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Mandibular condyle changes in rats with unilateral masticatory function

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All authors discussed the results and contributed to the final manuscript.

We declare that there is no conflict of interest regarding the publication of this manuscript.

Data availability statement.
The data that support the findings of this study are available from the corresponding author upon reasonable request.

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This article has been accepted for publication and undergone full peer review but has not been through the copyediting, typesetting, pagination and proofreading process which may lead to differences between this version and the Version of Record. Please cite this article as doi: 10.1111/ocr.12575

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source: https://doi.org/10.48350/168409 | downloaded: 3.5.2024

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Abstract

Objective: Certain malocclusions or unilateral tooth loss can lead to asymmetric functional load of the two mandibular sides during mastication and induce skeletal asymmetries to the condylar process of growing individuals. However, in adults, asymmetric function may have a different impact. The aim of the present study was to investigate three-dimensionally the effects of unilateral masticatory function on the condylar process morphology in growing and adult rats and the adaptive processes to differential condylar loading.

Materials and methods: Fifty-six growing and adult Wistar rats aged 4 and 26 weeks respectively were obtained. The maxillary right molars of the experimental animals were extracted and all animals were followed for 12 weeks. Three-dimensional images were obtained by an in-vivo microcomputed tomography (micro-CT) examination. The following measurements were studied: condylar process height, condylar base width, and condylar cross-sectional surface.

Results: While no differences were found with regards to condylar process height and base width, the cross-section of the condyle on the extraction side did not increase during growth in the young rats. No such differences were found in adults. Young rats had statistically significantly shorter condylar height, base width and cross-sectional surface than the adult rats and showed significant growth of these structures during the experimental period.

Conclusion: Condylar height and base width growth are not hindered by reduced occlusal function, contrary to the average cross-sectional surface, which implies that the condyle form of growing individuals becomes thinner while maintaining its length, in the absence of occlusal stimuli. The condyle of adult rats with extractions is less affected by occlusion changes.

Keywords: Occlusal function, mandibular condyle, condyle morphology, molar extraction, micro-CT

Introduction

The mandibular condyle, together with the glenoid fossa, condylar cartilage, articular disc and ligaments and masticatory muscles, composes the temporomandibular joint.[1] The main function of the temporomandibular joint is to articulate the mandible with the cranium and to allow mandibular rotary and translatory movements.[2]

The morphology of the temporomandibular joint is partly genetically determined but development of occlusion and masticatory function can affect growth and remodeling of the structures. In particular, condylar growth direction in growing individuals is closely related to mandibular displacement direction and deviation[3] and its morphology is also influenced by occlusal forces and tooth eruption.[4-5]

On the other hand, the adult temporomandibular joint has less adaptive and regenerative capability than the one of growing individuals and is unable to adjust to dental changes that may occur and a deformation of the articular surfaces can be seen.[6]

Unilateral edentulism, occlusal interferences, functional mandible deviations, malocclusions such as lateral open bite, unilateral cross bite or scissor bite and sucking or chewing habits all can lead to asymmetric functional load of the two mandibular sides during mastication. This may influence condylar growth, induce adaptive differences and skeletal asymmetries to the condylar process and the joint.[7-9]

Animal experimental studies on growing rats have shown that a low masticatory function induced by a soft diet leads to a lower bite force, to changes in the mandibular morphology and to a localized decline of growth of the condylar head in length and width.[10-16]

On the other hand, adult rats with no growth potential have shown condylar adaptive processes to a decreased masticatory function, but increasing age may diminish the capacity to adapt to altered function.[17-18]

Tooth loss also has an effect on the mandibular growth process. Molar extractions affect the masticatory function of rats, decreasing the mandibular and condylar head size[19-22], but the relationship between unbalanced occlusal support and condylar process development remains to be investigated.

Micro-computed-tomography (micro-CT) is a method to image and quantify bony structures through a three-dimensional reconstruction array first introduced by Feldkamp and coworkers.[23] Such examination has been proven to be a precise and effective way to investigate the three-dimensional structure and mineral density of bone in both in-vivo[24] and in vitro[25-26] studies.

Micro-CT has also been used to study condylar changes on rats with a diminished masticatory function.[22,27-30] To the best of our knowledge, however, there are only two references in the literature[31-32] in which an in vivo micro-CT examination was used to examine the effects of a decreased masticatory function on rats, induced by molar extraction and soft diet. Both studies though only used growing rats, did not analyze changes at the condylar process and did not evaluate the asymmetric functional load of the two mandibular sides, caused by unilateral maxillary molar extraction.

