

The effect of loss of occlusal support on mandibular morphology in growing rats

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ABSTRACT

Objective: To evaluate the effects of unilateral and bilateral premature loss of posterior occlusal support on mandibular bone dimensions in growing rats.

Materials and Methods: Thirty female Wistar rats (5 weeks old) were randomized into three groups: control, unilateral mandibular molar teeth extraction, and bilateral mandibular molar teeth extraction. After 8 weeks, animals were sacrificed and acrylic rapid-prototyped templates of the mandibles were constructed. Mandibular length, ramus height, intercondylar distance, and body weight were measured and analyzed by one-way analysis of variance (Tukey test as post hoc test; $\alpha = .05$).

Results: Mandibular length and intercondylar distance were significantly shorter in experimental animals, while no difference was observed for ramus height and body weight.

Conclusions: Unilateral and bilateral premature loss of posterior occlusal support in growing rats results in a smaller mandible at skeletal maturity. (*Angle Orthod.* 2012;82:242–246.)

KEY WORDS: Tooth loss; Mandible

INTRODUCTION

The mandibular condyle has been shown to be an important growth center within the facial skeleton.¹ Studies of craniofacial growth reveal that the mandible grows in parallel with the nasomaxillary complex to provide the basis for normal occlusal relationships.¹ Homeostasis of the temporomandibular joint (TMJ) form, function and, occlusal relationships is assured by normal functional demands present during and after natural growth.² However, the boundary separating normal adaptive responses from those resulting in disease is not completely understood.² Animal experiments have shown that condylar growth is highly sensitive to functional factors, which induce changes in

bone metabolism and expression of growth factors and other signaling molecules.³

Loss of occlusal support has been implicated in a wide range of clinical situations. The influence of tooth loss, especially loss of molar support, on the etiology of degenerative changes is a topic of long-standing controversy.⁴ Reduced occlusal support below the normal value, which is 12–14 pairs of contacting teeth in an adult, affects muscle activity, bite force, and jaw movements.⁵ Also, premature tooth loss often leads to space loss, alteration in the proper contact of the inclined planes of the teeth, and disturbance of masticatory function.⁶ Although tooth loss clearly affects masticatory function,^{5–6} the potential relationship between occlusal support and mandibular development remains to be investigated.

Since condylar growth is highly sensitive to functional factors, the purpose of this study was to evaluate the effect of unilateral and bilateral premature loss of posterior occlusal support on mandibular bone dimensions in growing rats. The research hypotheses are that (1) bilateral loss may impair mandibular growth bilaterally, (2) and unilateral loss may lead to mandibular growth impairment to the same side.

MATERIALS AND METHODS

Study Design and Surgical Procedures

The study was reviewed and approved by the institution's Ethics Committee on Animal Experiments.

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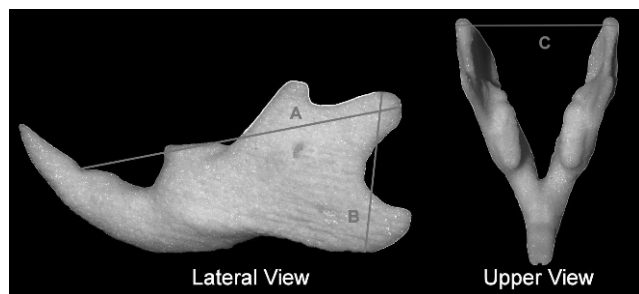


Figure 1. Measurements of anatomical distances. (A) Mandibular length, from the most distal point on the condyle articular surface to the most anterior point on the lingual aspect of the incisor alveolus. (B) Ramus height, from the most superior point on the condyle to the most inferior point on the angular process. (C) Intercondylar distance, as the greatest distance between the lateral surfaces of the condyles.

A sample size of 10 rats per group had been calculated using standard statistical criteria ($\alpha = .05$, $\beta = .20$), yielding a power of 80% for the primary outcome of the study, mandibular length. Thirty female Wistar rats (5 weeks old) (Multidisciplinary Center for Biological Research, State University of Campinas, Campinas, Brazil) were randomized into three groups: i) control; ii) unilateral mandibular molar teeth extraction, left side; and iii) bilateral mandibular molar teeth extraction. Rats were bred and kept under standard conditions, provided with water ad libitum and normal rat pellets in a 12-hour light-dark environment at a constant temperature of 23°C.

