2020-2021

# THÈSE

pour le

## DIPLÔME D'ÉTAT DE DOCTEUR EN MÉDECINE

**Qualification en Chirurgie Maxillo Faciale**

# Repeated unilateral injections of botulinum toxin in masticatory muscles in adult rats do not amplify condylar and alveolar bone loss nor modify the volume of the hypertrophic bone proliferation at enthesis

Les injections unilatérales et répétées de toxine botulique dans les muscles masticateurs de rats adultes n'amplifient pas la perte osseuse condylienne et alvéolaire et ne modifient pas le volume de la prolifération osseuse hypertrophique à l'enthèse.


**DECHAUFOUR Pierre**

Né le 13 décembre 1993 à Caen (14)


Sous la direction du Dr Jean Daniel Kün Darbois

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## Liste des abréviations

| | |
|---|---|
| BTX | Botulinum toxin |
| BV/TV | Trabecular bone volume/Total bone volume |
| Ct.Th | Cartilage thickness |
| En.Ct.Th | Enthesis cortical thickness |
| En. Th | Enthesis thickness |
| En. Vol | Enthesis volume |
| Micro CT | Microcomputed tomography |
| P-En. Ct. Th | Post enthesis cortical thickness |
| MCC | Mandibular condylar cartilage |
| SEM | Standard error of the mean |
| SD | Standard déviation |
| SNAP 25 | Synaptosomal associated protein 25kDa |
| TMJ | Temporo mandibular joint |
| UA | Uranyl acetate |
| VOI | Volume of interest |

# SUMMARY

# Repeated unilateral injections of botulinum toxin in masticatory muscles in adult rats do not amplify condylar and alveolar bone loss nor modify the volume of the hypertrophic bone proliferation at enthesis

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# INTRODUCTION

Botulinum toxin (BTX) is a bacterial metalloprotease produced by an anaerobic bacterium, *Clostridium Botulinum*. There are 7 serotypes of BTX (A to G), but only serotypes A and B are used in clinical practice. The most common serotype is the A. This neurotoxin is a specific ligand of the presynaptic membrane of neurons of the neuromuscular junction. It specifically degrades the SNAP-25 complex of proteins which is necessary for the release of acetylcholine at the axon end. In consequence, acetylcholine is not released from the presynaptic membrane and does not bind to postsynaptic neuromuscular receptors preventing the neurotransmission (1-3). It then leads to a skeletal muscle paralysis and atrophy which are fully reversible in a few month (4).

The use of BTX in orofacial clinical practice is now well established. It was discovered in the 19th century and was first used, as a therapeutic application, in 1977 in extra-ocular muscles injections for strabismus treatment (5). BTX is widely used nowadays to treat several diseases with muscular dysfunction such as bruxism, temporo-mandibular joint disorder, orofacial dystonia or myofacial pain (6, 7). It is also widely used for aesthetic purpose such as the management of facial wrinkles, brow ptosis, excessive gingival display, masseteric hypertrophy, platysmal banding, facial nerve paralysis, hypertrophic scars and keloids (8). A recent review supports the efficacy and safety of facial BTX injections in clinical practice (9). However, a few studies report mandibular bone consequences, such as a decreased bone density, in human after single or repetitive BTX injections in masticatory muscles (10, 11).

Bone remodeling is a balance between bone resorption and bone formation. Osteocytes are intraosseous cells that are sensitive to mechanical stimuli. They play a major role in the initiation and control of bone remodeling and bone homeostasis (12, 13). According to Moss functional matrix theory, muscle contractions are necessary for bone homeostasis and a muscle hypofunction leads to bone alterations such as osteopenia (14). It has been shown previously, in animal models, that intramuscular BTX injections lead to long bones

osteopenia (15, 16). It was also shown that single BTX injection in masticatory muscles induce mandibular bone loss at alveolar bone and condyle (16-19).

The occurrence of a bone hypertrophic metaplasia at the enthesis of *Mus. Digastricus* has also been showed to be linked to BTX injections in masticatory muscles of rats (18). Some cortical bone modifications of the *Mus. Digastricus* enthesis has also been previously reported in human (11). To our knowledge, this fact has not been investigated by any other author to date and the effects of repeated injections on this bone proliferation have thus not been analyzed. Repeated injections are currently performed in human clinical practice (20). In consequence, it would be of great interest to investigate the effects of such repeated BTX injections on that animal model.

The aim of the present study was to evaluate mandibular bone changes, especially on this bone proliferation, after unilateral repeated injections of BTX in temporal and masseter muscles in adult rats at 1, 2 and 3 months, using microcomputed tomography (microCT) to perform 2D and 3D analyses.

# MATERIAL AND METHODS

## Animals and experimental procedure

Eighteen weeks-old male Sprague-Dawley rats (n=24), weighing 564 ± 46 g, were used for the study (Janvier-Labs, Le Genest-Saint-Isle, France). They were acclimated for two weeks to the local vivarium conditions (24 °C and 12 h/12 h light dark cycle) where they were given standard laboratory food (UAR, Villemoison-sur-Orge, France) and water *ad libitum*.

Rats were randomized into 3 groups. Rats of the group 1 received a single BTX injection (Group 1, n=8), rats of the group 2 received two BTX injections (Group 2, n=8) and rats of the group 3 received three BTX injections (Group 3, n=8). Each injection was performed with a 4 weeks period after the prior injection. To ensure comparability among each group,

all rats were the same age at the first injection. Left sides were considered as a control side since no injections were performed there.

BTX injections were performed in each *Mus. Temporalis* and *Mus. Masseter* of the right side only. Each injection procedure was performed as follow: rats were anesthetized with isoflurane and received unilateral injection of type A BTX, 1UI for each muscle (Botox®, Allergan Inc., Irvine, CA, USA); three points of injections for each *Mus. Masseter* and two for each *Mus. Temporalis* were necessary as previously described (18, 19) (**Fig. 1**).