Therefore, the aim of this study was to investigate, in a three-dimensional way, the effects of unilateral masticatory function on the condylar process morphology of both growing and adult rats and the adaptive processes to differential loading of condyles. Our hypothesis was that unbalanced masticatory function induced by unilateral teeth loss may lead to changes in shape,

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volume and size of the condylar process in growing animals, and to reduced adaptation in adult rats in regards to occlusal changes, such as overloaded and unopposed condyles.

Material and methods

Animals

A total of fifty-six Wistar rats at two age groups, 4-week and 26-week-old were obtained and quarantined for 1 week before the start of the experiment. The animals were divided into young and adult group according to the age, each group having 16 experimental subjects, which had the maxillary right molars extracted, and 12 control subjects. Extractions were performed under anesthesia by intra-peritoneal injection of Ketamine 90 mg/kg and Xylazine 10mg/kg and supplemented by Buprenorphine 0.1 mg/kg SC 15 minutes before the surgery. The young control (YC), young experimental (YE), adult control (AC) and adult experimental (AE) groups were further divided by molar functionality: the right side of the experimental rats was defined as unopposed (YU and AU), due to the lack of occlusion (hypofunctional side), whereas the left side as overloaded (YO and AO), because of the anticipated higher functional forces applied (hyperfunctional side). All the animals were followed for 12 weeks, weighted at every micro-ct scan procedure, fed a soft diet and had water ad libitum. At the end of the experiment, they were sacrificed at the age of 16 and 38 weeks respectively. The experimental design was approved by the animal experimentation committee of the Canton of Geneva, GE/84/17.

Micro-CT

Three-dimensional digital images were obtained by an in vivo microcomputed tomography (micro-CT) examination using Quantum GX micro-CT imaging system, Perkin Elmer®, Waltham, Massachusetts, USA. We examined the rats at three different time-points representing the start of the experiment (T0), 1 month (T1) and 3 months (T2) follow up. The following settings were employed: FOV 60 mm, 90 kV, 88 µA, scan mode ‘high resolution’, scan time 4 min. Isoflurane anesthetic gas was administered at 5% and 1 l/min for the induction phase and at 3% and 1 l/min during the scan procedure. The scans were then reconstructed using the integrated scanner software by choosing the condylar process area as the region of interest with a voxel size 30 µm and a field of view (FOV) of 15,4 mm.

The scans were then exported in DICOM format, analyzed with Osirix® image analysis software, Geneva, Switzerland, and reoriented according to the base of the condylar process, defined by the AB line passing through the deepest points of the posterior mandibular notches, as previously described[28] (Figure 1). The following measurements were studied: condylar base width, condylar process height and condylar cross-sectional surface. The condylar base width was defined as the line passing through the deepest points of the posterior mandibular notches, while the condylar process height as the perpendicular distance between the condylar base and the highest point on the condylar head. Both these measurements were calculated using three-dimensional coordinates. To obtain the condylar cross-sectional surface, the cross-sectional surface of every slice parallel to the condylar base along the condylar process was measured. The average cross-sectional surface was calculated by dividing the sum of the surfaces by the

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number of surfaces. In this way we calculated the condylar process volume indirectly, using the cross-sectional surfaces of every slice as a ROI. The operator who performed the measurements was blinded as the rats had random numbers associated with them and the scans exported for the analysis did not contain the teeth, only the condyles.

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Statistical analysis

JASP statistics software (JASP Team (2020). JASP (Version 0.14.1)[Computer software].) was used to analyze the data with an analysis of variance (ANOVA) test. Since an ANOVA was performed, no Bonferroni correction was required. The dependent variables included the condylar process height, condylar base width and average condylar cross-sectional surface and were calculated as the difference between day 90 and day 0 of the experiment. Levene's Test for Equality of Variances was used to verify assumptions. The condyle height variable showed differences in variances thus a Kruskal-Wallis test (non-parametric) was used. The independent variables were Age (Young and Adult) and Experimental Group (Control and Extraction Groups) as well as their interaction. Tukey Post Hoc tests were performed in order to compare statistically significant independent variables and interactions of the ANOVA tests.

