

Bone changes in the mandible following botulinum neurotoxin injections

Chi-Yang Tsai*, Yi-Ming Shyr*, Wan-Chi Chiu* and Chi-Ming Lee**

*Graduate Institute of Dentistry and **Department of Radiology, Taipei Medical University and Hospital, Taiwan, Republic of China

Correspondence to: Chi-Yang Tsai, College of Oral Medicine, 250 Wu-Hsing Street, Taipei 11042, Taiwan, Republic of China. E-mail: cytsai@tmu.edu.tw

SUMMARY In this study, botulinum neurotoxin type A (BoTx/A) was injected into the temporalis and masseter muscles of growing rats to induce masticatory hypoactivity.

Sixty, 30-day-old, male Long-Evans rats were randomly divided into four groups. BoTx/A was bilaterally injected in the masseter muscles in group I, in the temporalis muscles in group II, and into both the masseter and the temporalis muscles in group III. Group IV served as the control in which saline was bilaterally injected into both muscles. Forty-five days after the injections, the rats were sacrificed. Observation of cortical bone thickness from bone biopsies of the right halves of the mandibles, evaluation of the volume of masseter and temporalis muscles with a plethysmometer, and scanning of bone mineral density (BMD) of the skull and mandibular bone structure with dual-energy X-ray absorptiometry were performed. One-way analysis of variance was employed to analyse measurements of muscle volume, BMD, and cortical bone thickness among the groups. The least square difference was then used to determine significance.

Reduced cortical bone thickness and BMD of the skull and mandibular bone structure were observed. The volumes of the temporalis and masseter muscles injected with BoTx/A were smaller. Masticatory hypofunction affects bone structure during development.

Introduction

Previous animal studies of masticatory hypofunction demonstrated less growth of the mandibular ramus in both the vertical and antero-posterior dimensions. The angular and condylar processes were also dimensionally smaller (Kiliaridis *et al.*, 1985, 1988; Kiliaridis and Shyu, 1988). One of the most common methods to reduce masticatory activities is to alter the dietary consistency in such experiments. Observations of mandibular radiographs from rats fed a soft diet showed reduced bone mass and thinner cortical bone thickness over the condylar process, alveolar process, condylar costa, and lower anterior border of the ramus (Bresin *et al.*, 1999). Recent animal studies also demonstrated anatomic changes and alterations of the craniofacial dimension after an injection of botulinum (Matic *et al.*, 2007; Tsai *et al.*, 2009).

In human studies, resorption of the alveolar process and decreases in bone mineral density (BMD) in the mandibular body region are often seen in patients with impaired masticatory function. This is due to an edentulous alveolar process for an extended period of time. However, it is not clear whether decreased masticatory function affects the bone structure of the mandible (Raadsheer *et al.*, 1996). Alterations in the skeletal structure may affect treatment progress, treatment results, and even stability in patients with growth potential who undergo orthodontic therapy.

The mandibular bone structure is composed mostly of cortical bone. The quantity of trabecular bone varies greatly among individuals (Kiliaridis *et al.*, 1996). Mandibular BMD and cortical bone thickness are correlated with masticatory function and occlusal force. Cortical bone measurements are critical to the scientific study of bone changes (Klemetti *et al.*, 1993).

Morphological changes during craniofacial growth have been investigated in animals using approaches such as altering food consistency and performing a myoectomy, myotomy, or denervation (von Wöern and Stoltze, 1978; Behrens and Johnston, 1984; Bouvier and Hylander, 1984; Kiliaridis *et al.*, 1985; Navarro *et al.*, 1995; Ulgen *et al.*, 1997). However, these methods possess some disadvantages and cannot totally eliminate the effects of tissue damage or nerve injury on bone growth (Kiliaridis *et al.*, 1985; Yamada and Kimmel, 1991).

Morphological changes in skeletal dimensions were demonstrated in previous studies using various methods to reduce muscle activity (Kiliaridis *et al.*, 1985; Mavropoulos *et al.*, 2005; Matic *et al.*, 2007). However, the effects on the internal structure of the craniofacial bone of utilizing a botulinum injection to reduce muscle activity as a minimally invasive approach have not been fully investigated.