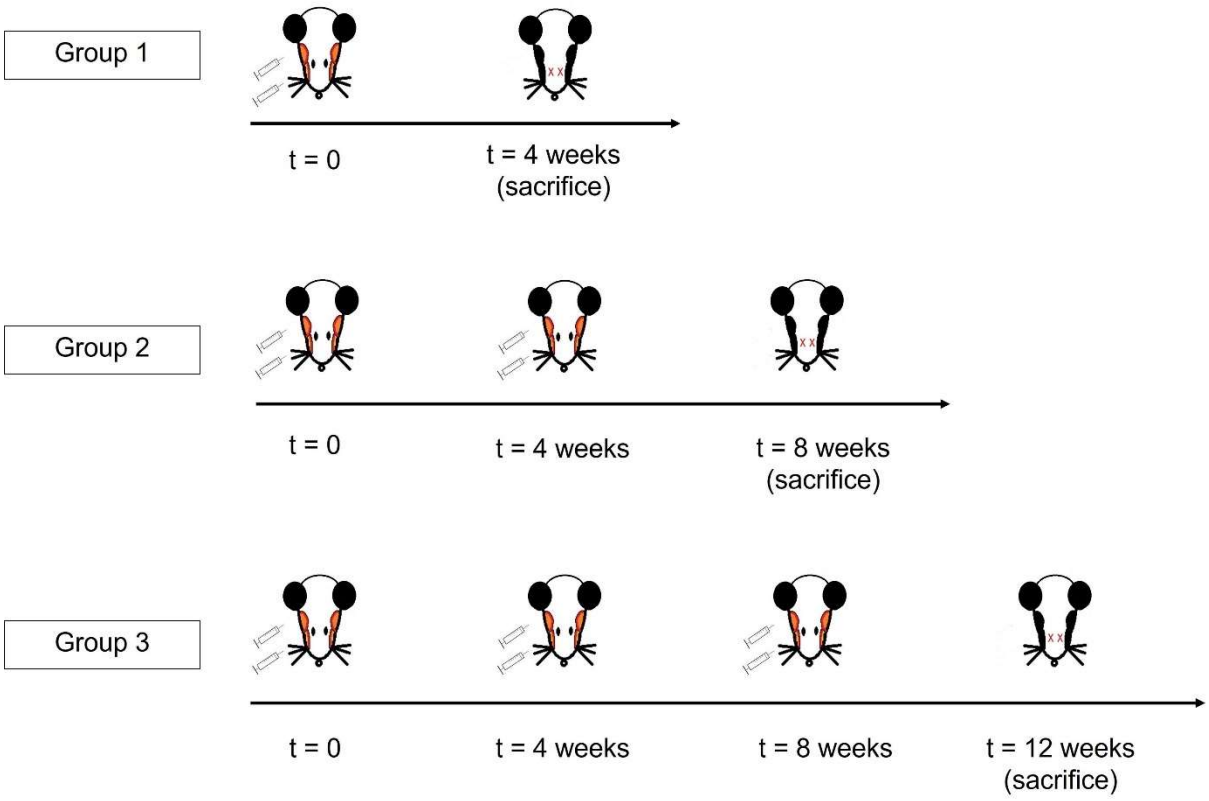


**Fig. 1.** Chart of the study.

Rats were weighed weekly and were sacrificed 4 weeks after their last injection of BTX. Facial skin was carefully dissected and removed to perform visual examination of right and left masticatory muscles. Hemimandibles were then dissected, defleshed and fixed in 10% formalin until use.

Animal care and experimental protocols were approved by the French Ministry of Research and were done in accordance with the institutional guidelines of the French Ethical

Committee (protocol agreement number 01732.01) and under the supervision of authorized investigators.

## Cartilage contrasting procedure

In order to allow articular cartilage analysis, a contrasting method was used as previously described (19). Briefly, condylar processes were separated from the rest of each hemimandible and immersed for 24 hours in uranyl acetate (UA). UA (Merck) was prepared as a 3% solution in 50° ethanol; the solution was filtered on a 0.2 µm syringe filter and stored at 4°C in the dark. The "stained" specimen was then rinsed during one hour in running tap water and transferred in formalin.

## Microcomputed tomography (microCT)

MicroCT of right and left hemimandibles was performed using a Skyscan 1172 X-ray computerized microtomograph (Bruker microCT, Kontich, Belgium) equipped with an X-ray tube working at 70 kV/ 100 µA. Bones were placed in plastic tubes filled with water to prevent desiccation. The tubes were fixed on a brass stub with plasticine. Analysis was done with a pixel size corresponding to 10.5 µm; the rotation step was fixed at 0.25° with a 0.5 mm aluminum filter. For each hemimandible, a stack of 2D-sections was obtained and reconstructed using Dataviewer software (Bruker) and analyzed using CTan software (Skyscan, release 1.13.11.0).

*2.1 Alveolar and condylar bone measurements*

To insure the quality and reproducibility of 3D measurements, reorientation of the alveolar and condylar sections was performed using Dataviewer software. Regions of interest for measurements were then manually selected on several 2D sections in order to create a 3D volume of interest (VOI) using CTan. At least ten sections were manually selected for the alveolar bone. Anterior limit was the first image where first molar roots and crown were

visible. Posterior limit was the last image where third molar crown and roots were observed. Teeth roots, alveolar canal and cortical bone were not included in the VOI which comprised only trabecular bone (**Fig. 2A**). For condylar bone, height sections were required. Anterior limit was the first image of trabecular bone visible when the posterior limit was the last image of trabecular bone visible. Cortical bone and subchondral bone were not included in the VOI (**Fig. 2B**). BV/TV was obtained with 3D measurements using CTan. According to guidelines for assessment of bone microstructure in rodents using MicroCT, trabecular bone volume (BV/TV) is the fraction of the VOI occupied by trabecular bone, expressed in % (21).

