Original Article

Altered mandibular growth under functional posterior displacement in rats

Arcelino Farias-Neto[a]; Ana Paula Varela Brown Martins[a]; Sidney Raimundo Figueroba[b]; Francisco Carlos Groppo[c]; Solange Maria de Almeida[d]; Célia Marisa Rizzatti-Barbosa[e]

ABSTRACT

Objective: To test the null hypothesis that there is no difference in mandibular growth between growing rats with posterior functional mandibular displacement and growing rats without functional mandibular displacement.

Materials and Methods: Twenty female Wistar rats (5 weeks old) were randomized into two groups: (1) control and (2) mandible posterior displacement in the occluded condition induced by an occlusal guiding appliance. After 8 weeks all animals were sacrificed, cone beam computed tomography scan images of the heads were taken using the classic I-CAT, and acrylic rapid-prototyped templates of the mandibles were constructed. Mandibular length, ramus height, and intercondylar distance were measured. Mandibular length and ramus height were submitted to the two-way analysis of variance, while intercondylar distance was analyzed by nonpaired Student's *t*-test.

Results: Mandibular length was bigger ($P < .0001$) in the control than in the experimental group, but no significant difference was found between the left and right sides ($P = .9380$). No significant differences were observed for ramus height and intercondylar distance.

Conclusions: The results of this study demonstrated that functional posterior displacement of the mandible in growing rats resulted in shorter mandibular length. (*Angle Orthod.* 2012;82:3–7.)

KEY WORDS: Craniofacial growth; Orthopedics; Occlusion

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INTRODUCTION

When the mandible is displaced from its physiologic position, the concomitant condylar displacement in the glenoid fossa may result in abnormal loading of the tissues in and around the joint, affecting the physiologic dynamics of condylar cartilage and expression of growth factors.[1] This feature has been exploited as the biologic basis for a dentofacial orthopedic therapeutic approach in the treatment of patients with maxillomandibular discrepancy.[2] Furthermore, it has been supposed that if an occlusal interference alters mandibular posture (functional malocclusion), the resultant condylar displacement could affect mandibular growth in a similar way to orthopedic appliances.[3]

The proliferating mesenchymal cells in condylar cartilage are the main source of chondrocytes and thus are responsible for condylar growth. In the rat model, mandibular advancement therapy accelerates and enhances condylar growth by accelerating the differentiation of mesenchymal cells into chondrocytes, leading to an earlier formation and increase in the amount of cartilage matrix and collagen type II.[4] A close correlation exists between the amount of collagen and the amount of bone formed in the condyles in response to forward mandibular positioning in growing rats.[5] In contrast, posterior displacement of the mandible demonstrated a decrease in the proliferation of chondrocytes and the amount of extracellular matrix.[6,7] However, the extent to which

[a] Graduate student, Department of Prosthodontics and Periodontology, Piracicaba Dental School, State University of Campinas, Piracicaba, São Paulo, Brazil.

[b] Graduate student, Department of Physiological Sciences, Piracicaba Dental School, State University of Campinas, Piracicaba, São Paulo, Brazil.

[c] Professor, Department of Physiological Sciences, Piracicaba Dental School, State University of Campinas, Piracicaba, São Paulo, Brazil.

[d] Professor, Department of Oral Diagnosis, Piracicaba Dental School, State University of Campinas, Piracicaba, São Paulo, Brazil.

[e] Professor, Department of Prosthodontics and Periodontology, Piracicaba Dental School, State University of Campinas, Piracicaba, São Paulo, Brazil.

Corresponding author: Dr Arcelino Farias-Neto, Piracicaba Dental School, Department of Prosthesis and Periodontology, Av. Limeira 901, Piracicaba, São Paulo, Brazil - CEP: 13414-903 (e-mail: saudeoral@yahoo.com.br)

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this can be achieved and whether it has any clinical significance are topics of long-standing controversy.[2] Due to the design of these experimental studies it is often not clear whether the observed effects are temporary or long lasting, and whether these changes are restricted at the cellular level in condylar cartilage or ultimately influence mandibular length.

Therefore, because functional posterior displacement of the mandible has shown to inhibit proliferative cells on condylar cartilage,[6] the hypothesis tested was that it impairs mandibular growth if induced during the developmental period. The purpose of this study was to evaluate the effect of posterior functional displacement of the mandible on mandibular length in growing rats.

