

Perspective Article

Neglecting Bone Health: A Critical Gap in Management of Muscle Spasticity with Botulinum Toxin in Spinal Cord Injury

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Abstract

Neuromuscular inhibitors have been quickly advanced from being used only for aesthetic purposes to being used as a treatment for musculoskeletal pain and muscle spasticity. This phenomenon stems from the diminished force exerted by muscles, which are essential for bone remodeling. In this context, it is hypothesized that botulinum toxin (BTX) might exert a direct influence on bone resorption. Although such treatments have the potential to provide patients with significant relief, bone loss occurring due to elective muscle paralysis has yet to be examined in clinical trials. The disuse model resulting from spinal cord injury, characterized by the absence of ground reaction and muscle forces, provides an ideal context for exploring the skeletal ramifications of intramuscular BTX injection. This approach enables an investigation into the intricate interplay between muscle and bone, encompassing the impact of spasticity on bone preservation, the potential positive and negative outcomes of BTX on bone metabolism, and the involvement of the autonomic nervous system in bone remodeling regulation. This paper presents a narrative review of research findings on the disturbance of the typical balance between muscles and bones caused by acute muscle paralysis from BTX, resulting in osteopenia and bone resorption.

Keywords: Bone, Botulinum Toxin, Spasticity, Spinal Cord Injury

Introduction

Intramuscular administration of botulinum toxin (BTX) exerts its effects by selectively impeding the release of acetylcholine at the neuromuscular junction, originating from presynaptic motor neurons. This inhibitory action

induces muscle paralysis, with BTX demonstrating a great specificity for motor nerve terminals upon muscle injection^{1,2}. These parameters may make it an optimal agent for inducing localized reduction in muscle activity, leading to subsequent muscle atrophy and diminished strength. Although BTX does not damage completely the motor neuron, the extent of recovery from exposure is dependent upon dosage, with limited recovery observed following higher doses. Within 10 days of moderate BTX exposure, recovery processes include dose-dependent neurogenesis, which is the production of new motor endplates via axonal sprouts from the original axon terminal³. Because new motor nerve terminals must form in order to make new neuromuscular connections, the muscle recovery following BTX injection is different from typical patterns of disuse⁴⁻⁶.

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The spinal cord injury (SCI) model is chosen for numerous reasons when studying the influence of BTX on bone. SCI is a severe condition that causes significant reduction in bone loading in humans. It is believed that the sympathetic nervous system may have a role in bone loss in this model due to the lack of mechanical stress; an observation already substantiated in animal models. Unlike hindlimb unloading, SCI incorporates some weight-bearing in the affected limb subsequent to BTX injection, notwithstanding the impaired muscle activity persisting for several weeks. The current literature seldom intersects, where the majority of reviews pertinent to BTX fail to address the associated risks of muscle atrophy and fibrosis. Multiple applications of BTX in muscle might lead to atrophy and fibrosis, through its effects on muscular mechanics. These effects might be either intramuscular or intermuscular and are believed to be influenced by alterations in collagen and the extracellular matrix. In earlier literature, the injection of BTX was initially perceived as entirely reversible, with the assumption that, even if it failed to ameliorate gait and function, it would pose no harm⁷⁻¹⁰.

Literature search

We conducted an electronic literature search in the Pubmed, Pubmed Central, and Scopus databases. We chose papers based on whether their titles addressed the themes of interest, specifically the changes in bone and muscle in spinal cord injury and the use of botulinum toxin. The keywords used were “osteoporosis”, “spinal cord injury”, “botulinum toxin”, “muscle mass”, “bone loss”, and “bone mineral density”. The search was limited to studies conducted in English and German languages, including clinical and randomized controlled trials, observational studies, cross-sectional diagnostic studies, and reviews. We omitted case reports and case series that lacked a properly structured intervention plan or outcome assessment. The complete textual articles were downloaded and perused. Two authors, namely YD and KP, conducted a thorough examination of the papers to ascertain if they satisfied the specified criteria for inclusion in this review. Information pertaining to the study population, design parameters, and major results related to bone loss and fractures were documented. The citations were examined separately to find any more trials that may have been missed during the initial literature search. If any such trials were found, the whole article for each citation was also obtained. We did not evaluate the methodological quality of the clinical trials included in the review using the Jadad scale. This could be seen as a limitation of our study. Conversely, YD and KP, scrutinized the research to reduce or eliminate any biases that could affect the results. There was unanimous agreement among the authors who reviewed the articles regarding the inclusion of articles.