The aim of this study was to use botulinum neurotoxin type A (BoTx/A) to induce masticatory hypofunction in growing rats in order to evaluate the effects of muscle

atrophy on bone changes in the mandible. It was hypothesized that an injection of toxin into the masseter and temporalis muscles would decrease the overall BMD of the skull and mandible. The cortical bone thickness in the posterior dental area around muscle attachment and insertion sites should be thinner than that of the control group.

Materials and methods

Ethical approval was obtained from the Institutional Animal Care and Use Committee at Taipei Medical University (IACUC approval no: LAC-96-0059).

Sixty, 30-day-old, male Long-Evans rats, with an average body weight of approximately 120 g, were used in this study. The rats were randomly assigned to four equal groups of 15. According to a pilot study, a sample size of fewer than 10 rats per group would result in lower statistical accuracy. BoTx/A (Botox®, Allergan Pharmaceuticals, Dublin, Ireland) was used in this research. Using pilot study data, a 0.9 per cent saline solution was used to dilute the BoTx/A to 25 U/ml. The rat muscles were injected with 2.5 ml of this solution. The injection contained 1.0 U BoTx/A.

Procedure

Under general anaesthesia, the rats were treated according to their random assignment to the following groups:

- Group I: BoTx/A was injected bilaterally into the masseter muscles, while the temporalis muscles received sterile saline;
- Group II: BoTx/A was injected bilaterally into the temporalis muscles, while the masseter muscles received sterile saline injections;
- Group III: BoTx/A was injected bilaterally into both the masseter and the temporalis muscles;
- Group IV (control): sterile saline was bilaterally injected into both muscles.

The masseter and temporalis muscle injections were performed by the same operator (W-CC), and all rats were kept in the same environment for 45 days. The rats were then killed and perfused, and the craniofacial region was carefully defleshed.

Measurements

Body weight: Each rat was weighed weekly to evaluate overall growth.

Muscle volume: After dissection of the masticatory muscles, the volumes of the right and left temporalis and masseter muscle specimens were independently measured with a plethysmometer (Diagnostic & Research Instruments, Taipei, Taiwan). A plethysmometer measures the volume of small objects by application

of the water displacement method. When a muscle specimen is immersed, the liquid surface in the measuring cylinder is displaced to a higher level, and the volume of the object is detected by the minute change in the electrical resistance of the liquid. Distilled water was used in the experiments, which was replaced after every measurement.

BMD: Dried skulls and mandibles were prepared after the soft tissue had been removed. BMD values of the skull and the mandible were obtained by dual-energy X-ray absorptiometry (DEXA, model: Eclipse, Norland Medical System, Fort Atkinson, Wisconsin, USA). DEXA was calibrated to ensure accuracy and precision with QC Phantom Scans. The scan mode was set for small objects with a resolution at 0.5×0.5 mm and the scan speed to 45 mm/second.

Cortical bone thickness: The right halves of the mandible were decalcified and dehydrated for observation of cortical bone thickness. The methods and references of incisions are described and illustrated in Figure 1. A thickness of 6 μ m per specimen was used, and three slices were prepared for each selected incision. Four sites at lines A and B were observed. The definition of each observation site is given in Figures 2 and 3.

The specimens were observed under an optical microscope (Zeiss, AxioScope 2, Jena, Germany) at $\times 10$ magnification. Digital photographs (Zeiss, AxioCam) of each observation site were taken, and the cortical bone thickness was analysed with graphic software (Zeiss, AxioVision).

Statistical analysis

One-way analysis of variance (ANOVA) was employed to analyse measurements of muscle volume, BMD of the skull and mandible, and cortical bone thickness at selected areas among the groups. *Post hoc* comparisons with the least significant difference identified significant variations and rankings. Descriptive statistics are presented as the mean \pm standard deviation (SD).

