EFFECTS OF BOTOX ON NON-INJECTED MUSCLES' MECHANICS AND MYOFASCIAL FORCE TRANSMISSION

by

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EFFECTS OF BOTOX ON NON-INJECTED MUSCLES' MECHANICS AND MYOFASCIAL FORCE TRANSMISSION

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Ferah Vardal, İstanbul, 2017

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ABSTRACT

EFFECTS OF BOTOX ON NON-INJECTED MUSCLES' MECHANICS AND MYOFASCIAL FORCE TRANSMISSION

Studies show that Botulinum toxin type A (BTX) injection causes a force decrease in the injected muscle. However, if BTX has effects beyond the injected muscle via diffusion and altered epimuscular myofascial force transmission (EMFT) is not known. It is hypothesized in this study that (1) BTX injection into the rat tibialis anterior (TA) has an impact on mechanics of adjacent extensor digitorum muscle (EDL) and (2) BTX has an impact on EMFT. The goal of this study was to test these hypotheses by measuring changes in total and passive forces exerted proximally and distally by the EDL muscle on both muscle length changes and muscle relative position changes. Two groups of Wistar rats were tested: Control (no BTX injected) and BTX (0.1 U of BTX injected to the mid-belly of the TA). Five days after injection, injection of BTX into the TA: (1) altered total and passive EDL forces exerted proximally and distally and after imposing muscle length changes. This does indicate that BTX changed EDL mechanics. The passive forces showed an increase (up to 13 folds) and the total forces showed a decrease (up to 81.7%). (2) Changed EMFT. EDL relative position changes towards the distal positions caused EDL total forces to significantly decrease also in the BTX group (64.1% decrease in BTX compared to Control) The findings indicate that BTX administration diminishes EMFT. Our findings support our hypotheses and improve our understanding of BTX effects on muscular mechanics and EMFT showing that BTX injected into one muscle affects the remainder of the compartment via leakage into other muscles and altered EMFT.

Keywords: Botulinum toxin type-A, epimuscular myofascial force transmission, muscular mechanics.

ÖZET

BOTOKSUN ENJEKTE EDİLMEYEN KASLARIN MEKANİĞİ VE MİYOBAĞDOKUSAL KUVVET İLETİMİ ÜZERİNE ETKİLERİ

Çalışmalar Botulinum toxin tip A (BTX) enjeksiyonunun, enjekte edilen kasta kuvvet azalmasına sebep olduğunu göstermektedir. Bununla birlikte, BTX'un enjekte edilen kasa difüzyon yoluyla etkileri ve değişen epimusküler myofasiyal kuvvet iletiminde (EMFT) rolü bilinmemektedir. Bu çalışmada, (1) sıçan Tibialis Anterior (TA) kasına BTX injeksiyonunun komşu extensor digitorum kasının (EDL) mekaniği üzerinde etkileri ve (2) BTX'un EMFT üzerine etkileri olduğu hipotez olarak öne sürülmüştür. Çalışmanın amacı, EDL kası tarafından proksimal ve distal olarak uygulanan toplam ve pasif kuvvetlerin değişimlerinin kas uzunluğu ve kas göreceli konum değişiklerine etkilerini ölçerek bu hipotezleri test etmektir. İki grup Wistar sıçanı test edilmiştir: Kontrol (BTX enjekte edilmemiştir), ve BTX (TA'nı n orta kısmından BTX enjekte edilmiştir). Enjeksiyondan beş gün sonra, BTX'un TA'ya enjekte edilmesi: (1) proksimal ve distal olarak uygulanan toplam ve pasif EDL kuvvetlerini uygulanan farklı kas uzunluklarında değiştirmiştir. Bu, BTX'un EDL mekaniğini değiştirdiğini gösterir. Pasif kuvvetler 13 kata kadar artmış ve toplam kuvvetler %81.7'ye kadar azalmıştır. (2) EMFT'yi değiştirmiştir. EDL göreceli pozisyon değişiklikleri distal pozisyonlara doğru EDL toplam kuvvetlerinin BTX grubunda da anlamlı olarak düşmesine sebep olmuştur. (Kontrol grubunda kıyasla BTX'da %64.1). Bulgular, BTX uygulamas{in{in EMFT'yi azalttığını göstermektedir. Sonuçlar, hipotezimizi desteklemekte, BTX'un kas mekaniği ve EMFT üzerindeki etkileri konusunda anlay{işımızı iyileştirmekte ve BTX'un bir kas iÃ§ine enjekte edilmesinin diğer kaslara sızıntı yaparak kalan kompartmanı etkilediğini ve EMFT'yi değiştirdiğini göstermektedir

Anahtar Sözcükler: Botulinum toxin tip A, epimusküler myofasiyal kuvvet iletimi, kas mekaniği.

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a_i	Curve Fitting Coefficients for Passive Force
b_i	Curve Fitting Coefficients of Total Force
U	Unit
y	Total Force
x	Muscle Tendon Complex Length
$\Delta EDL_{position}$	Displacement of Extensor Digitorum Longus
$\Delta LEDL$	Difference on Length of Extensor Digitorum Longus

LIST OF ABBREVIATIONS

ANOVA	Analysis of Variance
CP	Cerebral Palsy
BTX	Botulinum Toxin Type A
DT	Diffusion Tensor
ECM	Extracellular Matrix
EDL	Extensor Digitorum Longus
EHL	Extensor Hallucis Longus
EMFT	Epimuscular Myofascial Force Transmission
Hz	Hertz
kg	kilogram
mA	Milliampere
mg	Milligram
mm	Millimeter
ms	Millisecond
MR	Magnetic Resonance Imaging
p	level of Marginal Significance
TA	Tibialis Anterior

1. INTRODUCTION

1.1 Backround

In e.g., cerebral palsy (CP), an imbalance of signals from the central nervous system to the muscles and a lack of control of the stretch reflex due to exaggerated mechanoreceptor response results in muscle spasticity defined as a velocity-dependent response of muscle to passive stretching [1]. Muscle spasticity leads to an increase in muscleâs resistance to passive stretch [2] and causes increased muscle tone [3]. Patients with CP typically exhibit a limited range of joint motion, which causes impeded mobility. For children with CP, this affects their development unfavorably and has a major bad impact on their parents and on society as well. Consequently, management of spasticity is very important. Botulinum toxin type A (BTX) is a neurotoxin produced by the bacterium Clostridium botulinum, and is widely used in spasticity management worldwide. When injected, BTX causes partial muscle paralysis by blocking release of acetylcholine at the neuromuscular junction. This leads to two effects: (1) the signals from the mechanoreceptors are suppressed. (2) Force production of the muscle is decreased. The former effect helps spasticity management, thereby therapeutically, use of BTX is important in the treatment of different muscle spasticity disorders [4]. However, the latter alters mechanics of the spastic muscle. Although the resulting drop of muscle tone is regarded as a positive effect by some researchers [5,6], spastic muscle is often considered as weak. More importantly, how BTX treated muscle produces the motion is still a critical issue. Therefore, it is crucial to understand mechanics of muscle exposed to BTX. The total force exerted across muscle is the sum of active force and passive force. The active force is generated by the contractile proteins upon neural stimulation, whereas the passive force is developed by the stretching of connective tissue structures that are arranged within the muscle and along the contractile elements in the in active state. As a reduced passive resistance is regarded as a desired effect of BTX, it is important to test if that occurs by quantifying the change in muscleâs passive forces directly compared to BTX free condition. Skeletal muscle exerts force

