Jawbone Morphology in Rats with Extracted Maxillary Molars Reared on Powdered Diet

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Abstract

There have been numerous reports on the effects of the reduced masticatory function on jawbone growth, but the types of changes that occur during each period remain unclear. The objective of this study was to elucidate the effects of the reduced masticatory function on the jawbone over time. Micro-computed tomography (micro-CT) was used to scan the heads of rats that underwent extraction of all maxillary molars at the age of 5 weeks and were reared on a powdered diet, and the heads of control rats that did not undergo molar extraction and were reared on a solid diet. The changes in jawbone morphology up to 20 weeks were investigated in both groups.

There were no significant differences in the maxillary or mandibular size, but the mandibular ramus length was significantly smaller in the extraction group from 9 weeks to 20 weeks, while the mandibular angle was significantly larger from 7 weeks to 20 weeks. The mandibular area was also significantly smaller from 7 weeks to 20 weeks, the area of the mandibular notch was significantly larger from 7 weeks to 20 weeks, and the mandibular thickness was significantly smaller from 9 weeks to 20 weeks. These results suggest that the reduced masticatory function affects mandibular growth and development from 2-4 weeks after molar extraction.

Introduction

Malocclusion is known to be caused by both genetic and environmental factors, with environmental factors further including both pre-birth congenital factors and post-birth acquired factors (1). Over half of Japanese people today reportedly suffer from malocclusion (2, 3). According to Ito et al. (4), an investigation of bones from ancient Japanese people revealed that the incidence of malocclusion from prehistoric to historic times was lower, at 22.4%, in the incipient and initial Jomon periods (14,000-4,000 BCE) and 20.0% in the late and final Jomon periods (2,000-300 BCE), but then rose to 45.5% in the Kofun period (250-538 CE), 52.0% in the middle ages, 56.2% in the Edo period (1603-1868), and 80-90% today. Inoue (5) stated that the acquisition of the taste for soft, cooked foods in the diet, which constitutes one acquired factor, resulted in insufficient development of the masticatory organs, causing the size of jawbones to shrink between the prehistoric and contemporary eras. Searle (6) also reported that the maternal diet affected the tooth crown width in baby mice, suggesting that the tooth crown size in humans may have increased due to the shift in dietary contents in each era from nutritionally poor to nutritionally rich foods, and changes in dietary ecology. This may have resulted in an imbalance between tooth size and jaw size, increasing the incidence of malocclusion.

Animal experiments have also shown that the development of the jawbones, masseter muscle, and condylar process is delayed in animals reared on a powdered or liquid diet, but the types of changes that occur during each period remain unclear.
In this study, we used micro-computed tomography (micro-CT) to examine rats that had undergone extraction of the maxillary molars and had been reared on a powdered diet to minimize masticatory movement, and investigated changes in jawbone morphology in the same individuals, with the objective of elucidating the effects of the reduced masticatory function on the jawbone over time.

**Materials and Methods**

**Rats**

Four-week-old Wistar rats (12 males) were purchased from the Sankyo Labo Service and divided into two groups of 6 rats each.

(a) Extraction group: The maxillary molars of 5-week-old rats were all extracted by expansion of the socket with a probe and spoon excavator under general anesthesia with intraperitoneal pentobarbital (30mg/kg). After molar extraction, rats were reared on powdered standard chow (MF; Oriental Yeast Co., Ltd., Tokyo).

(b) Control group: Reared on solid standard chow. (Animal experiment approval number: No. AP09MD030)

**Micro-CT imaging**

The heads of all rats were scanned at 5, 7, 9, 12, 15 and 20 weeks by *in vivo* micro-CT (R_mCT®; Rigaku, Tokyo, Japan) under general anesthesia with intraperitoneal pentobarbital (30mg/kg). Imaging conditions were: tube voltage, 50kV; tube current, 90mA; magnification, 2x; measurement time, 17s; slice thickness, 0.800mm; and slice interval, 0.800mm.

**Body weight measurement over time**

Rats were weighed weekly from 4 weeks to 20 weeks.

**Jawbone measurements**

Micro-CT images were reconstructed in three dimensions and the cranial bones were observed. The maxillary complex and bilateral mandibles were measured using Image J (NIH, USA).

