



Histological Evaluation of Mandibular Condyle after Jaw Protrusive Stimulation in Rats

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Authors' contributions

This work was carried out in collaboration among all authors. Author TFS designed the study. Authors WFP, RPO, PFPA and ASP collected the data. Authors TFS and RPO analyzed the data. Authors TFS, CCOS and RPO drafted the article. All authors read and approved the final manuscript.

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ABSTRACT

Objective: The present study aimed to evaluate the cytological and histological behavior of the condyle of rats after mandibular advancement.

Materials and Methods: Thirty male Holtzman rats were randomly divided into three experimental and three control groups, with five animals each, aged five, nine, and thirteen weeks. The animals

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received flat tracks on the upper and lower incisors made of photopolymerizable composite resin, which resulted in the protrusion of the mandible. After thirty days, the animals were euthanized, the mandibles were dissected and photographed, the condyles were demineralized, paraffinized, sectioned, and stained in H&E. Cytological and histological analysis were performed using a light microscope. The collected data were tabulated using the SPSS 17.0 for Windows software and the Student's "t" test was used to compare the data.

Results: In the experimental group there was an increase in the number of cells in the proliferative layer and the mandibular size in five-week rats; increased number of fibrous layer cells in nine-week-old rats; and an increase in the proliferative layer in thirteen-week-old rats.

Conclusion: The protrusion of the mandible in rats of five, nine, and thirteen weeks of age promoted an increase in cell and area in different layers of the condyle. However, further studies are needed to better understand the adaptive responses generated in the condyle resulting from functional orthopedic stimuli.

Keywords: Angle class II; malocclusion; mandibular condyle; mandibular advancement osteogenesis; functional orthodontic appliances.

1. INTRODUCTION

Adaptive remodeling of the cartilage/bone of the mandibular condyles, in response to external stimuli of compression, tension and shear, is a condition that arouses the interest of several researchers. The condyle has the capacity for growth and multidirectional remodeling, in response to variations in position and function of the mandible [1], due to its structure formed by fibrocartilage composed of four layers. The most superficial layer, the articular zone, is composed of fibrous connective tissue. The polymorphic layer contains precursor cells for the underlying layers. The pre-hypertrophic layer is made up of chondroblastic cells in differentiation. The hypertrophic layer is formed by a cartilage layer with irregularly organized chondrocytes, areas with hypertrophic cartilage cells, and a deeper layer of mineralized cartilage. Underlying the cartilage there is an erosive layer where the transition from chondrogenesis to osteogenesis occurs [2-7].

The literature points out that the condylar region may be subject to remodeling to the detriment of external stimuli due to the high adaptive capacity of the cartilaginous zones, which would result in the potential to manipulate the growth of the mandible [8-12]. Many studies have evaluated skeletal and dentoalveolar changes during mandibular advancement therapies for the treatment of Class II malocclusions due to mandibular deficiency [13-19] with the use of fixed and removable functional orthopedic devices due to their ability to stimulate the growth of the mandible, especially in the region of the condyle and the mandibular fossa, restricting the anterior displacement of the maxilla, in addition, to generate dentoalveolar effects [20-24].

However, although functional orthopedic devices are effective in correcting the sagittal relationship of Class II malocclusions, the skeletal repercussions from these devices are not fully understood due to the wide variety of devices available and used in the studies, the different age groups evaluated, and different treatment and containment times [20,22,23,25-31].

Several human studies have shown that the changes produced by these devices result mainly from muscle, dentoalveolar and occlusal rearrangements [25,28,31-33]. Research shows that Herbst and Forsus devices are the ones with the most pronounced skeletal effects [34-36]. Animal studies show that the anterior repositioning of the mandible can accelerate the maturation of chondrocytes in young animals [9,10,37,38] and reactivate chondrogenesis at rest in adult animals [10,39,40], inducing morphological and histological changes in the anterior and posterior region of the condyle, formation of cartilaginous matrix and consequent increase in endochondral ossification. However, there are divergences especially concerning the age limit of response to orthopedic-functional therapies [8,9,41].

