UNIVERSIDADE ESTADUAL DE CAMPINAS
FACULDADE DE ODONTOLOGIA

ARCELINO FARIAS NETO

INFLUÊNCIA DE ALTERAÇÕES OCLUSAIS NA ARTICULAÇÃO TEMPOROMANDIBULAR E CRESCIMENTO MANDIBULAR: ESTUDO EM MODELO ANIMAL

TESE de DOUTORADO APRESENTADA À FACULDADE DE ODONTOLOGIA DE PIRACICABA DA UNICAMP PARA OBTENÇÃO DO TÍTULO DE DOUTOR EM CLÍNICA ODONTOLÓGICA, NA ÁREA DE: PRÓTESE DENTAL

ORIENTADORA: PROF^{A}. DR^{A}. CÉLIA MARISA RIZZATTI BARBOSA

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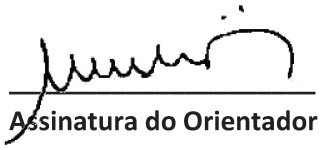

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RESUMO

A cartilagem articular do côndilo mandibular é responsável pelo crescimento ósseo endocondral durante o desenvolvimento mandibular. Ela depende do funcionamento adequado da articulação temporomandibular (ATM) para sua diferenciação e maturação. Trabalhos demonstram que a manipulação funcional da mandíbula foi capaz de alterar a dinâmica fisiológica dessa cartilagem. Nesse sentido, a protrusão diminuiria a ação de cargas sobre o côndilo mandibular, estimulando o crescimento endocondral, e de forma inversa, a retrusão aumentaria a pressão sobre a cartilagem, inibindo o crescimento. Essas técnicas têm sido utilizadas com relativo sucesso na ortopedia facial com o intuito de corrigir discrepâncias maxilo-mandibulares. Entretanto, alguns quadros patológicos presentes nas ATMs podem alterar o seu desenvolvimento normal. Um dos fatores etiológicos que pode ser associado à presença de alterações no côndilo mandibular é a oclusão dental. A hipótese formulada é de que a presença de instabilidade ortopédica causada por um fator oclusal durante a fase de desenvolvimento pode levar à deficiência do crescimento mandibular e alterações intra-articulares. Assim, este trabalho teve por objetivo avaliar, em modelo animal, alterações da oclusão dental sobre o crescimento mandibular e tecidos intra-articulares. O estudo foi randomizado e cego. Foram utilizadas 40 ratas Wistar com 5 semanas de idade divididas aleatoriamente em 4 grupos com o mesmo número de animais: controle, com interferência oclusal, com ausência dos molares inferiores unilateral e com ausência dos molares inferiores bilateral. Os animais foram acompanhados por 8 semanas, período que correspondeu a sua fase de maturação óssea. Após esse período, os animais foram sacrificados e realizou-se tomografia

computadorizada de feixe cônico (Cone beam) de suas cabeças para construção de protótipos de biomodelos, sobre os quais foram mensurados o comprimento da mandíbula, a altura do ramo mandibular e distância intercondilar. Em seguida, as articulações temporomandibulares foram cuidadosamente preparadas para análise imunohistoquímica dos níveis de colágeno tipo II, Fator de Crescimento Endotelial Vascular, e Interleucina 1β na cartilagem condilar. Os dados foram submetidos a análise estatística através do Software SPSS versão 17.0. As médias entre os grupos foram comparadas através do One-way Anova, enquanto as diferenças entre os lados da mandíbula foram avaliadas através do teste *t* de Student (α=0.05). A partir da análise dos resultados, observou-se que alterações oclusais podem afetar o desenvolvimento do osso mandibular, bem como alterar a expressão de Colágeno tipo II, Fator de Crescimento Endotelial Vascular e Interleucina 1β na cartilagem condilar. Diante do exposto, conclui-se que a oclusão dentária é capaz de interferir na dinâmica dos tecidos intra-articulares, sendo um fator importante durante o desenvolvimento craniofacial.

Palavras-chave: Articulação temporomandibular, Cartilagem condilar, Desenvolvimento mandibular, Oclusão.

ABSTRACT

The condylar cartilage regulates the endochondral ossification during mandibular development. Mechanical stimulus in the temporomandibular joint (TMJ) plays an important role in cell proliferation and differentiation of mandibular condyle. Studies have shown that functional mandibular displacement can affect TMJ cartilage dynamics. Mandibular advancement induces profound metabolic changes in the condyle and enhances growth. In contrast, mandibular retraction reduces growth. The overall picture emerging from the data is that unloading of the condyle increases growth, while loading reduces it. Therefore, dental occlusion could be one of the factors associated with the alteration of the TMJ growth. The hypothesis is that orthopedic instability caused by occlusal factors present during TMJ development can affect mandibular growth and intra-articular tissue. Thus, the purpose of this study was to evaluate the influence of dental occlusion on mandibular growth and intra-articular tissue in Wistar rats. The study was randomized and blinded. Forty 5 weeks old female Wistar rats composed the sample. The animals were randomly allocated to four groups with the same number of rats: (1) control, (2) occlusal appliance for functional posterior displacement of the mandible, (3) unilateral mandibular tooth extraction, (4) bilateral mandibular tooth extraction. The rats were sacrificed after 8 weeks, when they had achieved skeletal maturity. Immediately after death, the heads were fixed in 10% paraformaldehyde, and cone beam CT scan images were taken using the Classic I-CAT (Imaging Sciences International, Hatfield, PA, USA). The 3-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 for measurement of mandibular growth. Immunostaining was used for the detection of type II collagen, vascular endothelial growth factor (VEGF) and interleukin-1β. The data were processed with SPSS software (V 17.0 for Windows, SPSS Inc, Chicago, IL, USA). Differences among the groups were analyzed by one-way ANOVA (Tukey test as post-hoc test), while differences between sides were analyzed by non-paired Student's *t* test. Shapiro-Wilk and Levene tests were used to observe normality and variance homogeneity, respectively. Confidence level was set at 5%. The results of this study showed that dental occlusion is an important factor for the integrity of intra-articular tissues and to the healthy craniofacial development, emphasizing the importance of early treatment to normalize occlusion and create appropriate conditions for normal craniofacial development.

Key Words: Condylar cartilage, Mandibular development, Occlusion, Temporomandibular joint.

SUMÁRIO

INTRODUÇÃO

O côndilo mandibular é um dos principais locais de desenvolvimento ósseo dentro do esqueleto facial. Sua superfície encontra-se recoberta pela cartilagem condilar, a qual é responsável pelo crescimento ósseo endocondral durante o desenvolvimento mandibular e dependente do funcionamento adequado da articulação temporomandibular (ATM) para sua diferenciação e maturação. Ele provê um desenvolvimento local adaptativo de considerável significância clínica, visto que seu crescimento em direção póstero-superior regula o movimento ântero-inferior da mandíbula como um todo (Enlow e Hans, 1996). Conseqüentemente, o crescimento condilar deficiente pode resultar em assimetria facial ou retrognatia, com complicações diretas na aparência facial e função oclusal, como desvio da linha média e mordida cruzada. As principais causas de alteração do crescimento mandibular são as alterações degenerativas ou patológicas e o trauma de face que acometem as ATMs durante a infância e adolescência (Skolnick et al., 1994; Kambylafkas, 2005; Pirttiniemi et al., 2009).