SCI and Bone

Disuse is recognized as a causative factor of bone loss following SCI. The osteocytes located in the bone matrix are activated by mechanical loading signals, which either stimulate bone production or prevent bone resorption by the osteoblasts and osteoclasts, respectively. SCI leads to prolonged unloading and limited movement of the joints in the lower limbs, which can result in substantial loss of skeletal muscle mass. While unloading is an important contributor to the etiology of osteoporosis following SCI, hormonal changes and neural lesions also appear to play a role in this process. Innervation and neuropeptides play, in turn, a key role in normal bone remodeling. In the context of SCI, the denervation of sublesional bones emerges as a consequential outcome, which is linked to neural lesions. These factors are posited to wield considerable influence over the progression of bone loss subsequent to SCI. Osteoporosis following spinal cord injury (SCI) is the result of multiple variables, and it should not be just attributed to disuse. This is because it involves not only substantial bone loss caused by reduced mechanical stress, but also neurological abnormalities and hormonal alterations¹¹.

Jiang et al. propose that several hormonal mechanisms triggered by SCI may contribute to the development of osteoporosis. These mechanisms include: negative calcium balance caused by increased elimination of calcium by the kidneys and reduced absorption of calcium in the intestines; vitamin D deficiency, which plays a role in the development of SCI-induced osteoporosis; SCI's interference with gonadal function and inhibition of the bone-strengthening effects of sex hormones; hyperleptinemia after SCI, which may contribute to osteoporosis; pituitary suppression of thyroid-stimulating hormone (TSH), which may also contribute to bone loss after SCI; and insulin resistance and insulin-like growth factors (IGFs), which may be partially responsible for bone loss following SCI.

Additionally, the presence of catabolic variables, such as systemic inflammation or the use of medicines like steroids during the time of injury, also have a detrimental impact on bone metabolism. Thus, bone loss after SCI may be caused by an imbalance where there is an excessive production of osteoclasts compared to the necessary bone resorption, and/or a shortage of osteoblasts relative to the required ability to repair cavities. Furthermore, it is worth noting that while upper limbs typically experience loading and innervation after SCI, there is evidence to suggest that bone loss can also impact the upper extremities in individuals with paraplegia. This implies that hormonal changes may be associated with the development of osteoporosis following SCI¹².

SCI and muscle

SCI exerts a significant impact on skeletal muscle, primarily manifested through muscle disuse and spasticity. In extreme cases of disuse, as exemplified by SCI, the injury to the spinal cord results in a diminished capacity of muscle contractions, a phenomenon dependent upon the severity of

the injury (complete or incomplete SCI).

The European Working Group on Sarcopenia in Older People has classified sarcopenia into two categories: primary (related to aging) when no other cause is apparent save aging itself, and secondary (related to additional variables). There are three categories of secondary sarcopenia: activity-related, disease-related, and nutrition-related¹³. Sarcopenia is caused by a variety of pathophysiological mechanisms that follow different pathways in neurological diseases. These mechanisms include motor neuron loss, protein utilization deficits brought on by nutrient malabsorption, muscle atrophy from inactivity, thyroid-related hormonal imbalances, systemic cortisol stimulation, and insulin resistance¹⁴. Furthermore, it has been demonstrated that a shift toward more type 2 muscular fibers and significant atrophy of both types characterize the loss of muscle mass following SCI¹⁵⁻¹⁷. Muscle disuse in SCI may also result in muscle capillary decrease, which in turn leads to myopathic changes with fast type 2 fiber predominance. These findings may be modified based on the activity of the muscle facilitated either through spasms or an incomplete lesion, which may exert, in part, a protective effect. Spasticity, on the other hand, may result in fiber type transformation, making type 2 muscle fibers the predominant type^{17,18}.