*2.2 Condylar cartilage thickness measurements*

Condylar cartilage thickness (Ct.Th) measurement was performed on 2D sagittal sections obtained by using the cutting plane facility of the CTan sowtware. For each condyle, 12 measurement points were used i.e. 4 measurements on 3 different frontal sections were used. The first frontal section used was the most median section where cartilage thickness seemed the thickest. The other two sections were made 125 images in front and behind this first frontal section. These measurements were averaged and expressed in µm for each condyle. Articular cartilage was considered as the area comprised between the tide-mark and the external surface (**Fig. 2C**).

*2.3 Enthesis hypertrophic bone proliferation measurements*

The occurrence of a hypertrophic bone proliferation of the *Mus Digastricus* enthesis, on right sides of BTX injected animals, was expected as they had been previously described in this animal model (18). 2D and 3D measurements of those bone proliferations were performed for each hemimandible using CTan software. The following parameters were measured:

- enthesis volume (Enth.Vol. in $mm^3$), by manually selecting the volume of interest from the first image of visible bone hypertrophy to its last image.

- enthesis thickness (Enth.Th. in µm), which was defined as the longest height of each enthesis.
- enthesis cortical thickness (Enth.Ct.Th. in µm), which was defined as the thickness of underlying cortical bone.
- post enthesis cortical thickness (Post-Enth.Ct.Th in µm), which was defined as the thickness of underlying cortical bone measured at the first distal section without visible bone proliferation (**Fig. 2D-E**).

On left sides, where no bone proliferations were expected, two measurements were performed on the corresponding sections using anatomical reference points such as teeth roots, for equivalent enthesis cortical thickness and equivalent post enthesis cortical thickness.

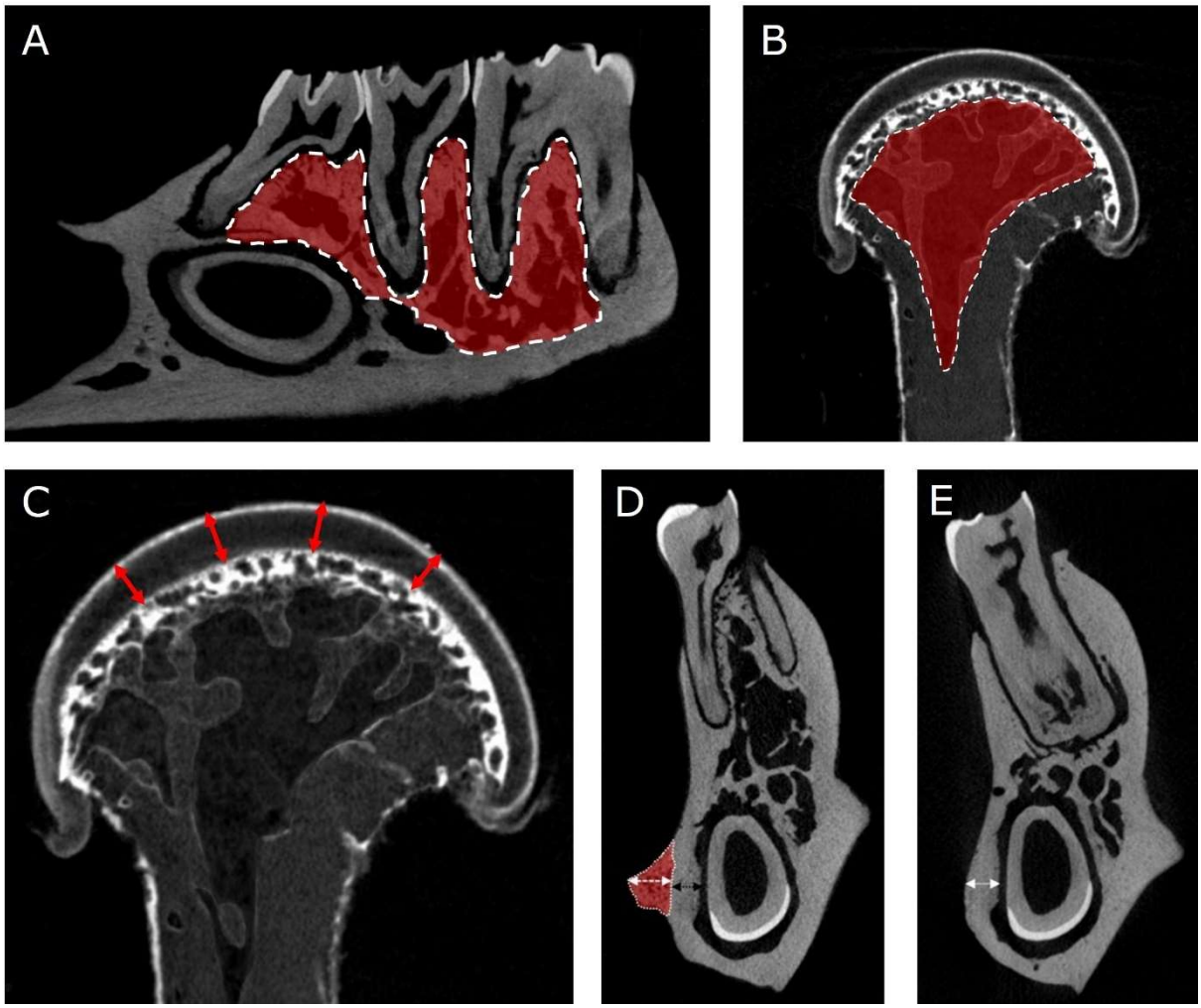


**Fig. 2.** MicroCT analysis, **A)** View of a region of interest used for 3D alveolar measurement of BV/TV. **B)** View of a region of interest used for 3D condylar measurement of BV/TV. **C)** View of a frontal section of right condylar process showing the distribution of the 4 points used for cartilage thickness (*Ct.Th*) measurement. **D)** View of a frontal section of a right hemimandible showing enthesis measurements: enthesis volume (*Enth.Vol*.) (outlined are), enthesis thickness (*En.Th*.) (white arrow), enthesis cortical thickness (*Enth.Ct.Th*.) (black arrow). **E)** View of a frontal section of a right hemimandible showing post enthesis cortical thickness (*Post-Enth.Ct.Th*) measurement.