Method error

Random and systematic errors of the condylar process height, condylar base width and condylar cross-sectional surface were evaluated on 30 repeated measurements, with a 1-month interval. Random error was calculated with Dahlberg's formula.[33]

The systematic error was assessed with a paired *t*-test, whereas the coefficient of reliability was calculated as described by Houston.[34]

Results

No systematic error was detected for any duplicated measurement. Coefficients of reliability were above 96.0% for all parameters, and low random errors were detected: 0.029 mm, 0.028 mm and 0.115 mm^2 for condylar process height, condylar base width and condylar cross-sectional surface, respectively.

No differences were seen between the groups as to the weight throughout the experimental period. One rat in the young control group and one in the adult control group died during the scanning procedure from the anesthesia. Table 1a shows the mean and standard deviation values of the unopposed and overloaded sides of the experimental animals at T2, as well as of the controls for the three measurements performed. Table 1b displays the mean and standard values of the measurements at the three time-points of the experiment.

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Control and experimental animals had no differences regarding the condylar process height and base width. However, young rats in the experimental group showed a smaller condylar cross-sectional surface compared to young control rats. This difference was not seen in adult animals.

Young animals had statistically significant shorter condylar process height and smaller base width and cross-sectional surface, and these values increased faster over the three-months experiment when compared to the adult group. (Figure 2). At T2 the growing rats almost reached the values of the adult rats at T0.

Condylar process height

No differences were found between control and experimental condyle process heights on both young and adult animals. The condylar process height of the growing animals showed a bigger increase over the three-month experiment compared to the condylar process height of the adult animals ($p<0.001$). On average the adult rats had 0.24mm condylar growth compared to 1.36mm in young rats (Table 2).

Condylar base width

Similarly to the condyle height, there was no statistically significant difference between control and experimental groups. Adult rats had significantly less growth (0.20mm) compared to young rats (1.85mm) in the same time period ($p<0.001$) (Table 3).

Condylar cross-sectional surface

In the adult group no statistically significant change occurred during the experiment whereas in the young group there was an increase in surface area ($p=0.026$). More specifically, only the young control group showed this increase of surface area of 0.25mm^2 ($p=0.043$), whereas the young experimental groups showed no increase.

Interestingly, rats in the control group had statistically significantly larger surface changes during the experimental period (0.09mm^2, $p=0.035$) than the experimental group. Analysis of the interaction of Age and Group variables reveals that young control (YC) rats have a larger surface change (0.19mm^2, $p=0.010$) compared to young experimental (YE). The young overloaded (YO) and young unopposed (YU) showed no difference. The YC groups surface change is also larger than all the adult groups (0.28mm^2, $p<0.001$). Furthermore, there is no statistically significant difference between YE group and AC ($p=0.371$) and AE groups ($p=0.385$) (Table 4).

As with condylar height and base width, adult rats had significantly larger average cross-sectional surface than young rats (1.81mm^2, $p<0.001$).

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Discussion

Young and adult rats have been used in several studies that aimed to compare condylar changes in animals with symmetrical and asymmetrical functional load. Soft diet and molar extraction are conditions leading to a lower masticatory function.[12,19-20] Although it has not been verified in this study, based on other studies[20-22,27-29,32], we assumed that extracting the maxillary right molars of young and adult rats could create a unilateral masticatory function, which in turn could cause changes to the condylar process.

Since two-dimensional x-rays have major disadvantages as compared to three-dimensional radiography, such as superimposition of anatomical structures, different angles of exposition between subjects and no possible reorientation[35], measurements taken from a three dimensional micro-ct were performed to evaluate the condylar changes.

Young rats were 4 weeks old, which corresponds to the initial period of usage of the teeth, which finish erupting at 21 days[36], while adult rats were 26 weeks old. Many studies report similar ages when evaluating young and adult rats.[10-18,21-22,27-29,31-32]

Due to the active growing phase, young rats showed statistically significantly lower values and a bigger increase of all condylar measurements, when compared to the adult rats. This is consistent with a previous report[17] that compared different masticatory functions on young, growing and mature rats, and with a study[30] that reported an increase of the length and width of rat condylar head from 2 to 7 months of age.

The results of our study reveal that no changes can be seen in condylar height, base width and cross-sectional surface of adult rats with lower masticatory function. This is in line with the study of Ödman[18], which reported that animals having a lower masticatory function induced by soft diet had a smaller mandible, but the condylar process area and the condylar process length didn't show a significant difference.

