ORIGINAL ARTICLE

Effect of multiple injections of botulinum toxin into painful masticatory muscles on bone density in the temporomandibular complex

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Abstract

Background: Adverse effects of masticatory muscle injections of Botulinum Toxin (Btx) have been noted in animal and, less dramatically, human studies.

Objective: Among women treated in multiple community-based private practices, to compare TMJ bone density and mandibular condylar volume between patients with myofascial TMJD receiving multiple masticatory muscle Btx treatments and similarly diagnosed women not receiving such treatment.

Methods: Cohorts consisted of women whose treatment charts indicated a diagnosis of myofascial TMJD: 35 received at least 2 Btx treatment cycles; 44 received none. Bone density at pre-specified regions of interest (ROI) was defined by grey scale values from Cone Beam CT, adjusting for a fixed density phantom included in each scan. Mean bone density and mandibular condyle volume were compared between groups. Dose-response effects were tested within the Btx-exposed group.

Results: The mean density of primary and secondary ROIs was similar between exposure groups, as was condylar volume. Among Btx-exposed women, increasing dose of Btx to the temporalis muscle was inversely proportional to the density of the trabecular area of the mandible body. Many Btx-exposed women received smaller doses of Btx to the masseter muscles than in most TMJD Btx clinical trials.

Conclusion: Masticatory muscle injections of Btx failed to produce clinically significant TMJ bone-related changes. Should Btx receive regulatory approval for treatment of myofascial TMJD, a phase IV study is recommended to evaluate potential adverse effects of Btx on bone and muscle when administered at higher doses and/or for more treatment cycles.

KEYWORDS

bone density, botulinum toxins, cone beam computed tomography, facial pain, masticatory muscles, temporomandibular joint dysfunction syndrome

1 | INTRODUCTION

Temporomandibular muscle and joint disorders (TMJD) involve pain and dysfunction in the masticatory muscles and/or temporomandibular joint. The most common subtype involves masticatory muscle pain, (ie myofascial TMJD). Conservative and reversible treatment modalities (eg medications, stabilisation splints, jaw exercises, dietary and habit modification) manage symptoms for many, but some individuals' symptoms are refractory to these conservative treatments.

Botox® (onabotulinumtoxinA: Btx; Allergan, Dublin, Ireland) is an FDA-approved treatment for other muscle and pain disorders (eg cervical dystonia and migraine). In the United States, it is increasingly used off-label to treat TMJD. According to a December 2019 press release by Allergan, Inc, it already has 28 indications worldwide, with approval for use in 100 countries.

Currently, clinical equipoise exists about the safety and efficacy of Btx injections for TMJD treatment. Small and underpowered clinical trials of Btx for TMJD pain show inconsistent clinical effects in the direction of benefit[1-5] when compared to placebo or more conservative treatments. Even less is known about the safety of Btx injections in TMJD patients, particularly concerning changes in bone density (but see[6]).

Btx causes muscle paresis and atrophy in injected muscles, typically lasting approximately three to 4 months[7] although the analgesic effect involves more than muscle paresis.[8,9] Through reduction of muscle force that stimulates bone remodelling, animal research has demonstrated that Btx can induce "disuse osteopenia," with significant changes in bone morphology and a reduction in condylar volume. In fact, Btx has been used for more than a decade to cause limb paresis in rodents and rabbits as a model of disuse osteopenia.[10,11] For example, one study using a rabbit model found that a single unilateral masseter muscle Btx injection produced "profound" morphological changes and bone loss in the mandibular condyle, as well as bone loss in the alveolar region[12,13] similar to that found in other more recent rodent and rabbit studies.[14-16] In addition to the impact of muscle paresis on bone, Btx may also have direct effect on bone resorption. Preclinical research in mice has demonstrated that Btx rapidly induces the increase of a bone resorption promoter (receptor activator of nuclear factor κ-B ligand, RANKL).[17] The cellular and molecular nature of the muscle-bone remodelling relationship as it pertains to potential Btx therapeutic use needs to be better understood, to consider development of protective interventions to guard against possible adverse bone resorption.[14]

Despite concerning evidence from the animal literature, the clinical significance of isolated findings of masticatory muscle Btx effects on bone in human studies[6,18-22] remains unclear. Small effects seen from one or perhaps two treatments may not adequately represent any clinically important risks associated with long-term, repeated injection cycles in individuals suffering from chronic TMJD.