and transmits it to the bone to produce joint movement. Transmission of force occurs in ways: (i) myotendinous way, via the aponeurosis and (ii) myofascial way [7], via the muscleâs epimuscular connections to the surrounding muscles and non-muscular tissues within a muscle compartment. Muscle forces are needed for locomotion and maintenance of body balance implicating their importance for movements. Intramuscularly, many structural elements such as actin, myosin, transsarcolemmal proteins, endo-, peri-, and epimysium as well as tendons and aponeurosis play an important role in force production at the muscle level. However, regarding the latter force transmission way, inter- and extramuscular connective tissues such as compartmental fascia, septa, membranes and collagen reinforced neurovascular tracts are important structural elements that play a role at the compartment level. These structures affect force production of muscle [8,9] as well as the force exerted at the tendon [10]. On the other hand, BTX has been shown to change the force-length properties of the injected muscles [11]. This is important as effects of BTX on the muscleâs mechanics are central. Yaraskavitch et al. [12] showed that BTX causes a more pronounced muscle force reduction at shorter muscle lengths compared to longer. However, those researchers did not address the two highly relevant issues. They did not measure muscle passive forces, hence could not show if BTX reduces muscleâs passive resistance. Moreover, they did not assess if the length range the muscle can produce active force is actually increased. Without testing these issues directly, BTX effects on muscular mechanics remain unclear and unspecific. In addition, the force-length properties of neighboring non-injected muscles were also shown to be influenced suggesting that BTX passes through the fascia to neighboring muscles [12]. However, this effect can be considered as incomplete, since epimuscular myofascial force transmission might play a potential influence on this process. However, this needs to be studied

1.2 Skeletal Muscle

1.2.1 General Structure

Muscle is a tissue composed of cells i.e., muscle fibers and the extracellular matrix and its contraction produces movement via force generation. Skeletal muscles attached to bones produce movements in the body parts under voluntary control, where the contraction of the other types of muscles (namely cardiac muscle and smooth muscle) occurs involuntarily in the body [13]. The skeletal muscle is the most abundant tissue in the body, which performs both dynamic and static work. While locomotion and positioning of the body segments occurs in dynamic work, body posture is maintained by static work [14].

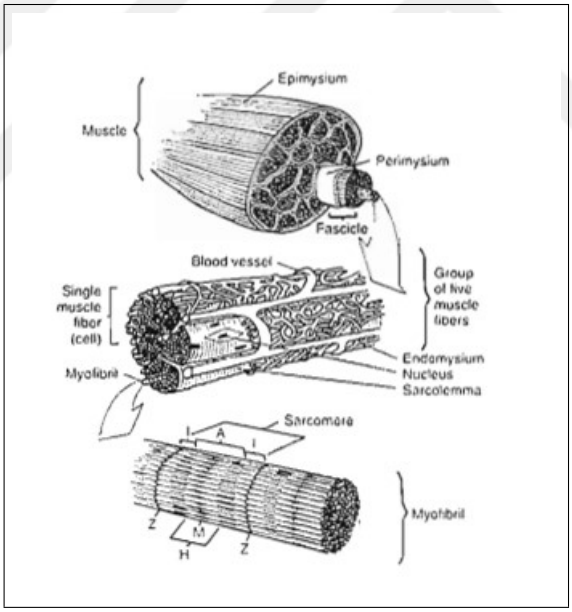

Figure 1.1 Schematic diagram of structural organization of the skeletal muscle (top), the muscle fibers (middle), and the myofibrils which are contractile elements of the muscle (bottom) [14].

There is a hierarchical structural organization in the skeletal muscle (see Figure 1.1). This involves sarcomeres, myofibrils, muscle fibers and fascicles (i.e., bundles of muscle fibers) arranged into the muscle belly. On the other hand, the muscle is hierarchically divided also by intramuscular connective tissues, which includes three different components namely the epimysium (muscle fiber level), the perimysium (fascicle level) and the endomysium (the whole muscle belly level). Epimysium is the sheath of fibrous

connective tissue, which surrounds the surface of the whole muscle. The perimysium groups the individual muscle fibers into bundles known as a fascicle. The endomysium wraps each individual muscle fiber in which this layer consists of blood vessels, nerves and lymphatics. The parallel elastic components of muscle are endomysium, perimysium, epimysium and sarcolemma act as connective tissue. Contracting muscle produces force and transmits it to the bone through also these components in addition to the tendons [14].

1.2.1.1 Muscle Fiber. All skeletal muscles are composed of numerous muscle fibers, long cylindrical cells having hundreds of nuclei in thickness of 10 to 100 μm . These muscle fibers consist of several hundred to several thousand myofibrils which are surrounded by a plasma membrane named the sarcolemma [14]. Each myofibril appears as a serial array of sarcomeres, which consist of thin (actin), thick (myosin), elastic (titin) and inelastic (nebulin) filaments. The former two myofillaments act in active muscle contraction (see Figure 1.2). While thin filaments are composed of actin proteins attaching to Z disc at the ends of the sarcomere, thick filaments are composed of myosin proteins that are holding connections with actin proteins for the working of the contractile machinery. This side-by-side relationship is maintained by titin proteins in which they serve as binding sites for myosin and links thick filaments to the Z disc. Nebulin acts as a template for the thin filament assembly [13,14]. Sliding of the actin filaments into the spaces between the myosin filaments generates active force [15]. Muscle contraction results in the forces generated by the interaction of myosin heads over the actin filaments. This leads to formation of cross-bridges via which, shortening of the sarcomere occurs without altering the lengths of individual filaments

Through this mechanism, the typical repeating arrangement of the myofilaments in a sarcomere results in force generation, which is closely associated with the instantaneous muscle length. Length-tension diagram of an individual contracted sarcomere is influenced by the sarcomere length as this characterizes the amount of actin-myosin overlap (see Figure 1.3). At extremely long sarcomere lengths (the point D in Figure 1.3), no actin-myosin overlap is observed. Hence, the sarcomereâs force production is

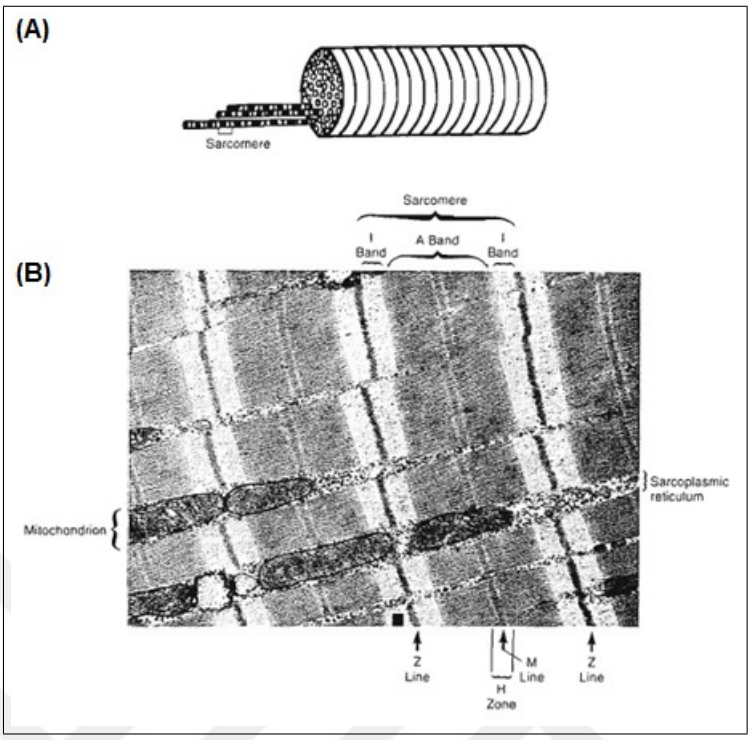

Figure 1.2 Single muscle fiber with protruding myofibrils (A) and electron microscope image of a human skeletal muscle (B) [14].