Trabalhos que demonstram que a manipulação funcional da mandíbula é capaz de alterar a dinâmica fisiológica dessa cartilagem (Rabie et al., 2003; Cholasueksa et al., 2004; Rabie et al, 2004). Nesse sentido, a protrusão diminuiria a ação de cargas sobre o côndilo, estimulando o crescimento (Rabie et al., 2003), e de forma inversa, a retrusão aumentaria a pressão sobre o côndilo, inibindo o crescimento (Cholasueksa et al., 2004; Rabie et al, 2004)). Essas técnicas têm sido utilizadas com relativo sucesso na ortopedia facial com o intuito de se corrigir discrepâncias maxilo-mandibulares (Meikle, 2007). Além disso, o conhecimento atual também nos permite estabelecer que um fator oclusal que cause

transmissão de cargas não-fisiológicas à ATM devido ao posicionamento instável do côndilo na fossa pode alterar sua dinâmica funcional com reações histológicas evidentes (Von den Hoff JW e Delatte, 2008). Entretanto, devido ao curto tempo de observação e à idade dos espécimes empregados na maioria dos experimentos realizados, o seguinte questionamento permanece sem resposta:

- Uma alteração oclusal que ocorra durante o período de crescimento e afete o posicionamento estável do côndilo na fossa é capaz de afetar o desenvolvimento saudável do osso mandibular?

A hipótese formulada é de que a presença de instabilidade ortopédica causada por um fator oclusal durante a fase de desenvolvimento pode levar à deficiência do crescimento ósseo mandibular e alteração dos tecidos intra-articulares. De fato, se for confirmada a hipótese do presente estudo, que propõe um modelo animal de alteração oclusal plausível de ser verificado em humanos, ficará demonstrado que fatores oclusais influenciam nas alterações das ATM, o que poderá fornecer um respaldo ético e clínico a essa questão na medida em que se assume que, uma vez removida a alteração oclusal, tais problemas poderão ser prevenidos ou solucionados.

A apresentação deste trabalho foi no formato alternativo de tese de doutorado, de acordo com as normas estabelecidas pela deliberação 002/06 da Comissão Central de Pós-Graduação da Universidade Estadual de Campinas.

O artigo correspondente ao Capítulo 1 encontra-se aceito para publicação no periódico *The Angle Orthodontist*, conforme documento relacionado no Anexo 1. Os artigos correspondentes aos Capítulos 2 e 3 foram submetidos para publicação nos periódicos *The Angle Orthodontist* (anexo 2) e *American Journal of Orthodontics and Dentofacial Orthopedics* (anexo 3), respectivamente. O trabalho foi aprovado pelo Comitê de Ética em Experimentação Animal da Universidade Estadual de Campinas (Anexo 4).

CAPÍTULO 1

Altered mandibular growth under functional posterior displacement in rats

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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 fictional mandibular displacement.

Materials and Methods: Twenty female Wistar rats (5 weeks old) were randomized into two groups: (1) control, (2) mandible posterior displacement in the occluded condition induced by an occlusal guiding appliance. After 8 weeks all animals were sacrificed, cone beam CT 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 intercondilar distance were measured. Mandibular length and ramus height were submitted to the two-way ANOVA, while intercondilar distance was analyzed by non-paired Student's t test.

Results: Mandibular length was bigger ($P < .0001$) in control than experimental group, but no significant difference was found between left and right sides ($P = 0.9380$). No significant differences were observed for ramus height and intercondilar distance.

Conclusions: The results of this study demonstrated that functional posterior displacement of the mandible in growing rats resulted in shorter mandibular length.

Key words: Craniofacial growth; Orthopedics; Occlusion.

Introduction

When the mandible is displaced from its physiological 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 dentofacial orthopedic therapeutic approach in the treatment of patients with maxilo-mandibular 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 responsible for condylar growth. In 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 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 mandible posterior functional displacement 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 ten rats per group had been calculated using standard statistical criteria (α=.05, β=.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, (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 23ºC. 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 eight weeks (Figure 1).

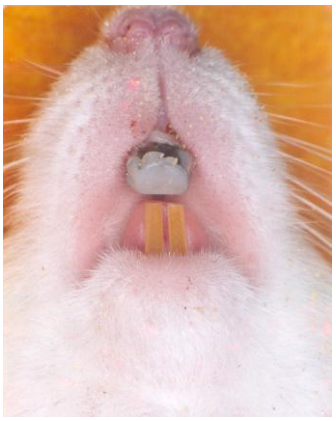

Figure 1. Occlusal guiding appliance attached to the maxillary incisors to induce posterior displacement of the condyles in the occluded condition.

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 min under anesthesia similarly to the rats that received the guiding appliances. 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 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 CT scan images were taken using the Classic I-CAT (Imaging Sciences International, Hatfield, PA, USA). The 3-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 TMJ and the intra-articular tissues were preserved for further investigation. The following anatomical 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; (C) intercondilar distance, as the greatest distance between the lateral surfaces of the condyles. Measurements were made by two independent observers at an interval of four weeks, and the averaged data were used to calculate the distances. Examiners were blinded to study groups.

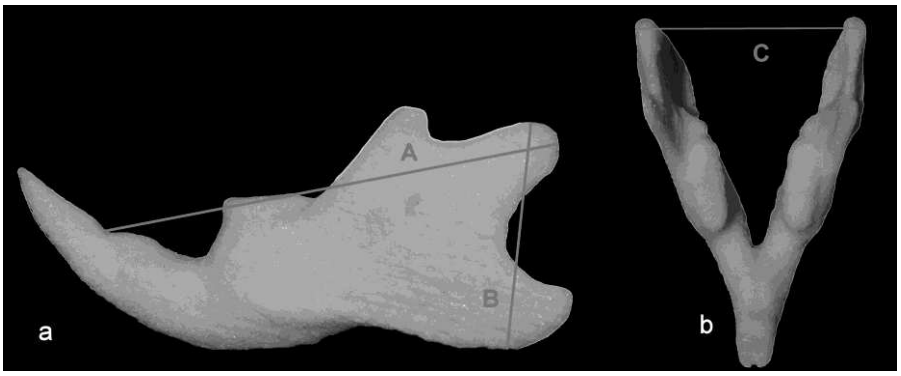

Figure 2. Measurements of mandible templates. (a) Lateral view: A, mandibular length, from the most distal point on the condyle articular surface to the most anterior point on the lingual aspect of the incisor alveolus; B, ramus height, from the most superior point on the condyle to the most inferior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

Statistical analysis

The data were processed with SPSS software (V 11.0 for Windows, SPSS Inc, Chicago, IL, USA). 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's 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.[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 ANOVA (Tukey test as post-hoc test) considering the sides (left and right) and groups (1 and 2). Intercondilar distance and body weight were analyzed by non-paired 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 on Table 1. Confidence level was set at 5%.