Bouvier[17] on the other hand, reported that adult rats fed a soft/hard diet had a small condylar width deficit compared to animals fed a hard diet. Nevertheless, the gross dimensions were measured with calipers and not radiographically. Moreover, the rats of this study were 16-weeks-old and were younger compared to the ones used in our study, which were 26-week-old. If the rats would have been left to live more, there might be no differences between the groups. The different measurement techniques and ages of the animals could therefore explain the differences between the studies.

Several experiments[10-12,15-16] reported that a low masticatory function led to smaller mandible, ramus height and condylar head dimensions in growing rats. Our findings showed no differences in condylar height and base width of young control and experimental rats. No differences in condylar height between rats with different masticatory functions were also seen in the study of Enomoto.[27]

Instead, a larger condylar cross-sectional surface was detected in growing control animals, while the young experimental group experienced no statistically significant change. The condylar process growth of the experimental rats was therefore slowed down due to the extractions. Since the condylar process height and condylar base width grew similarly between control and experimental young rats, the difference in the cross-sectional surface between the groups may be explained by a change of the condylar shape, in that it must become thinner in order to see this difference in the surface. This change of the condylar shape is in accordance with the results of previous studies[22,31-32], that reported a smaller condylar size, bone volume, condylar thickness width, and condylar process neck width in the extraction and soft diet groups compared to the control. It could be speculated that if the young experimental rats would be left to grow until the adult age, they would have a smaller condylar average surface than the adult control animals.

The rats in our study were fed soft food because after the surgery in the extraction group, the masticatory capacity is reduced due to the operation for a limited time[37] and we did not want differences to appear between the groups because of the operation itself. This, though, could have affected the load on the condyles, since no intensive mastication was needed and there could be a systematic underestimation of the results, since the control rats were also fed a soft diet. Since the loads on the occlusion and on the condyles would have been higher, differences between extraction and non-extraction sides could have appeared. Several studies[10,15-16,31] compared mandibular changes in rats fed diets with different physical consistencies and showed that rats fed a hard diet had greater condylar length, width and depth compared to animals in the soft diet group. Kiliaridis[12] compared changes in the size of the condyle in rats fed soft or hard diet and found that a low masticatory function leads to decreased growth of the condylar head, which was though not measured in the present study. Enomoto[27] reported on different measurements, the only of them comparable with the ones of our study was the condylar height, where no differences were seen. Abo[31] found no differences in the condylar process neck width, which can be compared to the condylar base width of your study. All the mentioned studies though performed different measurements compared to the one used in the current experiment.
The study which evaluated the most similar measurements, was the one by Denes[28], which compared mandibular condylar growth in rats fed hard and soft diet with or without bite-blocks. The findings suggest that the soft diet did not affect the length of the condylar process but a smaller cross-sectional condylar area was seen in the animals with a reduced masticatory function. We could speculate, therefore, that if our control group would have had a normal hard diet, the results could have been different, showing an even greater difference between the experimental and control groups. This could also explain the lack of difference between control and experimental animals regarding the condylar base width and process height.

Nakano[29] evaluated the differences on rats with different masticatory loads between right and left side, caused by a maxillary occlusal splint that shifted the mandible to the left during closure. The results showed that the experimental animals developed smaller mandibles than the controls and that the right side of the experimental rats was significantly longer than the left one, resulting in an asymmetric mandible. A partial recovery was seen, in an attempt to restore the symmetry, although the size difference at the end of the experiment was still present. An explanation for the different results with our study might be that the mandibular lateral shift generated by the occlusal splint caused an asymmetric unilateral displacement while in our study only a functional asymmetry was induced due to the unilateral molar extraction. Therefore, the mandible of the rats of our study could have had possibilities to adapt to an asymmetric loading without any necessity to adaptation of a functional shift. Moreover, the changes were measured on the entire mandibular length, whereas in our study only the condylar process was studied.