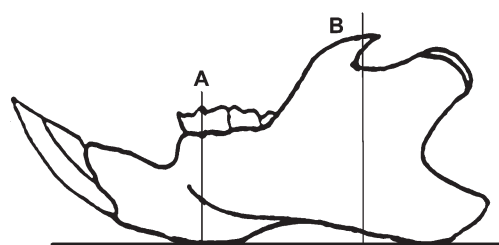


Figure 1 Incision lines for cortical bone thickness measurements. (A) A perpendicular line from the central cusp of the first molar; (B) a perpendicular line from the most-superior point of the coronoid process to the horizontal reference line; a line connecting the lowest point of incisal alveolar process and the lowest point of the angular process of the mandible.

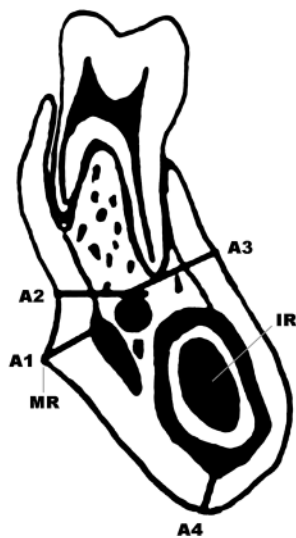


Figure 2 Definition of observation sites at incision A. A1, the most prominent point of the masseteric ridge (MR) perpendicular to the inner cortical surface; A2, a tangent line of the upper border of the mandibular canal perpendicular to the outer cortical surface on the lateral side; A3, a tangent line of the upper border of the mandibular canal perpendicular to the outer cortical surface on the medial side; A4, the inferior most point of the lower mandibular border perpendicular to the inner cortical surface; IR, incisor root.

Results

Method error

The experimental error was calculated using the formula $SE^2 = \sum d^2 / 2n$ (Dahlberg and Lander, 1948). Ten observation sites for the muscle volume, BMD, and cortical bone thickness were randomly selected and measured twice by the same operator (Y-MS). The experimental error was 0.024 ml for muscle volume, 0.0023 g/cm² for BMD, and 0.25 μ m for cortical bone thickness.

Body weight

The average (\pm SD) weight gain over the 45 days was 276.46 (\pm 41.06) g for group I, 274.54 (\pm 35.36) g for group II, 263.67 (\pm 36.48) g for group III, and 265.12 (\pm 27.05) g for group IV. There was no evidence of growth deficits and no statistical differences among the groups.

Volumes of the temporalis and masseter muscles

The lowest significant volumetric measurement of the masseter muscle was found in group III in which both the masseter and the temporalis muscles were injected with Botox (Tables 1A and 1B).

The lowest temporalis muscle volumes were observed in groups II and III ($P < 0.001$). The percentage difference compared with the control group (IV) was calculated for the results that showed significance, i.e., the volume of group III was 34.02 per cent less than that of the control, while the

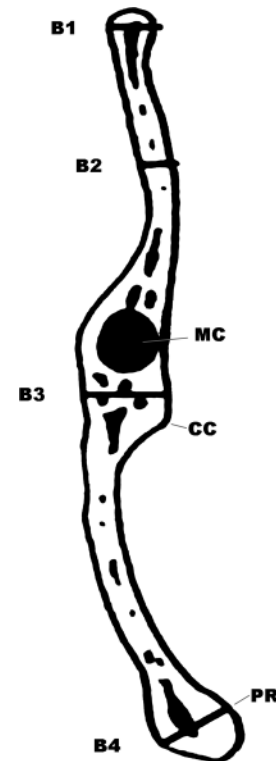


Figure 3 Definition of observation sites at incision B. B1, a line connecting the most prominent points of the lateral and medial surfaces of the coronoid process; B2, the midpoint between the top of the coronoid process and the upper border of the mandibular canal (MC), perpendicular to the lateral and medial bone surface; B3, the midpoint between the lower border of the MC and condylar costa (CC), perpendicular to the lateral and medial bone surface; B4, a line connecting the most prominent point of the pterygoid ridge (PR) on the medial to the most prominent point of the lower mandibular border on the lateral bone surface.

temporalis muscle volume was 31.78 per cent less in group II and 47.20 per cent less in group III.