Table 1. Size of method error in the measurements (ME) and statistical significance between registrations. Mean (M), standard-deviation (SD), registrations (I and II).

Linear measurements (mm)	*ME*	*M (SD) I*	*M (SD) II*	*Difference P*
Mandibular length – right side	0.14	26.69 (0.56)	26.68 (0.56)	P = 0.49
Mandibular length – left side	0.15	26.68 (0.59)	26.68 (0.56)	P = 0.93
Ramus height – right side	0.15	11.91 (0.32)	11.91 (0.31)	P = 0.91
Ramus height – left side	0.14	11.87 (0.34)	11.88 (0.38)	P = 0.8
Intercondilar distance	0.16	18.84 (0.37)	18.89 (0.42)	P = 0.42

Results

The intra-class 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 bigger ($P < .0001$) in control group, but no significant difference was found between left and right sides (P = 0.938). No significant difference was observed between groups (P = 0.0509) nor between sides (P = 0.734) considering the ramus height. The intercondilar distance did not show significant difference (P = 0.3069). No significant difference (P = 0.081) was observed regarding body weight. Functional posterior displacement during developmental period altered mandibular bone morphology at grown age.

Table 2. Measurements of anatomical distances (mm) and body weight (g). Mean (M), standard-deviation (SD).

	Mandibular length		*Ramus height*		*Intercondilar distance*	*Body weight*
	Right	*Left*	*Right*	*Left*		
Study group	*M (SD)*	*M (SD)*	*M (SD)*	*M (SD)*	*M (SD)*	*M (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	26.3* (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 < 0.05.

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 up to 13 weeks old. 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 morphological changes. The sample was composed solely by 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 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 nonphysiological 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 anatomical differences between rats an 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, mandibular retractive force resulted in anteroposterior mandibular growth inhibition, accompanied by less proliferation of chondroblasts and irregularity of bone formation in the condyle after four 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 extra-oral 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 provide 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 extra-oral appliances, such as the chin cup, may have psychological consequences and affect social living.

It is hypothesized that the occlusal interference at the physiological 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 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 where 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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CAPÍTULO 2

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

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Abstract

Objective: The aim of this study was to evaluate the effect unilateral and bilateral premature loss of posterior occlusal support on mandibular bone dimensions in growing rats.

Materials and Methods: Thirty female Wistar rats (5 wk old) were randomized into three groups: control, unilateral mandibular molar teeth extraction, and bilateral mandibular molar teeth extraction. After 8 wk, animals were sacrificed and acrylic rapid-prototyped templates of the mandibles were constructed. Mandibular length, ramus height, intercondilar distance and body weight were measured and analyzed by one-way ANOVA (Tukey test as post-hoc test), while differences between sides were analyzed by non-paired Student's t test (α=.05).

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

Conclusions: The results of this study demonstrated that unilateral and bilateral premature loss of posterior occlusal support in growing rats resulted in a smaller mandible at grown age.

Key words: Tooth Loss, Craniofacial Growth, Mechanical Loading.

Introduction

Traditionally, the mandibular condyle has been held as an important growth center within the facial skeleton.[1] Studies of the craniofacial growth revealed that the mandible grows in parallel with the nasomaxillary complex to provide the basis for normal occlusal relationships.[1] Homeostasis of 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,[3] the purpose of this study was to evaluate the effect of 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, and (2) unilateral loss may lead to mandibular growth impairment restrained to the same side.

Materials and methods

Study design and surgical procedures

The study was reviewed and approved by the Ethics Committee on Animal Experiments, University of Campinas, Brazil (# 1841-1). A sample size of ten rats per group had been calculated using standard statistical criteria (α=.05, β=.20), yielding a power of 80% for the primary outcome of the study, mandibular length. Thirty female Wistar rats (5 wk old) were randomized into three groups: control, unilateral mandibular molar teeth extraction – left side, 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 23ºC.

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 mouth opened through the use of two rubber bands. Hollemback 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 non-traumatic needles. Control rats underwent a sham operation, which aimed to maintain maximum jaw opening for 10 min 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 wk after tooth extraction (13 wk old), when they had achieved skeletal maturity.[7]

Measurement of anatomical distances

Immediately after death, the heads were fixed in 10% paraformaldehyde, and cone beam CT scan images were taken using the Classic I-CAT (Imaging Sciences International, Hatfield, PA, USA). The 3-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 TMJ and intra-articular tissues were preserved for further investigation at a later moment. The following anatomical distances were measured on both sides of the mandible as shown in Figure 1: (A) mandibular length, from the most distal point on the condyle articular surface to the most anterior point on the incisor alveolus (11); (B) ramus height, from the most superior point on the condyle to the most inferior point on the angular process; (C) intercondilar distance, as the greatest distance between the lateral surfaces of the condyles. Measurements were made by two independent observers at an interval of four weeks, and the averaged data were used to calculate the distances.

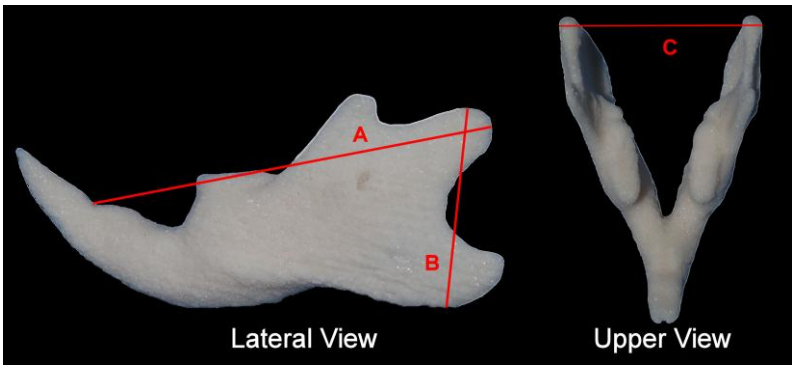

Figure 1. Measurements of mandible templates. (a) Lateral view: A, mandibular length, from the most distal point on the condyle articular surface to the most anterior point on the incisor alveolus; B, ramus height, from the most superior point on the condyle to the most inferior point on the angular process. (b) Upper view: C, intercondilar distance, as the greatest distance between the lateral surfaces of the condyles.

Statistical analysis

The data were processed with SPSS software (V 17.0 for Windows, SPSS Inc, Chicago, IL, USA). 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's 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.[8] Ten mandibles were randomly selected for the evaluation of method error. The size of the method error in the measurements and statistical significance between registrations are shown on Table 1.