All rats were anesthetized by an intramuscular injection (10% ketamine and 2% xylazine, 2:1, 0.1 mL/100 g) before tooth extraction. Rats were positioned on a surgical apparatus designed to keep the mouth open through the use two rubber bands. A Hollembach 3ss was used to make the syndesmotomy, disconnecting the surrounding gingiva of the mandibular molars. Teeth were removed with a curved mosquito forceps, and sockets were closed with 5-0 nylon thread sutures using nontraumatic needles. Control rats underwent a sham operation, which aimed to maintain maximum jaw opening for 10 minutes under anesthesia. To detect signs of malnutrition that could presumably affect growth, animals' body weight was registered at inception and weekly during the study period. All animals were sacrificed with an overdose of sodium pentobarbital (60 mg/kg; intraperitoneal injection) 8 weeks after tooth extraction (13 weeks old), when they had achieved skeletal maturity.⁷

Measurement of Anatomical Distances

Mandibular dimensions were measured as previously described.⁸ Immediately after death, the heads were fixed in 10% paraformaldehyde, and cone beam computed tomography scan images were taken using the Classic I-CAT (Imaging Sciences International,

Hatfield, Pa). The three-dimensional images of rats' skulls were exported in multfile Digital Imaging and Communications in Medicine (DICOM) format, and acrylic rapid-prototyped templates of the mandibles were constructed. Thus, the anatomy of the TMJ and intra-articular tissues was preserved for further investigation at a later moment. The following anatomical distances were measured on both sides of the mandible templates with an electronic digital caliper: mandibular length, from the most distal point on the condyle articular surface to the most anterior point on the incisor alveolus (Figure 1A); ramus height, from the most superior point on the condyle to the most inferior point on the angular process (Figure 1B); and intercondylar distance, as the greatest distance between the lateral surfaces of the condyles (Figure 1C). Measurements were made by two independent observers at an interval of 4 weeks, and the averaged data were used to calculate the distances.

Statistical Analysis

The data were processed with SPSS software (version 17.0 for Windows, SPSS Inc, Chicago, Ill). The measurements of the two independent observers were submitted to the Intra-Class Correlation test. The size of the method error in measuring the anatomical distances was calculated with the Dahlberg formula: $ME = \sqrt{\sum d^2 / 2n}$, where d is the difference between the two registrations of a pair, and n is the number of double registrations.⁹ Ten mandibles were randomly selected for the evaluation of method error. The size of the method error in the measurements and the statistical significance between registrations are shown on Table 1.

Before carrying out the statistics, mandibular measurements were controlled for body size through the division of the linear measurements by the raw body weight. Mandibular length, ramus height, intercondylar distance, and body weight were analyzed by one-way analysis of variance (Tukey test as post hoc test). Shapiro-Wilk and Levene tests were used to observe normality and variance homogeneity, respectively. Confidence level was set at 5%.

RESULTS

The intraclass correlation index (ICC = 0.9996, $P < .0001$) showed excellent reproducibility between the two observers. Table 2 shows the measurements of anatomical distances and body weight. Mandibular length was significantly shorter ($P < .05$) on both sides of the mandible in the unilateral and bilateral extraction groups, but no difference was observed between sides in each group. Intercondylar distance was significantly shorter after unilateral ($P < .001$) and bilateral ($P < .005$)

Table 1. Size of Method Error in the Measurements and Statistical Significance Between Registrations^a

Linear Measurements, mm	ME	Mean (SD) I	Mean (SD) II	Difference <i>P</i>
Mandibular length (right side)	0.14	26.69 (0.56)	26.68 (0.56)	<i>P</i> = .49
Mandibular length (left side)	0.15	26.68 (0.59)	26.68 (0.56)	<i>P</i> = .93
Ramus height (right side)	0.15	11.91 (0.32)	11.91 (0.31)	<i>P</i> = .91
Ramus height (left side)	0.14	11.87 (0.34)	11.88 (0.38)	<i>P</i> = .8
Intercondylar distance	0.16	18.84 (0.37)	18.89 (0.42)	<i>P</i> = .42

^a ME indicates method error in the measurements such that $ME = \sqrt{\sum d^2/2n}$, where *d* is the difference between the two registrations of a pair, and *n* is the number of double registrations; SD, standard deviation; I, registration I; II, registration II.

tooth extraction than in the control group, but no difference was found between them. No significant difference was observed regarding ramus height and body weight.

DISCUSSION

The results of this study support the first research hypothesis that bilateral premature loss of posterior occlusal support may lead to mandibular growth impairment. The experimental animals exhibited a significantly shorter mandibular length and intercondylar distance at skeletal maturity. These results are interesting, since only one previous study¹⁰ had investigated this relationship. In that study,¹⁰ the authors observed no difference in mandibular length 6 months postextraction in hamsters, but found a medial shift of the mandible on the extracted side that resembled the reduced intercondylar distance observed in our study. This disagreement may be related to differences between animal species and length of follow-up period. However, although in our study rats were followed for a shorter period, they were killed when they had already achieved skeletal maturity. Thus, it is unlikely that a catch-up growth behavior would have been observed after that.

Ramus height was not affected by loss of occlusal support. This is in agreement with the results of Yokoyama et al.¹¹ The authors believed that some decrease in ramus height would have occurred after a longer period of observation, but in our study rats were followed for 56 days, in contrast to the 28 days of that study, and no ramus height difference persisted. During mandibular development in humans, the

condyle's upward and backward growth movement regulates the anterior and inferior displacement of the mandible. This movement is necessary to increase mandibular anteroposterior length and craniofacial vertical dimension.¹ Anatomical differences between rats and humans suggest that the vertical growth component is not so prominent in rats, which could explain why ramus height was not affected.