Currently, it is believed that in cases of mandible skeletal Class II, the mandibular protrusion devices have a good indication, even in young adults. However, the literature points out that orthopedic treatment are not effective after growth has ceased. The present study is trying to better elucidate the events resulting from experimental mandibular advancement in the mandibular condyle region. The aim of this study was to assess the cytological and histological behavior of the condyle region of *Holtzman* rats at different stages of development five (pre-

pubertal period), nine (post-pubertal period), and 13 (adult) weeks of age against after jaw protrusion stimulation.

2. MATERIALS AND METHODS

2.1 Study Design and Animals

A sample of 30 male *Holtzman* rats was randomly divided into three experimental groups and their respective controls. Sample size calculation was performed using data from our pilot study. Taking into consideration a population variability of 11% (standard deviation) and difference the 40% between groups, accepting alpha and beta errors of 0.05 and 0.20, respectively, a number of 5 animals per group was considered necessary. The *Holtzman* rat was selected for this study because this specie is commonly used in orthodontics research and, consequently, would make it possible to compare our results with other studies.

All animals were kept under the same conditions of ambient temperature and humidity, with food (softened feed) and water ad libitum. Experimental and control groups were formed by animals in different periods of development: Group One (G1): five weeks (pre-pubertal period); Group Two (G2): nine weeks (post-pubertal period); and Group Three (G3): 13 weeks (adults) of age. This study was approved by the local Committee on Animal Research and Ethics (protocol no 032/2018).

The animals were anesthetized with an intraperitoneal injection of Ketamine (60 mg/kg) and Xylazine (10 mg/kg) for the construction of flat tracks in the upper and lower incisor teeth,

made of composite resin to promote mandibular advancement. Control groups did not receive any orthodontic devices or flat tracks and were kept in physiological occlusion. After thirty days, the animals were euthanized, the mandibles were dissected and fixed in 10% formaldehyde.

2.2 Macroscopic Morphometric Analysis

Half-mandibles on the left side were photographed and the morphometric analysis of the mandibular length was performed with ImageJ®. Firstly, the condylion point and point lia were identified. Then, the line Co-lia was drawn using the "straight" function of the software and the measurement of the mandibular length was obtained. The measurements were performed by the same evaluator, blindly, and the average of the left mandibular lengths of the experimental and control groups was calculated [42].

2.3 Cytological and Histological Analysis

The half-mandibles were demineralized in 7% nitric acid for 24 hours, then the condyles were sectioned over the Ic-Rd line and passed by the paraffinization process [42]. Tissue sections of 5 µm thick parallel to the sagittal plane of the mandibular condyle were obtained and stained in hematoxylin and eosin. The most central histological section of each mandibular condyle was photographed using a camera (UCMOS03100KPA; USeries CMOS Camera) attached to a Nikon Eclipse E200 optical microscope.

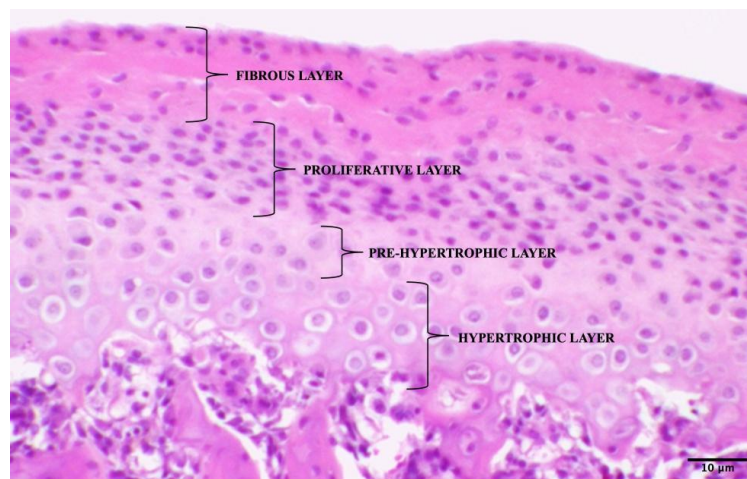


Fig. 1. Identification of the four layers of the condylar cartilage: fibrous layer, proliferative layer, pre-hypertrophic and hypertrophic layer. 100x magnification