Given the alarming findings from animal studies and potentially concerning findings from limited human subject studies, more research on Btx safety for masticatory muscle injections to treat TMJD is of public health importance. The current observational cohort study of patients seen in community practices using Btx off-label for relief of muscle-based TMJD pain was designed to test the effect of such treatment on (1) TMJ bone density, (2) mandibular condylar volume and (3) to evaluate the relationship between Btx dose and bone density and volume.

2 | METHODS

This cohort study was approved by the Institutional Review Board (IRB) of the New York University School of Medicine (i14-00946) on 21 January 2015. The IRB-approved protocol (including, as detailed below, Cone Beam CT, DEXA, Medical record abstraction, recruitment processes and enrolment interviews), informed consent processes and documents were consistent with US Department of Health and Human Services, Office of Human Research Protections Federal Policy for the Protection of Human Subjects ("The Common Rule").

2.1 | Participants

Women over age 18 who had been diagnosed by their treating clinician with a myofascial TMJD-related problem were recruited from two regions of the United States, that is near New York University (NYU) College of Dentistry in Manhattan and near University of California in Los Angeles (UCLA). A variety of recruitment methods were used: (a) A small number of cooperating clinicians who used Btx to treat patients with myofascial TMJD pain directly queried recently treated women to determine if they were interested in speaking with research personnel at NYUCD to learn about a study for which they might be eligible, (b) Web-based and notices describing the study appeared on the site of the TMJ Association, a patient advocacy and support group, and (c) Google Adword searches for "facial pain" and "Botox" were optimised to show listings directing individuals to a webpage where an IRB-approved notice describing the study and contact information was provided.

Eligible Btx-exposed women had to have clinical-chart-documentation of at least two cycles of Btx treatment in the past year for myofascial TMJD pain. Eligible unexposed women had to have clinical-chart-documentation of myofascial TMJD pain with receipt of or discussion of treatment. Unexposed women could not have any history of Btx treatment for any reason.

All potential participants were verbally consented before eligibility screening and enrolled after satisfying eligibility criteria, and reviewing and signing a full informed consent and authorisation to obtain medical records. Medical records showing proof of myofascial TMJD diagnosis and treatment for self-referred patients who were not pre-screened by participating clinicians could not provide proof of diagnosis and treatment until completion of the consent process and initial enrolment. Therefore, these individuals were informed that they would be withdrawn by the Principal Investigator if clinical

records confirming self-reported diagnosis and treatment history were not received. Study-related procedures commenced after verification of myofascial TMJD diagnosis and treatment (with or without Btx) via medical records.

2.2 | Measures

2.2.1 | Botox injection protocols

Medical records were abstracted for all participants to obtain primary and secondary diagnoses, and details on treatments received. The timing, dose and location of Btx injections for exposed participants were recorded. When injection procedure remained unclear, study clinical personnel sought clarification from the clinician's office.

2.2.2 | Cone Beam CT (CBCT) of the TMJ Complex

Three-dimensional imaging of the TMJ complexes and the mandibular second premolar areas were conducted using an Accuitomo 170 CBCT unit (J. Morita Mfg. Corp., Kyoto, Japan). This unit uses exposure parameters of 90KV and 5mA for a standard mode and a flat panel detector with a 14-bit dynamic range grey scale. It displays images in true three dimensions based on an isotropic voxel size of 250 μm for the selected FOV (120 × 170 mm). The scan time was 17.5 seconds for a 360-degree rotation around the head/area of interest. Image data were exported in DICOM-2 format.

OnDemand 3D software (Cybermed, Inc, Seoul, Korea) was used to quantify bone density at specified regions of interest (ROI) in the condyle and body of the mandible. The software outputs minimum, maximum and mean of grey scale values (GSV) within each ROI. Cortical ROIs tended to be homogeneous, and the three GSV measures were the same. Trabecular bone, on the other hand, was a heterogeneous combination of areas of greater and lesser density, and the three measures in these areas (ie minimum, maximum, mean) did not agree. As well, some measurements of cortical bone were not heterogeneous. In these last two cases, we used the maximum GSV for the ROI. While the mean value would be more stable, we avoided inaccuracy in this measure that might be introduced, for example, by entrained air included in the ROI.