MATERIALS AND METHODS

The study was reviewed and approved by the Ethics Committee on Animal Experiments, University of Campinas, Brazil (1841-1). 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. Twenty female Wistar rats (5 weeks old) were randomized into two groups: (1) control and (2) posterior mandibular displacement. 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 animals were anesthetized by an intramuscular injection (10% ketamine and 2% xylazine, 2:1, 0.1 mL/100 g). To induce posterior displacement of condyles in the occluded condition, an occlusal guiding appliance[6] was attached to the maxillary incisors for 8 weeks (Figure 1). The disto-occlusion was confirmed by visual examination of molar relationship with the rat in occlusion while anesthetized. Control rats underwent a sham operation, which aimed to maintain maximum jaw opening for 10 minutes under anesthesia similar to the rats that received the guiding appliances. To detect signs of malnutrition that could presumably affect growth, the body weight of the animals was registered at inception and weekly during the study period.

All animals were killed with an overdose of sodium pentobarbital (60 mg/kg; intraperitoneal injection) 8 weeks after treatment (13 weeks old), when they had achieved skeletal maturity.[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 multifile Digital Imaging and Communications in Medicine (DICOM) format, and acrylic rapid-prototyped templates of the mandibles were constructed at the Technology of Information Center, Campinas, Brazil. Thus, the anatomy of the temporomandibular joint (TMJ) and the intra-articular tissues were preserved for further investigation. The following anatomic distances were measured on both sides of the mandible templates with an electronic digital caliper (Figure 2): (A) mandibular length, from the most distal point on the condyle articular surface to the most anterior point on the incisor alveolus[9]; (B) ramus height, from the most superior point on the condyle to the most inferior point on the angular process; and (C) intercondylar distance, as the greatest distance between the lateral surfaces of the condyles. Measurements were made by two independent observers at an interval of 4 weeks, and the averaged data were used to calculate the distances. Examiners were blinded to study groups.

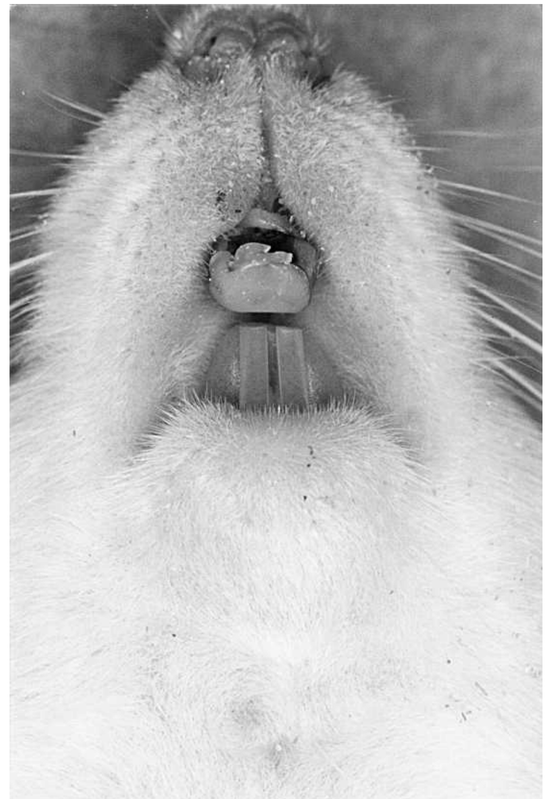

Figure 1. Occlusal guiding appliance attached to the maxillary incisors to induce posterior displacement of the condyles in the occluded condition.

Statistical Analysis

The data were processed with SPSS software (V 11.0 for Windows, SPSS Inc, Chicago, Ill). The measurements of the two independent observers were submitted to the intraclass correlation test. The size of the method error in measuring the anatomic distances was calculated with the Dahlberg formula: ME $= \sqrt{[\sum d^2 / 2n]}$,

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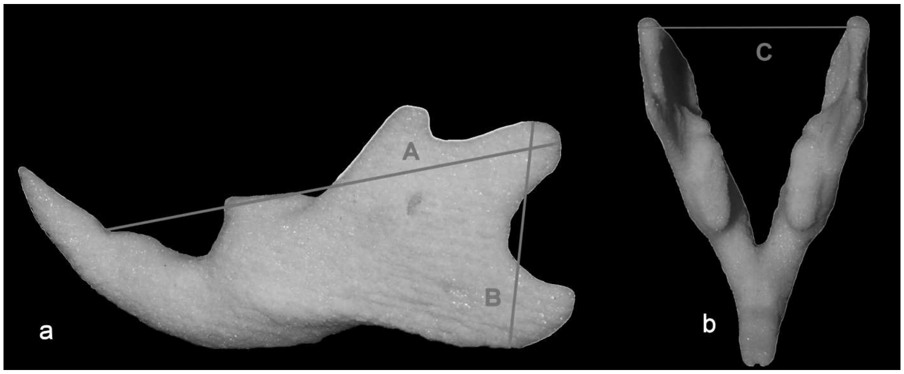