The four layers of the condylar cartilage were identified in the histological examination - fibrous layer, proliferative layer, pre-hypertrophic and hypertrophic layer. The analysis of the thickness and area of the layers was performed using photographs in 100x magnification (three fields) as follows: fibrous, proliferative, hypertrophic layers (total of the pre-hypertrophic and hypertrophic layers) and total of three previous layers (Fig. 1). The cell count of cartilage layers was performed in 400x magnification (six fields): fibroblasts and undifferentiated cells from the fibrous layer; densely packed undifferentiated cells of the proliferative layer; immature and mature chondrocytes from the pre-hypertrophic and hypertrophic layers. The arithmetic mean of the fields was calculated for each animal. All measurements and counts were performed with ImageJ® by a single trained and blind researcher. The examiner performed the calibration process and carried out the analyzes twice to verify satisfactory intra-examiner agreement (Kappa = 0.83).

2.4 Statistical Analysis

The collected data were tabulated using SPSS 17.0 for Windows. Data showed normal distribution ($p > 0.05$) and Student "t" test was

applied to them. The level of significance was 5% (95% confidence interval).

3. RESULTS

3.1 Evolution of the Animals' Weight

There was a significant weight gain in control (77%) and experimental (47.62%) groups of five weeks of age at the end of the experiment. In the nine-week group, weight gain was 19.70% in the control group, while in the experimental group there was no significant weight gain. In the 13-week group, there was a weight gain of 14.30% in the control group and weight loss of 2.10% in the experimental group (Table 1).

3.2 Macroscopic Morphometric Analysis

In the macroscopic analysis of the mandible, there was a statistically significant difference between experimental and control groups at five weeks of age ($p = 0.044$), with mean mandibular lengths of 22.34 and 21.50, respectively, and a mean difference of 0.842 between the groups. The groups of nine and 13 weeks showed no significant difference between the experimental and the control (Table 2).

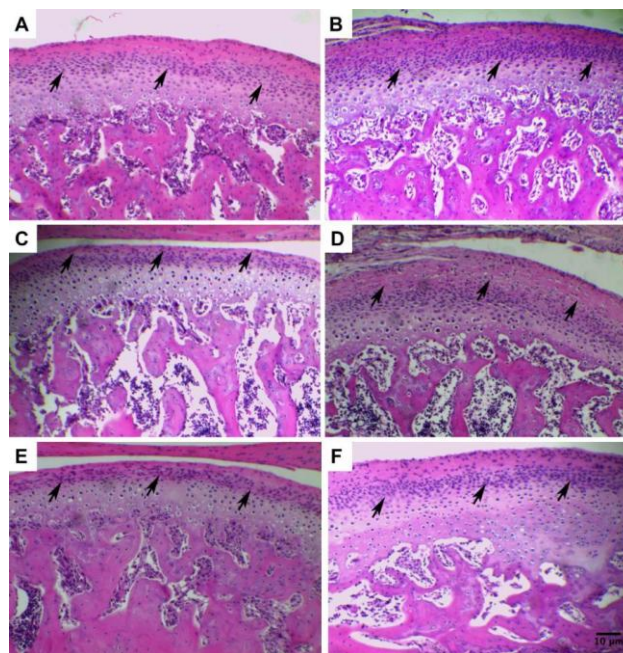


Fig. 2. Photomicrographs of left mandibular condyles of Holtzman rats. Five-week-old group with natural growth (A) and group submitted to mandibular protrusion (B). Arrows indicate the proliferative layer. In the nine-week-old group with natural growth (C) and the group submitted to mandibular protrusion (D). Arrows indicate the fibrous layer. In the 13-week-old group with natural growth (E) and the group submitted to mandibular protrusion (F). Arrows indicate the proliferative layer. 100x magnification

Table 1. Comparison of the initial average weight versus the final average weight (in gram)