**Measurements (Figure 1):**

(a) Maxillary size (distance between the labial-side tip of the alveolar process between the maxillary incisors and the anterior-most point of the frontonasal suture)

(b) Mandibular size (distance between the labial–side anterior–most point of the alveolar process between the mandibular incisors and the condylar process)

(c) Mandibular ramus length (length of line drawn from the top of the condylar process perpendicularly to the mandibular plane)

(d) Mandibular angle (angle formed between the line joining the posterior–most point of the mandibular angle with the bottom of the condylar process and the mandibular plane)

(e) Mandibular area (area of external surface of the mandible)

(f) Area of mandibular notch (area surrounded by the coronoid process, mandibular notch, and condylar process)

(g) Mandibular thickness (distance between the buccal and lingual sides at the point corresponding to the apex between the first and second molars in coronal section)

Means and standard deviations were calculated for each group.

**Statistical analysis**

Mann-Whitney U-test was used for statistical analysis.

**Results**

**Changes in body weight over time**

Figure 2 shows the changes in body weight from 4 weeks to 20 weeks. There were no significant differences in body weight between the extraction and control groups at any point.

**Jawbone measurements**

Figure 3 shows the three-dimensional reconstructed images at 20 weeks, and Figure 4 shows measurements of the maxillary size, mandibular size, mandibular ramus length, mandibular angle, mandibular area, area of the mandibular notch, and mandibular thickness, and obtained three-dimensional reconstructed images from these measurements.

(a) Maxillary size and (b) Mandibular size

Maxillary and mandibular sizes increased over time in both the extraction and control groups, and there were no significant differences between the groups at 5, 7, 9, 12, 15 or 20 weeks.

(c) Mandibular ramus length and (g) Mandibular thickness

Mandibular ramus length and mandibular thickness increased over time in both the extraction and control groups, but was significantly smaller in the extraction group when compared with the control group from 9 weeks to 20 weeks.

(d) Mandibular angle, (e) Mandibular area and (f) Area of mandibular notch
The mandibular angle, mandibular area and area of the mandibular notch decreased over time in both the extraction and control groups, but were significantly larger in the extraction group when compared with the control group from 7 weeks to 20 weeks: this difference tended to increase over time.

**Discussion**

Normal maxillofacial growth and development are believed to occur if normal occlusion and sufficient masticatory functions are present during the growth period. In pediatric dental practice, however, patients may be encountered with both malocclusion and congenitally missing teeth or early tooth loss due to caries or trauma.
There have been numerous studies of the effects of a reduced masticatory function on jawbone growth, and it has been reported that the jawbones of mice fed soft food are smaller, and that extraction of the maxillary molars of rats reduces the mandibular bone mass (7-9). In this study, we extracted all the maxillary molars of rats and fed them a standard powdered diet to investigate the effects of the marked reduction in the masticatory function at a standard nutritional level on jawbone development at various time points. Rats were selected as experimental animals because, among other advantages, they can be reared under identical conditions. The masticatory function was reduced by extracting the maxillary molars, which can easily be removed in a short time, at the age of 5 weeks, after the third molar had erupted.

In vivo micro-CT was used to investigate the development of the jawbones over time, and rats were scanned under general anesthesia, enabling jawbone measurements to be made in the same individuals.

Body weight measurements exhibited the same tendency to increase in both groups, and no significant differences between the two groups were evident during the experimental period, indicating that there were no differences in nutritional status. This indicates that growth differences in jawbone morphology between the two groups were not the result of nutritional status, but were due to the presence or absence of maxillary molars and a certain type of diet, that is, the activity of the masticatory muscle.

Jawbone measurements showed no significant differences in maxillary size between the two groups. Growth of the maxillary complex depth is dependent on the growth of the base of the skull, and this mainly occurs by synchondrosis, which exhibits a nervous-system type pattern (10,11), meaning it may be less susceptible to the effects of differences in the masticatory function, which constitutes an acquired environmental factor. The effects of the masticatory function on the growth of the neurocranium, i.e., the calvaria and the cranial base, have been investigated in a number of studies by means such as removal of the masticatory muscle (12), molar extraction (13) and variations in food hardness (14-16); all of these studies have found that it has no effect on the neurocranium.

Moore (15) reported that in one-month old rats, the viscerocranium had grown to approximately 75% of its size in mature rats, and the size of the neurocranium had reached 93%, meaning that neurocranial growth was already complete in 5-week-old rats at the start of our experiment. Our study also found no differences in maxillary size between the two groups.

Although there were no significant differences in the mandibular size between the two groups, the mandibular ramus size was significantly smaller in the extraction group from 9 weeks to 20 weeks, and the mandibular angle was significantly larger from 7 weeks to 20 weeks. The mandibular area was also significantly smaller in the extraction group from 7 weeks to 20 weeks, the area of the mandibular notch was significantly larger in the extraction group from 7 weeks to 20 weeks, and the mandibular thickness was significantly smaller in the extraction group from 9 weeks to 20 weeks.