trivial. At low sarcomere lengths (the point A in Figure 1.3), the thick filaments correspond with each other and limit actin-myosin overlap. This causes sarcomere force to decrease as the muscle contracts and attains shorter lengths. The sarcomere reaches its optimum length (the points B and C in Figure 1.3) by generating maximum amount of force as a consequence of maximal number of cross-bridges formed between the actin and myosin myofilaments.

1.3 Force Transmission in Muscle

?? explains length tension curve for active and passive forces. Structural alterations in sarcomere result in changes in tension when muscle fiber is contracted. While the changes in active forces (tension) occur by the contractile elements of muscle, passive forces reflect stiffness of muscle and occur by the help of series elastic components. The curve in Figure 1.4 exhibits that stretching muscle from its resting length lead to increase in passive force and reduction in active force [14].

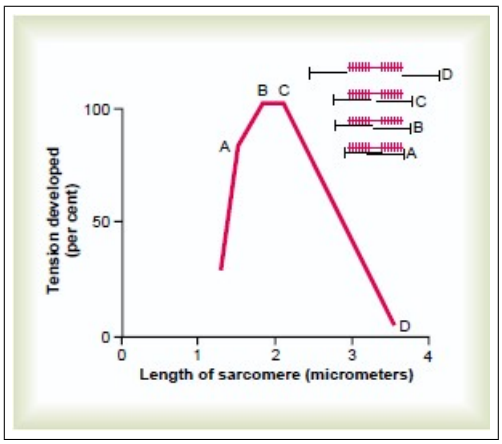

Figure 1.3 Length-tension diagram of an individual contracted sarcomere [13].

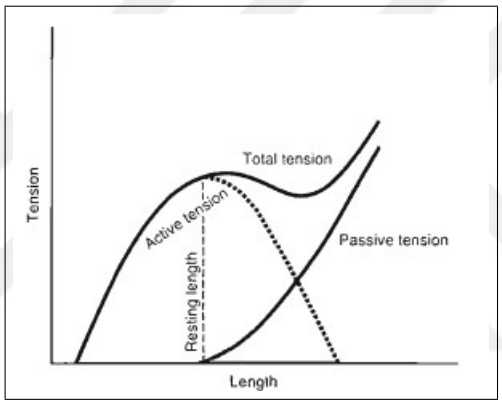

Figure 1.4 The active and passive length-tension curve [14].

1.3.1 Myotendinous Force Transmission

Myotendinous force transmission is the major and well recognized pathway in which force transmission occurs from muscle fibers to bone by means of the specialized myotendinous junctions at the aponeurosis in the muscle belly level and beyond that via the muscleâs tendon [16]. As muscle fibers fuse with the tendon tissue, their diameter decrease up to 90% leading to considerable amount of increased force transmission capacity per cross-sectional area, per muscle fiber [17]. Myotendinous junction structures at the ends of the muscle fibers play an important role for the longitudinal force transmission to the tendon tissue. However, other mechanisms have been also shown to play crucial roles in muscular force transmission since muscle fibers in their natural environment are interacting with the extracellular matrix along their full peripheral

lengths and the muscle belly is not an isolated entity. Instead, it has connections to the surrounding structures, which are capable of transmitting substantial amounts of force without reaching the muscleâs tendon. This mechanism is referred to as myofascial force transmission and is addressed in the following section.

1.3.2 Myofascial Force Transmission

Apart from the transmission of force from the ends of muscle fibers onto the tendon (i.e., myotendinous force transmission), transsarcolemmal proteins in between the extracellular matrix and the muscle fibers provide a mechanical interaction with the muscle fibers, where generated force is transmitted from the myofibers onto extracellular space via myofascial force transmission. Intramuscular connective tissues representing the extracellular matrix, however, are also not isolated, but are continuous with the extramuscular connective tissues, which contains collagen reinforced neurovascular tracts and compartmental boundaries that play an important role in continuity and force transmission between muscular and non-muscular structures [18]. Two different myofascial force transmissions were named in relation to the major contributions of the myofascial structures on such force transmission process:

1. **Intramuscular myofascial force transmission:** In intramuscular myofascial force transmission, transmission of force occurs onto the endomysium and from there the force is transmitted onto the remainder intramuscular connective tissue stroma, which comprises intricate network of endomysial tunnels, and perimysium and epimysium in the higher levels [7]. There are connections between the trans-sarcolemmal molecules of the muscle fiber by means of e.g., laminin and dystrophin molecules in the basal lamina to the collagen meshwork and from there onto the endomysium. It has been shown that these mentioned pathways play crucial role in intramuscular myofascial transmission [19]. In addition, existing collagen fibers and specialized junctions have an impact on muscle function and adaptation, and may stand important roles in mechano-transduction [20].

2. **2. Epimuscular myofascial force transmission:**Force transmission occurs between a muscle and its surrounding tissues, which also includes other muscles and both in the same muscle compartment and beyond. Force in this kind for myofascial force transmission is needed to be transmitted outside its epimysium. Two potential paths are proposed for epimuscular myofascial force transmission:

    - *Intermuscular myofascial force transmission:* Transmission of force occurs between the linked intramuscular connective tissue stromata of the adjacent muscles by means of e.g., their shared epimysia or via the neurovascular tracts which inter connect even distant muscles, named intermuscular myofascial force transmission [7].
    - *Extramuscular myofascial force transmission:* Extramuscular connective tissues such as neurovascular tract (i.e, blood and lymph vessels, nerves) and compartments delimitating connective tissue (i.e, general fascia, intermuscular septa) support this kind of myofascial transmission of muscular force to non-muscular structures [7]

    Epimuscular myofascial force transmission is the term, which refers to both inter- and extramuscular myofascial force transmission in combination.