The influence of spasticity/spasms on bone in individuals with SCI

Whether the presence of spasticity supports the preservation of lower-extremity bone mass has been an ongoing debate, for which research is conflicted¹⁹. When compared to flaccid people, those with spasticity are characterized by higher bone mineral density (BMD)²⁰, while other studies²¹ have observed a strong link between BMD and the degree of spasticity as determined by the modified Ashworth scale. Nevertheless, the existing evidence from currently available studies is deemed insufficient to substantiate the assertion that spasticity aids SCI patients in preserving their BMD²¹.

Bone/Muscle interaction and the role of sympathetic nervous system

When a specific threshold strain is applied to bone through force, bone synthesis is triggered. Conversely, a decline in muscle force below a predetermined set point, as observed in conditions like muscle immobilization or paralysis, leads to the loss of bone tissue.

Frost's mechanostat hypothesis posits that the bone's ability to add or remove tissue enables it to dynamically regulate strains, hence allowing the bone to adapt its strength in response to external forces²².

Under physiological conditions the main forces are produced by contractions of the muscles. Damage to the neuronal tissue inside the spinal canal after a SCI impairs motor function and causes restricted muscular contractions, which are mostly experienced by most SCI patients as

spasms and abnormal spinal reflexes²³. Recent research indicates that the sympathetic nervous system provides nerve supply to bone tissue, suggesting that it functions as a mediator of mechanical loading in bone²⁴. It is thought that this innervation inhibits the growth of new bone while promoting bone resorption. One might anticipate gains in bone growth and strength through this pathway following SCI, due to the likely decrease in sympathetic nerve activity^{23,24}. Moreover, the osteoblast surface's β -adrenergic receptors provide additional evidence for the sympathetic nervous system's impact on bone metabolism. For example, non-selective β -adrenergic pathway blocker propranolol therapy has been shown to improve bone mass in mice models. Interestingly, it has also been demonstrated that non-selective pharmacological blockage of the β -adrenergic system, accomplished by oral propranolol administration, prevents bone loss caused by a lack of mechanical stress in the tail-suspended rat model²⁵.

Discussion

Tissue deformations in bone, which are responsible for maintaining bone homeostasis over loading, are caused by a combination of ground reaction forces and muscle contraction. During hindlimb suspension, casting, neurectomy, or spinal cord injury (SCI), animals are incapable of employing their hindlimbs for weight bearing. Presently, disuse models involve either the complete elimination of all ground response and muscle forces (spinal cord injury) or the elimination of ground reaction forces while muscle contraction remains active (tail suspension and casting)²⁶.

Rodent hindlimbs have been utilized as a model to investigate the interaction between muscle and bone through intramuscular injection of BTX. The swift development of muscle wasting and subsequent reduction in bone density is a result of the muscle suppression caused by BTX, with the hypothesis that diminished muscular loading contributes to the observed skeletal effects. The study sought to examine the effects of botulinum toxin-induced muscular inhibition on the skeletal structure of mice, including both control and tail-suspended groups. The tail suspension was employed as a control to replicate the decreased gravitational loading induced by BTX. Significantly, the reduced bone health observed in the hindlimbs of animals hanging by their tails after receiving BTX injections indicates that the muscle inhibition caused by BTX has extra consequences on skeletal muscles, apart from the changes caused by altered gravity loading. These data further emphasize the concept that muscle loss directly affects bone health²⁷.

The detrimental effects of BTX on the skeleton, as found in cage control animals, are consistent with prior studies investigating the influence of botulinum toxin-induced muscle inhibition on skeletal well-being. After receiving a sole injection of BTX, experiments conducted on juvenile rats have shown a significant decrease in muscular strength of more than 80% within a short span of a few days²⁸. When

adult rabbits are injected with BTX, their muscle force is reduced by 70% after four weeks²⁶. Similarly, studies on rats and mice have shown that a single BTX injection into their quadriceps and gastrocnemius muscles leads to a decrease in bone mass at the tibia within a few weeks²⁹.

Researchers compared the effects of unloading and reloading on the bone mass of the hindlimbs in mice that were normal versus mice that were *Gja1* haplo-insufficient. The timing and degree of recovery after BTX injection-induced restoration of muscle function were investigated in this study. Irrespective of *Gja1* status, bone loss exhibited only partial reversibility upon muscle recovery, with complete restoration of bone mass remaining elusive within the 12-week observation period. Notably, *Gja1* haplo-insufficient mice responded more gradually to Botox injections in terms of skeletal system effects compared to their wild-type counterparts³⁰.