Some radiologists continue to be sceptical about the use of CBCT-based voxel measures as a proxy for bone density.[23,24] There is little argument that voxel-based measures are not equivalent to Hounsfield units for absolute measures of density, but our goal was to examine *relative differences* between exposed and unexposed cohort group density equivalence. As such, absolute measures were not necessary. Even so, a concern remained about day-to-day and site-to-site variability in grey scale readings. To address these concerns, we included in each set of images a **phantom**, a small capsule containing a fixed concentration of dipotassium phosphate that produces an x-ray signature of known density in each set of images. It

was included by taping the phantom to the skin overlying the cheekbone of each participant prior to imaging.

Phantom values differed between NYU and UCLA, even though the same Accuitomo model was used. Phantom densities averaged 1564.5 at NYU (SD = 165.5) and 1099.4 (SD = 176.3) at UCLA, and Phantom values also differed between days within a site. While day-to-day variation from a given scanner was smaller than that between the two scanners, both measures indicate variable calibrations on different days. To mitigate these variabilities, phantom values were subtracted from each ROI value (or, used as a covariate) for analysis. Some early studies used phantoms of questionable reliability (29 in NY and 1 in LA) for two major reasons: some early models could leak, and some early analysis could have ROIs that included entrained air (as the phantoms are homogeneous materials, ROIs with standard deviations greater than 0 were suspicious). All other phantom values were trusted. As a result, phantom values for the 30 studies deemed unreliable based on the above criteria were imputed to have the average value of the trusted phantoms from that site. The imputed values adjust only for the average difference between the unit in NYU versus UCLA, while the trusted values (n = 49) also adjust for day-to-day variability.

ROIs in the mandibular condyle

After the centre of the condyle was localised on the axial plane, four ROIs on each condyle were identified. The first three were defined as 1 mm^2 ellipses at the cortical borders of, respectively, the anteromedial (hereafter, anterior), postero-lateral (hereafter, posterior) and superior poles of the condyle. The final ROI was an area radiating from the centre of the condyle to the furthermost border safely within the trabecular compartment. The anterior and superior ROIs are the principal articular surfaces of the mandibular condyle and most likely to exhibit the flattening, erosion and/or hyperostotic cortical morphology and osteophyte formation that are attributed to increases in loading of the temporomandibular joint complex.[25,26] The posterior ROI was used as an internal control, as this surface bears the least load. The trabecular ROI was used to evaluate the cancellous component of the condyle, where it was hypothesised that the earliest changes in loading might be most apparent.[27-31] Thus, the superior and trabecular ROIs were selected a priori as the primary measures for analysis.

ROIs in the body of the mandible

Consistent with several other studies,[32-35] we evaluated density of cortical and cancellous bone of the mandible, standardised by using the mental foramen as a reference point as it is a stable anatomic landmark that is easy to identify and can be standardised.. Four density measurements were made on left and right mandibles—three 1 mm^2 ROIs at, respectively, the inferior cortical border of the mandible, the buccal and lingual cortices at the alveolar crest of the premolar, and one 3 mm^2 ellipse in the trabecular bone below the apex of the tooth and above the mental foramen. The primary ROIs here are the two regions of alveolar bone and the trabecular area.

The radiology co-investigator trained two research assistants in the use of OnDemand software for identifying and scoring density at specified ROIs. All scorers were blind to the exposure status of participants providing CBCT imaging data. In the course of the study, the radiology co-investigator [AT] rescored 8 CBCT studies originally scored by a research assistant. Three of these studies were done on exposed patients, age range 23-66 years. Inter-rater correlations ranged from 0.61 to 0.98, median = 0.82, depending on which of the 9 ROIs were measured. Thus, high levels of agreement were seen between raters when scoring these CBCT studies.

Volumetric measurements of each mandibular condyle used segmentation analysis (Mimics-9.0; Materialise NV Technologielaan, Leuven, Belgium). Using the 3D volumetric rendering mode, the condyle was reconstructed in the coronal plane and the ROI defined by identifying the neck of the condyle where the ellipsoid shape becomes most circular. Once the condyle was segmented, condylar volume was calculated using the dedicated tool. While there is no absolute method to calculate volume of non-geometric shapes on three-dimensional radiographic images, this method appears to be most reliable.[36,37] Fewer of these observations were analysed than were for density analyses, because the quality of some studies was considered inadequate. Given that only one individual was trained to use Mimics, inter-rater reliability studies were not done.