1.3.3 Characteristics of Muscle Involved in Epimuscular Myofascial Force Transmission

Due to epimuscular myofascial force transmission, muscle relative position has great importance on the force exerted as well. Earlier animal experiments showed that for determination of isometric force, relative position of muscle with respect to surrounding connective tissues and adjacent muscles is a determinant on its own: e.g., proximo-distal force difference (see below for the addressing of this key epimuscular myofascial force transmission effect) varies with the relative position of muscle. Such difference is a direct measure of net amount of muscle force transmitted via epimuscular myofascial pathways [21,22]. Fully isolated muscle has proximally and distally directed equal forces. While proximally directed myofascial load is joined into the force exerted

at the distal tendon, it did not integrate itself at the force exerted onto the proximal tendon and vice versa [9]. Proximo-distal force difference is equal to net amount of epimuscular myofascial force transmitted from the muscle. This also represents the net amount of myofascial loads that act on the muscle belly. Those forces are distributed along the epimysium and can impose effects internally within the muscle due to connective tissue continuity. Yucesoy et al. [9] showed that the difference between the total force exerted at the distal and proximal tendon of a muscle is accompanied by length differences in sarcomeres within muscle fiber. This is a consequence of those myofascial loads. Their findings indicate that while the proximal total force is less than the distal total force, sarcomeres located proximally within muscle fibers exhibited shorter lengths than the sarcomeres distally in muscle fiber. As length of the sarcomeres near the distal tendon was beyond their optimal length, not only the active sarcomeres exert force at the distal tendon but also intracellular passive elements and cytoskeleton play role in force development. However, lengths of sarcomeres near the proximal aponeurosis were below the optimum length in which force exerted at the proximal aponeurosis is predominantly the cause of active force. Overall, they explain the higher distal force compared to proximal force with the higher contribution of extracellular matrix and intracellular structures on sarcomeres, which is affected to produce the proximo-distal force differences shown due to epimuscular myofascial force transmission. In recent years, magnetic resonance imaging (MRI) technique provided a powerful tool for in vivo assessment of EMFT via sarcomere length heterogeneity along human muscle. Yaman et al.23 studied effects of EMFT on sarcomere length distribution in human muscle lower leg in vivo. Their results demonstrated that fiber direction strain distributions significantly differed in all muscle groups in comparison to reference values. Moreover, fiber direction strains were above the reference values not only in gastrocnemius muscle but also in muscle groups having unchanged lengths. These muscle groups (i.e, soleus, deep flexors, peroneal and anterior crural compartment) having high fiber direction strain are expected to be isometric but they exhibited strains. Therefore, this result indicated that gastrocnemius muscle transmits force to these muscle groups pointing out the existence of epimuscular myofascial force transmission via sarcomare length heterogeneity [23]. Pamuk et al. [24] studied muscle fiber direction local tissue deformations within the human medial gastrocnemius mus-

cle combining MRI and diffusion tensor (DT) imaging based tractography in passive condition. Their major finding indicated that inhomogeneity of muscle fiber direction strain in which lengthened proximal and shortened distal track segments was observed in all subjects (see Figure 1.5). The authors also reported that substantial along fiber-shear strains showing shearing between medial gastrocnemius muscle as evidence for major mechanical interaction between extracellular matrix and the muscle fibers that affecting deformation along muscle fibers. Karakuzu et al. [25] studied submaximal plantar flexion activity on length changes of human medial gastrocnemius muscle in vivo combining MRI and DT imaging based tractography. Their results indicated that submaximal plantar flexion activity resulted in heterogenous distributions of strain within the human medial gastrocnemius muscle indicating that muscle fiber sarcomeres may attain different lengths and distinct force production capacities. These studies imply epimuscular myofascial force transmission in human muscle in vivo.

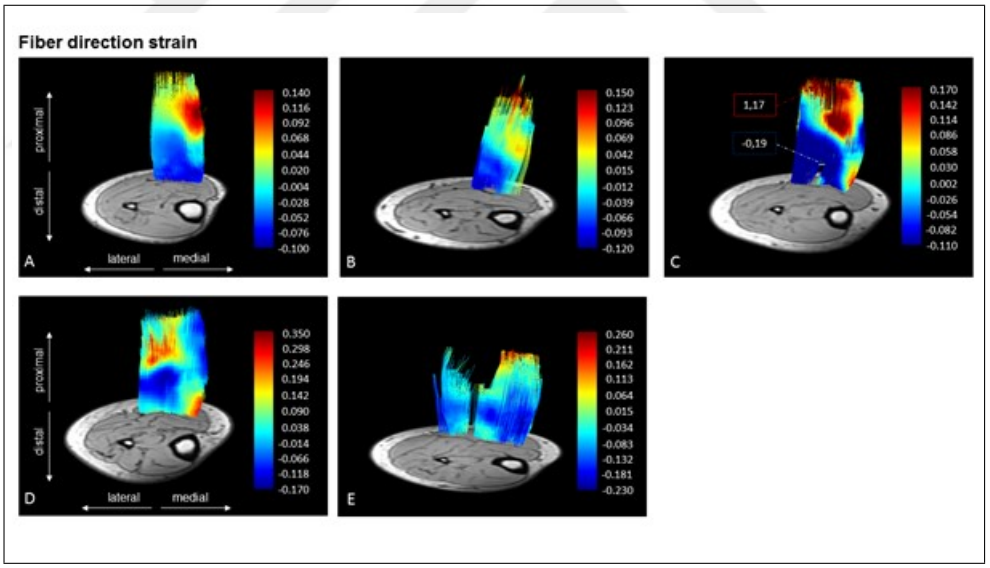

Figure 1.5 Serial distribution of fiber direction strain for subjects A-E in passive test conditions [24]. The general pattern demonstrating locally lengthened and shortened parts within gastrocnemius tracts for each subject. Local shortening was shown as negative values whereas local lengthening was shown as positive values

1.4 Anatomy of Rat Lower Leg

In the anterior crural compartment of the rat leg, mechanical characteristics of muscle and inter-, and extramuscular tissues have an interaction, which play important roles in force transmission. Anterior crural compartment is located at the lower leg and envelopes Tibialis Anterior (TA), Extensor Digitorum Longus (EDL), and Extensor Hallucis Longus (EHL) muscles. TA is the biggest of three and directs the dorsiflexion and inversion of the foot. EHL tendon extends the big toe and EDL distal tendon separates into the four digits, which extend the toes (Figure 1.6). Surgical disruption at the rat anterioral crural compartment have influence on EDL muscle length force characteristics from proximal tendon of muscle via myofascial force transmission [26]

