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Dose Dependent Effects of Intramuscular Botulinum Neurotoxin on Passive Mechanical Properties of Skeletal Muscle

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INTRODUCTION: Botulinum Neurotoxin Type A (BoNT) is used clinically to relieve hypertonic or overactive muscles, and is often administered to individuals with neurological injuries such as Cerebral Palsy (CP) or stroke (1). However, more recently, clinicians have questioned the efficacy of intramuscular BoNT to improve musculoskeletal outcomes in CP populations (2). Indeed, our lab has demonstrated that BoNT injections increase passive muscle stiffness in individuals following a stroke (4). These findings contradict the clinical use of BoNT intramuscularly, especially in cases where stiffness is already exacerbated, such as that in CP and post-stroke. The purpose of our investigation was to assess the influence of clinically relevant doses of BoNT (2 Units (U), 4U & 6U/kg) on skeletal muscle in healthy juvenile mice. We hypothesize that BoNT will result in a dose-dependent reduction of muscle mass and altered *ex vivo* muscle passive mechanics in healthy juvenile mice.

METHODS: 30 C57Bl6 mice (15M, 15F) were injected with either 2U, 4U or 6U/kg of BoNT (n=5/each sex) into the hindlimb posterior compartment (medial and lateral gastrocnemius). All injections were performed on the left limb, leaving the right limb as a non-injected control. All experimental procedures were approved by Drexel University's IACUC. At 8 weeks post-injection, the lateral gastrocnemius muscles were dissected and tested bilaterally. Passive muscle mechanical testing was performed *ex vivo*, where the Lateral Gastrocnemius was suspended in an experimental bath, measured at resting length, and stretched incrementally by 5% muscle length, to a maximum strain of 130%. Passive forces were collected and normalized to physiological cross-sectional area (PCSA), calculated from direct measurement of muscle mass and length. All data is reported as mean ± SEM. Mass ratios (BoNT/Control) and stress-strain relationships were analyzed via an ANOVA (dose) and Three-Way ANOVA (strain, dose, and limb), respectively, with a priori significance of 0.05.

RESULTS: 8-weeks following BoNT injection pooled gastrocnemius muscle masses (medial & lateral) were 22% lower compared to control ($p=0.007$) . Calculated stress-strain curves represent an assessment of the muscle's passive mechanical properties irrespective of muscle size. Muscle stress-strain relationships demonstrated an upward shift in BoNT-injected muscles that was dose dependent. That is, the stress was greater in BoNT muscles as compared to healthy controls across the entire strain continuum (i.e., 10%, 20% & 30%) and this increase was dose dependent ($p<0.05$). Maximal stress was 39%, 59%, and 83% greater in muscles-injected with 2 U, 4U, and 6U, respectively, compared to the contralateral control muscles.

DISCUSSION: Our data demonstrates clinically relevant doses (2-6U/kg) of BoNT reduced muscle mass, and altered skeletal muscle mechanical properties to exhibit higher stresses 8 weeks after a single BoNT injection. BoNT has been administered to millions of individuals, and remains to be used to inhibit hypertonic or spastic muscles, despite a growing body of evidence against intramuscular BoNT. These findings highlight the need to further explore clinically relevant dosing, the influence of repeated doses, and how these changes alter material properties and muscular function overall. Lastly, the mechanism of these changes is relatively unknown, whereas future investigation will strive to pinpoint the mechanisms responsible for BoNT-induced changes in muscle size, and mechanical properties. In conclusion, BoNT-injection results in elevated stress-strain curves, and ultimately a stiffer less compliant muscle at rest.

SIGNIFICANCE/CLINICAL RELEVANCE: These findings are clinically relevant because intramuscular BoNT injections are a clinical tool used in both pediatric and adult population, despite a growing number of studies highlighting the negative consequences on the musculoskeletal system.

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