## Statistical analysis

Statistical analysis was performed using the Systat statistical software release 13.0 (Systat Software Inc., San Jose, CA). All microCT measurements were expressed as mean ± standard deviation (SD). Body weight was expressed as mean ± standard error of the mean (SEM). Differences among groups were analyzed by a non-parametric ANOVA (Kruskall-Wallis) and between groups by the Mann and Whitney's *U* test. Data from right

and left hemimandibles were compared using a paired *t*-test. Differences were considered significant when $p < 0.05$.

# RESULTS

## Body weight and anatomic muscle examination

No significant difference in body weight was evidenced within each group during the course of the study except between group 1 and group 3, 4 weeks after the first injections of BTX (rats weighing respectively 559 g and 617g $p<0.035$) (**Fig.3**).

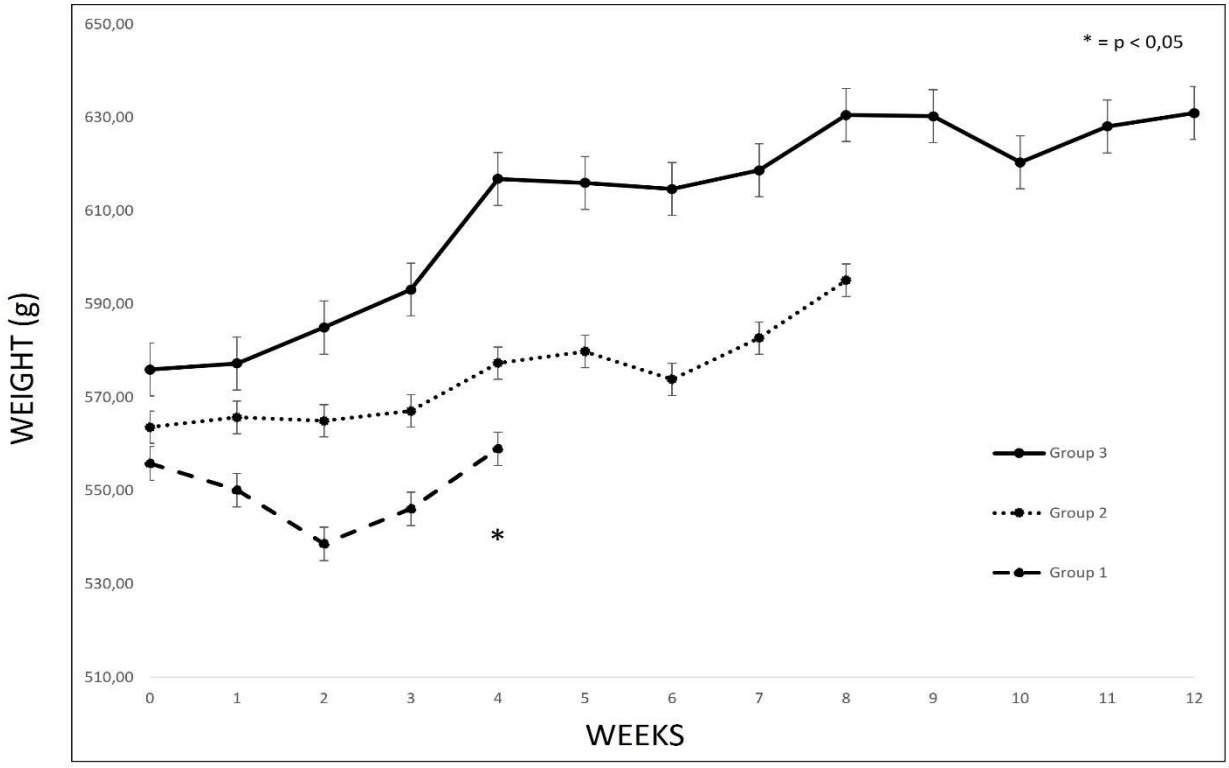


**Fig. 3.** Evolution of body weight with significant differences between group 1 and group 3 at four weeks. Data expressed in mean with standard error of the mean (SEM).

Visual examination during dissection revealed that all rats of all groups presented a *Mus. Masseter* and *Mus. Temporalis* amyotrophy at the right side (**Fig. 4A-B**).

One rat of group 3 was sacrificed 8 weeks after the beginning of the study. He had started to lose weight 2 weeks before and was sacrificed for ethical purpose since no weight regain had been observed. Post sacrifice examination revealed severely overgrown superior incisors (**Fig. 4C**).