Mechanical modulation of the condylar cartilage has shown that TMJ overloading inhibits condylar growth.³ The development of a smaller mandible may be the result of condyles' reduced growth potential caused by overloading of the TMJ due to loss of posterior occlusal support. In rats, the chewing movement is described as cutting by central incisors and strong grinding by molars. Each dental arch presents two incisors and six molars (three on each side of the arch). Between incisors and molars there is a long toothless space called diastema. Thus, in the unilateral extraction group mastication was predominantly unilateral, whereas in the bilateral group incisal cutting prevailed. Biomechanical studies have shown that these conditions may act as an overloading factor on the TMJs.¹²

Normal development of the mandible and nasomaxillary complex is necessary to provide the basis for healthy occlusal relationships.¹ Based on our findings, we propose that at a certain moment during postnatal growth, occlusion and craniofacial growth become interdependent, working in a two-way mechanism in which stable occlusion is necessary to achieve healthy mandibular development and vice versa. Thus, mandibular growth impairment may also be related to the new muscle balance and altered force vectors established

Table 2. Measurements of Anatomical Distances and Body Weight

	Mandibular Length, mm		Ramus Height, mm		Intercondylar Distance, mm	Body Weight, g
	Right	Left	Right	Left		
Study Group	Mean (SD)	Mean (SD)	Mean (SD)	Mean (SD)	Mean (SD)	Mean (SD)
Control	27.08 (0.4)	27.05 (0.4)	12.03 (0.29)	11.96 (0.27)	19.11 (0.29)	247.7 (13.8)
Unilateral extraction	26.36* (0.72)	26.40* (0.61)	12.15 (0.32)	11.65 (0.31)	18.12* (0.38)	250.6 (19.2)
Bilateral extraction	26.42* (0.41)	26.36* (0.41)	11.88 (0.35)	11.98 (0.22)	18.40* (0.28)	250.9 (13.7)

* Significantly different from control at *P* < .05; equality of means was assessed by one-way analysis of variance with Tukey test as post hoc test. SD indicates standard deviation.

after teeth extraction. It is well known that muscle forces have a strong influence on mandibular growth and morphology. According to Moss's functional matrix theory, it is the investing soft tissues, especially the masticatory muscles, and the forces exerted by them that serve as the primary impetus for craniofacial growth and development.¹³

The second research hypothesis was not supported. Mandibular length was similarly affected on both sides in rats submitted to unilateral tooth extraction. Thus, the belief that a greater increase in mechanical forces takes place on the extracted side and is followed by hypogrowth restrained to the same side¹⁴ was not confirmed. Also, the inhibition of mandibular growth observed in this study was not related to the surgical procedure or the bone metabolism around the extraction site. Our results show that unlike most extremity joints, the left and right TMJs are connected through the mandibular bone in such a way that alterations in one side have effects on the opposite side. This is in agreement with a previous study on the expression of sulfated glycosaminoglycans, which is commonly found in tissues exposed to loading, in which no difference between the extracted and nonextracted side was found.¹⁵ In addition, there was a transient increase in bone metabolism¹¹ and type II collagen¹⁶ on the extracted side, which returned to the normal levels in a few days. We speculate that the transient increase in metabolic activity on the extracted side is part of the adaptation process of the mandibular condyle to changes in functional loading, which is followed by redistribution of functional loads to both TMJs a few days after balance disruption as an initial approach of the masticatory system to sustain the nonphysiological forces.

Rats are a widely accepted model for the study of mandibular growth.^{9,11,14} In this study, the sample was composed solely of female rats because this gender seems more prone to condylar cartilage remodeling due to occlusal alteration.⁹ Rats were followed from 5 up to 13 weeks old, spanning the transition from early puberty (5 weeks) to young adulthood (9 weeks). At 13 weeks old, skeletal maturity has been achieved, and rat bones continue growing at a reduced rate.⁷ Thus, animals were followed during a meaningful period of body development suitable for observation of bone morphologic changes. Animal growth was not compromised by nutrient intake, as evidenced by no significant difference for body weight among the groups. Further, mandibular measurements were controlled for body size before carrying out the statistics.

To summarize, this study showed that premature loss of posterior occlusal support resulted in mandibular growth impairment. Obviously, our results are very limited from a clinical point of view. Although studies

using rodents provide insights into the basic mechanisms of how masticatory function may influence craniofacial growth, anatomic differences in dental morphology, TMJ, and masticatory function between rats and humans make it difficult to extrapolate these findings to patients. It is possible that the same alteration of masticatory function might have a different impact on mandibular growth in species with different masticatory systems. However, this study suggests that posterior occlusal support is an important element for healthy mandibular development, emphasizing the importance of early treatment in normalizing occlusion and creating appropriate conditions for normal occlusal development.

CONCLUSION

- Unilateral and bilateral premature loss of posterior occlusal support in growing rats results in the development of a smaller mandible at skeletal maturity.

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