Table 1. Size of method error in the measurements (ME) and statistical significance between registrations. Mean (M), standard-deviation (SD), registrations (I and II).

Linear measurements (mm)	*ME*	*M (SD) I*	*M (SD) II*	*Difference P*
Mandibular length – right side	0.14	26.69 (0.56)	26.68 (0.56)	P = 0.49
Mandibular length – left side	0.15	26.68 (0.59)	26.68 (0.56)	P = 0.93
Ramus height – right side	0.15	11.91 (0.32)	11.91 (0.31)	P = 0.91
Ramus height – left side	0.14	11.87 (0.34)	11.88 (0.38)	P = 0.8
Intercondilar distance	0.16	18.84 (0.37)	18.89 (0.42)	P = 0.42

Mandibular measurements were controlled for body size before carrying out the statistics through the division of the linear measurements by the raw body weight. Mandibular length, ramus height, intercondilar distance and body weight were analyzed by one-way ANOVA (Tukey test as post-hoc test), while differences between sides were analyzed by non-paired Student's *t* test. Shapiro-Wilk and Levene tests were used to observe normality and variance homogeneity, respectively. Confidence level was set at 5%.

Results

The intra-class correlation index (ICC = 0.9996, P < 0.0001) showed excellent reproducibility between the two observers. Table 2 shows the measurements of anatomical distances and body weight. Mandibular length was significantly shorter (P<0.05) on both sides of the mandible in the unilateral and bilateral extraction groups, but no difference was observed between sides in each group. Intercondilar distance was significantly shorter after unilateral (P<0.001) and bilateral (P<0.005) 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.

Table 2. Measurements of anatomical distances (mm) and body weight (g). Mean (M), standard-deviation (SD).

	Mandibular length		*Ramus height*		*Intercondilar distance*	*Body weight*
	Right	*Left*	*Right*	*Left*		
Study group	*M (SD)*	*M (SD)*	*M (SD)*	*M (SD)*	*M (SD)*	*M (SD)*
Control	27.08	27.05	12.03	11.96	19.11	247.7
	(0.4)	(0.4)	(0.29)	(0.27)	(0.29)	(13.8)
Unilateral ext.	26.36*	26.40*	12.15	11.65	18.12*	250.6
	(0.72)	(0.61)	(0.32)	(0.31)	(0.38)	(19.2)
Bilateral ext.	26.42*	26.36*	11.88	11.98	18.40*	250.9
	(0.41)	(0.41)	(0.35)	(0.22)	(0.28)	(13.7)

* Significantly different from control at $P < 0.05$.

Discussion

Rats are a widely accepted model to the study of mandibular growth.[8-10] In this study, the sample was composed solely by female rats because this gender seems more prone to condylar cartilage remodeling due to occlusal alteration.[8] Rats were followed from 5 up to 13 wk old, spanning the transition from early puberty (5 wk old) to young adulthood (9 wk old).[20] At 13 wk 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.

The results of this study support the research hypothesis that premature loss of posterior occlusal support may lead to mandibular growth impairment. Experimental animals exhibited significantly shorter mandibular length and intercondilar distance at grown age. These results are interestingly new, since only one previous study[11] had investigated this relationship. In that study,[11] the authors observed no difference in mandibular length 6 months post-extraction in hamsters, but found a medial shift of the mandible on the extracted side which resembled the reduced intercondilar 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 hard to believe 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 Yokohama et al.[9] 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 28 days of that study and no ramus height difference persisted. During mandibular development in humans, 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 6 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; while in the bilateral group incisal cutting prevailed. Biomechanical studies have shown that these conditions act as overloading factors to the TMJs.[12]

Normal development of the mandible and the nasomaxillary complex are necessary to provide the basis for healthy occlusal relationships.[1] Based on our findings, we suppose that at a certain moment during post-natal growth, occlusion and craniofacial growth become interdependent, working in a two-way mechanism where stable occlusion is necessary to achieve healthy mandibular development and vice-versa. Thus, mandibular growth impairment may be also related to the new muscle balance and altered force vectors established after teeth extraction. It is well known that muscle forces have strong influence on mandibular growth and morphology. According to Moss' 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]

Mandibular length was similarly affected on both sides in rats submitted to unilateral tooth extraction. Thus, the inhibition of mandibular growth observed in this study was not related to the surgical procedure nor bone metabolism around the extraction site.

Also, the belief that a greater increase in mechanical forces takes place on the extracted side and is followed by hypogrowth restrained to the same side[10] was not confirmed. Our results showed 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, where no difference between extracted and non-extracted side was found.[14]

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, 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. It is possible that the same alteration of masticatory function might have a different impact on craniofacial 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 to normalize occlusion and create appropriate conditions for normal occlusal development.

Conclusion

Unilateral and bilateral premature loss of posterior occlusal support in growing rats resulted in the development of a smaller mandible at grown age.

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Disclosure of financial conflicts of interest

All authors have no conflicts of interest. The authors have full control of all primary data and they agree to allow the journal to review their data if requested.

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CAPÍTULO 3

The effect of posterior tooth loss on the expression of type II collagen, IL-1β and VEGF in the condylar cartilage of growing rats.

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Abstract

Purpose**:** since loss of posterior teeth has been implicated in the etiology of degenerative changes, this study evaluated the effect of unilateral and bilateral loss of posterior occlusal support on the expression of type II collagen, interleukin-1β and VEGF in the condylar cartilage of rats.

Materials and methods: thirty female Wistar rats (5 weeks old) were randomized into three groups: (1) control, (2) unilateral mandibular molar teeth extraction – left side, (3) bilateral mandibular molar teeth extraction. All animals were sacrificed 8 weeks after tooth extraction and the temporomandibular joints were prepared for immunohistochemical analysis. Immunostaining was used for the detection of type II collagen, IL-1β and VEGF in the condylar cartilage.

Results: abnormal TMJ loading due to bilateral loss of posterior occlusal support resulted in increased expression of IL-1β ($P < 0.01$) and VEGF ($P < 0.01$). The expression pattern of the investigated proteins was different when loss of occlusal support was unilateral, including differences between functional and non-functional sides. Experimental animals exhibited significantly increased expression of IL-1β ($P < 0.05$) and type II collagen ($P < 0.01$) on the functional side when compared to controls. The expression of VEGF was higher on the non-functional side than the functional side ($P < 0.01$) when comparing the differences between sides.

Conclusion: abnormal TMJ loading due to loss of posterior occlusal support alters the expression of type II collagen, IL-1β and VEGF in the condylar cartilage of rats. The expression pattern of these proteins is different when loss of occlusal support is bilateral or unilateral, including differences between functional and non-functional sides.