Group	Initial average weight ± SD	Final average weight ± SD	Weight gain (%)	“t” value	“p” value	Average difference	Confidence Interval Minimum Maximum	
G1C	166 ± 12	294.40 ± 19.36	77	-30.47	0.000*	-128.40	-140.10	-116.70
G1E	126 ± 20.35	186 ± 32.06	47.62	-2.92	0.043*	-60	-117.09	-2.91
G2C	269.80 ± 22.61	323.20 ± 21.48	19.70	-6.52	0.003*	-53.40	-76.15	-30.65
G2E	250.40 ± 22.65	262.40 ± 40.23	4.70	-1.17	0.309	-12	-40.59	16.59
G3C	310.40 ± 13.69	354.80 ± 17.07	14.30	-7.68	0.002*	-44.40	-60.46	-28.34
G3E	323.60 ± 13.89	316.80 ± 20.77	-2.10	0.74	0.502	6.80	-18.80	32.40

Note: *Level of significance 5% (p<0.05). Paired “t” test

Table 2. Average lengths of the left half-mandibles (mm) of Holtzman rats from control and experimental groups of different ages

Group	Average lengths of the left half-mandibles Controle ± SD	Average lengths of the left half-mandibles Experimental ± SD	“t” value	“p” value	Average difference	Confidence Interval Minimum Maximum	
G1	21.50 ± 0.41	22.34 ± 0.57	-2.455	0.044*	-0.842	-1.654	-0.031
G2	26.43 ± 3.33	29.87 ± 1.70	-1.865	0.104	-3.440	1.845	-7.802
G3	30.03 ± 1.14	31.80 ± 1.54	-2.064	0.073	-1.769	-3.744	0.207

Note: * Level of significance 5% (p<0.05)

Table 3. Histological and cytological comparisons of control (G1C) and experimental (G1E) groups of five-week-old group (pre-pubertal period). Thickness (mm) and area (µm²)

Variable	Control Average ± SD	Experimental Average ± SD	“t” value	“p” value	Average Difference	Confidence Interval Minimum Maximum	
Fibrous layer thickness	6.58 ± 2.38	10.56±3.51	-1.938	0.101	-3.98	-9.00	1.04
Proliferative layer thickness	7.48 ± 0.56	7.74 ± 2.56	-0.218	0.833	-0.25	-2.96	2.45
Hypertrophic layer thickness	23.15 ± 6.38	27.52 ± 13.00	-0.674	0.519	-4.36	-19.30	10.57
Three-tier thickness	37.21 ± 5.11	34.47 ±2.82	1.046	0.326	2.73	-3.29	8.75
Fibrous layer cells	39.43 ± 7.46	49.99± 6.35	-2.033	0.088	-10.56	-23.27	2.14
Proliferative layer cells	68.80 ± 6.81	81.73 ± 9.44	-2.483	0.038*	-12.93	-24.94	-0.92
Hypertrophic layer cells	60.90± 14.56	64.60± 17.40	-0.365	0.725	-3.70	-27.10	19.70
Fibrous layer área	718.79±237.09	947.66 ± 136.99	-1.499	0.185	-228.86	-602.55	144.82
Proliferative layer area	1095.50 ± 123.71	1055.90 ± 57.47	0.586	0.577	39.60	-120.30	199.50
Hypertrophic layer area	2014.37 ± 604.85	2141.63 ± 707.49	-0.306	0.768	-127.26	-1087.17	832.65
Three-tier área	3824.64 ± 361.19	3658.20 ± 391.22	0.614	0.562	166.43	-497.38	830.25

Note: * Level of significance 5% (p<0.05)

Table 4. Histological and cytological comparisons of the control (G2C) and experimental (G2E) groups of nine weeks of age (post-pubertal period). Thickness (mm) and area (μm^2)