2.2.3 | Systemic bone mineral density

Bone mineral density (BMD) of the left hip and lumbar spine was assessed in both NY and Los Angeles for each participant using a fan-beam Hologic Discovery densitometer (Bedford, MA). The Hololgic unit was chosen because the recalibration routine appears better and reliability stronger over time compared to several other widely available units or manufacturers.[38-40]

2.3 | Statistical approach

The exposed and unexposed cohorts were planned for a size of 50 participants each, in order to detect a moderate effect (at least 0.5 SD) in a t test for independent samples with type 1 and 2 error rates of 5 and 20%, respectively. The distribution of ROI intensities was examined for skew, and mean values were compared between groups with ANOVA. Comparisons were pursued as crude values, values adjusted for phantom intensities, and values adjusted for phantom intensities as well as left hip DEXA values. This last adjustment was to control for the possible confounding effects of differences in systemic bone density.

Race and ethnicity differences between cohorts were evaluated with chi-square statistic or the Fisher exact test.

For Btx-exposed participants, treatment dose was evaluated as a function of each muscle injected and the patient's primary diagnosis.

Means were then compared with ANOVA, and post hoc comparisons were evaluated with the Tukey HSD procedure.

Pearson's correlation coefficients were computed to evaluate dose-response functions.

All analysis employed IBM SPSS (v. 25, IBM Corp., Armonk, NY). Exact P-values are shown except when P was <.001. For primary ROIs, "significant" implies a P-value <.05. For other ROIs, critical P-values for Bonferroni corrections were also computed and are shown as a note on each table.

3 | RESULTS

3.1 | Sample composition

The full consent process, including receipt of a signed consent form, was completed by 114 women (41 Btx-exposed and 73 Btx-unexposed). Unlike those referred by a study-affiliated clinician, for individuals who had contacted the study in response to web postings or other indirect methods, their clinical diagnosis and Btx treatment status had to be verified via medical record after initial enrolment. Thirty-four of these individuals were then withdrawn because they were unable to provide verifying information.

Of the remaining 80 individuals, 79 completed a CBCT study. One CBCT study could not be used, because it did not cover all relevant ROIs, and another participant failed to complete a DEXA but agreed that we could use her other data. Thus, 79 participants were included in the analysis: 35 who had received Btx injections to the masticatory muscles at least twice in the prior year and 44 who had never received Btx injections to the masticatory muscles but had received other treatments. Because participants were treated by a diverse group of clinicians in the community, specific diagnostic procedures could not be confirmed for every participant. Instead, notes in the clinical record were reviewed, and "myofascial TMD," "myofascial TMJD," "masticatory muscle pain" or other reference to pain in the masticatory muscles were accepted. Although all participants had a TMJD diagnosis, the most common primary diagnosis was headache (n = 20); 9 had primary myofascial TMJD, and the remaining 6 included primary TMJ arthritis, capsulitis and dystonia. Two patients received 2 Btx treatments in the last year, 15 received 3, 11 received 4, and 7 received 5.

The sample was mostly white/Caucasian (60.8%), and similar in Btx-exposed (51.4%) and Btx-unexposed (68.2%) participants (Chi-square = 1.65, P > .10). Hispanic ethnicity was endorsed by 13.9% of the sample (5.7% Btx-exposed and 20.5% Btx-unexposed; Fisher's exact test, P = .10).

As shown in Table 1, exposed and unexposed cohorts were not different in age, worst facial pain (0-10 scaling) or BMD scores in the left hip or lumbar spine. Participants who had received Btx injections reported a much longer (P < .001) duration of facial pain than those not receiving Btx.

TABLE 1 Mean (SD) demographic and clinical characteristics of the sample, by exposure status

| | N | Age | Worst Pain | Pain duration | DEXA BMD (T-score) | |
					(left hip)	(lumbar spine)
Not exposed	44	41.2 (14.3)	8.1 (1.7)	8.9 (4.6)	−0.53 (1.11)	−0.68 (1.04)
Exposed	35	41.2 (12.6)	7.8 (1.4)	13.8 (10.1)	−0.58 (1.01)	−0.34 (1.1)
P-level		.99	.42	<.001	.85	.17

3.2 | Botox dose

Table 2 displays the dosage employed at the most recent treatment (summed over sides) as well as the cumulative dose over the last year (Table 3) in exposed participants, broken down by primary diagnosis. Focusing on the masseter muscle, the typical participant with a primary diagnosis of TMD received about 40 U (20 U each at the right and left masseter) at the most recent treatment, (Table 2), while the typical headache patient received only about half this dose, and remaining participants received an intermediate dose. By contrast, migraine patients who had comorbid myofascial TMJD received larger doses of Btx to their temporalis muscles than primary TMJD or other primary diagnoses and were also more likely to receive injections to muscles in the back of the head and neck, as part of the standard protocol for frequent migraine.[41]