Animal studies using EMG and muscle biopsies have revealed subclinical adverse effects that appear as aberrant alterations in muscle and bone structures. The disruption of the normal muscle-bone equilibrium, brought about by the impaired release or transport of specific biochemical factors due to repeated acute muscle paralysis from BTX, leads to osteopenia and bone resorption^{26,30}.

It is reasonable to predict that the gradual recovery of muscle function in the injected leg would, at the very least, rebalance the relationship between bone formation and resorption. This would stop the sudden loss of bone that was observed in the first four weeks. Expected results involve an increased creation of bone compared to its breakdown, resulting in a simultaneous restoration of bone density as muscle strength is completely returned. Findings from human studies substantiate the notion that regained muscle function has the potential to enhance bone formation relative to resorption³¹.

Tang et al. were the first to report that injection of BTX, acute sarcopenia, and acute osteopenia known as “osteosarcopenia,” are significantly and consistently linked³². The prospect of repeated BTX injections poses the potential for progressive declines in bone morphology, physiology, and function. Conclusions regarding the long-term effects on tiny mammals’ developing bones are difficult to make, though, especially when it comes to children with cerebral palsy who need repeated injections during their formative years. A maximum follow-up of 28 weeks was seen in the analysed trials³², and only one study examined the effects of two injections of BTX separated by one month³³. The finding that certain bone properties had not entirely regenerated six months after injection is very concerning. This raises questions about the long-term response of the injected muscle and related bone segment. Given that this scoping review demonstrates that osteosarcopenia can occur in experimental small animals even with a single injection, more investigation is necessary to determine whether BTX treatment may cause reductions in bone health in cerebral palsy patients³².

Hong et al. demonstrated that BTX-induced atrophy of

the muscles used for chewing can affect the integrity of the outer layer of bone in the lower jaw in both young women and women who have gone through menopause. The impact of reduced thickness of masticatory muscles on the quality of cortical bone at the sites where the muscles attach was more noticeable in post-menopausal women, as compared to younger women. Furthermore, the decreased thickness of the muscles used for chewing was observed to impact the formation of the cortical bone in the condyle in both age groups³⁴.

Moreover, BTX can directly influence bone resorption, in addition to the effect of muscular paresis on bone. BTX administration in mice resulted in an upregulation of receptor activator of nuclear factor κ -B ligand (RANKL), a promoter of bone resorption, as observed in preclinical studies^{35,36}. Furthermore, this manipulation of the masseter muscle resulted in an augmentation of the gene expression related to indicators of bone resorption. Significantly, the messenger RNA (mRNA) of RANKL exhibited a 4.2-fold rise in mandibular head samples within two days following the injection of BTX on the experimental side, in comparison to the control side³⁶.

Due to their near closeness, muscles and bones can interact with each other through biochemical factors such as IL-6, TGF- β , TNF- α , VEGF, glutamate, calcitonin gene-related peptide (CGRP), or substance P. These substances are released during muscle contraction and contribute to the maintenance of bone homeostasis. The release or transportation of biological substances into the nearby muscle/bone environment may be decreased due to BTX’s reduction in the full spectrum of normal muscular contractions (small intensity, high frequency (twitch), and big intensity contractions³⁶.

Carpentier et al. analyzed basic research studies that used animal models and stated that there exists a non-mechanically induced mechanism that leads to osteopenia, at least partially. Botulinum toxin injections promote bone resorption and thus cause osteopenia, permanently changing bone structure, while bone development and healing may also be affected. The cumulative evidence points to the conclusion that intramuscular injections of BTX have the capacity to modify both the metabolism and structure of bone³⁷.

For example, individuals, like those who have had a stroke, who receive many BTX injections may gradually have muscular atrophy not just in the injected muscles but also in nearby or distant muscle groups. This overall weakness adds to compromised bone health, hence elevating the susceptibility to falls and fractures³⁸. Hence, the utilization of proper ankle-foot orthoses (AFOs) for walking becomes particularly valuable for these individuals.