According to Yamada (17), rats fed a solid diet bite their food 2-3 times with the incisors and then chew it with the molars for several seconds, whereas those fed a powdered diet only chew it with the molars for a few seconds, with a clear difference in muscle activity by the masticatory
Fig. 4 Jawbone measurements

(a) Maxillary size
(b) Mandibular size
(c) Mandibular ramus length
(d) Mandibular angle
(e) Mandibular area
(f) Area of mandibular notch
(g) Mandibular thickness
muscle. In this study, all maxillary molars were extracted and rats were also fed a powdered diet; thus, muscle activity would have been even lower than that reported by Yamada (17). Yamada (17) reported that although there were no differences in the mandibular size between developing rats reared on a solid or powdered diet, the mandibular ramus length was significantly smaller in rats fed a powdered diet after 30 days and the mandibular thickness was significantly smaller after 120 days when compared with those of rats fed a solid diet, which was consistent with the results of the present study.

Okamoto et al. (18) bred two strains of mice with different mandibular sizes and found that the large mandible trait was dominant, but that the mandibular ramus length was intermediate, suggesting that genetic factors play a major role in determining mandibular size and that the determinant gene is probably located on mouse chromosome 11 (19). This suggests that genetic factors play a greater role in determining mandibular size, while environmental factors play a greater role in determining mandibular ramus length. In our study, we also found that there were no significant differences in the mandibular size between the two groups, but that the mandibular ramus length was significantly smaller in the extraction group, probably because of the influence of molar extraction and a powdered diet.

Moss et al. (20) proposed that bones are formed by function, with the morphology of the coronoid process, condylar process and mandibular angle in humans being strongly affected by the function of the masticatory muscle in the form of the temporal, masseter, and medial and lateral pterygoid muscles, respectively. Washburn (21), Horowitz et al. (21) and Moss et al. (22) resected the temporal muscle in rats, and reported that this suppressed the growth of the coronoid process, for which function of the temporal muscle is a vital factor. Horowitz et al. (23) and Kanda (24) also reported that resection of the masseter muscle reduced the mandibular ramus length. In this study, all the maxillary molars were extracted and rats were fed a powdered diet, which minimized masticatory movements involving opening and closing movements of the mouth, and the muscle activity of the temporal, masseter, and inner and outer pterygoid muscles would have been greatly reduced when compared with the control group. This may have suppressed the growth of the mandible and the coronoid process, reducing the growth of the mandibular ramus and resulting in a smaller mandibular ramus length.

The mandibular angle also became narrower in rats in the control group as their masticatory muscle activity increased with increasing age, but in the extraction group, the reduction in activity of the masseter muscle, the most important masticatory muscle, meant that the tuberositas masseterica in the mandibular angle, the point of origin of the masseter muscle, occupied a smaller area, resulting in a lower muscle mass. This may have suppressed growth in the mandibular angle region, meaning that as the rats increased in age, it remained at the same obtuse angle as at birth. In the mandibular notch region, activity of the inner pterygoid, outer pterygoid and temporal muscles was also low, which may have resulted in little addition of bone to the mandibular notch and its strongly depressed shape.

Significant differences in the mandibular ramus length, mandibular angle, mandibular area, the area of the mandibular notch, and mandibular thickness between the two groups all became evident from 2 to 4 weeks after extraction. This suggests that the masticatory function affected the growth and development of the mandible at a comparatively early stage. Our results support the theory of Moss et al. (20) that bones are formed by function, and may also be understood as an experimental confirmation of the importance of a normal masticatory function during childhood growth and development.

Conclusions

We used in vivo micro-CT to scan the heads of rats that underwent extraction of all maxillary molars at 5 weeks of age and were reared on a powdered diet. Control rats did not undergo molar extraction and were reared on a solid diet. We then compared the growth of their cranial and maxillofacial morphology. We obtained the following results.

1. There were no significant differences between the two groups with regard to body weight changes up to 20 weeks, with no reduction in the nutritional status despite the reduction in masticatory activity as a result of the extraction of all maxillary molars and feeding on powdered standard chow.
2. There were no differences in maxillary size between the two groups.
3. Although there were no significant differences in mandibular size between the two groups, the mandibular ramus length was significantly smaller in the extraction group from 9 weeks to 20 weeks, and the mandibular angle was significantly larger from 7 weeks to 20 weeks. The
mandibular area was also significantly smaller from 7 weeks to 20 weeks, the area of the mandibular notch was significantly larger from 7 weeks to 20 weeks, and the mandibular thickness was significantly smaller from 9 weeks to 20 weeks.

These results suggest that the masticatory function affected mandibular growth and development at a comparatively early stage.

Acknowledgments

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