BMD values of the skull and mandible

Group III had significantly lower BMDs (Table 2A). The percentage differences between groups III and IV for both the skull and the mandible were calculated and are shown in Table 2B. The BMD of the skull and mandible in group III was 6.21 and 5.71 per cent less than that of group IV, respectively.

Cortical bone thickness

Cortical bone thickness at A1, A2, and A4 of groups I, II, and III were significantly less than those of group IV (Tables 3A and 3B). Similarly, the thickness at B1, B3, and B4 of groups I, II, and III was significantly less (Tables 4A and 4B).

Discussion

BoTx/A was used in this study rather than other invasive methods such as myoectomy or surgical denervation. The

Table 1A Statistical analysis of muscle volume. Group I: BoTx/A in the masseter and saline in the temporalis muscle; group II: BoTx/A in the temporalis and saline in the masseter muscle; group III: BoTx/A in both the masseter and the temporalis muscles; group IV: saline in both the masseter and the temporalis muscles (asterisks indicate significant results compared with group IV).

	Group I		Group II		Group III		Group IV		Analysis of variance
	Mean	Standard deviation (SD)	Mean	SD	Mean	SD	Mean	SD	
Masseter right	0.624	0.138	0.641	0.006	0.441***	0.008	0.688	0.010	$F = 12.996^{***}$; I, II, IV > III
Masseter left	0.633	0.139	0.649	0.007	0.458***	0.007	0.675	0.007	$F = 12.014^{***}$; I, II, IV > III
Temporalis right	0.242	0.007	0.191***	0.006	0.152***	0.004	0.276	0.003	$F = 11.635^{***}$; I > II, III; IV > II, III
Temporalis left	0.252	0.006	0.193***	0.005	0.145***	0.003	0.287	0.004	$F = 17.330^{***}$; I > II, III; IV > II, III

*** $P < 0.001$.

Table 1B Percentage muscle volume differences compared with group IV.

	I-IV%	II-IV%	III-IV%
Temporalis mean	NS	NS	34.02
Masseter mean	NS	31.78	47.20

NS, not significant.

reason for this was because the invasive methods might damage peripheral tissues and cause undesirable dysfunction, e.g., scar tissue might decrease blood flow and denervation might affect the muscles adjacent to the target muscle (Zucman, 1960; Gardner *et al.*, 1980). Alteration of food consistency is another method which is assumed to reduce masticatory function in animals that are fed a soft diet (Kiliaridis *et al.*, 1988). However, quantitative evidence between diet consistency and specific muscle function is difficult to obtain. Moreover, changing food consistency is not practical during clinical use.

BoTx/A acts on a specific muscle without undesirable side-effects. It blocks the action potential transmission in neuromuscular junctions by inhibiting acetylcholine release. The nerves and muscle structures are not damaged by the toxin, and function is restored after neutralization of the toxin (de Paiva *et al.*, 1999).

BoTx/A paralyses the target muscle for a duration of 4–6 weeks in rodents (Filippi *et al.*, 1993; Ma *et al.*, 2004). In the present study, BoTx/A was given to rats 30 days after birth. Rats undergo puberty 35 days after birth and attain maturity at approximately 60 days. The toxin was given before the growth spurt in order to observe the effects of the drug on muscle and skeletal development.

Bilateral injections were used to avoid compensatory growth effects that occurred when using unilateral injections in a pilot study. With unilateral injections of BoTx/A as the toxin-injected side had to function alongside the saline-

injected side the desired differences in masticatory functions might not be apparent.

Overall body weight

Overall growth of the rats was not altered after local injections of BoTx/A into the masticatory muscles. Reduced masticatory function by the toxin did not alter the systemic functions of the rats. These results are in agreement with the rabbit study of Matic *et al.* (2007) and the rat study of Tsai *et al.* (2009) in which overall growth was not affected by regional injections of BoTx/A.

Muscle volume

Measurements of muscle volume for both sides showed no significant difference, indicating that manual operating errors were small.

The masseter muscles in group III showed the smallest volumetric measurements, 28.49 per cent less than those of group I. The same observation was found for temporalis muscle volume which was 22.64 per cent less than that of group II. The same toxin dose was incorporated in every muscle. The ‘additive effect’ was generated when muscles with the same function were medicated. Both the temporalis and masseter muscles control the function of mouth closing; therefore, the greatest changes were found in group III, with toxin injections in both muscles.