Introduction

Theoretical models of degenerative temporomandibular joint (TMJ) disease predict that mechanical overloading is the major direct cause of condylar cartilage breakdown[1]. Biomechanical factors such as loss of posterior teeth and unilateral chewing have been implicated in the etiology of degenerative TMJ disease through absolute or relative overloading of joint structures[2]. However, this assumption is usually based on autopsy and skull studies where ageing was a confounding factor, since tooth loss and signs of osteoarthritis increase with age. Recently, examination of contemporary human skull material reported an association of tooth loss and degenerative changes in females at higher ages only[3].

Type II collagen is the main type of collagen that forms the framework of the cartilage matrix in the adult condyle[4]. The load-bearing functions of cartilage are mainly provided by the viscoelastic property of collagen fiber network and the osmotic pressure due to the presence of proteoglycans[4]. Degenerative changes are characterized by progressive degradation of the cartilage matrix and progressive loss of mechanical properties[5]. Interleukin 1β (IL-1β) reduces matrix production, diminishes chondrocyte proliferation, and stimulates the chondrocytes to release proteases responsible for cartilage degradation such as matrix metalloproteinases[6]. Vascular endothelial growth factor (VEGF) also regulates the production of matrix metalloproteinases and its tissue-inhibitors[7]. As degenerative changes progress, it is expected a decreased expression of type II collagen in the condylar cartilage due to matrix degradation, as shown in two studies of surgically-created disc displacement in rabbits[8,9]. Interestingly, unilateral extraction of teeth led to

higher levels of type II collagen, and differences between extracted and non-extracted sides were not clear[10]. Also, it has not been investigated if bilateral tooth extraction affects the expression of type II collagen in the same way as unilateral extraction.

Since loss of posterior teeth may lead to degenerative changes[2], the purpose of this study was to evaluate the effect of unilateral and bilateral loss of posterior occlusal support on the expression of type II collagen, IL-1β and VEGF in the condylar cartilage of growing rats. The research hypothesis is that abnormal functional loading of the TMJ due to loss of posterior occlusal support may alter the expression of the investigated proteins. Also, it is hypothesized that protein expression may differ between bilateral and unilateral loss, including differences between extracted (non-functional) and non-extracted (functional) sides.

Materials and methods

Study design and surgical procedures

The study was reviewed and approved by the Ethics Committee on Animal Experiments, University of Campinas, Brazil (# 1841-1). Thirty female Wistar rats (5 weeks old) were randomized into three groups: (1) control, (2) unilateral mandibular molar teeth extraction – left side, (3) 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 23ºC. All animals 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

mouth opened through the use two rubber bands. Hollemback 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 non-traumatic needles. Control rats underwent a sham operation, which aimed to maintain maximum jaw opening for 10 min 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).

Tissue preparation

The right TMJ of all groups and the left TMJ of the unilateral extraction group were prepared for immunohistochemical analysis. Immediately after death, the heads were fixed in 10% paraformaldehyde for 3 days, and then decalcified in 10% EDTA (Ethylenediamine Tetraacetic Acid) for 30 days. After that, the heads were carefully dissected along the middle sagittal plane into two halves and tissues were removed until the areas surrounding the temporomandibular condyle were exposed. Any excess tissues were removed and specimens were embedded in paraffin with the ramus parallel to the surface of the block. Serial sections of 5 μm were cut through the TMJ at the parasagittal plane using a rotary microtome (Leica RM 2155) and mounted on TESPA-coated glass slides. Sections were left to dry.

Immunohistochemistry

Sections were submerged in 3% H_2O_2 for 10 minutes to block endogenous peroxidase activity. After washing, sections were incubated with Proteinase K (10ug/ml, Sigma, Missouri, USA) for 30 minutes at 37°C for protease digestion. Sections were then washed and incubated in normal blocking serum (sc-2023, Santa Cruz Biotechnology, California, USA) for 30 minutes, followed by incubation with primary goat anti-IL-1β antibody (M-20, Santa Cruz Biotechnology), anti-type II collagen antibody (C-19, Santa Cruz Biotechnology) or anti-VEGF antibody (A-20, Santa Cruz Biotechnology), overnight under 4°C. After washing, sections were incubated with biotinylated secondary antibody (sc-2023, Santa Cruz Biotechnology) for 30 minutes at 37°C, followed again by washing. AB enzyme reagent (sc-2023, Santa Cruz Biotechnology) was applied for one hour at 37°C and washed with 1x TBS plus 0.1% tween-20 before dipping in 3,3-Diaminobenzidine Tetrahydrochloride (DAB, Sigma) for five minutes to identify the binding sites. Brown staining indicated positive binding. Sections were then counterstained with Mayer Haematoxylin for background staining. In order to evaluate for non-specific binding, substitution of the primary antibody with blocking serum (sc-2023, Santa Cruz Biotechnology) was performed as negative control.

Quantitative analysis

Brown staining that localizes the expression of type II collagen, IL-1β and VEGF was quantified at a magnification of X180 via a true-color red-green-blue (RGB) computer-assisted image analyzing system with a digital camera (Leica DC 300 V 2.0) and Leica

Qwin (Version 2.4) software. The amount of area was quantified within a fixed measurement frame of 1044 x 766 pixels. The middle one-third of the mandibular condylar cartilage was selected for analysis[10]. Measurements were made by the same blinded investigator while viewing both the immunostaining of interest and the corresponding negative control.

Statistical analysis

The data were processed with SPSS software (V 17.0 for Windows, SPSS Inc, Chicago, IL, USA). Statistical significance of differences among groups was determined by one-way ANOVA (Tukey test as post-hoc test), while differences between functional and non-functional sides in the unilateral extraction group were analyzed by Student's t test. Shapiro-Wilk and Levene tests were used to observe normality and variance homogeneity, respectively.

Results

Neither postoperative complications nor behavioral changes were observed. The rats returned rapidly to their normal diet and showed no loss of weight during the experimentation. No brown staining was found in any of the negative control sections. Thus, all brown color in test sections was interpreted as specific antibody binding. Expression of type II collagen, IL-1β and VEGF are show on Figure 1.

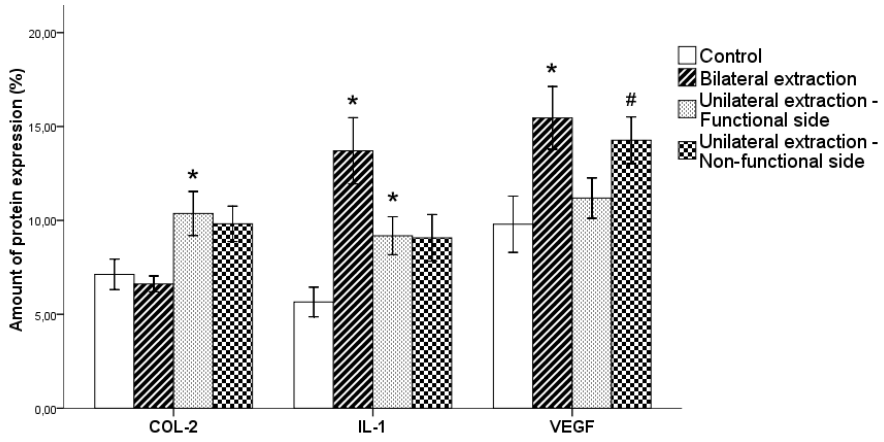

Figure 1. Expression of type II collagen, IL-1β and VEGF in the condylar cartilage of rats eight weeks after teeth extraction. *Significantly different from control. #Significantly different from the opposite side. P < .05.