Variable	Control Average \pm SD	Experimental Average \pm SD	“t” value	“p” value	Average Difference	Confidence Interval Minimum Maximum	
Fibrous layer thickness	5.24 \pm 2.12	8.24 \pm 4.70	-1.172	0.279	-3.00	-9.05	3.05
Proliferative layer thickness	7.43 \pm 2.54	7.21 \pm 1.91	0.156	0.880	0.22	-3.06	3.50
Hypertrophic layer thickness	19.74 \pm 4.85	19.94 \pm 9.12	-0.044	0.966	-0.20	-10.85	10.45
Three-tier thickness	30.25 \pm 7.21	34.81 \pm 7.53	-0.978	0.356	-4.56	-15.32	6.19
Fibrous layer cells	26.54 \pm 6.56	39.16 \pm 8.43	-2.447	0.044*	-12.62	-24.82	-0.42
Proliferative layer cells	56.30 \pm 9.15	64.80 \pm 15.53	-1.054	0.323	-8.50	-27.10	10.10
Hypertrophic layer cells	61.36 \pm 12.40	65.43 \pm 28.61	-0.292	0.778	-4.06	-36.22	28.09
Fibrous layer área	540.64 \pm 137.85	942.20 \pm 365.68	-2.059	0.079	-401.56	-862.82	59.70
Proliferative layer area	856.41 \pm 254.57	875.47 \pm 267.00	-0.116	0.911	-19.06	-399.51	361.39
Hypertrophic layer area	1850.62 \pm 426.15	1784.58 \pm 727.70	0.175	0.865	66.03	-803.63	935.71
Three-tier área	3331.06 \pm 612.03	3707.26 \pm 869.52	-0.729	0.490	-376.20	-1597.27	844.87

Note: *Level of significance 5% ($p < 0.05$)

Table 5. Histological and cytological comparisons of the control (G3C) and experimental (G3E) groups of 13 weeks of age (adult period). Thickness (mm) and area (μm^2)

Variable	Control Average \pm SD	Experimental Average \pm SD	“t” value	“p” value	Average Difference	Confidence Interval Minimum Maximum	
Fibrous layer thickness	5.76 \pm 2.33	6.93 \pm 1.87	-0.868	0.411	-1.16	-4.24	1.92
Proliferative layer thickness	5.93 \pm 1.39	6.17 \pm 1.57	-0.251	0.808	-0.23	-2.40	1.93
Hypertrophic layer thickness	19.16 \pm 3.60	27.45 \pm 13.21	-1.353	0.239	-8.29	-24.47	7.88
Three-tier thickness	30.80 \pm 3.43	40.56 \pm 14.08	-1.504	0.199	-9.75	-27.03	7.52
Fibrous layer cells	37.43 \pm 14.07	37.53 \pm 7.30	-0.014	0.989	-0.10	-16.45	16.25
Proliferative layer cells	68.66 \pm 5.64	66.53 \pm 22.12	0.209	0.840	2.13	-21.41	25.67
Hypertrophic layer cells	56.13 \pm 13.85	55.33 \pm 13.22	0.093	0.928	0.80	-18.94	20.54
Fibrous layer área	667.68 \pm 197.12	729.94 \pm 182.60	-0.518	0.618	-62.26	-339.37	214.85
Proliferative layer area	745.07 \pm 93.67	909.14 \pm 78.19	-3.007	0.017*	-164.06	-289.90	-38.23
Hypertrophic layer area	1581.89 \pm 364.77	2091.33 \pm 934.21	-1.136	0.289	-509.44	-1543.71	524.82
Three-tier área	2994.65 \pm 510.02	3730.42 \pm 955.88	-1.519	0.167	-735.77	-1853.10	381.55

Note: *Level of significance 5% ($p < 0.05$)

3.3 Cytological and Histological Analysis

3.3.1 Pre-pubertal group

Histological analysis of condylar cartilage layers thickness did not reveal any significant difference between experimental and control groups; however, an increased thickness was noticed in the experimental group in the fibrous layers, and hypertrophic (Table 3). Similarly, evaluation of the area of each layer did not show any significant difference between experimental and control groups, however, there was a greater area in the fibrous layers of the experimental group and hypertrophic (Table 3).