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Despite the initial assumption that the loading difference between the extraction and the non-extraction side could influence differently the condylar process, the condyles were similarly affected on both sides in rats submitted to unilateral molar extraction, even though the masticatory function was asymmetrical. We can speculate that since the left and right condylar processes are connected through the mandibular bone, they adapt to the unilateral masticatory function by redistributing the functional loads to both sides, in an attempt to sustain the unbalanced forces. This is in accordance with a previous study[38] on the expression of sulfated glycosaminoglycans, which is found in tissues exposed to loading, in which there were no differences between the extraction and non-extraction sides. No differences between right and left condyles in the experimental rats, were also found in the report of Farias-Neto[21], which compared mandibular morphology after unilateral or bilateral mandibular molar extractions. The study showed that mandibular length and intercondylar distance were shorter in both extraction groups compared to control animals but there was no difference between unilateral and bilateral extraction groups. Another possible reason of our findings could be the reduced bilateral masticatory function in the animals which suffered unilateral extraction of their molars, resulting in less functional loading of their condyles both in the extraction and the non-extraction side.

Both dietary consistency and molar extraction play a role in generating condylar changes. The results of the present study suggest that unilateral molar extraction and subsequent unbalanced masticatory function changes the form of the condylar process of young rats, causing a decrease of the cross-sectional surface and the overall growth of the condyle. Our findings support the theory of Moss[39], which advocates that bone is formed by function and reveals the importance of a normal masticatory function during childhood for a correct growth and development of the mandibular condyle.

Conclusions

This study aimed to detect condylar process changes on adult and young rats with unilateral maxillary molars extraction. After a 12 week-period, adult animals showed no condylar changes. On the other hand, the extractions affected the morphology of the condylar process of growing rats, influencing right and left sides similarly. While condylar height and base width growth were not hindered, reduced functional forces led to a smaller condylar cross-sectional surface. This implies that young rats with induced asymmetrical extractions have a thinner condylar process bilaterally compared to controls. Appropriate bilateral masticatory function is therefore beneficial for a normal growth of the mandibular condyle.

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Table 1. Descriptive statistics

(a) Mean values within the groups

	Average of Average surface (mm^2)						**Average of Condyle base width (mm)**						**Average of Condyle height (mm)**					
	AC	AO	AU	YC	YO	YU	AC	AO	AU	YC	YO	YU	AC	AO	AU	YC	YO	YU
Mean	2.694	2.851	2.737	2.260	2.198	2.170	7.878	7.894	7.964	6.423	6.376	6.360	6.308	6.255	6.322	5.260	5.219	5.182
Standard Deviation	0.310	0.310	0.260	0.205	0.208	0.184	0.333	0.388	0.385	0.849	0.798	0.840	0.184	0.276	0.247	0.588	0.643	0.597
Minimum	2.051	2.121	2.085	1.900	1.640	1.689	7.200	7.127	7.181	4.646	4.795	4.708	5.960	5.681	5.705	4.245	4.162	4.200
Maximum	3.269	3.439	3.150	2.718	2.870	2.613	8.576	8.638	8.917	7.787	7.915	8.110	6.728	6.884	6.866	6.200	6.211	6.177

Table 1 (a) Descriptive statistics. Mean within the groups. AC: adult control, AO: adult overloaded, AU: adult unopposed, YC: young control, YO: young overloaded, YU: young unopposed.

(b) Mean values at T0, T1 and T2

	Average of Average surface (mm^2)			**Average of Condyle base width (mm)**			**Average of Condyle base width (mm)**		
	1 day	30 days	90 days	1 day	30 days	90 days	1 day	30 days	90 days
Mean	2.487	2.413	2.537	6.573	7.233	7.605	5.314	5.812	6.131
Standard Deviation	0.377	0.368	0.362	1.262	0.750	0.520	0.883	0.500	0.367
Minimum	1.900	1.689	1.640	4.646	6.021	6.408	4.162	5.000	5.331
Maximum	3.338	3.439	3.301	8.691	8.638	8.917	6.642	6.840	6.884

Table 1 (b) Descriptive statistics. Mean values at TO, T1 and T2. T0: 1 day, T1: 30 days, T2: 90 days

Table 2. ANOVA – Condylar process height

Cases	**Sum of Squares**	**df**	**Mean Square**	**F**	**p**
Group	0.125	1	0.125	2.963	0.086
Age	87.451	1	87.451	2075.803	< .001
Exp. Days	33.485	2	16.742	397.411	< .001
Group * Age	0.035	1	0.035	0.829	0.363
Group * Exp. Days	0.032	2	0.016	0.385	0.681
Age * Exp. Days	17.129	2	8.565	203.297	< .001
Group * Age * Exp. Days	0.035	2	0.017	0.415	0.661
Residuals	12.849	305	0.042		