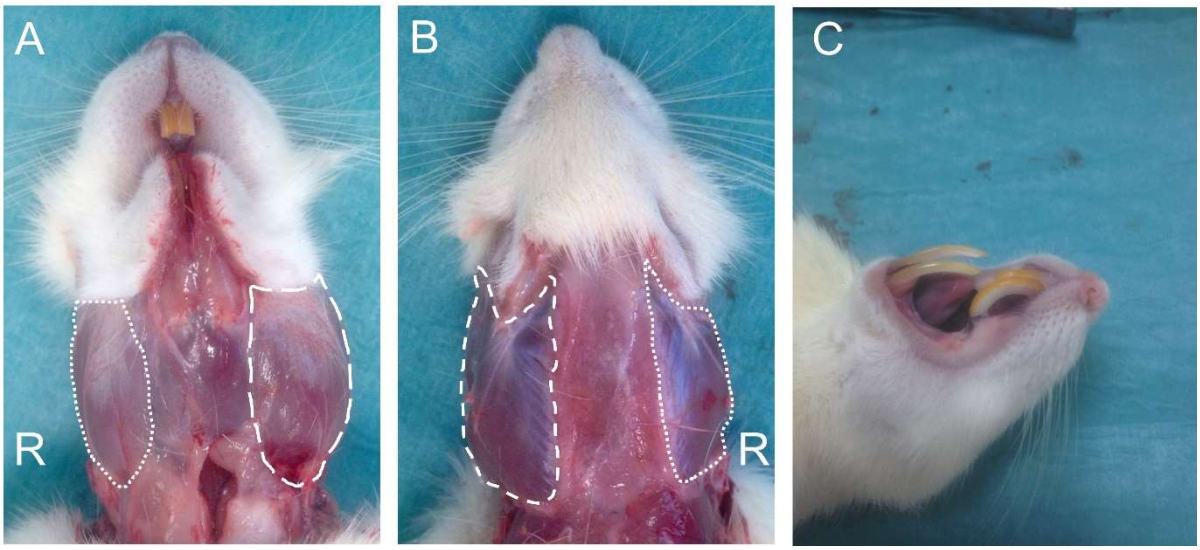


**Fig. 4.** Macroscopic aspect of muscles after BTX injections; **A)** right *Mus. Masseter* (white dotted line) and left *Mus. Masseter* (white dash line); **B)** right *Mus. Temporalis* (white dotted line) and left *Mus. Temporalis* (white dash line); **C)** Overgrown superior incisors on sacrificed rat.

## MicroCT analysis of alveolar and condylar bone measurements

All microCT measurements are summarized in **Table I.** Bone loss was evidenced on the right sides of alveolar and condylar bone. Alveolar BV/TV was found with an average -8% reduction at the right sides during the all study i.e. -6%, - 9% and -10% for group 1, 2 and 3 respectively (**Fig. 5A**). Condylar BV/TV was found with an average -30% reduction at the right sides during the all study (**Fig. 5B**). Interestingly a time-related discrepancy could be observed between condylar and alveolar results (**Fig. 6A-B**). No difference within the same sides between group 1, group 2 and/or group 3 could be observed for condylar measurements whereas a significant increase of right and left alveolar BV/TV occurred between group 1 and group 3.

| | Group 1 left side | Group 1 right side | Group 2 left side | Group 2 right side | Group 3 left side | Group 3 right side |
|---|---|---|---|---|---|---|
| BV/TV Alveolar (%) | 52.0 ± 5.0 [b] | 45.6 ± 5.1 [a, b] | 56.7 ± 4.5 | 48.0 ± 5.3 [a] | 60.7 ± 7.1 | 51.5 ± 6.5 [a] |
| BV/TV Condylar (%) | 66.7 ± 6.3 | 33.5 ± 4.5 [a] | 69.4 ± 5.5 | 40.4 ± 3.6 [a] | 67.6 ± 12.9 | 39.6 ± 6.8 [a] |
| Cartilage thickness (µm) | 161 ± 25 [b] | 194 ± 37 [b, c] | 132 ± 30 | 151 ± 27 | 118 ± 25 | 128 ± 27 |
| Enthesis volume (mm3) | 0 | 0.198 ± 0.21 | 0 | 0.172 ± 0.178 | 0 | 0.211 ± 0.131 |
| Enthesis thickness (µm) | 0 | 284 ± 195 | 0 | 240 ± 188 | 0 | 285 ± 111 |
| Enthesis cortical thickness (µm) | 452 ± 52 | 533 ± 95 | 421 ± 55 | 533 ± 95 [a] | 448 ± 35 | 503 ± 57 |
| Post enthesis cortical Thickness (µm) | 396 ± 57 | 614 ± 111 [a] | 451 ± 72 | 717 ± 103 [a] | 443 ± 55 | 740 ± 60 [a] |

[a] Indicates a significant difference with the left side of the same group, $p < 0.05$
[b] Indicates a significant difference with the same side of the group 3, $p < 0.05$
[c] Indicates a significant difference with the same side of the group 2, $p < 0.05$

**Table I:** Bone morphometric parameters**.** All data were expressed as mean ± standard deviation (SD).

## MicroCT analysis of condylar cartilage thickness measurements

After UA impregnation, articular cartilage was clearly visible. No significant difference could be evidenced when comparing cartilage thickness between the right and left sides at group 1, group 2 and group 3. A trend to a thicker cartilage thickness on right sides (BTX sides), with a decreasing difference over time, was however noticed (**Fig. 6C**). Cartilage thickness significantly decreased with time on both sides (Right side = - 35%, Left side = - 27%)

## MicroCT analysis of enthesis hypertrophic bone proliferation measurements

Hypertrophic bone proliferation at the *Mus. Digastricus* enthesis was only detectable on right sides of BTX injected animals. Enthesis volume and enthesis thickness remained

unchanged with time. It is important to note that enthesis volume and thickness measurements presented high standard errors of the mean. A significantly thicker enthesis cortical thickness was found on the BTX injected sides only in group 2. However, a trend towards thicker enthesis cortical thickness is to note in group 1 and group 3. Post enthesis cortical thickness was found significantly and largely thicker on BTX injected sides throughout the study (**Fig. 5C and 6D**). No other difference could be evidenced between and among each group.