3.3 | Effect of Btx exposure status on bone density (adjusted GSV)

Table 4 shows the GSV for each ROI. Exposure groups were compared on mean GSV with three models; first, as a function of raw GSV, second, when the GSV was adjusted for the best available phantom, and finally, when GSV was adjusted for the phantom and systemic bone density in the left hip. Results for these three models are shown in the final columns of the Table. Explained variance (eta^2) is shown in the three columns at the end the first row of each group comparison within ROI; P-values are then shown in the second row of those columns.

As shown in Table 4, the groups did not differ in the density of any primary ROI in the condyle. Patients exposed to Btx did show larger GSVs than patients unexposed to Btx at both the left and right alveolar lingual area of the mandible. However, these differences were in the opposite direction of that predicted.

Table 4 shows that the density of one secondary ROI, the left posterior pole of the condyle, showed significantly lower GSVs among exposed than unexposed participants. A similar pattern was not, however, found for the right posterior pole of the condyle.

3.4 | Effect of Btx treatment dose on bone density

Table 5 displays Pearson's correlation coefficients relating the dose of Btx received at last treatment prior to CBCT imaging and

phantom-corrected GSVs in each ROI among the exposed cohort. Among primary ROIs, masseter dose was inversely proportional to the density of the right, but not the left, superior pole of the condyle ($r = −.41$). Similarly, the density of left (and to a lesser extent, the right) trabecular region of the mandible was inversely proportional to the dose of Btx injected into the temporalis muscle. None of the dose/response correlations for secondary ROIs reached levels of significance. We also evaluated correlations between density and the cumulative dose during the past 6 months and during the past year. They produced similar findings and are not detailed here.

3.5 | Effect of Btx exposure on the volume of the mandibular condyle

In Table 6, the volume of the left and right condyles is shown by exposure group status. Groups were statistically similar on the left and right sides.

3.6 | Effects of Btx treatment dose on condylar volume

Condylar volume was unrelated to the dose of Btx delivered (at either the last treatment or cumulative over the last year) to either the left or right masseter ($r = −.08$, $P > .10$; $r = −.12$ $P > .10$, respectively) or temporalis ($r = −.09$, $P > .10$; $r = −.05$, respectively), muscles (see Table 7).

4 | DISCUSSION

The present study failed to provide evidence of clinically significant changes in density or volume of the temporomandibular complex in women who received at least two (and most typically three) Btx treatment cycles for myofascial TMJD in the past year. The failure to detect a group difference might be attributed to a small sample, but measures of explained variance, which do not vary with sample size, also indicated small effects. Similar TMJ density between those exposed and not exposed to Btx is also reported by Hong.[6]

The most prominent effect was a relationship between temporalis muscle dosing and a reduction of bone density in the trabecular compartment of the mandibular condyle. This is consistent

TABLE 2 Botox Dose (U) at Last Treatment by Chief Complaint and Muscle (*summed over sides*)

	Headache (N = 20)					Myofascial TMD (N = 9)					Other (N = 6)					P-level
	M	SD	Med	Min	Max	M	SD	Med	Min	Max	M	SD	Med	Min	Max	
Temporalis	39.2[a]	9.2	40.0	15	50	16.7[b]	15.0	20.0	0	40	5.0[b]	12.2	0.0	0	30	<.001
Masseter	19.0[a]	10.7	20.0	10	60	42.4[b]	20.5	40.0	20	70	32.8[a,b]	11.0	32.5	16	50	0.001
Corrugator	20.7[a]	9.3	20.0	0	30	1.8[b]	5.3	0.0	0	16	2.5[b]	6.1	0.0	0	15	<.001
Procerus	8.5[a]	4.0	10.0	0	15	0.4[b]	1.3	0.0	0	4	0.0[b]	0.0	0.0	0	0	<.001
Occipitalis	30.3[a]	13.6	30.0	0	60	5.1[b]	10.3	0.0	0	26	0.0[b]	0.0	0.0	0	0	<.001
Cervical paraspinal	22.0[a]	12.4	20.0	0	50	2.2[b]	6.7	0.0	0	20	5.8[b]	14.3	0.0	0	35	<.001
Trapezius	33.0[a]	18.1	30.0	0	70	4.4[b]	13.3	0.0	0	40	6.7[b]	16.3	0.0	0	40	<.001

Note: Values with the same superscript across rows indicate homogeneous subsets via Tukey's HSD test. When a mean value has the same superscript across groups (Headache, Myofascial TMD or Other), values are not significantly different from one another; when they have different superscripts, they are significantly different; and when no superscripts appear in a row, none of the mean values are significantly different from one another.