The temporalis muscles showed a greater reduction in volumetric measurements than the masseter muscles, as seen in the comparison of group III with group IV (Table 1B). An apparent decrease in the percentage of temporalis muscle volume was noted. This was due to the anatomically smaller and thinner temporalis muscles being injected with the same dose of BoTx/A.

The findings with regard to muscle volume are similar to those of previous research in which reduced muscle function decreased the thickness and weight of the muscles (Rauch and Hamdy, 2006; Matic *et al.*, 2007).

Table 2A Statistical analysis of bone mineral density (BMD) of the skull and mandible. Group I: BoTx/A in the masseter and saline in the temporalis muscle; group II: BoTx/A in the temporalis and saline in the masseter muscle; group III: BoTx/A in both the masseter and the temporalis muscles; group IV: saline in both the masseter and the temporalis muscles (asterisks indicate significant results compared with group IV).

	Group I		Group II		Group III		Group IV		Analysis of variance
	Mean	Standard deviation (SD)	Mean	SD	Mean	SD	Mean	SD	
Skull BMD	0.168	0.001	0.163	0.001	0.151*	0.001	0.161	0.001	$F = 8.053^{***}$; I, II, IV > III
Mandible BMD	0.139	0.001	0.139	0.001	0.132**	0.001	0.140	0.001	$F = 2.947^*$; I, II, IV > III

* $P < 0.05$; ** $P < 0.01$; *** $P < 0.001$.

Table 2B Percentage differences of bone mineral density (BMD) compared with group IV.

	I~IV%	II~IV%	III~IV%
Skull BMD	NS	NS	6.21
Mandible BMD	NS	NS	5.71

NS, not significant.

BMD of the skull and mandible

Decreases in BMD and bone mineral content in the limb skeletal structure after quadriceps or gastrocnemius muscles were injected with BoTx/A have been reported (Chappard *et al.*, 2001; Rauch and Hamdy, 2006; Warner *et al.*, 2006). Similar results were found in the present study in which a significant decrease in BMD was noted which affected the skull and mandible, especially around the attachment sites of the muscles. The attachment sites for the temporalis muscles are the temporal fossa in the skull and the coronoid process in the mandible. The masseter muscle attaches to the zygomatic process in the skull and to the mandibular angular lower border and mesenteric ridge in the mandible.

Group III showed the smallest BMDs over the skull and mandible. No statistical differences were found between groups I, II, and IV (control group). In groups I and II, the saline injection into the muscles might have caused a compensatory effect on the BoTx/A-injected muscles. As a result, the attachment sites overlying the toxin-injected muscles possessed normal physiological mechanical stimulation and generated normal BMD values. In group III, where both masticatory muscles were treated, function was simultaneously reduced. Significant decreases in skull and mandibular BMD values were observed.

Bresin *et al.* (1999) found a reduction in computed tomography radiographic bone mass and bone density in rats fed a soft diet. They concluded that the reduction in BMD was correlated with muscle attachment sites, which concurred with the current findings. Chappard *et al.* (2001) stated that removing normal mechanical loading will

decrease the mineral content in specific unit cross-sectional areas, thus altering the internal skeletal structure.

Cortical bone thickness

The deep masseter muscle attaches to the masseteric ridge and the area superior to the masseteric ridge in the mandible. The insertion site for the superficial masseter muscle is over the lower border of the mandibular body. A1, A2, and A4 (Figure 2) are mechanical stimulation-bearing areas when the muscle contracts. In toxin-injected muscles, inadequate mechanical stimulation was found during growth; therefore, the physiological deposition of cortical bone decreased. A3 is located in the medial mandibular body where there are no muscle attachments. The cortical bone thickness was insignificantly affected in this area.

The coronoid process is the attachment site for the temporalis muscles. Cortical bone thickness was reduced in groups II and III in which the temporalis muscles were injected with BoTx/A. B4 showed a decrease in cortical thickness overlying the lower border of the mandibular angular process where the masseter muscles attach.