Cell counting showed a significant difference in the number of cells in the proliferative layer, between the experimental and control group, with a higher average in the experimental group (Figs. 2A and 2B). In the fibrous layers and hypertrophic an increase in the number of cells was noticed in the experimental group (Table 3), however, the difference between the groups was not statistically significant.

3.3.2 Post-pubertal group

In the histological analysis of thickness and area of the condylar cartilage layers, there was no significant difference between the experimental and control groups, of nine weeks of age. There was also a decrease in the thickness and area of the hypertrophic layer, in control and experimental groups of nine weeks of age, when compared to the five-week-old group (Table 4).

Cell counting of fibrous layer was significantly higher in the experimental group (Figs. 2C and 2D). There was also an increase in the number of cells in the proliferative layer in the experimental group, but with no statistical significance.

3.3.3 Adult group

The thickness of the condylar cartilage layers did not show any significant difference between the experimental and control groups, however, there was a greater thickness of fibrous layers, hypertrophic and three layers in the experimental group. There was an increase in the area of the fibrous layers, proliferative, hypertrophic and sum of the three layers in the experimental group when compared to the control, the

difference being significant only in the proliferative layer ($p = 0.017$) (Figs. 2E and 2F). Regarding the cell count in the condylar cartilage layers, no significant difference was observed between the experimental and control groups (Table 5).

4. DISCUSSION

The use of functional appliances at an early age to improve sagittal and vertical jaw relationships, through functional orthopedics, aims to promote the redirection of bone growth to a favorable occlusion pattern [43]. In this sense, to understand the real repercussions of mandible after functional orthopedic stimulation is an important way to create improves on clinical treatments. The present study showed, in the experimental groups, there was an increase in the number of cells in the proliferative layer and the mandibular size in pre-pubertal period rats; increase in the number of fibrous layer cells in post-pubertal period rats and increase in area of the proliferative layer in adult rats. These events suggest an adaptive response of the condyle cartilage induced by a functional orthopedic protrusion of the mandible, resulting in stimulation of cell proliferation in different sites at the different ages studied.

Pre-pubertal group findings demonstrated mandible growth stimulated by the modification of the occlusal posture. Thus, the increase in the number of cells in the proliferative layer can result in a greater number of cells in differentiation, which can result in greater production of endochondral tissue and impact on animals' jaws size. The literature points out that there was no statistical difference between the thickness of the proliferative layer of one, four, 12, or 36-week rabbits in natural growth, indicating that, during natural growth, the proliferative layer remains active, and it provides cells to fibrocartilage when requested [44]. Our results suggest that, although the thickness and area of the proliferative layer did not increase in the experimental group, the mandibular advancement can have promoted an increase in the number of cells in this layer with greater proliferative activity.

Western Blot analyzes revealed an expression of type II collagen and SRY-Box Transcription Factor 9 (SOX9) gradually decreased from one to 32 weeks of age and, an expression of Runt-related Transcription Factor 2 (Runx2) and type X collagen increased from one to four weeks of

age and that was assembled at high levels from four to 12 weeks of age [44]. These findings showed an increase in chondrogenesis regulators and subsequently an increase in osteogenesis regulators, which is consistent with endochondral ossification growth. In the present study, an increase in the number of cells in the proliferative layer may be related to an increase in chondrogenic activity and greater cell availability to a hypertrophic layer, and, consequently, increased ossification activity.

Several experimental studies have shown increased cell proliferation markers such as Sox9, Proliferating Cell Nuclear Antigen (PCNA), Parathyroid Hormone-related Protein (PTHrP), Ribonucleic Acid messenger (mRNA) and type II collagen in the proliferative layer of animals in a mandibular protrusion [9,40,45]. These markers are related to the early differentiation of mesenchymal cells into chondrocytes and the early onset of chondrogenesis. Analyzes performed in rats, at the peak of growth, showed an increase in type X collagen mRNA expression and type X collagen detection in the hypertrophic zone, after functional stimulation of the mandible, in addition to a possible indication of increased endochondral ossification [11,12,46]. These findings support that mandibular advancement could promote increased cell proliferation and differentiation in certain condyle areas and it could result in the development or acceleration of the transition from chondrogenesis to osteogenesis.