TABLE 3 Botox Dose (cumulative U) over the Last Year by Chief Complaint and Muscle (*summed over sides*)

	Headache (N = 20)					Myofascial TMD (N = 9)					Other (N = 6)					P-level
	M	SD	Med	Min	Max	M	SD	Med	Min	Max	M	SD	Med	Min	Max	
Temporalis	120.8[a]	45.3	120.0	40	200	23.1[b]	25.8	20.0	0	72	18.3[b]	36.0	0.0	0	90	<.001
Masseter	57.4	28.2	60.0	10	120	87.9	53.4	85.0	24	180	70.5	33.7	73.5	16	120	.28
Corrugator	65.7[a]	37.8	80.0	0	120	4.4[b]	10.7	0.0	0	32	7.0[b]	17.1	0.0	0	42	<.001
Procerus	26.9[a]	14.9	30.0	0	50	0.9[b]	1.8	0.0	0	4	0.0[b]	0.0	0.0	0	0	<.001
Occipitalis	93.0[a]	47.3	110.0	0	160	7.8[b]	16.1	0.0	0	44	3.3[b]	8.2	0.0	0	20	<.001
Cervical paraspinal	68.0[a]	42.6	60.0	0	160	2.2[b]	6.7	0.0	0	20	24.2[a,b]	59.2	0.0	0	145	<0.001
Trapezius	105.5[a]	66.1	120.0	0	200	10.1[b]	20.3	0.0	0	51	20.0[b]	49.0	0.0	0	120	<.001

Note: Values with the same superscript across rows indicate homogeneous subsets via Tukey's HSD test. When a mean value has the same superscript across groups (Headache, Myofascial TMD or Other), values are not significantly different from one another; when they have different superscripts, they are significantly different; and when no superscripts appear in a row, none of the mean values are significantly different from one another.

TABLE 4 Mean (SD) GSV for Each Condylar and Mandibular ROI, Comparing Groups Exposed and Unexposed to Btx (n = 78)

ROI	N	Mean (SD)	Crude	A1	A2
Condyle					
Anterior pole L					
Exposed	35	800.9 (226.5)	0.01	0.01	0.01
Unexposed	43	860.7 (296.1)	P:.33	0.42	0.41
Anterior pole R					
Exposed	35	726.2 (205.3)	0.04	0.04	0.04
Unexposed	42	831.4 (278.4)	0.07	0.10	0.08
Posterior pole L					
Exposed	34	638.1 (228.9)	0.09	0.10	0.09
Unexposed	43	794.0 (256.9)	0.007	0.005	0.008
Posterior pole R					
Exposed	35	708.7 (268.3)	<0.01	<0.01	<0.01
Unexposed	42	696.0 (240.2)	0.83	0.56	0.65
Superior pole L					
Exposed	35	782.2 (256.0)	0.02	0.02	0.01
Unexposed	43	854.9 (256.4)	0.22	0.27	0.36
Superior pole R					
Exposed	35	803.1 (242.3)	<0.01	<0.01	<0.01
Unexposed	42	844.9 (223.4)	0.43	0.68	0.71
Trabecular L					
Exposed	35	477.6 (161.8)	0.05	0.04	0.04
Unexposed	43	561.2 (211.9)	0.06	0.07	0.07
Trabecular R					
Exposed	35	474.5 (128.5)	<0.01	<0.01	<0.01
Unexposed	42	470.3 (150.3)	0.90	0.72	0.72
Mandible					
Inf border L					
Exposed	32	2158.6 (407.4)	<0.01	<0.01	<0.01
Unexposed	40	2202.3 (441.2)	0.67	0.96	0.94
Inf border R					
Exposed	33	2173.3 (426.0)	0.02	<0.01	<0.01
Unexposed	40	2280.8 (453.6)	0.30	0.48	0.51
Alveolar Buccal L					
Exposed	34	1886.8 (401.7)	<0.01	0.02	0.02
Unexposed	42	1832.5 (419.2)	0.57	0.22	0.19
Alveolar Buccal R					
Exposed	34	1871.8 (385.4)	<0.01	<0.01	0.01
Unexposed	42	1849.2 (461.6)	0.82	0.48	0.42
Alveolar Lingual L					
Exposed	34	1562.5 (347.1)	0.02	0.08	0.10
Unexposed	42	1466.5 (364.7)	0.25	0.02	0.006
Alveolar Lingual R					
Exposed	34	1626.4 (348.9)	0.03	0.10	0.10
Unexposed	42	1495.0 (404.6)	0.14	0.007	0.006