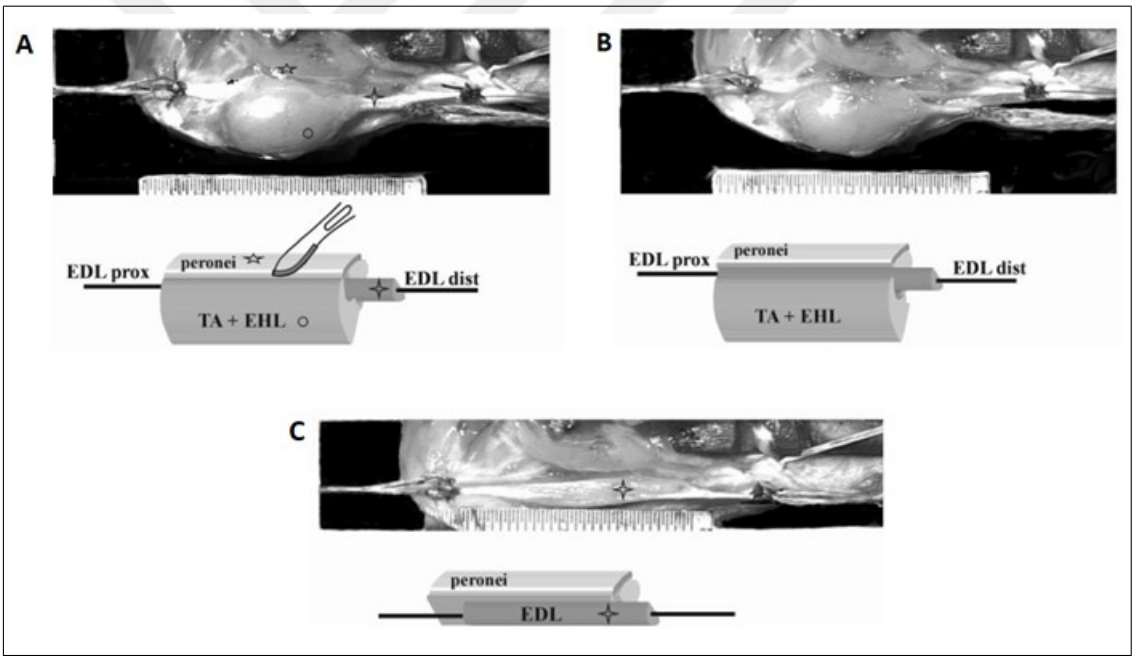

Figure 1.6 The rat anterioral crural compartment [26]. Muscles at the rat anterioral crural compartment named as extensor digitorum muscle (EDL), extensor hallucis longus (EHL) muscle, and tibialis anterior (TA) muscle (A) Anterior crural compartment after removal of biceps femoris muscle, (B) Anterior crural compartment after full lateral fasciotemy, (C) Image represents after EDL was fully isolated from TA+EHL

1.5 Botox in Muscle Mechanics

Clostridium botulinum produces Botulinum type-A (BTX) neurotoxin, which has a paralytic impact on muscles by blocking the release of acetylcholine at the neuromuscular junctions. Therefore, BTX limits force in muscle fibers and leads to impact on movement control due to its mode of action alters gamma efferent motor nerve terminal. Because of this effect, BTX has been used to temporarily treat various muscle spasticity disorders. It has previously been shown that injection of BTX on lateral rectus muscle of the cat exhibits a functional decrease in twitch and tetanic tension without changing muscle-speed characteristics [27]. Another study demonstrated that after injection, BTX leakage leads to penetrate fascia exhibiting the force decrease in plantaris muscle across the long muscle lengths. Such a leakage and force decrease suggest that non-target muscles might be influenced by BTX injections [12]. However, authors did not investigate whether this decrease might be influenced from epimuscular myofascial force transmission or not. Yaraskavitch and his colleagues made an important contribution to the literature by proposing effects of BTX on injection site and adjacent tissue for reconstructive surgery applications. This study is especially important which provides theoretical knowledge for further clinical studies [12]. However, considering the presence of intermuscular myofascial force transmission, regardless of whether BTX did diffuse or not, the force decrease in adjacent muscle is expected due to decreased muscle force in Soleus muscle in which epimuscular myofascial force is diminished.

1.6 Objective of the Study

The specific aim of this study is to test the hypotheses that (1) the effects of BTX injection into the rat Tibialis anterior (TA) muscle has an impact on mechanics of its neighboring EDL muscle. (2) BTX has an impact on epimuscular myofascial force transmission (EMFT). In order to test these hypotheses, BTX was administered to the TA muscle and changes in total and passive forces exerted proximally and

distally by the EDL muscle were measured. Additionally, effects of muscle length and relative position changes imposed were assessed by keeping the BTX injected TA and its neighbor EHL muscles at fixed lengths. Therefore, any effect of BTX on mechanics of a muscle at constant length is not due to muscle length changes, but will reflect how EMFT is affected in a unique way.

2. MATERIALS AND METHODS

2.1 Animals

10 male Wistar rats (5 for control and 5 for BTX groups) with the body mass weighing 300-330 grams were used. Surgical and experimental protocols were in strict agreement with the guidelines and regulations concerning animal welfare and experiments set forth by Turkish law, and approved by the Ethical Committee at Boğaziçi University.

2.2 Experimental Animal Model to Assess Effects of BTX on Muscular Mechanics and EMFT

Following a 1 mg/kg intraperitoneal dose of ketamine injection on rats to impose sedation, a circular region approximately 15-mm radius away from the center of patella was shaved. Palpation allowed to locate the TA muscle with the ankle kept in maximal

plantarflexion and the knee angle at approximately 90°. The patella was marked from the center and a second marker was placed at a point 10 mm distal along the tibia. A line segment was drawn between the 2 markers to determine injection location which was 5 mm lateral. All injections were made in a depth of 3 mm corresponding to the superficial half of the TA muscle. Each of 100-U vial of vacuum-dried botulinum toxin type A (BTX) was reconstituted with 0.9% sodium chloride. A single intramuscular BTX injection at a total dose of 0.1 U (injection volume was 20 μl) was applied for the BTX group. For the control group, only 20 μl of 0.9% saline solution was injected. All these injections were applied 5 days prior to testing. Standard cages and thermally regulated animal room with 12-h dark light cycle were used to keep animals separately until the day of the experiment.

2.3 Experimental Surgery Procedure

Intraperitoneal injection of urethane (1.2 ml of 12.5% solution per 100 g body mass) applied for the animal anesthesia and more doses up to 0.5 ml were given, if necessary. In the end of the experiments, an overdose of urethane solution applied for animal euthanasia. A heated pad (Homoethermic Blanket Control Unit; Harvard Apparatus, Holliston, Massachusetts) used for prevention of animal hypothermia during both surgery and subsequent data collection. The integrated rectal thermometer allowed monitoring and controlling body temperature at 37 °C by setting the temperature of heating pad stable. By removing the skin and biceps femoris muscle of the left hindlimb, the anterior crural compartment comprising the EDL and TA muscles are brought into open. A limited distal fasciotomy was applied to remove the retinaculae. However, the connective tissues of muscle bellies within the anterioral crural compartment were left intact in order to allow inter-muscular mechanical interactions. The knee joint angle of 120 °and the ankle joint angle of 100 °in combination was selected as the reference position. A silk thread was used to tie 4 distal tendons of the EDL muscle, at this reference position. Matching markers were located on the distal tendons of EDL and TA and on the lower leg at the fixed locations. Afterwards, the TA and distal EDL tendons were cut as distally as possible in order to allow long enough tendon material for successive attachment to the force transducers. The proximal EDL tendon was reached by opening the femoral compartment and was cut loose from the femur with the osteo-tendinous attachment left intact by extracting also a small part of the lateral femoral condyle. By suturing Kevlar threads to (a) the proximal tendon of the EDL muscle, (b) the tied distal tendons of the EDL muscle and (c) the distal tendon of TA muscle, the connection of the force transducers with the muscle tendons was provided. The dissection of sciatic nerve by freeing of other tissues and shearing of all nerve branches were performed within the femoral compartment. Afterwards, the sciatic nerve was cut proximally.

2.4 Experimental Set-Up

The animal was placed in the experimental set-up (Figure 2.1). Metal clamps were used to fix the foot in the maximal plantarflexion position (at 180 °), which allowed the free passage of the Kevler threads to distal force transducers. Metal clamps were used also to fix the femur maintaining the knee angle at 120 °. Kevlar threads from the proximal and distal EDL tendons as well the TA distal tendon were connected to different force transducers. A bipolar silver electrode was used to support the distal end of the sciatic nerve.

