loss from the condyle and alveolar bone in ovariectomized rodents, but did not measure condylar width [22,29]. Condylar width in the Ov/S group was slightly decreased, suggesting that ovariectomy or estrogen deficiency also impacted bone remodeling of the mandibular condyle as well as endocortical bone resorption and cortical porosity. However, cortical bone mineral loss and cortical porosity were not evaluated in the present study. Future studies will need to investigate these issues using micro-CT and histological evaluation. At the study baseline, body weight was greater in the Ov/S group than in the NO/S group. Ovariectomized rats show a rapid increase in body weight because of changes in body composition and abdominal fat deposition, as well as bone mineral loss due to estrogen deficiency [30]. Comparisons of condylar width between the Ov/S and Ov/RMA groups demonstrated atrophic changes in the condyle in both groups, although they were more rapid and significant in the Ov/ RMA group. This result suggested that proper masticatory activity is very important for perimenopausal women.

The RMA procedure seems to offer a good model of reduced masticatory activity for comparisons of tooth abrasion between NO/S and NO/RMA based on qualitative analysis. Another advantage of the RMA procedure is its reversibility. Tooth extraction, bite-raising appliances and grinding of the molars have been introduced to achieve malocclusion or hypofunction models in rodents [31-35]. However, these procedures involve irreversible pathological changes to masticatory activity. When weekly tooth grinding using a diamond point is stopped, normal occlusion or masticatory activity is able to recover. Other models of reduced masticatory activity have been established by changing only the consistency of the diet, i.e. use of a powdery, paste or liquid diet [11,13-19]. Bruxism is a normal activity for rodents, in order to keep the incisors sharp. Changing the consistency of food would therefore not stop the routine bruxism in rodents, whereas feeding soft food while reducing routine bruxism might provide a suitable model of reduced masticatory activity. The RMA procedure employed in the present study includes both artificial incisor grinding and powder feeding, and reduced bruxism in rats through reduced abrasion of the molars. This RMA model could thus be considered a true model of reduced masticatory activity, at least in theory. However, quantitative analysis of tooth abrasion and physiological evaluation in RMA and control groups, and comparisons between the RMA model and a model involving supply of a soft diet alone will be needed in future studies.

It was noteworthy that condylar growth in the NO/RMA group, consid- ered to represent the adolescent female state without proper masticatory activity, stopped or was poor at 2 weeks after imposition of the RMA procedure. Vaid et al. [11] compared condylar width between rats fed a soft diet consisting of powdered food and those fed solid food for 8 weeks, and found that condylar width was smaller in the former group than in the latter. Kato et al. [16] demonstrated that condylar width was greater in rats fed a solid diet than in those fed a liquid diet. However, their measurements included the bone and chondral thickness of the condyle. These results sug- gested the importance of proper mastication for growth of the mandibular condyle in adolescence. In contrast, in a study of domestic pigs, Lindsten et al. [12] reported that food consistency had no consequences for the size of the TMJ condyles, and demonstrated no correlation between tooth abrasion and condylar morphology. Differences in the condylar response to food consistency may exist between rodents and other mammals.

Various limitations to the present study should be considered. First, the masticatory system in rodents may differ from that in humans, as men- tioned above. In humans, bruxism is an abnormal activity and induces tooth abrasion, tooth fracture, tooth wear, TMJ disorders and other pathologies. However, bruxism in rodents is a normal activity to protect masticatory and bite function. Therefore, it is debatable whether rodents can provide a suitable model of human dentofacial conditions. Second, only condylar width was studied. Other measurement items such as condylar height, thickness of the cartilage and bone mineral density should also be evalu- ated. Future studies will need to include these items along with soft-tissue evaluations such as volume, blood flow and elasticity of the muscle using other modalities. Third, this study was limited to a relatively short period of 8 weeks, and was concluded when condylar width in the NO/S group reached a plateau. A longer observation period might have provided other interesting results. Fourth, physiological evaluations regarding reductions of bruxism are needed to clarify the effects of the RMA procedure. These present limitations will need to be addressed in future studies.

In conclusion, masticatory activity acts to maintain proper growth of the mandibular condyle in the adolescent growth phase and inhibits atro- phic changes to the mandibular condyle in the aged/postmenopausal phase. In addition, reduction of masticatory activity has an immediate impact on growth and atrophy of the mandibular condyle.

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Conflict of interest
The authors have no conflict of interest to declare in association with this study.
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