TABLE 4 (Continued)

ROI	N	Mean (SD)	Crude	A1	A2
Trabecular L					
Exposed	34	646.0 (268.7)	0.03	0.03	0.02
Unexposed	42	740.6 (262.23)	0.13	0.14	0.19
Trabecular R					
Exposed	34	717.3 (236.8)	0.02	0.02	0.02
Unexposed	42	793.8 (303.7)	0.23	0.26	0.20

Note: Bolded ROIs indicate primary ROIs per analytic plan. Eta^2 (row 1 per ROI) and P-values (row 2 per ROI) are derived from models that are crude (unadjusted) or adjust for phantom values (column A1), or both phantom and hip DEXA scores (column A2), respectively. P-levels shown are prior to correction for multiple comparisons; the critical level for Bonferroni corrected significance is $P < .003$ for secondary ROIs.

TABLE 5 Correlations (R) Between Btx Unit Doses Injected at the last treatment into Masseter and Temporalis Muscles and the phantom-corrected GSV of Each Condylar and Mandibular ROI

	Temporalis			Masseter		
ROI	R	P-value	n	r	P-value	n
Condyle						
Anterior pole L	.22	.21	35	−.07	.67	35
Anterior pole R	.06	.74	35	−.02	.91	35
Posterior pole L	.20	.26	34	−.22	.21	34
Posterior pole R	.33	.06	35	−.42	.01	35
Superior pole L	.18	.31	35	−.01	.96	35
Superior pole R	.30	.08	35	−.41	.01	35
Trabecular L	.14	.42	35	.01	.93	35
Trabecular R	.18	.31	35	−.13	.45	35
Mandible						
Inf border L	.18	.33	32	.08	.67	32
Inf border R	.14	.43	33	−.11	.55	33
Alveolar Buccal L	.11	.54	34	−.18	.31	34
Alveolar Buccal R	−.03	.87	34	−.06	.74	34
Alveolar Lingual L	.02	.93	34	.13	.47	34
Alveolar Lingual R	.06	.74	34	−.01	.97	34
Trabecular L	−.44	.01	34	−.18	.31	34
Trabecular R	−.31	.08	34	−.20	.27	34

Notes: Bold indicates primary measures. P-levels are not corrected for multiple comparisons; the critical level for Bonferroni corrected significance is $P < .003$ for secondary ROIs.

with our earlier report, in which radiologists' review of CBCT images identified osteopenia confined to the trabecular region of the condyle of TMJD patients who had received 2 or more masticatory muscle Btx treatments for their pain.[22] Both studies are consistent with micro CT studies showing more remodelling in the trabecular than the cortical bone of the mandibular condyle[42] in humans.

As a community study, the current report revealed a variable use of Btx for myofascial TMJD in a variety of clinical practices. It would appear that masseter muscle Btx dosing is markedly lower in the community than in clinical trials of Btx for myofascial TMJD pain, for example.[43] or as recommended in treatment guidelines.[44] Of note, we cannot claim that our community sample is a representative one. It was biased towards primary headache cases and away from primary myofascial pain cases. Individuals for whom TMJD treatment was a secondary goal of Btx treatment typically received lower doses of Btx than patients who had a primary diagnosis of TMJD. Compared to protocols customised in clinical trials for treating myofascial TMJD patients (ie typically, 25-50 IU Units for each masseter muscle[1-5] in general recommended treatment guidelines[44] and especially in single treatment sessions where even low-dosed patients (temporal: 10 UI per side; masseter 30) received average sided injections, Btx-exposed participants in our study received low Btx doses in their masseter muscles. This is not to suggest that the doses used here fell below a clinically detectable level. In our survey of these patients not detailed in the results section, Btx-exposed subjects were more likely to report facial muscle spasms and paresis, and less likely to report "stiff jaw" than unexposed patients, suggesting that the doses