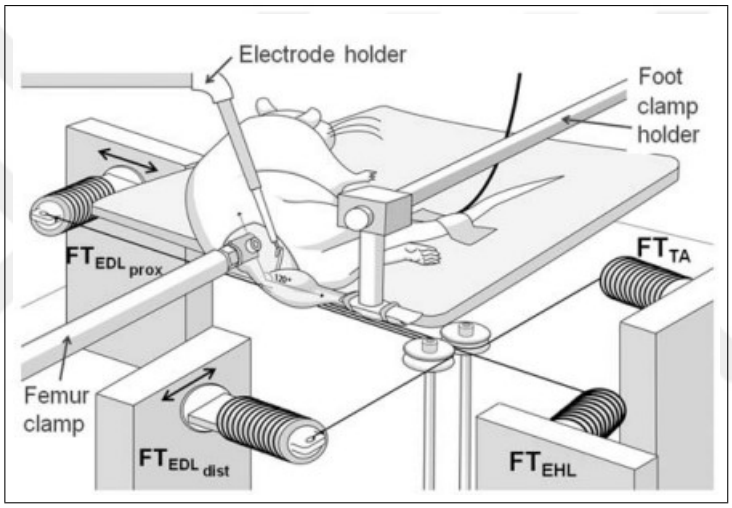

Figure 2.1 The schematic representation of the experimental set-up [10]. The proximal tendon of EDL muscle ($EDL_proximal$), the tied distal tendons of the EDL muscle (EDL_distal) and the distal tendons of TA muscle connected to distinct force transducers.

2.5 Experimental Conditions and Procedure

Experiments were performed at the room temperature (26 °C). To prevent dehydration, isotonic saline solution was used for irrigating tendons and muscles during the surgery and regularly during the force measurements. The sciatic nerve was stimulated supramaximally to maximally activate the TA and EDL muscles (STMISOC; BIOPAC Systems, Goleta, California, USA) by means of constant current implemented as 2. The EDL muscle was set to a target length or position. Subsequently, 2 twitches were

evoked followed by a tetanus (the muscles were activated with 400 ms pulse train at 100 Hz frequency). Following the end of the tetanus, another twitch was applied after 200 ms. To let the recovery of the muscles and avoid fatigue and potentiation, after each stimulation, a waiting period of 2 minutes was allowed.

2.5.1 The Effects of BTX on Mechanics of EDL Muscle

The distal TA tendon was always kept in the reference position during the entire experiment. Therefore, the TA length was not manipulated. Regarding the EDL muscle, either the proximal force transducer was repositioned in the proximal direction or the distal force transducer was repositioned in the distal direction to impose different EDL lengths: proximal and distal lengthening conditions, respectively. For the EDL as well as the TA muscles, total and passive forces were simultaneously recorded at different EDL muscle-tendon complex lengths. By increasing EDL length from the starting position with 1 mm increments, it was stretched up to 2 mm over the optimum length. $\Delta LEDL$ proximal and $\Delta LEDL$ distal are expressed as a deviation from the related optimum length.

2.5.2 The Effects of BTX on EMFT

To test the effects of BTX on EMFT, two different assessments were conducted: (1) The changes in proximal and distal EDL forces as a function of different EDL muscle relative positions were studied. For that purpose the EDL was restrained (i) at short length (i.e., 6 mm below at long length) and (ii) at long length (i.e., 2 mm over the length at which highest highest EDL total force measured). As muscle force changes as a function of muscle length, keeping the EDL length constant and changing its position exclusively allowed a unique test of EMFT: any changes to be shown in EDL forces cannot be ascribed to muscle length effects, but are due to EMFT. EDL proximal and distal force transducers were moved by 5 mm proximally to change EDL muscle position. From this proximal starting position ($\Delta EDL_{position} = 0mm$) on, EDL

position was altered with 1 mm increments through the 8 mm distal ending direction ($\Delta EDL_{position} = 8mm$) by moving both of the proximal and distal force transducers. Afterwards, EDL total and passive forces were recorded. (2) The changes in distal TA forces were studied as a function of different EDL muscle lengths. The force transducer of the TA muscle and proximal force transducer of the EDL muscle were kept at their reference positions and hence muscle-tendon complex length of the TA was always constant. In this condition, the distal EDL force transducer position was changed distally in 1 mm increments to alter EDL length exclusively. Similar to (1), also in (2) the target muscle (i.e., the TA) is kept at constant length and any changes in TA force cannot be ascribed to muscle length effects, but are due to EMFT.

2.6 Statistics

2.6.1 Data Processing

Total isometric forces were determined when tetanic plateau was reached for 200 ms interval and 150 ms after tetanic stimulation whereas muscle passive isometric forces were determined 100 ms after the second twitch. By applying a least squares criterion for total force data, muscle complex tendon length or muscle position fitting with a polynomial function was described:

$$y = b_0 + b_1 x + b_2 x^2 \ldots\ldots b_n x^n \tag{2.1}$$

where y stands for total force and x stands for muscle tendon complex length or $\Delta EDL_{position}$ b_0, b_1, b_2 $\ldots b_n$ are coefficients used for polynomial fitting procedure. Data for passive isometric forces through muscle position were calculated in the same way. For muscle-tendon complex lengths, passive muscle force data were fitted an exponential function described below by applying a least squares criterion:

$$y = e^{a_1 + a_2 x} \tag{2.2}$$

where y stands for passive force and x represents passive muscle-tendon complex length. a_1 and a_2 are coefficients used for polynomial fitting procedure. These polynomials were used to calculate mean values and standard deviations for each EDL muscle complex length and $\Delta EDL_{position}$.

2.6.2 Statistical Analysis

2.6.2.1 Statistics to Assess Effects of BTX on TA and EDL Muscle Mechanics .

Two-way ANOVA for repeated measures for factors of EDL length and animal group was performed separately to assess effects of BTX on TA and EDL muscle mechanics in proximal and distal lengthening conditions. Data considered significant if $p < 0.05$. Bonferroni post-hoc testing applied for the data demonstrating significant main effects to assess significant within-factor force differences. Regarding to different EDL muscle-tendon complex lengths, reduction in total forces expressed as percentage for TA and EDL muscles were found to locate mean force differences between control and BTX groups.

2.6.2.2 Statistics to Assess Effects of BTX on EMFT. Two-way ANOVA for repeated measures for factors of ÎEDLposition and animal group was performed separately to assess the effects of BTX on EDL muscle position. Data considered significant if $p < 0.05$. Bonferroni post-hoc testing applied for the data demonstrating significant main effects to assess significant within-factor force differences. Spearman rank correlation coefficient was calculated to test whether reductions in EDL proximal and distal total forces have a correlation with each EDL muscle relative position through BTX administration. $p < 0.05$ was considered significant.

3. RESULTS

3.1 Effects of BTX on Mechanics of EDL Muscle

ANOVA (factors: EDL length and animal group) showed significant main effects of both factors on EDL proximal total forces and a significant interaction after proximal lengthening. Post-hoc testing showed significant major effects of BTX for all muscle lengths except at $-8mm$ (Figure 3.1A) leading to a force decrease (e.g., at $\Delta LEDL = 0mm$ by 76.9%). ANOVA (factors: EDL length and animal group) also showed significant main effects of both factors on EDL proximal passive forces and a significant interaction after proximal lengthening. Post-hoc testing showed significant effects of BTX between $1mm \leq \Delta LEDL \leq 2mm$ leading to a force increase (e.g., at $\Delta LEDL = 2mm$ by about 13 folds).