Figure 2. Measurements of mandible templates. **(a)** Lateral view: A, mandibular length, from the most distal point on the condyle articular surface to the most anterior point on the lingual aspect of the incisor alveolus and B, ramus height, from the most superior point on the condyle to the most inferior point on the angular process. **(b)** Upper view: C, intercondylar distance, as the greatest distance between the lateral surfaces of the condyles.

where d is the difference between the two registrations of a pair, and n is the number of double registrations.[10] Ten mandible templates were randomly selected for the evaluation of method error.

Mandibular measurements were controlled for body size before carrying out the statistics through the division of the linear measurements by the raw body weight. The results of mandibular length and ramus height were submitted to the two-way analysis of variance (Tukey test as post-hoc test) considering the sides (left and right) and groups (1 and 2). Intercondylar distance and body weight were analyzed by nonpaired Student's *t*-test. Shapiro-Wilk and Levene tests were used to observe normality and variance homogeneity, respectively.

The size of the method error in the measurements and statistical significance between registrations are shown in Table 1. The 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 anatomic distances and body weight. Mandibular length was bigger ($P <$ .0001) in the control group, but no significant difference was found between the left and right sides ($P =$ .938). No significant difference was observed between groups ($P =$ .0509) nor between sides ($P =$ .734) considering the ramus height. The intercondylar distance did not show significant difference ($P =$ .3069). No significant difference ($P =$ .081) was observed regarding body weight. Functional posterior displacement during the developmental period altered mandibular bone morphology at grown age.

DISCUSSION

Numerous studies have used rats as an experimental model to the study of TMJ condylar cartilage and mandibular growth.[4–6] In the present study, growing female rats were followed from 5 to 13 weeks of age. The experimental period spanned the transition from early puberty (5 weeks old) to young adulthood (9 weeks old). At 13 weeks old skeletal maturity has been achieved, but rat bones still continue to grow, albeit at a reduced rate.[8] Thus, animals were followed during a meaningful period of body development suitable for observation of bone morphologic changes. The sample was composed solely of female rats because this gender seems more prone to condylar cartilage remodeling due to occlusal alteration.[10]

The guiding appliance used to induce functional posterior displacement of the mandible established a disto-occlusion in the rat without changing the vertical dimension, so the animal was able to open and close the mandible normally, while occluding the posterior teeth during mastication.[6] For this reason, it was expected that animal growth would not be compromised

Table 1. Measurement Error (ME), Meand and Standard Deviation of Linear Measurements for Group 1 and 2

Linear Measurements (mm)	ME, mm	Group 1 Mean (SD)	Group 2 Mean (SD)	Difference
Mandibular length – right side	0.14	26.69 (0.56)	26.68 (0.56)	$P =$ .49
Mandibular length – left side	0.15	26.68 (0.59)	26.68 (0.56)	$P =$ .93
Ramus height – right side	0.15	11.91 (0.32)	11.91 (0.31)	$P =$ .91
Ramus height – left side	0.14	11.87 (0.34)	11.88 (0.38)	$P =$ .8
Intercondylar distance	0.16	18.84 (0.37)	18.89 (0.42)	$P =$ .42

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Table 2. Measurements of Anatomic Distances and Body Weight

Study Group	Mandibular Length, mm		Ramus Height, mm		Mean Intercondylar Distance (SD), mm	Mean Body Weight (SD), g
	Mean Right (SD)	Mean Left (SD)	Mean Right (SD)	Mean Left (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)
Experimental	26.30* (0.42)	26.31* (0.47)	11.79 (0.3)	11.79 (0.4)	18.96 (0.33)	237.6 (10.2)

* Significantly different in the same column at $P < .05$.

by nutrient intake, as evidenced by no significant difference for body weight between groups. Further, mandibular measurements were controlled for body size before carrying out the statistics.