Bilateral extraction

Abnormal TMJ loading due to bilateral loss of posterior occlusal support resulted in increased expression of IL-1β (P < 0.01) (Figure 2) and VEGF (P < 0.01) (Figure 3).

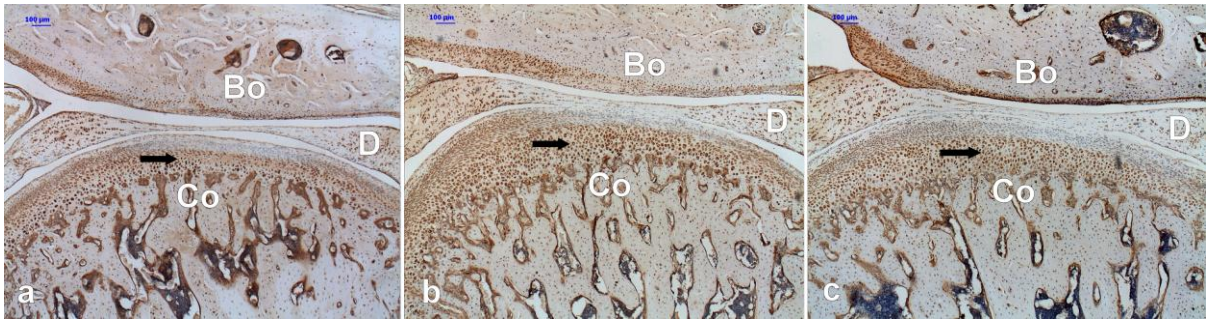

Figure 2. Brown staining indicates the increased expression of IL-1β in the condylar cartilage after bilateral (B) and unilateral teeth extraction (C) when compared to control (A). Co, condyle; Bo, bone; D, articular disc.

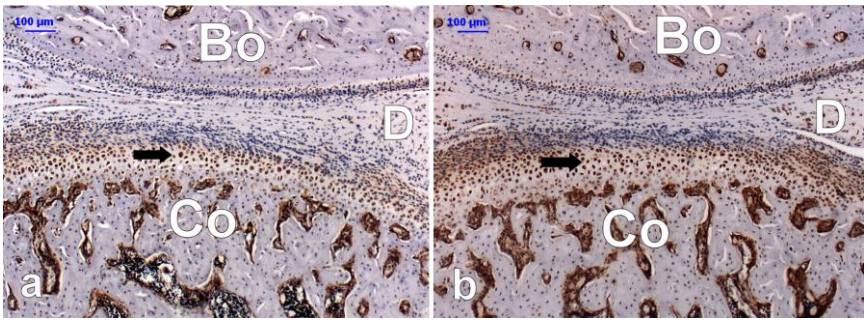

Figure 3. After bilateral extraction (B), observe the higher expression of VEGF and the increased thickness of the mature layer in condylar cartilage (arrow) when compared to control (A). Co, condyle.

Unilateral extraction

The expression pattern of the investigated proteins after unilateral loss of occlusal support differed from that of bilateral loss, including differences between functional and non-functional sides. Experimental animals exhibited significantly increased expression of IL-1β ($P < 0.05$) (Figure 2) and type II collagen ($P < 0.01$) (Figure 4) on the functional side when compared to controls. The expression of VEGF was higher on the non-functional side than on the functional side ($P < 0.01$) when comparing the differences between sides.

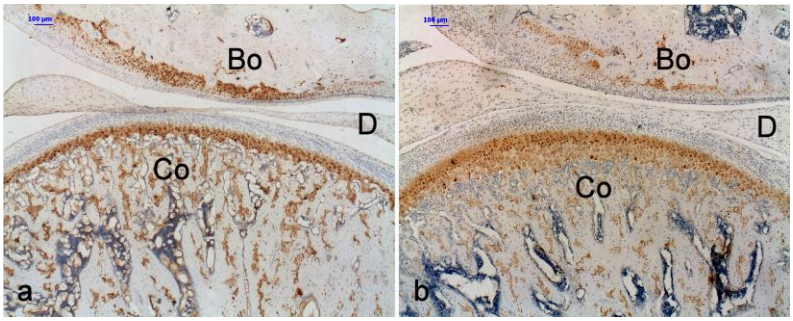

Figure 4. Brown staining indicates the increased expression of type II collagen in the condylar cartilage after unilateral teeth extraction (B) when compared to control (A). Co, condyle; Bo, bone; D, articular disc.

Discussion

The results of this study support the research hypothesis that abnormal functional loading of the TMJ due to loss of posterior occlusal support affects the expression of type II collagen, IL-1β and VEGF. Also, the expression pattern of these proteins seems to be different when occlusal support loss is bilateral or unilateral. The sample was composed solely by growing female rats to avoid age as a comorbid factor for TMJ degenerative disease and because this gender seems more prone to condylar cartilage remodeling due to occlusal alteration[11].

Bilateral extraction

The results of our study support the involvement of IL-1β and VEGF in condylar cartilage remodeling due to abnormal TMJ loading. However, the expression of IL-1β under non-physiological loading is not completely understood. While in our study mechanical overloading up-regulated IL-1β, similar result was observed after decreased articular loading induced by bilateral masseteric resection in growing rats[12]. In both cases, catabolic degradation was above normal levels, suggesting that loads within a physiological range are necessary for maintenance of cartilage integrity and growth. The increased expression of VEGF is in agreement with the results of Tanaka et al.[13], who observed abundant presence of VEGF in the mandibular condyle after mechanically induced TMJ osteoarthrosis. In that study, the percentage of VEGF immunopositive chondrocytes significantly increased with the period of applied mechanical stress. During mechanical

overloading, reduced oxygen tension activates the hypoxia-induced transcription factor-1, which is linked to the expression of VEGF[14].