In the present study, pre-pubertal animals went through the peak of growth. The largest mandibular size was observed in the animals that received protrusive stimulus of the mandible. Such evidence points out that functional orthopedic stimulation seems to be related to the mandibular growth increase when it coincides with the peak of growth. The period between five and nine weeks in rat growth resembles the adolescent growth spurt in humans, indicating condylar growth pattern similarity between laboratory rats and humans [11,38].

During natural condylar growth, undifferentiated cells proliferate and differentiate into chondrocytes (leading to appositional growth), they mature into hypertrophic chondrocytes and produce cartilage matrix and it will be replaced by endochondral bone tissue [11,47]. The significant increase in the undifferentiated

mesenchymal cells of the proliferative layer and the thickness and area increase of fibrous and hypertrophic layers in pre-pubertal animals of the experimental group may signal a greater metabolic activity committed to increase and accelerate the processes of differentiation of the hypertrophic layer resulting in increased cartilage and subsequent endochondral ossification, even with no statistical significance. Studies based on gene expression of condylar cartilage cells in mice have shown that cartilage progenitor cells are mesenchymal cells that were diverted to chondrogenesis in response to mechanical load [48]. These cells, when isolated, have the potential to transform into bone tissue and fatty tissue like mesenchymal stem cells [49]. The conversion to cartilage depends on the magnitude, duration, and direction of the applied mechanical force [50].

Post-pubertal group features demonstrate an adaptive response of the fibrocartilaginous tissue in temporomandibular joint (TMJ) protection and about supporting traction forces during mandibular advancement. The collagen type I (mainly in the fibrous layer) and type II (in the mature and hypertrophic layers) distribution may indicate the main type of load: tension in the fibrous zone and compression in mature and hypertrophic zones [51]. The literature indicated that physical elongation of the fibrous layer caused by mandibular advancement and reorientation of mesenchymal cells towards the center of contraction can trigger enhanced differentiation and maturation of chondrocytes, resulting in increased synthesis of type X collagen and, subsequently, in production bone [12,46]. Our results suggest that alterations found in the fibrous layer represent responses to tensions generated and transmitted to fibrocartilage in experimental condyles.

Additionally, condylar cartilage regional and local heterogeneity suggests higher loads cartilaginous tissue experiences in thicker regions, revealed in pig condylar cartilage. The presence of the fibrous zone can reduce its deformation under compression and friction force on the cartilage surface [44]. In addition, the surface zone (density and thickness) constitution transmits and supports the traction and compression forces, protecting mechanical load changes [44,51,52]. Huang et al. (2020) demonstrated that 12-week-old female rabbit condyles subjected to static mechanical pressure had significantly trabecular bone damage compared to condyles

of 32-week-old female rabbits and control animals, and it was associated with a decrease in thickness of the fibrous layer in younger animals [44].

The reduction in the hypertrophic area in the post-pubertal group may result from cartilage degradation in this area and because of a consequent increase in endochondral ossification. In rats up to four-month-old occur endochondral bone formation and, from nine months onwards occurs osteon-type osteogenesis around the blood vessels in the limit between calcified cartilage and subchondral bone [4]. During natural growth, in 56-day-old rats, underlying bone tissue cartilage thickness is proportional, indicating a chondrogenesis predominance with a moderate transition to osteogenesis; whereas in adaptive remodeling, in rats of the same age, after 21 days of mandibular advancement, a transition from chondrogenesis to osteogenesis is accelerated in the erosive zone, where the degradation of hypertrophic chondrocytes and surrounding cartilage and its matrix can be observed and the replacement by endochondral bone formation is also observed [11]. These findings support that the presence of a thick area of proliferating chondrocytes and a reduction in the thickness of the hypertrophic and erosive layers are directly related to the process of replacement by ossification.