While the clinical benefits of functional appliance therapy remain controversial,[2] there is a large body of evidence showing that the condylar growth is highly adaptable to functional factors, which induce changes in bone metabolism and expression of growth factors and other signaling molecules.[1] Studies have been published about molecular markers for condylar growth under mandibular advancement and different diet consistencies,[11] but further investigations for posterior displacement are necessary. A previous experiment has shown that functional posterior displacement of the condyles with this guiding appliance led to a decrease of proliferative cells in condylar cartilage of grown rats after 4 days,[6] but the bone morphology was not analyzed. Although there was some adaptation of cartilaginous cells on day 14, the authors believed that even if the displacement had persisted, the nonphysiologic stress would still occur. In the present study, the appliance was used in growing rats for 8 weeks and resulted in shorter mandibular length on both sides. These results indicate that TMJ loading changes were not restricted at the cellular level, but ultimately influenced a response manifested as the development of a smaller mandible. Indian hedgehog (Ihh) signaling from prehypertrophic chondrocytes has been implicated in the control of chondrocyte maturation by way of feedback control at the articular surfaces.[12] It is the mechanotransduction mediator in condylar cartilage that perceives mechanical strain and converts it into growth.[11] We speculate that the TMJ abnormal loading from posterior displacement negatively influenced Ihh expression, and consequently inhibited cellular proliferation.

In rats, the TMJ exhibits a flat glenoid fossa with no articular eminence. The TMJ is relatively loosely connected to allow condyle movement in a combination of superoinferior, anteroposterior, and mediolateral direction. The chewing movement of rodents is described as cutting by central incisors and strong grinding by molars.[13] Despite anatomic differences between rats and humans, studies using rodents provide insights into the basic mechanisms of mandibular growth and may orient future clinical research. Different therapeutic approaches have been proposed for the treatment of skeletal Class III malocclusions with no consensus about the best moment to begin the orthopedic treatment (infancy or adulthood), or if it should be treated surgically. A systematic review of the effectiveness of early orthopedic treatment in Class III subjects with different orthopedic appliances found only one randomized clinical trial on this outcome.[14] In growing rats, a mandibular retraction force resulted in anteroposterior mandibular growth inhibition, accompanied by less proliferation of chondroblasts and irregularity of bone formation in the condyle after 4 weeks, and no catch up growth behavior after treatment.[15,16] In adult rats, continuous compressive force on the mandibular condylar cartilage decreased the proliferation of chondrocytes and the amount of extracellular matrices after 7 days.[7] In young monkeys, constant retraction force applied to the mandible resulted in growth disturbance and condylar remodeling.[17] Resorption occurred at the posterior surface of the condyle and the posterior wall of the glenoid fossa, while apposition was observed at the anterior surface.

Unlike previous studies,[15–17] no extraoral mechanical force was exerted to retract rat mandible. While the level of external force utilized to reduce mandibular growth is not clear, in this study the guiding appliance attached to the maxillary incisors passively guided mandible posterior displacement under the action of the muscular apparatus during mastication and occlusion. This is in contrast to previous observations that suppression of normal growth was achieved in rabbits subjected to heavy retraction forces, but not to those of mild degree.[18] The resultant growth inhibition provides some basis for further research in the use of intraoral appliances as an alternative in the treatment of anteroposterior mandibular excess at an early stage. Preventive orthopedic treatment avoids the need for surgical correction at the end of growth period. In addition, the use by children and adolescents of extraoral appliances, such as the chin cup, may have psychological consequences and affect social living.

It is hypothesized that the occlusal interference at the physiologic position causes the deviation of the mandibular posture, as mandible may shift to seek a more stable position (maximum intercuspation) in order to evade interference. The resultant altered

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mandibular posture is accompanied with condylar displacement in the glenoid fossa, which may consequently lead to growth disturbance.[3] In this study, the guiding appliance acted as an occlusal interference at the anterior region, shifting mandible to a more retruded position while occluding. Thus, it was possible to evaluate the interaction between dental occlusion and growth as a singular relationship, in contrast to inherent implications of cross-sectional studies and the multifactorial etiology of growth disturbances. The presented results support the hypothesis that condylar position in the glenoid fossa plays a key role for healthy mandibular development.[3]

To summarize, we found that functional posterior displacement of the mandible in growing rats resulted in a response manifested as the development of a smaller mandible. Occlusion played a major role in craniofacial development, highlighting the need of preventive treatment for functional malocclusion. Obviously, the results of this study are very limited from a clinical point of view. There are anatomic differences in dental morphology, TMJ, and masticatory function between rats and humans that make it difficult to extrapolate these findings to patients. Nevertheless, this study revisited an old subject, the manipulation of condylar growth in rats, in a new way where the condyles were repositioned via an appliance not previously used for posterior positioning. The advantage of this system is extraneous force needs not to be applied, nor is any invasive surgery required, simulating a situation more plausible to be found in clinical practice. Furthermore, the study looked for the actual mandibular length, the relevant parameter, rather than the usual and possibly inaccurate surrogate, condylar cartilage activity.

CONCLUSION

- Functional posterior displacement of the mandible in growing rats results in the development of a smaller mandible at a grown age.

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