TABLE 6 Comparison of Left and Right Condylar Volume Among Btx-Exposed and Btx-Unexposed Participants

	Study group	N	Mean	Std. Deviation	Std. Error Mean
Condylar volume left (mm^3)	Exposed	29	1290.6	561.0	104.2
	Unexposed	41	1324.9	471.8	73.7
Condylar volume right (mm^3)	Exposed	29	1290.4	500.9	93.0
	Unexposed	41	1339.8	514.7	80.4

TABLE 7 Correlation of Left and Right Condylar Volume with Masticatory Muscle Btx Doses among Btx-Exposed Participants

		Temporalis Dose	Masseter Dose
Condylar volume left (mm^3)	Pearson Correlation	−0.087	−0.075
	Sig. (2-tailed)	0.473	0.540
	N	70	70
Condylar volume right (mm^3)	Pearson Correlation	−0.053	−0.121
	Sig. (2-tailed)	0.664	0.318
	N	70	70

experienced here produced effects consistent with expectations of Btx treatment. Nevertheless, these smaller doses may have limited the appearance of bony changes in the temporomandibular complex. This finding is consistent with recommendations by De la Torre Canales, et al[43] that lower doses of Btx be recommended if considering Btx treatment, since lower doses are associated with lower rates of adverse events.

Other human studies have reported bony changes using a similar and more powerful pre-post design than the current study, but their clinical significance is unknown. For example, in a retrospective cohort study without random assignment, Hong et al[6] reported reduced condylar density following two Btx injections within a 6-month period. Lee et al[21] reported that bone volume of the mandibular gonial angle area was reduced significantly more in patients receiving two sets of masticatory muscle injections 4 month apart than a group receiving a single injection. Another small study[18] reported grey scale non-uniformity increased in condylar and alveolar bone after masticatory muscle Btx treatment and that 6 of their 12 treated patients showed condylar bone increase or decrease, depending on the specific area. While all of these studies showed statistical significance, the clinical significance of all these findings is uncertain.

In contrast, a single and disturbing case report describes a woman suffering from oromandibular dystonia who was treated quarterly for more than a year with a massive unilateral dose of 140 U of Btx. MRI and CT evaluation pre- and post-treatment revealed that the treatment caused severe unilateral condylar degeneration.[19] While the dosing was far beyond any dosing seen at a single point in time in our community-based study, it shows the possibility for Btx to cause clinically significant bone degradation. Importantly, human studies need to better understand the potential direct role of Btx on bone resorption,[14,17] aside from its role in reducing muscle force on bone.

Human subjects' studies on the risks of injecting masticatory muscles with Btx must also consider potential adverse effects on the muscles themselves, which has never been examined systematically in humans over repeated injections. In a rabbit study,[45] multiple cycles of Btx treatment were accompanied by the loss of contractile muscle and replacement with fatty infiltrates, reflecting permanent changes in the ability of masticatory muscles to exert force needed for necessary function. Such permanent changes to injected muscles have been observed in humans when Btx has been used to treat piriformis syndrome.[46] Could Btx

treatment cause such changes to masticatory muscles? If so, might this have clinically significant consequences, over the long term, by permanently reducing the muscle load required for bone re-modelling? Might the fatty infiltrates themselves cause functional problems related to chewing harder foods, even if pain is not a limiting factor?

As long as Btx remains an off-label treatment, at least in the United States, these concerns remain moot. Long-term, off-label treatment at regular intervals will likely be unusual, given high treatment costs. Nevertheless, in other countries, treatment may already be available, and the currently regulatory situation in the United States can change. An in-progress Phase 3 clinical trial (NCT03223298) suggests that new indications for Btx, including myofascial TMJD, are being pursued. If successful, safety concerns should grow. Unless specialised imaging of muscle and bone are conducted among patients who receive Btx treatment over long periods, true cumulative adverse effects will remain unknown. Given potential irreversible impact on muscle, small adverse changes associated with a single treatment cycle may accumulate until they become clinically significant. Thus, should treatment of myofascial TMJD with Btx become an approved indication, it is *strongly recommended* that a Phase IV study be conducted using CT/CBCT and MRI to rule out plausible long-term and potential clinically significant adverse changes to the bone and muscle.

CONFLICT OF INTEREST
The authors report no conflicts of interest.

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