Nevertheless, there is promising news concerning the utilization of BTX in the context of heterotopic ossification (HO)³⁹. Neuroinflammatory cytokines activate BMP signalling pathways, which enhance bone formation in response to a traumatic injury. It is postulated that an intervention that temporarily inhibits the transmission of signals between nerves and muscles, such as with

BTX, might decrease the formation of abnormal bone in unintended areas. This analysis considers the importance of neural signalling pathways in the process of fracture repair and the series of events known as the osteogenic cascade that is triggered during the healing of a fracture. This cascade includes inflammation, angiogenesis, chondrogenesis, and osteogenesis⁴⁰. Additional research is necessary to ascertain the effectiveness of muscular paralysis as a potential therapy method once HO has begun to advance or once has reached a mature stage.

Generally, the impact of BTX in medical management is influenced by the geographical distribution of research papers. This distribution reveals common findings regarding worldwide research activity, with a limited representation of many low-income countries or regions lacking specialized medical centers. Nevertheless, it is crucial to ensure extensive participation from numerous Eurasian countries in order to achieve widespread dissemination of scientific findings, particularly in the field of medicine, on a global scale⁴¹.

The limitations of our paper stem from a scarcity of studies, which restricted our analysis to human bone outcomes and secondary examination of animal datasets. We also did not assess the methodological rigor of the clinical trials included using the Jadad scale. This aspect could be perceived as a constraint of our investigation.

The relationship between bone loss and the dosage and frequency of administration of BTX is currently unknown. Meanwhile, clinicians should contemplate administering lesser doses whenever feasible and closely monitor individuals for alterations in skeletal structures resulting from repeated injections over extended durations⁴².

Conclusion

People with SCI have many complications related to the locomotor system, especially regarding bone and muscle. This condition carries a significant risk for the development of osteoporosis. The occurrence of bone loss during neurologic injury is influenced by multiple factors and is determined by both the duration and severity of the neurologic injury. Spasticity is frequently occurring after SCI, and currently, one of the primary treatments is the use of BTX. However, BTX affects bone mineral density, mainly due to several cellular and molecular alterations. It is important, then, to keep in mind that the effect of the BTX on the bone of the patient with SCI is potentially aggravating the osteoporosis that may be associated with SCI. Additional longitudinal research is necessary to have a comprehensive grasp of this phenomenon in human populations.

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Authors' contributions

Yannis Dionyssiotis and Konstantinos Prokopidis have given substantial contributions to the conception and reviewed the articles included in the manuscript, authors Andrea Olascoaga Gomez de Leon, Roberto Coronado Zarco, Melina Longoni and Nicola Minocchio to acquisition, analysis, and interpretation of the data. All authors have participated in drafting the manuscript, authors Yannis Dionyssiotis, Belgin Erhan and Calogero Foti revised it critically. All authors read and approved the final version of the manuscript.

References

1. Brin MF. Botulinum toxin: chemistry, pharmacology, toxicity, and immunology. *Muscle Nerve Suppl* 1997; 6:S146–S168.
2. Pearce LB, First ER, Borodic GE. Botulinum toxin: death versus localized denervation. *J R Soc Med* 1995; 88:239–240.
3. Huang W, Foster JA, Rogachefsky AS. Pharmacology of botulinum toxin. *J Am Acad Dermatol* 2000;43:249–259.
4. Hassan SM, Jennekens FG, Veldman H. Botulinum toxin-induced myopathy in the rat. *Brain* 1995;118(Pt 2):533–45.
5. de Paiva A, Meunier FA, Molgo J, Aoki KR, Dolly JO. Functional repair of motor endplates after botulinum neurotoxin type A poisoning: biphasic switch of synaptic activity between nerve sprouts and their parent terminals. *Proc Natl Acad Sci U S A* 1999;96:3200–5.
6. Duchen LW. Changes in motor innervation and cholinesterase localization induced by botulinum toxin in skeletal muscle of the mouse: differences between fast and slow muscles. *J Neurol Neurosurg Psychiatry* 1970;33:40–54.
7. Cocco A, Albanese A. Recent developments in clinical trials of botulinum neurotoxins. *Toxicon* 2018;147:77–83.
8. de Paiva A, Meunier FA, Molgo J, Aoki KR, Dolly JO. Functional repair of motor endplates after botulinum neurotoxin type A poisoning: biphasic switch of synaptic activity between nerve sprouts and their parent terminals. *Proc Natl Acad Sci USA* 1999;96:3200–5.
9. Kaya CS, Yilmaz EO, Akdeniz-Doğan ZD, Yucesoy CA. Long-term effects with potential clinical importance of Botulinum Toxin Type-A on Mechanics of Muscles Exposed. *Front Bioeng Biotechnol* 2020;30:8:738.
10. Multani I, Manji J, Tang MJ, Herzog W, Howard JJ, Graham HK. Sarcopenia, Cerebral Palsy, and Botulinum Toxin Type A. *JBJS reviews* 2019;7(8):e4.
11. Dionyssiotis Y. Spinal cord injury-related bone impairment and fractures: an update on epidemiology and physiopathological mechanisms. *Journal of Musculoskeletal & Neuronal Interactions* 2011; 11(3):257–265.
12. Jiang SD, Jiang LS, Dai LY. Mechanisms of osteoporosis in spinal cord injury. *Clinical Endocrinology* 2006; 65(5):555–565.