Recently, a study in female rabbits, pointed out that cartilage thickness (pre-hypertrophic, hypertrophic, and total layers) rapidly decreased from four weeks of age and was maintained at the same level at the ages of 12 and 32 weeks. Furthermore, the subchondral bone thickened with age, and changes in its architecture occurred four weeks after the thinning of the fibrocartilage [44]. These data point to a rapid decrease in chondrogenic activity and an increase in osteogenic activity during condyle development. Similarly, in the present study, a decrease in the hypertrophic layer was noted in the nine-week group, which may suggest the occurrence of an increase in ossification.

The findings of the adult group may indicate that functional stimulation contributes to the activation of cytological and histological activity in rats considered adults. Some research evidenced the reactivation of proliferation, maturation, and chondrogenesis as a result of functional stimulation in rats aged after a pubertal growth

spurt [39,40]. In addition, Xiong et al. (2004) observed greater mandibular length in adult rats (120 days of age) after 30 days of mandibular advancement compared to controls [53]. The presence of a larger area of the proliferative layer and the increase the mandibular length, thickness, and area of the three-layer, fibrous and hypertrophic layers in the experimental group, in the present study, suggests that cytological and architectural activations of the condyle against the stimulus of protrusion in rats after pubertal growth spurt may be related to the induced remodeling or growth stimulus of the mandible.

The fibrocartilage of the mandibular condyle, unlike the cartilage of the long bone growth plate, remains for life with the role of absorbing and distributing forces during the normal function to maintain joint homeostasis. Recent studies have demonstrated the presence of stem cells in the fibrocartilage of the mandibular condyle with potential for spontaneous chondrogenic differentiation (which may participate in cartilage repair) and expression of different genes, proteins, and surface markers according to cell differentiation degree [54-56]. Studies have shown that a single mesenchymal stem cell was able to generate cartilage, bone, and organize a hematopoietic microenvironment when transplanted in vivo [55] and that the therapeutic application of exogenous Wnt canonical inhibitory sclerostin (SOST) maintained the pool of mesenchymal stem cells and repaired rabbit TMJ cartilage [57]. These findings indicate that these pluripotent cells remain in the joint and can be activated or reactivated when adequate stimuli are provided.

It is important to point out that the animals submitted to mandibular protrusion had less weight gain at the end of the experiment. This fact may be related to lower food intake or a masticatory hypofunction because of the functional device since some studies indicate that masticatory hypofunction can change the measurements of mandibular length and height of the ramus and mandibular body [2,42, 58]. In the present study, it was possible to infer that the animals that received the device for mandibular protrusion had lower food intake due to chewing limitations resulting from the modification of the dental and occlusal pattern, which impacted their weight difference. However, even with the smallest weight gain, the experimental groups showed differential cytological and histological activity in comparison to controls.

The present study evaluated the mandibular condyles of rats through macroscopic analysis of the mandible and microscopic analysis (cytological and histological) of the non-mineralized cartilage, in histological sections stained by H&E. The limitation is related to the non-evaluation of mineralized areas through specific staining and the non-performance of immunohistochemical and molecular analyzes in the condylar layers. Future research in the field of functional orthopedic mandibular growth stimulation may help to elucidate the biological processes related to orthopedic stimuli of the jaws and help orthodontists in conducting clinical treatments. Immunohistochemical and molecular analyzes of mandibular condyle fibrocartilage can help to identify pathways of activation and differentiation of mesenchymal cells and to quantify cartilage production and endochondral bone formation. Additionally, studies with condyle cartilage stem cells could contribute to the understanding of the phenomena involved in mandibular growth or in remodeling of the mandible condyle. These perspectives could help the comprehension of the response of the mandible condyle to functional orthopedic stimuli and their impact on growth at different ages of activation.

5. CONCLUSION

The protrusion of the mandible in rats of pre-pubertal, post-pubertal and adult promoted an increase in the number of cells and the area in different layers of the condyle.

CONSENT

It is not applicable.

ETHICAL APPROVAL

This study was approved by the local Committee on Animal Research and Ethics (protocol no 032/2018) of the Universidade Federal dos Vales do Jequitinhonha e Mucuri (UFVJM). The authors declare that the present study was conducted in accordance with the ethical standards of animal research.

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COMPETING INTERESTS

Authors have declared that no competing interests exist.

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