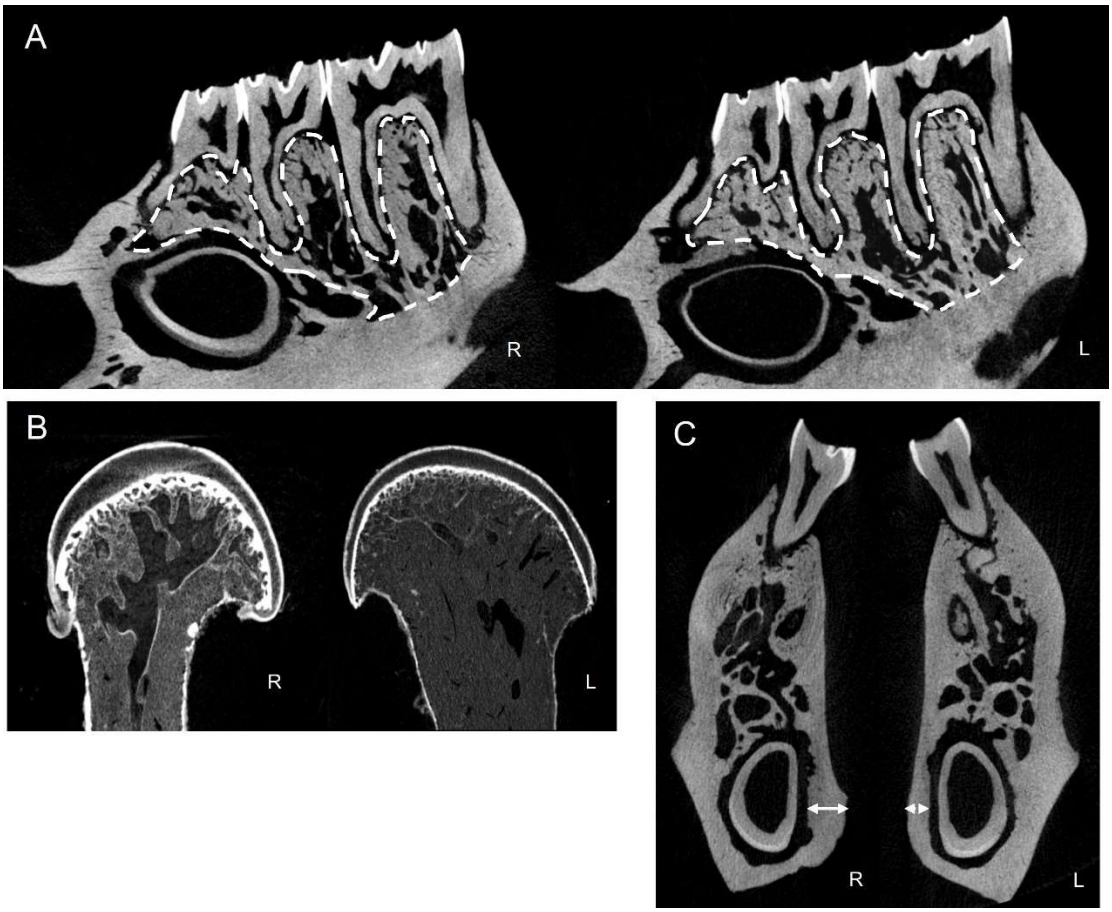


**Fig. 5.** MicroCT analysis as seen on 2D sections; **A)** Sagittal sections of right (R) and left (L) hemimandibles in a rat from the group 1 showing difference at the alveolar bone; **B)** Frontal sections of right (R) and left (L) hemi condylar head from a rat from the group 3 showing a marked trabecular bone loss; **C)** Frontal sections of right (R) and left (L) hemimandibles in a rat from the group 2 at the first distal section without visible enthesis. Note the increasing thickness of the cortical from the right side (white arrow) comparing to left side (white dotted arrow).