In rabbits, unilateral removal of teeth[10] and surgically-created disc displacement[9] resulted in increased and decreased expression of type II collagen in the condylar cartilage, respectively. The results of our study showed no difference for the level of type II collagen after bilateral teeth extraction. Besides differences between animal models, these contrasting results suggest that the type of loading is an important factor in type II collagen expression. Basically, three types of loading can be distinguished: compression, tension, and shear. Tensile forces correspond more to fibroblastic activity, leading to the production of type I collagen, while compressive forces tend to be correlated with chondrocytes and the increased production of type II collagen[15]. During joint loading the cartilage layers are sheared to adapt their shape to the incongruent articular surfaces. Excessive shear, however, can cause a fatigue, which irreversibly may lead to damage of cartilage. Furthermore, excessive shear stress is associated with a breakdown of joint lubrication through a reduction of hyaluronic acid molecular weight[4]. We speculate that bilateral symmetrical loss of posterior teeth may keep mandibular stability, since both TMJs will be similarly loaded. However, this would be accompanied by increased shear stress. Is has been shown that loss of posterior occlusal support leads to a noticeable cranial condyle movement during clenching[16]. This may lead to a more intimate contact between the articular surfaces, causing excessive shear stress.

Unilateral extraction

In contrast to bilateral tooth loss, the increased expression of IL-1β after unilateral extraction was accompanied by an increase in type II collagen. This different response was probably due to differences in the nature and magnitude of the forces applied to the TMJs in these distinct biomechanical situations. As mentioned before, bilateral symmetrical loss of posterior teeth might still keep mandibular stability, but would be accompanied by increased shear stress. On the other hand, unilateral loss may cause mandibular instability, leading to higher compressive loads on the non-functional side than on the functional side[17]. We suppose that as compressive forces increase on the non-functional side, non-physiological tension loads of the same magnitude occurs on the functional side as a consequence. This could explain why no difference was found for IL-1β and type II collagen between sides. This is in agreement with a previous study on the expression of sulfated glycosaminoglycans, which is commonly found in tissues exposed to loading, where no difference between sides was reported[18]. In addition, a transient increase in bone metabolism[19] and type II collagen[10] was observed on the non-functional side, returning to levels similar to the functional side in few days. We speculate that the transient increase in metabolic activity on the non-functional side was part of the adaptation process of the mandibular condyle to changes in functional loading, which was followed by redistribution of functional loads to both TMJs few days after balance disruption as an initial approach of the masticatory system to sustain the non-physiological forces.

The increased level of type II collagen may be the result of increased synthesis rate by the chondrocytes in response to the non-physiological loads following unilateral loss of

occlusal support. According to Dijkgraaf et al.[5], if a primary mechanical insult disturbs the balance between synthesis and degradation of extracellular matrix components, cartilage degradation occurs. Initially, cartilage degradation will be counteracted by attempts at repair. The initial repair stage is characterized biochemically by an increased synthesis of extracellular matrix components and DNA, accounting for proliferation, mitosis and increased metabolic activity of the chondrocytes[5]. Thickening of condylar cartilage was observed after posterior teeth extraction as a signal of this proliferative response[18,20].

VEGF plays a key role controlling not only chondrocyte metabolism, but also angiogenesis present during inflammation and new bone formation[13]. Mandibular advancement has shown to increase the expression of VEGF, with subsequent neovascularization and bone formation in rats[21]. During unilateral chewing, the ipsilateral condyle is almost limited to rotation, while the contralateral condyle accomplishes rotational and translational movements. Thus, we speculate that the higher level of VEGF on the non-functional side was related to the greater extension of the translational movement accomplished by the condyle on the same side. It is well known that muscle forces have strong influence on natural bone remodeling. From the insertion to aponeurosis or periosteum, muscles exert force on bones; as a result, bone remodels to attain the shape that can best withstand the mechanical loads applied[22].

To summarize, abnormal TMJ loading due to loss of posterior occlusal support increased the expression of IL-1β, type II collagen and VEGF in the condylar cartilage of rats. The expression pattern of these proteins was different when loss of occlusal support was bilateral or unilateral, including differences between functional and non-functional

sides. These differences were probably related to the type of mechanical forces applied in each situation. Obviously, the results of this study are very limited from a clinical point of view. Although studies using rodents provide insights into the basic mechanisms of how occlusion may influence the condylar cartilage, 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. It is possible that the same occlusal alteration might have a different impact on the TMJs of species with different masticatory systems. However, this study suggests that occlusal support is an important element for the integrity of the condylar cartilage.

Conclusions

Abnormal TMJ loading due to loss of posterior occlusal support alters the expression of type II collagen, IL-1β and VEGF proteins in the condylar cartilage of rats. The expression pattern of these proteins is different when loss of occlusal support is bilateral or unilateral, including differences between functional and non-functional sides.

Acknowledgments

This study was supported by the National Council for Scientific and Technological Development (CNPq), Ministry of Science and Technology, Brazil (grant number 470454/2009-1).

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CONCLUSÃO

Apesar das diferenças anatômicas entre a morfologia dentária, articulações e função mastigatória de humanos e roedores tornarem difícil a extrapolação desses resultados para pacientes, estudos em roedores fornecem um certo discernimento sobre a influência da oclusão dentária sobre a articulação temporomandibular e os tecidos intra-articulares. Assim, ficou demonstrado que a presença de instabilidade ortopédica causada por um fator oclusal durante a fase de desenvolvimento pode levar à deficiência do crescimento ósseo mandibular e alteração dos tecidos intra-articulares.

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ANEXO 1

De: sjlindau@vcu.edu
Assunto: Manuscript040411-241R Decision Letter
Data: Qua, Maio 18, 2011 1:07 pm
Para: saudeoral@yahoo.com.br
CC: saudeoral@fop.unicamp.br

Dear Dr. Farias-Neto,

I am pleased to inform you that your manuscript "Altered mandibular growth under functional posterior displacement in rats" has been accepted for publication. We look forward to publishing your contribution in The Angle Orthodontist.

Allen Press will e-mail instructions to you in about 6 weeks telling you how to view a galley proof of your article on the Internet in PDF format. These proofs will look like your article as it will appear in print. The proofs will contain changes that occurred in the printing process (editing, typesetting or layout).

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Again, congratulations on your work and thank you for your contributions to the orthodontic literature. I look forward to seeing your article in print. If you have any questions, please be sure to contact us.

Sincerely,

Robert J. Isaacson, DDS, MSD, PhD
Editor-in-Chief, The Angle Orthodontist
Professor Emeritus
University of Minnesota
Virginia Commonwealth University