Table 2. ANOVA – Average of condylar process height (mm). Type III Sum of squares. Exp.: Experimental

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Table 3. ANOVA – Condylar base width

Cases	Sum of Squares	df	Mean Square	F	p
Group	0.001	1	0.001	0.010	0.922
Age	174.415	1	174.415	1523.689	< .001
Exp. Days	55.052	2	27.526	240.465	< .001
Group * Age	0.225	1	0.225	1.966	0.162
Group * Exp. Days	0.042	2	0.021	0.182	0.834
Age * Exp. Days	36.258	2	18.129	158.374	< .001
Group * Age * Exp. Days	0.068	2	0.034	0.297	0.743
Residuals	34.913	305	0.114		

Table 3. ANOVA – Average of condylar base width (mm). Type III Sum of squares. Exp.: Experimental

Table 4. ANOVA – Average condylar cross-sectional surface

Cases	Sum of Squares	df	Mean Square	F	p
Group	0.012	1	0.012	0.196	0.658
Age	21.012	1	21.012	345.169	< .001
Exp. Days	0.862	2	0.431	7.078	< .001
Group * Age	0.589	1	0.589	9.680	0.002
Group * Exp. Days	0.135	2	0.067	1.108	0.331
Age * Exp. Days	0.450	2	0.225	3.696	0.026
Group * Age * Exp. Days	0.132	2	0.066	1.085	0.339
Residuals	18.566	305	0.061		

Table 4. ANOVA – Average of condylar cross-sectional surface (mm^2). Type III Sum of squares. Exp.: Experimental

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Figure 1. Three-dimensional reconstruction of the mandible and condylar measurements