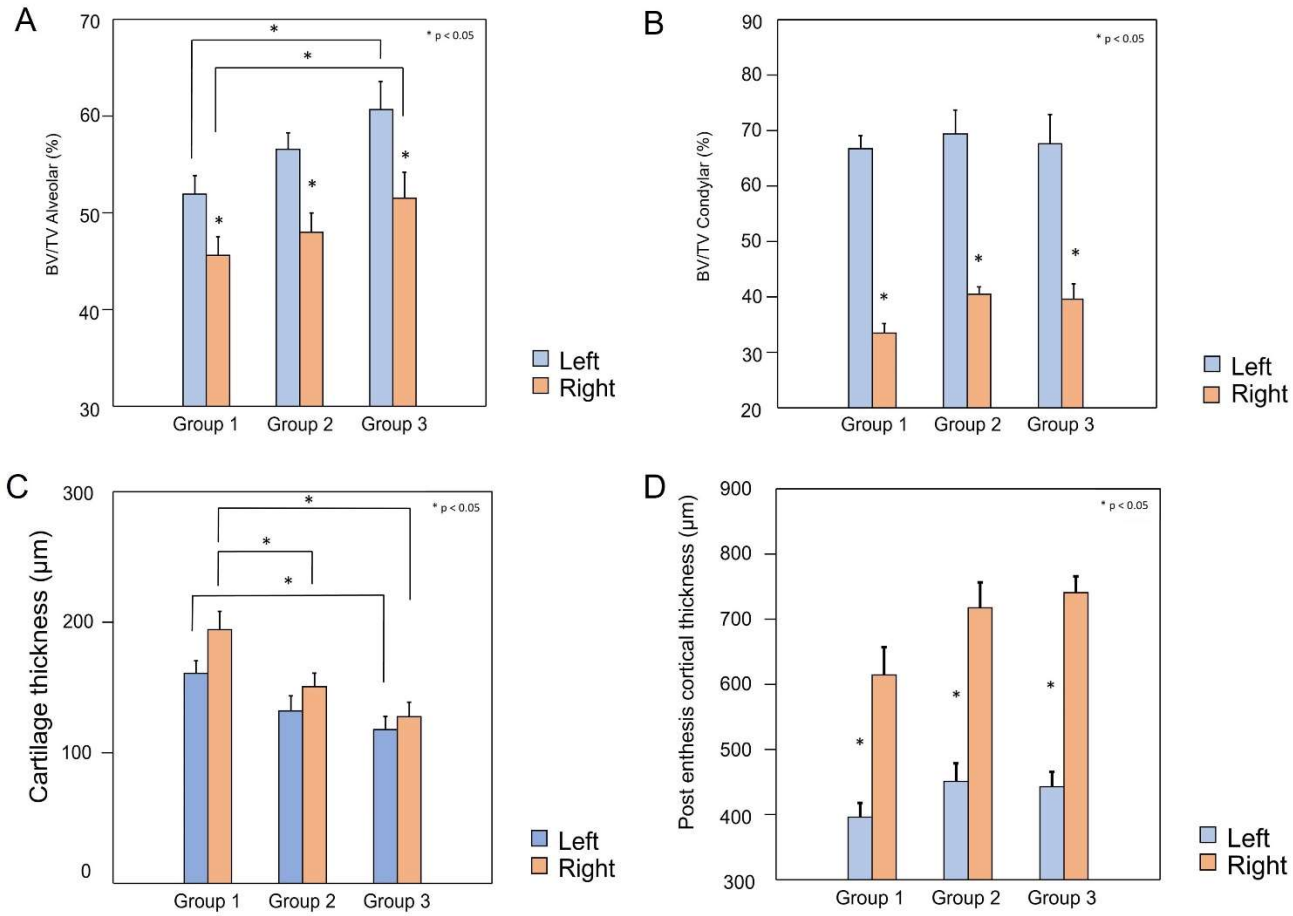


**Fig. 6.** Injected *vs* uninjected side comparisons; **A)** Trabecular bone volume in the alveolar bone (in %) with significant increase of right and left alveolar BV/TV occurred between group 1 and group 3; **B)** Trabecular bone volume in the condylar bone (in %) with a constant -30% reduction at the right sides during the all study; **C)** No significant differences between left side and right side in cartilage thickness, but a significant time related decrease; **D)** Post enthesis cortical thickness was found significantly thicker on BTX injected sides.

# DISCUSSION

Mandibular bone changes after BTX uni or bilateral injections into masticatory muscles in adult and growing animal have been described by a few authors (17). To date only a few studies have analyzed mandibular bone changes in human after single or repeated BTX injections (11, 22, 23). The present study aimed at studying the effects of multiple BTX injections on mandibular bone in animal since it had not been been studied to date.

In our study, the control group was the non injected side (left side). Previous works on comparable animal models didn't show any significant difference between the non-injected and saline injection sides used as control (18, 24, 25).

Rodent incisors grow continuously and must be used regularly to keep them worn down and sharp. If alimentation and the wear of incisors decrease, a vicious circle is established where the animal will starve. Indeed, overgrown incisors that are not used enough may prevent animals from normal gnawing. In our study, one rat was sacrificed because of overgrown incisor. No relevant case was found in the literature after BTX use. We hypothesized that an imbalance in mastication could explain this result.

Muscular loading plays an essential role in maintaining bone volume and density (26). Mechanical forces exerted by the masticatory muscles stimulate the mandibular bone and alveolar process to maintain the underlying bone and teeth state. Alveolar bone mineral density is thus considered as correlated with masticatory function (27).

In the present work, we found a decrease of alveolar trabecular bone density (alveolar BV/TV) on the BTX injected side as reported in many previous studies in adult rabbits or rats (18, 25, 28). To our knowledge alveolar bone loss is not found in only one study of the literature in which *Mus. Temporalis* were not injected and animals were sacrificed early i. e. two weeks after injection (29). Surprisingly, we evidenced an increase of alveolar bone density with time and repeated injections of BTX in BTX and control sides. A time-related increase of alveolar BV/TV in adult rats cannot be excluded, however it has never been

described in the literature. A hypothesis to explain that finding could be that a greater muscle activity occurs in non-injected sides in order to compensate the lack of muscle activity of BTX side, leading to an increased bone density with time in non-injected sides.

A decrease of condylar trabecular bone density (condylar BV/TV) on the BTX injected sides was also evidenced in the present study. Those results are also in line with previous studies (18, 24). We showed a greater decrease of condylar BV/TV when comparing with alveolar BV/TV. This fact is constantly reported in previous studies with same animal models (17, 18, 29, 30). Interestingly, we did not evidence any change with time for condylar BV/TV unlike alveolar BV/TV even if results in adult rats suggest dose dependent deleterious changes in mandibular bone (30). We can hypothesize that this finding could be due to an earlier bone resorption occurring at condyles. This fact seems to be supported by other studies (29). It could be explained by the presence of secondary cartilage with osteoblasts and osteocytes in the subchondral bone (31). In addition, studies on rodent have shown a higher mechanical stress in the condylar region (32). Shi and al showed a decrease of nearly 30% of BV/TV of the trabecular bone in condylar region after one bilateral injections in *Mus. Masseter* of BTX on female rats (33). They demonstrated that condylar degradation resulted from decreased mechanical loading after BTX injection-induced masseter atrophy.

Furthermore, the cartilage thickness showed a decrease after repeated injections of BTX. This suggest a time and dose dependance link. This is consistent with other previous work where triple bilateral injections of BTX into the *Mus. Masseter* led to a greater decrease in cartilage thickness with a reduction in chondrocyte proliferation and differentiation that may explain these results. (34). The mandibular condylar cartilage (MCC) is formed by fibrocartilage and has the unique capacity to adapt to loading changes (35, 36). By using BTX, this loading decrease, due to the decrease of *Mus. Masseter* and *Mus. Temporalis* activity, and result in an increased cells apoptosis in the MCC and subchondral bone (37).

An outstanding consequence was this hypertrophic bone proliferation at an antagonistic muscle enthesis of *Mus. Masseter* and *Mus. Temporalis*. This bone thickening at the digastric fossa was found only on the BTX side. Previous work has hypothesized that this

bone proliferation was the enthesis of the *Mus. Digastricus*, according to this concept that increased muscle activity leads to increased bone remodeling (18, 38, 39). From this perspective, after paralysis of the elevator muscles of the mandible, an increase in the activity of the depressor muscles, such as *Mus. Digastricus*, could correspond to this bone proliferation.

Our results showed a heterogeneous and non-time dependent distribution of enthesis volume and thickness. The thickness of the bone cortical at the muscle insertion followed the same distribution with a consistent aspect in time, despite the repetition of BTX injections. To our knowledge, only one study found this bone proliferation in human, using 3D study of the lingual side of the mandibular symphysis (11).

A relevant finding was this thickening of the enthesis adjacent cortical. As observed below, no time dependance link was found. To date, no study has shown similar results. Recent study on *Mus. Masseter* enthesis indicates that local mechanical environments, as muscle activity, generate differences in enthesis morphology and bone modification (40).

We can hypothesize that increasing muscle activity insertions, as enthesis of a depressor muscle such *Mus. Digastricus* after paralysis of elevator muscles, may lead to an increase of cortical thickness at close to the insertions of this muscle. This remains to be more investigated in future studies, with more interest on the mandibular symphysis bone changes when studying effect of botulinum toxin in human or animal.