13. Cruz-Jentoft AJ, Baeyens JP, Bauer JM, Boirie Y, Cederholm T, Landi F, et al. & European Working Group on Sarcopenia in Older People Sarcopenia: European consensus on definition and diagnosis: Report of the European Working Group on Sarcopenia in Older People. *Age and Ageing* 2010;39(4):412–423.
14. Dionyssiotis Y, Skarantavos G, Petropoulou K, Galanos A, Rapidi CA, Lyritis GP. Application of current sarcopenia definitions in spinal cord injury. *Journal of Musculoskeletal & Neuronal Interactions* 2019; 19(1):21–29.
15. Castro MJ, Apple DF Jr, Staron RS, Campos GE, Dudley GA. Influence of complete spinal cord injury on skeletal muscle within 6 months of injury. *J Appl Physiol* 1999; 86:350–8.
16. Olsson MC, Kruger M, Meyer LH, et al. Fiber type-specific increase in passive muscle tension in spinal cord-injured subjects with spasticity. *J Physiol* 2006;577:339–52
17. Scelsi R. Skeletal Muscle Pathology after Spinal Cord Injury: Our 20 Year Experience and Results on Skeletal Muscle Changes in Paraplegics, Related to Functional Rehabilitation *Basic Appl Myol* 2001;11:75–85.
18. Verdijk LB, Dirks ML, Snijders T, Prompers JJ, Beelen M, Jonkers RA, et al. Reduced satellite cell numbers with spinal cord injury and aging in humans. *Medicine and science in sports and exercise* 2012;44(12):2322–2330.
19. Biering-Sørensen F, Hansen B, Lee BS. Non-pharmacological treatment and prevention of bone loss after spinal cord injury: a systematic review. *Spinal Cord* 2009;47(7):508–518.
20. Eser P, Frotzler A, Zehnder Y, Schiessl H, Denoth J. Assessment of anthropometric, systemic, and lifestyle factors influencing bone status in the legs of spinal cord injured individuals. *Osteoporos Int* 2005;16:26–34.
21. Löfvenmark I, Werhagen L, Norrbrink C. Spasticity and bone density after a spinal cord injury. *J Rehabil Med* 2009;41:1080–1084
22. Frost HM. The mechanostat: a proposed pathogenic mechanism of osteoporoses and the bone mass effects of mechanical and nonmechanical agents. *Bone Miner* 1987;2(2):73–85.
23. Rittweger J, Gerrits K, Altenburg T, Reeves N, Maganaris CN, de Haan A. Bone adaptation to altered loading after spinal cord injury: a study of bone and muscle strength. *Journal of musculoskeletal & Neuronal Interactions* 2006;6(3):269–276.
24. Takeda S, Elefteriou F, Levasseur R, Liu X, Zhao L, Parker KL, et al. Leptin regulates bone formation via the sympathetic nervous system. *Cell* 2002;111:305–17.
25. Levasseur R, Sabatier JP, Potrel-Burgot C, Lecoq B, Creveuil C, Marcelli C. Sympathetic nervous system as transmitter of mechanical loading in bone. *Joint Bone Spine* 2003;70(6):515–519.
26. Warner SE, Sanford DA, Becker BA, Bain SD, Srinivasan S, Gross TS. Botox induced muscle paralysis rapidly degrades bone. *Bone* 2006;38(2):257–264.
27. Warden SJ, Galley MR, Richard JS, George LA, Dirks RC, Guildenbecher EA, et al. Reduced gravitational loading does not account for the skeletal effect of botulinum toxin-induced muscle inhibition suggesting a direct effect of muscle on bone. *Bone* 2013;54(1):98–105.