ANEXO 2

THE ANGLE ORTHODONTIST
ONLINE MANUSCRIPT SUBMISSION AND PEER REVIEW

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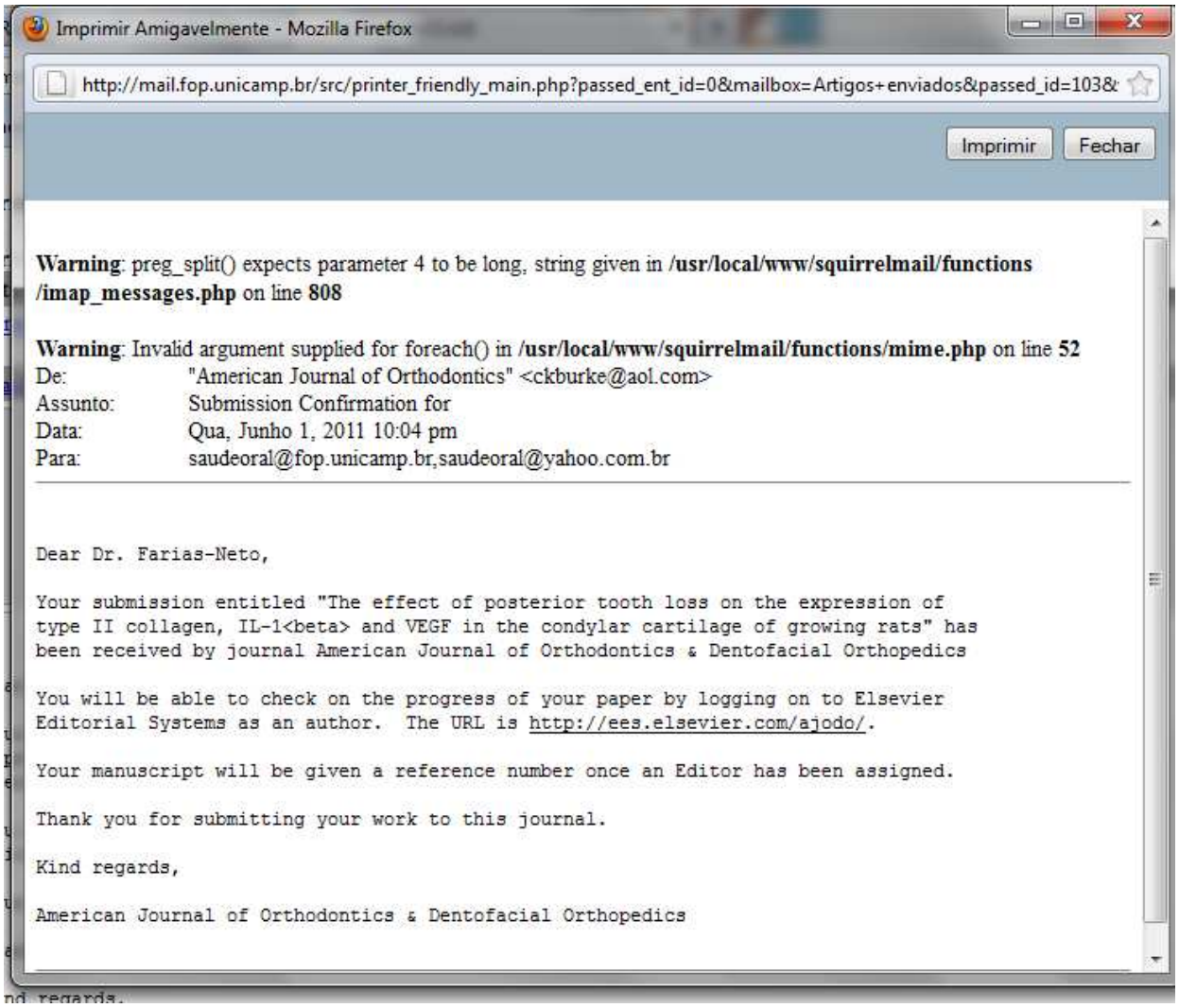
Detailed Status Information

Manuscript #	060711-373
Current Revision #	0
Submission Date	2011-06-07 21:56:08
Current Stage	Initial QC Started
Title	The effect of occlusal support loss on mandibular morphology in growing rats
Running Title	Premature tooth loss and growth
Manuscript Type	Original Article
Special Section	N/A
Corresponding Author	Arcelino Farias-Neto (Piracicaba Dental School)
Contributing Authors	Ana Paula Martins , Célia Rizzatti-Barbosa
Financial Disclosure	I have no relevant financial interests in this manuscript.
Abstract	Objective: The aim of this study was to evaluate the effect unilateral and bilateral premature loss of posterior occlusal support on mandibular bone dimensions in growing rats. Materials and Methods: Thirty female Wistar rats (5 wk old) were randomized into three groups: control, unilateral mandibular molar teeth extraction, and bilateral mandibular molar teeth extraction. After 8 wk, animals were sacrificed and acrylic rapid-prototyped templates of the mandibles were constructed. Mandibular length, ramus height, intercondilar distance and body weight were measured and analyzed by one-way ANOVA (Tukey test as post-hoc test), while differences between sides were analyzed by non-paired Student's t test (α=.05). Results: Mandibular length and intercondilar distance were significantly shorter in experimental animals, while no difference was observed for ramus height and body weight. Conclusions: the results of this study demonstrated that unilateral and bilateral premature loss of posterior occlusal support in growing rats resulted in a smaller mandible at grown age.
Key Words	tooth loss, mandible
Conflict of Interest	**I have no conflict of interest that I should disclose.**

ANEXO 3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Warning: Invalid argument supplied for foreach() in /usr/local/www/squirrelmail/functions/mime.php on line 52
De: "American Journal of Orthodontics" <ckburke@aol.com>
Assunto: Submission Confirmation for
Data: Qua, Junho 1, 2011 10:04 pm
Para: saudeoral@fop.unicamp.br,saudeoral@yahoo.com.br
Dear Dr. Farias-Neto,
Your submission entitled "The effect of posterior tooth loss on the expression of type II collagen, IL-1<beta> and VEGF in the condylar cartilage of growing rats" has been received by journal American Journal of Orthodontics & Dentofacial Orthopedics
You will be able to check on the progress of your paper by logging on to Elsevier Editorial Systems as an author. The URL is http://ees.elsevier.com/ajodo/.
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Kind regards,
American Journal of Orthodontics & Dentofacial Orthopedics

ANEXO 4

UNICAMP

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Comissão de Ética na Experimentação Animal
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CERTIFICADO

Certificamos que o Protocolo nº **1841-1**, sobre **"Influência de alterações das articulações temporomandibulares induzidas por alteração oclusal no desenvolvimento mandibular: estudo em modelo animal"**, sob a responsabilidade de **Profa. Dra. Célia Marisa Rizzatti Barbosa / Arcelino Farias Neto**, está de acordo com os Princípios Éticos na Experimentação Animal adotados pelo Colégio Brasileiro de Experimentação Animal (COBEA), tendo sido aprovado pela Comissão de Ética na Experimentação Animal – CEEA/Unicamp em **04 de maio de 2009**.

CERTIFICATE

We certify that the protocol nº **1841-1**, entitled **"Influence of temporomandibular joint changes induced by occlusal changes"**, is in agreement with the Ethical Principles for Animal Research established by the Brazilian College for Animal Experimentation (COBEA). This project was approved by the institutional Committee for Ethics in Animal Research (State University of Campinas - Unicamp) on **May 4, 2009**.

Campinas, 04 de maio de 2009.

Profa. Dra. Ana Maria A. Guaraldo
Presidente

Fátima Alonso
Secretária Executiva

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