ANOVA (factors: EDL length and animal group) showed significant main effects of both factors on EDL distal total forces and a significant interaction after proximal lengthening. Post-hoc testing showed significant major effects of BTX for muscle lengths at which EDL length is greater than â 5 mm (Figure 3.1B) leading to a force decrease (e.g., at $\Delta LEDL = 0mm$ by 81.7%). Minimal total force reduction was 67.4% observed at -4 mm EDL length. ANOVA also showed significant main effects on EDL distal passive forces and a significant interaction after proximal lengthening. Post hoc testing showed significant effects of BTX between $0mm \leq \Delta LEDL \leq 2mm$ leading to a force increase (e.g., at $\Delta LEDL = 2mm$ by about 5 folds).

ANOVA (factors: EDL length and animal group) showed significant main effects of both factors on EDL proximal total forces and a significant interaction after distal lengthening. Post-hoc testing showed significant major effects of BTX for muscle lengths greater than -7 mm (Figure 3.2A) leading to force decrease (e.g., at at $\Delta LEDL = -2mm$ by 77.6%). ANOVA (factors: EDL length and animal group) also showed significant main effects of both factors on EDL proximal passive forces and

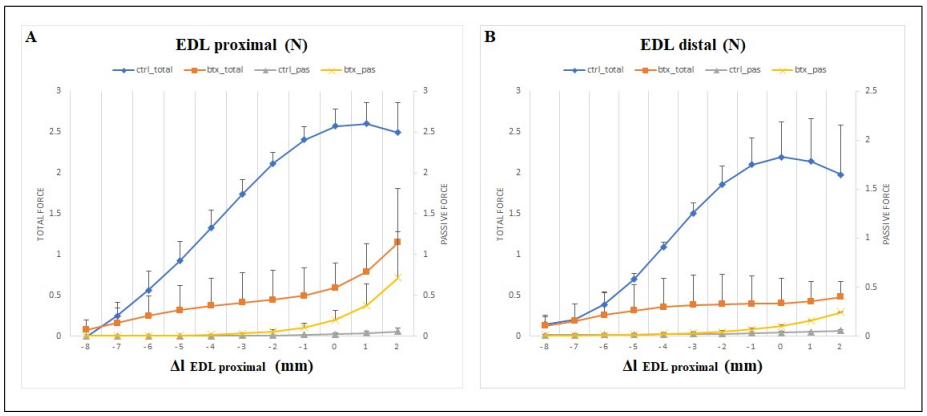

Figure 3.1 EDL length force characteristics between animal groups after proximal lengthening. Both total and passive muscle forces are shown as mean value ± SD for control and BTX animal groups. Proximally (A) and distally (B) exerted total and passive forces are obtained after proximal lengthening

a significant interaction after distal lengthening. Post hoc testing showed significant effects of BTX between $1mm \leq \Delta LEDL \leq 2mm$ leading to force increase (e.g at $\Delta LEDL = 2$ mm by about 8 folds).

ANOVA (factors: EDL length and animal group) showed significant main effects of both factors on EDL distal total forces and a significant interaction after distal lengthening. Post hoc testing showed significant major effects of BTX for muscle lengths at which EDL length is greater than -7 mm (Figure 3.2B) leading to force decrease (e.g at $= 1mm$ by 74.8%). Post-hoc testing showed significant effects of BTX between $1mm \leq \Delta EDL \leq 2mm$ leading to a force increase (e.g., at $\Delta EDL = 2$ mm by about 2 folds).

3.2 Effects of BTX on EMFT

3.2.1 Effects of Relative Position: EDL at Short Length

ANOVA (factors: $\Delta EDL_{position}$ and animal group) at the short length condition showed significant main effects of both factors on EDL total proximal forces (Figure 3.3A) and a significant interaction. EDL relative position changes towards the dis-

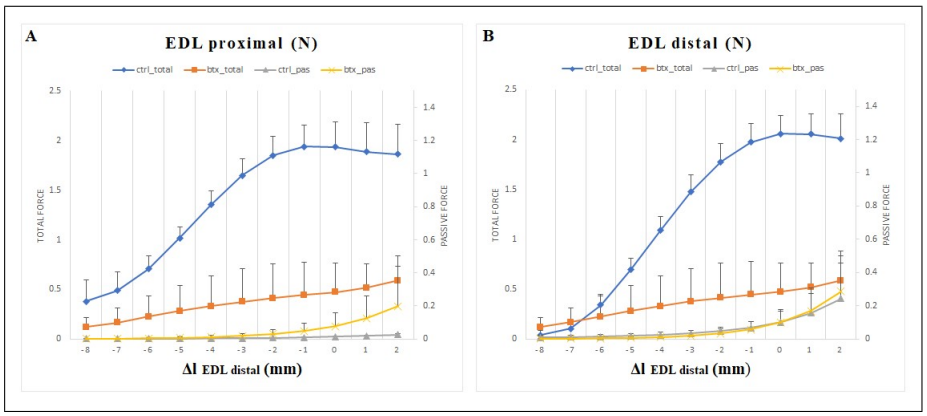

Figure 3.2 EDL length force characteristics between animal groups after distal lengthening. Both total and passive muscle forces are shown as mean value ± SD for control and BTX animal groups. Proximally (A) and distally (B) exerted total and passive forces are obtained after distal lengthening.

tal positions caused EDL total proximal forces to decrease in both control and BTX groups. Post hoc testing showed significant effects of BTX for $4mm \leq \Delta EDL_{position} \leq 8mm$(force drop at $\Delta EDL_{position} = 8mm$ equals to 60.4%). A perfect significant negative correlation was found between force reductions (correlation coefficient= -1.0 and $p = .0001$) when $\Delta EDL_{position}$ changed from proximal to distal location. ANOVA (factors: $\Delta EDL_{position}$ and animal group) also showed significant main effects of both factors on EDL passive proximal forces and a significant interaction. Post-hoc testing showed significant effects of BTX for all $\Delta EDL_{position}$ leading to force increase (e.g., force increase at $\Delta EDL_{position} = 0mm$ was about 3 folds).

ANOVA (factors: ÎEDLposition and animal group) at the short length condition showed significant effects of both factors on EDL total distal forces (Figure 3.3B) and a significant interaction. Post hoc testing showed significant effects of BTX for $3mm \leq \Delta EDL_{position} \leq 8mm$ (force drop at $\Delta EDL_{position} = 8mm$ equals to 64.1%). A perfect significant negative correlation was found between force reductions (correlation coefficient= -1.0 and $p = .0001$) when $\Delta EDL_{position}$ changed from proximal to distal location. ANOVA (factors: $\Delta EDL_{position}$ and animal group) also showed significant main effects of both factors on EDL passive distal forces, but no significant interaction. The passive force increase after BTX injection was 0.9−fold, on average.