Although toxin-injected masseter and temporalis muscles do not attach to the region where B3 is located, reduced thickness was noted. This finding is in agreement with the conclusions of Bresin *et al.* (1999) that not only direct loading affects bone growth but also indirect loading such as tension or bending forces from the functioning of several muscles might contribute to alterations in skeletal development. Chappard *et al.* (2001) stated that removing normal mechanical loading will decrease the mineral content in the unit cross-sectional area, thus altering the internal skeletal structure.

According to Schönau (1998), the biomechanical use of muscle plays an important role in bone development. Pubertal rats were used in this study, and cortical bone thickness was reduced as a result of masticatory hypofunction after BoTx/A injections. These morphological changes conform to the functional matrix theory (Moss and Rankow, 1968) which states that reduced muscle function is an epigenetic factor in the craniofacial region that affects bone structure, which is assumed to be a non-genetic component, during development (Tsai *et al.*, 2009).

Table 3A Statistical analysis of cortical thickness at incision line A in group I: BoTx/A in the masseter and saline in the temporalis muscle; group II: BoTx/A in the temporalis and saline in the masseter muscle; group III: BoTx/A in both the masseter and the temporalis muscles; group IV: saline in both the masseter and the temporalis muscles.

	Group I		Group II		Group III		Group IV		Analysis of variance
	Mean	Standard deviation (SD)	Mean	SD	Mean	SD	Mean	SD	
A1	18.82	1.31	18.10	1.18	18.44	1.02	22.16	2.55	$F = 9.23^{***}$; I, II, III< IV
A2	9.58	0.74	8.89	0.64	9.55	2.01	11.24	1.81	$F = 3.42^{*}$; I, II, III< IV
A3	10.61	1.05	9.16	1.66	9.25	1.99	11.80	3.74	$F = 2.03$
A4	16.16	2.23	17.06	1.90	16.76	2.20	19.58	3.10	$F = 3.37^{*}$; I, II, III< IV

* $P < 0.05$; *** $P < 0.001$.

Table 3B Percentage differences of cortical bone thickness at A compared with group IV.

	I~IV%	II~IV%	III~IV%
A1	15.07	18.32	16.79
A2	14.77	20.91	15.04
A3	NS	NS	NS
A4	17.47	12.36	14.40

NS, not significant.

BoTx/A was injected into the temporalis and masseter muscles to reduce the masticatory loading of growing rats in this study. The normal systemic growth of the rats was not altered by local injections of the toxin. Muscle paralysis following a BoTx/A injection induced muscular disuse, resulting in muscle atrophy (volumetric reduction) and bone structure alterations (decreases in BMD and cortical bone thickness).

Deep bite patients often exhibit strong masticatory muscles, brachyfacial profiles, hypodivergency with a prominent mandibular border, and even a hyperplastic alveolar housing (Waltimo *et al.*, 1994). It is clinically difficult to open the bite or move teeth along the ridge due to the high muscle tension and dense alveolar structure. According to the result of this study, with the aid of BoTx/A, it is possible to reduce muscle activity and bone density in such patients and so shorten treatment time. When treating patients with excessive bite force such as clenching, bruxism, or hypertrophic masticatory muscles, taking muscle activities into consideration can help clinicians make comprehensive diagnoses and treatment plans to attain ideal treatment results. BoTx/A is now commonly used in cosmetic medicine (Park *et al.*, 2003) and in treating hemifacial spasms, cervical dystonia, and spasticity (Jankovic and Orman, 1987; Jankovic *et al.*, 1990; Grazko *et al.*, 1995). Nevertheless, clinical use of BoTx/A in the dental field has not yet been explored or established. The results of this study indicate that the toxin not only alters muscle activity but also affects the underlying bony structure.

Relationships between quantitative measurements of reduced muscle activity and skeletal modifications were not observed in the present study. Future research that utilizes

electromyography in either animal or human studies is essential to provide quantitative scientific information on the potency of BoTx/A and its influences on bone structures.

