Patrick Haubruck

Dr. med.

Botulinum Neurotoxin A injection influences muscle biomechanics during stretching of the gastrocnemius muscle-tendon unit

An *in vivo* animal study

Promotionsfach: Orthopädie

Doktorvater: Priv.-Doz. Dr. med. Arash Moghaddam-Alvandi

Botulinum Neurotoxin A is used as an adjunct to physical therapy (e.g. stretching) to modulate muscle-tendon unit stiffness and reduce spastic tone, thereby improving patient function. However, only limited basic science studies have examined the influence of Botulinum Neurotoxin A on the *in vivo* passive biomechanics of skeletal muscle. So far the influence of Botulinum Neurotoxin A injection on the myotatic or stretch reflex and *in vivo* modulus is not known. The current study used Botulinum Neurotoxin A to chemically denervate the gastrocnemius muscle-tendon unit and quantify *in vivo* neurological contributions to skeletal muscle tone after repetitive, non-traumatic stretching. Furthermore the influence of chemical denervation on muscle's passive biomechanical properties was addressed. Evaluation of the post-denervation myotatic reflex as well as the elastic modulus are introduced as new modalities in assessing the influence of Botulinum Neurotoxin A injection on passive muscle biomechanics. This study expands upon previously published literature and will help to enhance the knowledge how chemical denervation impacts passive muscle biomechanics. Here it is hypothesized that injection of Botulinum Neurotoxin A modulates *in vivo* skeletal muscle tone and disrupts the myotatic or stretch reflex, thereby decreasing muscle stiffness during stretching.

Mouse gastrocnemius muscle was injected with Botulinum Neurotoxin A (n = 18) or normal saline solution (n = 18). One week post-injection active muscle force generation testing and passive, non-destructive, *in vivo* load relaxation, was tested, consisting of three stretching protocols, to examine how the muscle-tendon unit behaves after chemical denervation with Botulinum neurotoxin A. Throughout passive testing tension was recorded continuously for 210 sec, followed by a recovery time of 180 sec between each displacement interval. After

load relaxation testing was conducted biometrical muscle parameters were assessed and the animals subsequently sacrificed. T-tests and two-way repeated-measures ANOVA with Bonferroni post-hoc comparisons were used for statistical comparisons between and within groups. Linear regression analysis determined *in vivo* stiffness and *in vivo* modulus.

Each muscle-tendon unit in the Botulinum Neurotoxin A group produced less active force (average 0.491 ± 0.310N) compared to the saline-injected controls (average 1.738 ± 0.126N, $p<0.05$) and therefore proved successful injection. Passive tension was significantly lower throughout the load-displacement time-history for all stretching protocols ($p < 0.05$). The average muscle-tendon unit stiffness of the Botulinum neurotoxin A injected group was significantly decreased (0.531 ± 0.061 N/mm), compared to the saline injected group (0.780 ± 0.037 N/mm, $p < 0.05$). Injection of Botulinum neurotoxin A impaired passive muscle recovery (15% *vs.* 35% recovery to pre-stretching baseline, $p < 0.05$) compared to saline controls. Intrinsic elasticity of the muscle-tendon unit for peak and equilibrium tension was decreased by 7.13% and 5.44%, respectively, and thereby showing a non-significant response to chemical denervation. Denervated gastrocnemius muscle-tendon unit wet weight and volume was significantly decreased (24.3% and 26.8%, respectively, $p < 0.05$) if compared to saline control muscles.

Chemical denervation reduces neuronal tone in the muscle. Thus, influencing the contractile properties and muscle-tendon unit *in vivo* soft-tissue passive biomechanical properties. Injection of Botulinum Neurotoxin A significantly attenuates stiffness of the muscle-tendon unit. Furthermore, passive forces generated by displacing the muscle-tendon unit are significantly lower. This may facilitate physical therapy by decreasing required forces to stretch the muscle-tendon unit. However, it is also possible that Botulinum neurotoxin A injection may alter the structure of skeletal muscle; thus modulating the *in vivo* passive biomechanical properties of the muscle-tendon unit. The successful use of Botulinum Neurotoxin A injections as an adjunct to physical therapy may be in part attributed to the disruption of the stretch reflex; in particular the efferent arm (and possibly the afferent arm as well) of the myotatic stretch reflex is affected by chemical denervation and thereby modulating *in vivo* passive muscle properties. Furthermore, results of this study indicate that injection of Botulinum Neurotoxin A modifies the elastic modulus of the *in-situ* muscle-tendon unit differently than previously published in reports of the impact of this drug on both *ex-vivo* muscle fiber bundles and *ex-vivo* single muscle fibers. The findings suggest *in vivo* compensating mechanisms that offset the elevating effect on the elastic modulus through the increased extracellular passive load bearing structures subsequent to chemical denervation.

The findings of this study may provide a possible explanation of the clinical success of adjunct Botulinum Neurotoxin A injections to stretching during physical therapy in patients with spasticity.