# CONCLUSION

The aim of our study was to investigate the mandibular bone changes after repeated injections of BTX. Our results show a decrease of trabecular bone density in alveolar and condylar regions after masticatory muscles paralysis as reported before in previous studies.

However, iterative injections of BTX didn't amplify bone loss compared to single injections. Only cartilage thickness of the condylar head shows a decrease with time and repeated injections.

Concerning the bone proliferation at the enthesis of *Mus. Digastricus*, no time related association was found. It seems to be a variable and heterogeneous distribution, unrelated to the number of BTX injections. It seems to be a consistent and stable increase cortical thickness in regard to the enthesis and adjacent cortical.

This study aimed to get closer to current clinical practice by studying bone changes after repeated injections of BTX.

In addition, hypertrophic bone metaplasia at the insertions of *Mus. Digastricus* should be more considered in future studies when BTX is used in masticatory muscles.

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# DECHAUFOUR Pierre


## Les injections unilatérales et répétées de toxine botulique dans les muscles masticateurs de rats adultes n'amplifient pas la perte osseuse condylienne et alvéolaire et ne modifient pas le volume de la prolifération osseuse hypertrophique à l'enthèse.


RÉSUMÉ

**Introduction** : La toxine botulique (BTX) est une métalloprotéase bactérienne qui entraine une paralysie musculaire. Elle est utilisée chez l'homme, à des fins thérapeutiques, par injections dans les muscles masticateurs. Il a été démontré que cette toxine induisait une perte osseuse mandibulaire au niveau de l'os alvéolaire et du condyle avec l'apparition d'une prolifération osseuse au niveau de l'enthèse du *Mus. Digastricus*, après injection unique de BTX chez l'animal. L'objectif de cette étude était d'évaluer les changements osseux mandibulaires, en particulier sur cette prolifération osseuse, après injections unilatérales répétées de BTX dans les muscles temporaux et masséters chez des rats adultes.

**Matériel et méthodes :** Des rats mâles adultes (n = 24) ont été randomisé en 3 groupes : une injection (groupe 1 ; n = 8), deux injections (groupe 2 ; n = 8) et trois injections (groupe 3 ; n = 8). Chaque injection a été réalisée 4 semaines après l'injection précédente. Chaque rat a reçu des injections de BTX dans le *Mus. Masseter* et *Mus. Temporalis*. Le côté gauche a été considéré comme le côté témoin. Les rats ont été sacrifiés 4 semaines après leur dernière injection. La micro-tomographie (microCT) a été utilisée pour effectuer des analyses 2D et 3D.

**Résultats** : Une perte osseuse a été mise en évidence sur les côtés droits de l'os alvéolaire et condylien. La diminution du volume osseux a atteint - 6 %, - 9 % et - 10 % pour l'os alvéolaire pour les groupes 1, 2 et 3 respectivement et - 30 % pour l'os condylien pour tous les groupes. Après 3 mois, la densité de l'os alvéolaire a augmenté autant du côté gauche non injecté que du côté droit injecté, tandis que la densité de l'os condylien est restée constante dans tous les groupes. L'apparition de cette prolifération osseuse au niveau de l'enthèse du *Mus. Digastricus* a été mise en évidence sur les côtés injectés de BTX. Aucune modification de cette prolifération osseuse n'a été constatée en fonction du nombre d'injections.

**Conclusion :** Les injections répétées de BTX dans les muscles masticateurs entraînent une perte osseuse condylienne et alvéolaire mandibulaire majeure qui n'augmente pas avec le temps. Elles conduisent également à l'apparition d'une prolifération osseuse à l'enthèse du *Mus. Digastricus* qui ne dépend pas du nombre d'injections.



**Mots-clés : Enthèse ; Toxine botulique ; Injections répétées ; Mandibule ; Muscles masticatoires ; Animal ; Perte osseuse**


## Repeated unilateral injections of botulinum toxin in masticatory muscles in adult rats do not amplify condylar and alveolar bone loss nor modify the volume of the hypertrophic bone proliferation at enthesis


ABSTRACT

**Introduction:** Botulinum toxin (BTX) is a bacterial metalloprotease that induces muscle paralysis. It is used in human for therapeutic purpose in masticatory muscles injections. It has been shown that single BTX injections in masticatory muscles in animal induce mandibular bone loss at alveolar bone and condyle with the occurrence of a bone hypertrophic metaplasia at the enthesis of *Mus. Digastricus*. The aim of the present study was to evaluate mandibular bone changes, especially on this bone proliferation, after unilateral repeated injections of BTX in temporal and masseter muscles in adult rats.

**Material and methods:** Mature male rats (n = 24) were randomized into 3 groups: One injection (Group 1; n = 8), two injections (Group 2; n = 8) and three injections (Group 3; n = 8). Each injection was performed 4 weeks after the prior injection. Each rat received BTX injections in right *Mus. Masseter* and *Mus. Temporalis*. Left side was considered as the control side. Rats were sacrificed 4 weeks after their last injection. Microcomputed tomography (microCT) was used to perform 2D and 3D analyses.

**Results:** Bone loss was evidenced on the right sides of alveolar and condylar bone. Decrease in bone volume reached – 6%, -9% and -10% for alveolar bone for group 1,2 and 3 respectively and -30% for condylar bone for all groups. After 3 months, alveolar bone density increased in both control left side and injected right side whereas condylar bone density remained constant in all groups and for both left and right side. The occurrence of a bone hypertrophic metaplasia at the *Mus. Digastricus* enthesis was evidenced on BTX injected sides. No modification of this bone proliferation was noticed in relation with the number of injections.

**Conclusion:** BTX repeated injections in masticatory muscles lead to major mandibular condylar and alveolar bone loss that doesn't increase with time. They also lead to the occurrence of an enthesis bone proliferation that is not dependent on the number of injections



**Keywords: Enthesis; Botulinum toxin; Repeated injections; Mandible; Masticatory muscles; Animal; Bone loss**



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