Conclusions

BoTx/A was utilized to induce muscle disuse in order to evaluate the influence of masticatory muscle function on the craniofacial bone structure in growing rats. Alterations of craniofacial bone BMD and cortical bone thickness were observed. Toxin-injected muscles showed reduced volumetric measurements compared with the control group. Skull and mandibular BMD values and cortical bone thickness at muscle attachment sites of the mandible showed significant decreases.

Acknowledgement

Special thanks go to Wen-Tien Hsiao, radiologist of the Department of Radiology of Taipei Medical University and Hospital, for his generous assistance and technical support.

References

- Behrents R G, Johnston L E Jr. 1984 The influence of the trigeminal nerve on facial growth and development. *American Journal of Orthodontics* 85: 199–206
- Bouvier M, Hylander W L. 1984 The effect of dietary consistency on gross and histologic morphology in the craniofacial region of young rats. *The American Journal of Anatomy* 170: 117–126
- Bresin A, Kiliaridis S, Strid K G. 1999 Effect of masticatory function on the internal bone structure in the mandible of the growing rat. *European Journal of Oral Sciences* 107: 35–44
- Chappard D, Chennebault A, Moreau M, Legrand E, Audran M, Basle M F. 2001 Texture analysis of X-ray radiographs is a more reliable descriptor of bone loss than mineral content in a rat model of localized disuse induced by the *Clostridium botulinum* toxin. *Bone* 28: 72–79
- Dahlberg G, Lander E. 1948 Size and form of the foot in men. *Acta Genetica et Statistica Medica* 1: 115–162
- de Paiva A, Meunier F A, Molgo J, Aoki K R, Dolly J O. 1999 Functional repair of motor endplates after botulinum neurotoxin type A poisoning: biphasic switch of synaptic activity between nerve sprouts and their parent terminals. *Proceedings of the National Academy of Sciences of the United States of America* 96: 3200–3205
- Filippi G M, Errico P, Santarelli R, Bagolini B, Manni E. 1993 Botulinum A toxin effects on rat jaw muscle spindles. *Acta Otolaryngologica* 113: 400–404

Table 4A Statistical analysis of cortical thickness at incision line B in group I: BoTx/A in the masseter and saline in the temporalis muscle; group II: BoTx/A in the temporalis and saline in the masseter muscle; group III: BoTx/A in both the masseter and the temporalis muscles; group IV: saline in both the masseter and the temporalis muscles.

	Group I		Group II		Group III		Group IV		Analysis of variance
B1	11.67	2.48	9.71	2.00	9.43	1.61	12.52	1.42	$F = 4.28^*$; II, III < IV
B2	6.49	1.66	6.34	0.83	6.03	1.16	6.83	1.82	$F = 0.39$
B3	29.54	2.21	26.81	2.95	24.25	3.76	28.91	1.68	$F = 5.24^{**}$; III < I, IV
B4	19.94	2.15	24.33	4.16	19.05	3.17	26.11	2.26	$F = 8.77^{***}$; I, III < II, IV

* $P < 0.05$; ** $P < 0.01$; *** $P < 0.001$.

Table 4B Percentage differences in cortical bone thickness at B compared with group IV.

	I-IV%	II-IV%	III-IV%
B1	NS	22.44	24.68
B2	NS	NS	NS
B3	NS	NS	16.11
B4	23.63	NS	27.03

NS, not significant.