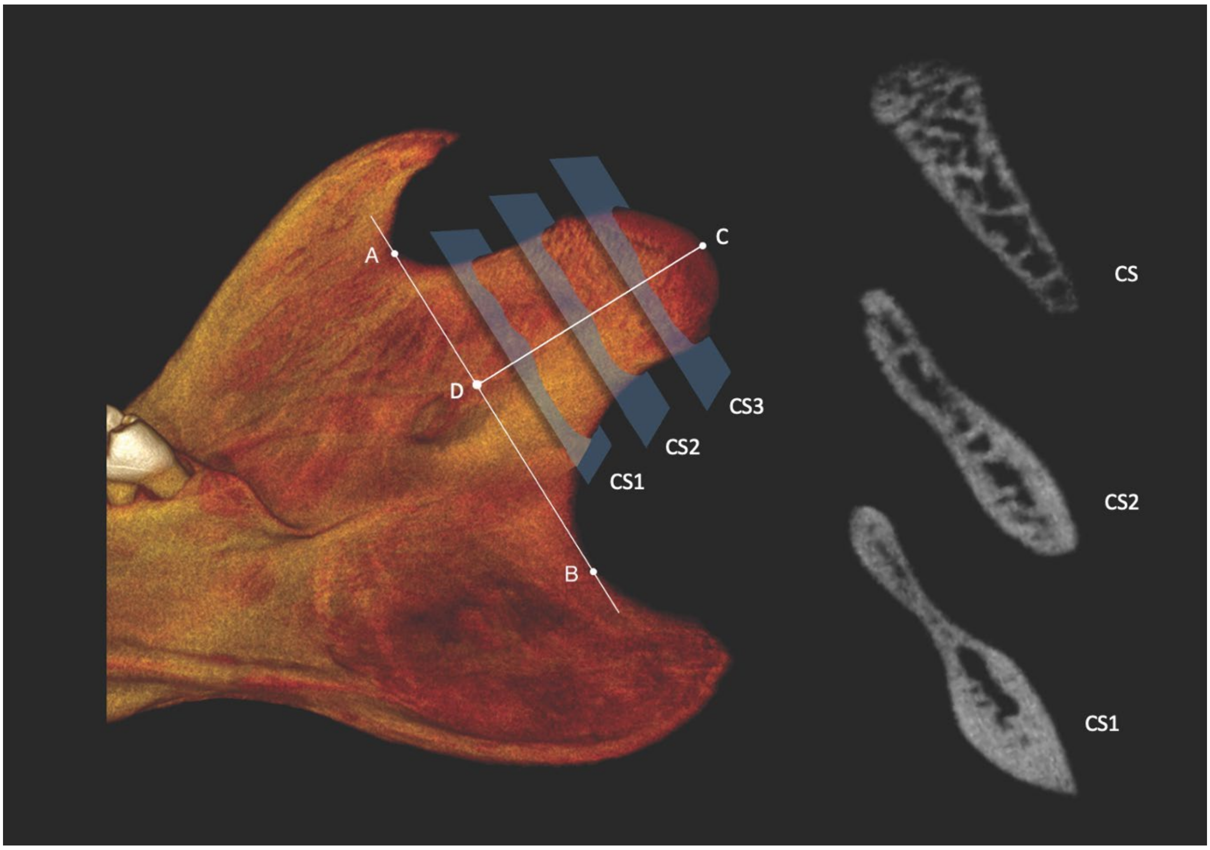

Figure 1. A: deepest point in the coronoid-condylar notch; B: deepest point in the condylar-angular notch; Line A-B: Condylar base width; C: highest point of the condylar head; D: intersection point between the condylar base width and the perpendicular line to it passing through C point; Line C-D: Condylar process height; CS: Cross-sectional surface; CS1, CS2, CS3: example of three condylar process slices parallel to the condylar base used to calculate the average condylar surface.

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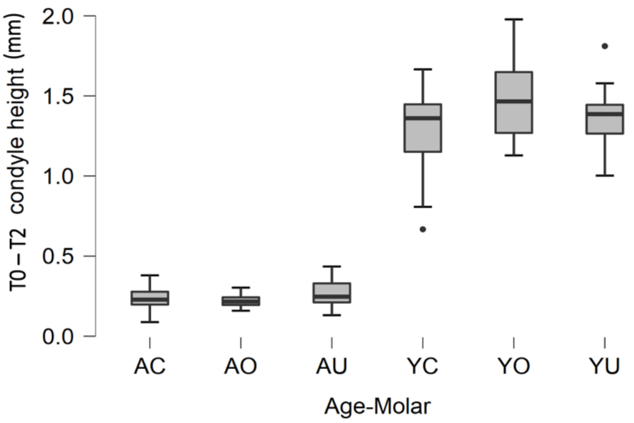
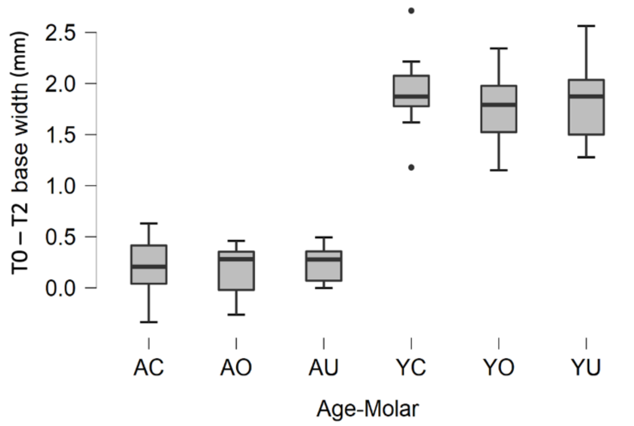
Figure 2. Adult and young condylar measurements growth between T0 and T2.

(a) Measurements showing the growth of condylar process heights



(b) Measurements showing the growth of condylar base widths



(c) Measurements showing the growth of condylar average surface

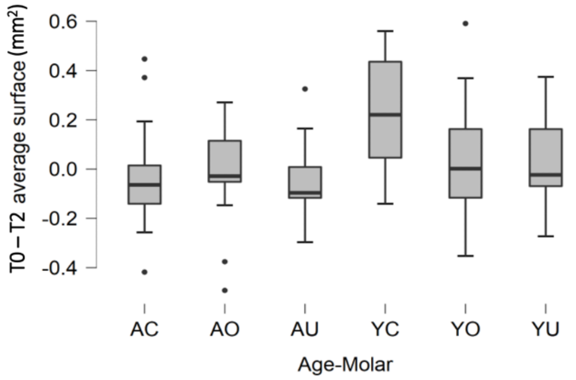


Figure 2. AC: Adult control; AO: Adult overloaded; AU: Adult unopposed; YC: Young control; YO: Young overloaded; YU: Young unopposed; Condylar process height (mm); Condylar base width (mm); Condylar average surface (mm²).