28. Ma J, Elsaidi GA, Smith TL, Walker FO, Tan KH, Martin E, Koman LA, Smith BP. Time course of recovery of juvenile skeletal muscle after botulinum toxin A injection: an animal model study. *Am J Phys Med Rehabil* 2004; 83:774–780
29. Rauch F, Hamdy R. Effect of a single botulinum toxin injection on bone development in growing rabbits. *Journal of Musculoskeletal & Neuronal Interactions*, 2006;6(3):264–268.
30. Grimston SK, Silva MJ, Civitelli, R. Bone loss after temporarily induced muscle paralysis by Botox is not fully recovered after 12 weeks. *Annals of the New York Academy of Sciences* 2007;1116:444–460.
31. Bloomfield SA, and Jackson WJ. Bone mass and endocrine adaptations to training in spinal cord injured individuals. *Bone* 1996;19: 61–68.
32. Tang MJ, Graham HK, Davidson KE. Botulinum Toxin A and Osteosarcopenia in Experimental Animals: A Scoping Review. *Toxins* 2021; 13(3): 213.
33. Vegger, J.B.; Bruel, A.; Thomsen, J.S. Vertical Trabeculae are Thinned More Than Horizontal Trabeculae in Skeletal-Unloaded Rats. *Calcif. Tissue Int.* 2015; 97:516–526.
34. Hong SW, Kang JH. Decreased mandibular cortical bone quality after botulinum toxin injections in masticatory muscles in female adults. *Sci Rep.* 2020, 27;10(1):3623.
35. Raphael KG, Janal MN, Tadinada A, Santiago V, Sirois DA, Lurie AG. Effect of multiple injections of botulinum toxin into painful masticatory muscles on bone density in the temporomandibular complex. *Journal of oral rehabilitation* 2020;47(11):1319–1329.
36. Balanta-Melo J, Toro-Ibacache V, Torres-Quintana MA, Kupczik K, Vega C, Morales, C, et al. Early molecular response and microanatomical changes in the masseter muscle and mandibular head after botulinum toxin intervention in adult mice. *Annals of anatomy = Anatomischer Anzeiger: official organ of the Anatomische Gesellschaft* 2018;216:112–119.
37. Carpentier VT, Gracies JM, Razakarivony A, Lagnau P, Savard E. Alteration of bone metabolism and microarchitecture by intramuscular injections of botulinum toxin: A systematic review. *Annals of Physical and Rehabilitation Medicine* 2017;60 Supplement:e26.
38. Chang MA. Clinical commentary: Systemic and subclinical adverse effects of repeated botulinum toxin a injections to decrease spasticity in adults affected by stroke undergoing rehabilitation. *J Neurol Neuromed* 2016;1(6): 58–60.
39. Ausk BJ, Gross TS, Bain SD. Botulinum Toxin-induced Muscle Paralysis Inhibits Heterotopic Bone Formation. *Clinical Orthopaedics and Related Research* 2015;

- 473(9):2825–2830.
40. Aydin A, Memisoglu K, Cengiz A, Atmaca H, Muezzinoglu B, Muezzinoglu US. Effects of botulinum toxin A on fracture healing in rats: an experimental study. *J Orthop Sci* 2012;17(6):796-801.
41. Coraci D, Maccarone MC, Ragazzo L, et al. Botulinum toxin in the rehabilitation of painful syndromes: multiperspective literature analysis, lexical analysis and systematic review of randomized controlled trials. *Eur J Transl Myol* 2024;34(2).
42. Moussa MS, Bachour D, Komarova SV. Adverse effect of botulinum toxin-A injections on mandibular bone: A systematic review and meta-analysis. *J Oral Rehabil* 2024;51(2):404-415.