- Gardner D E, Luschei E S, Joondeph D R 1980 Alterations in the facial skeleton of the guinea pig following a lesion of the trigeminal motor nucleus. *American Journal of Orthodontics* 78: 66–80
- Grazko M A, Polo K B, Jabbari B 1995 Botulinum toxin A for spasticity, muscle spasms, and rigidity. *Neurology* 45: 712–717
- Jankovic J, Orman J 1987 Botulinum A toxin for cranial-cervical dystonia: a double-blind, placebo-controlled study. *Neurology* 37: 616–623
- Jankovic J, Schwartz K, Donovan D T 1990 Botulinum toxin treatment of cranial-cervical dystonia, spasmodic dysphonia, other focal dystonias and hemifacial spasm. *Journal of Neurology, Neurosurgery, and Psychiatry* 53: 633–639
- Kiliaridis S, Shyu B C 1988 Isometric muscle tension generated by masseter stimulation after prolonged alteration of the consistency of the diet fed to growing rats. *Archives of Oral Biology* 33: 467–472
- Kiliaridis S, Engstrom C, Thilander B 1985 The relationship between masticatory function and craniofacial morphology. I. A cephalometric longitudinal analysis in the growing rat fed a soft diet. *European Journal of Orthodontics* 7: 273–283
- Kiliaridis S, Engstrom C, Thilander B 1988 Histochemical analysis of masticatory muscle in the growing rat after prolonged alteration in the consistency of the diet. *Archives of Oral Biology* 33: 187–193
- Kiliaridis S, Bresin A, Holm J, Strid K G 1996 Effects of masticatory muscle function on bone mass in the mandible of the growing rat. *Acta Anatomica (Basel)* 155: 200–205
- Klemetti E, Vainio P, Lassila V, Alhava E 1993 Cortical bone mineral density in the mandible and osteoporosis status in postmenopausal women. *Scandinavian Journal of Dental Research* 101: 219–223
- Ma J *et al.* 2004 Time course of recovery of juvenile skeletal muscle after botulinum toxin A injection: an animal model study. *American Journal of Physical Medicine & Rehabilitation* 83: 774–780

- Matic D B, Yazdani A, Wells R G, Lee T Y, Gan B S 2007 The effects of masseter muscle paralysis on facial bone growth. *Journal of Surgical Research* 139: 243–252
- Mavropoulos A, Ammann P, Bresin A, Kiliaridis S 2005 Masticatory demands induce region-specific changes in mandibular bone density in growing rats. *Angle Orthodontist* 75: 625–630
- Moss M L, Rankow R M 1968 The role of the functional matrix in mandibular growth. *Angle Orthodontist* 38: 95–103
- Navarro M, Delgado E, Monje F 1995 Changes in mandibular rotation after muscular resection. Experimental study in rats. *American Journal of Orthodontics and Dentofacial Orthopedics* 108: 367–379
- Park M Y, Ahn K Y, Jung D S 2003 Botulinum toxin type A treatment for contouring of the lower face. *Dermatologic Surgery* 29: 477–483
- Raadsheer M C, Kiliaridis S, Van Eijden T M, Van Ginkel F C, Prahl-Andersen B 1996 Masseter muscle thickness in growing individuals and its relation to facial morphology. *Archives of Oral Biology* 41: 323–332
- Rauch F, Hamdy R 2006 Effect of a single botulinum toxin injection on bone development in growing rabbits. *Journal of Musculoskeletal & Neuronal Interactions* 6: 264–268
- Schönau E 1998 The development of the skeletal system in children and the influence of muscular strength. *Hormone Research* 49: 27–31
- Tsai C, Chiu W, Liao Y, Tsai C 2009 Effects on craniofacial growth and development of unilateral botulinum neurotoxin injection into the masseter muscle. *American Journal of Orthodontics and Dentofacial Orthopedics* 135: 142.e1–e6
- Ulgen M, Baran S, Kaya H, Karadede I 1997 The influence of the masticatory hypofunction on the craniofacial growth and development in rats. *American Journal of Orthodontics and Dentofacial Orthopedics* 111: 189–198
- von Wowern N, Stoltze K 1978 Histoquantitation on small jaw specimens. *Scandinavian Journal of Dental Research* 86: 193–199
- Waltimo A, Nyström M, Könönen M 1994 Bite force and dentofacial morphology in men with severe dental attrition. *Scandinavian Journal of Dental Research* 102: 92–96
- Warner S E, Sanford D A, Becker B A, Bain S D, Srinivasan S, Gross T S 2006 Botox induced muscle paralysis rapidly degrades bone. *Bone* 38: 257–264
- Yamada K, Kimmel D B 1991 The effect of dietary consistency on bone mass and turnover in the growing rat mandible. *Archives of Oral Biology* 36: 129–138
- Zucman J 1960 Studies on the vascular connections between periosteum, bone, and muscle. *British Journal of Surgery* 48: 324–328