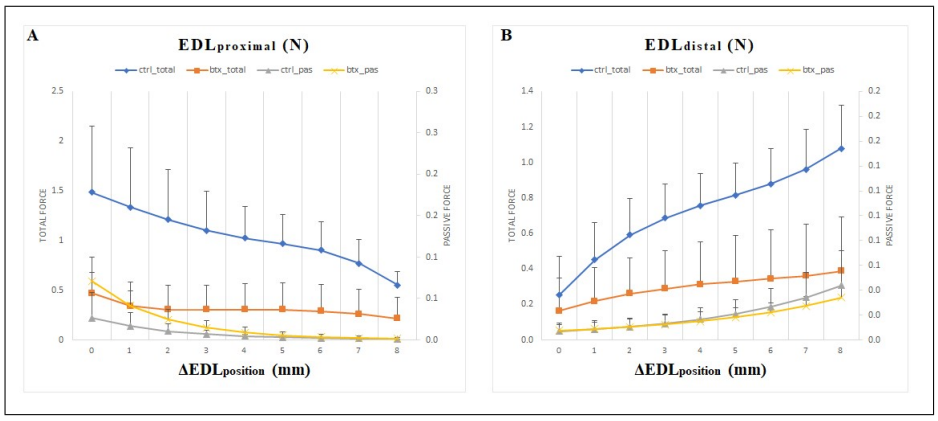

Figure 3.3 Forces of EDL muscle as a function of different muscle positions at the short length. Both total and passive muscle forces are shown as mean value ± SD for control and BTX animal groups. EDL proximal (A) and EDL distal (B) forces are obtained at the reference length. $\Delta EDL_{position} = 0mm$ indicates the proximal starting position and $\Delta EDL_{position} = 8mm$ indicates the distal ending position

3.2.2 Effects of Relative Position: EDL at Long Length

ANOVA (factors: $\Delta EDL_{position}$ and animal group) at the long length condition showed main significant effects of both factors on EDL total proximal forces (Figure 3.4A) and a significant interaction. Post-hoc testing showed significant effects of BTX for $5m \leq \Delta EDL_{position} \leq 8m$ (force drop at $\Delta EDL_{position} = 8mm$ equals to 58.4%). A high significant negative correlation was found between force reductions (correlation coefficient= $-.950$ and $p = .0001$) when $\Delta EDL_{position}$ was changed from the proximal to distal location. ANOVA (factors: $\Delta EDL_{position}$ and animal group) also showed significant effects of both factors on EDL passive proximal forces and a significant interaction. Post-hoc testing showed significant effects of BTX for all $\Delta EDL_{position}$ leading to a force increase (e.g., force increase at $\Delta EDL_{position} = 0mm$ was about 5 folds).

ANOVA (factors: $\Delta EDL_{position}$ and animal group) at the long length condition showed a significant effect of only BTX injection on EDL total distal forces (Figure 3.3B), but no significant effects of $\Delta EDL_{position}$ or a significant interaction. The mean force decrease for $\Delta EDL_{position}$ studied was 70.74%. No significant correlation was found between the force reductions when $\Delta EDL_{position}$ changed from proximal to distal location (correlation coefficient= $-.633$ and $p = .67$). ANOVA (factors: $\Delta EDL_{position}$

and animal group) also showed significant main effects of on EDL passive distal forces, but no significant interaction. The passive force increase after BTX injection was 1.5-fold, on average.

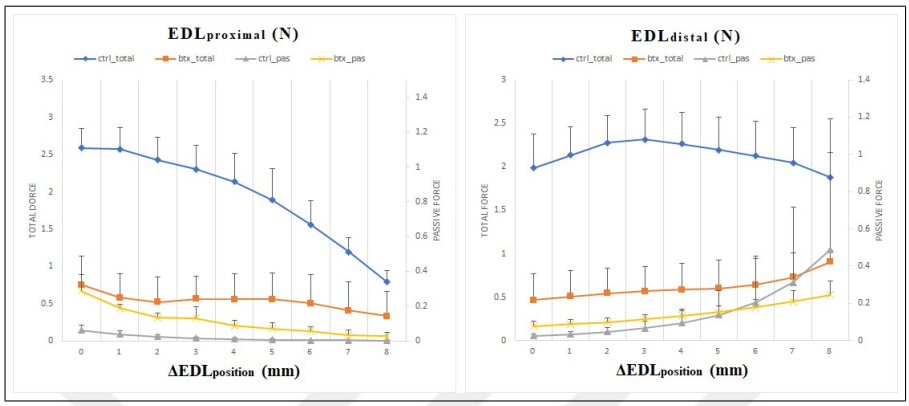

Figure 3.4 Forces of EDL muscle as a function of different muscle positions at the long length. Both total and passive muscle forces are shown as mean value $\pm SD$ for control and BTX animal groups. EDL proximal (A) and EDL distal (B) forces are obtained at the optimal length. $\Delta EDL_{position} = 0mm$ indicates the proximal starting position and $\Delta EDL_{position} = 8mm$ indicates the distal ending position.

3.2.3 The Effects of Muscle Length: TA at Reference Position

ANOVA (factors: EDL length and animal group) showed significant main effects of only BTX injection on total forces of the restrained TA muscle. The mean total force decreases for the EDL lengths studied were 71.8% (Figure 3.5A) for proximal lengthening and 74.6% (Figure 3.5B) for distal lengthening conditions. ANOVA (factors: EDL length and animal group) showed significant main effects of only BTX injection on passive forces of the restrained TA muscle. TA passive force increased significantly for the proximal lengthening condition by 1.5 folds (Figure 3.5A) and for the distal lengthening condition 1.2 folds (Figure 3.5B) on average, respectively.

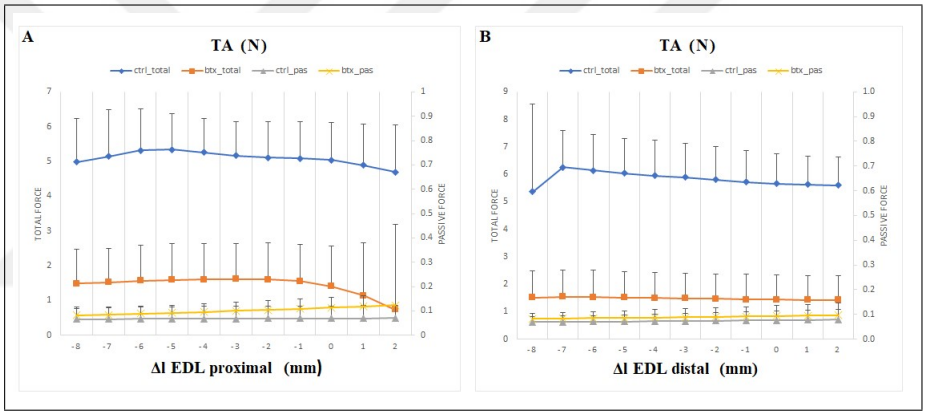

Figure 3.5 Forces of TA muscle as a function of increasing EDL muscle length. Both total and passive muscle forces are shown as mean value ± SD for control and BTX animal groups. TA total and passive forces are obtained after EDL proximal lengthening (A) and EDL distal lengthening (B).

4. DISCUSSION

The main aims of this project were to understand the effects of BTX (1) on EDL muscle mechanics and (2) on EMFT. In order to address the former, by keeping the distal TA tendon in the reference position, either proximal or distal EDL force transducers were repositioned in order to impose different EDL lengths (i.e., proximal and distal lengthening conditions). In order to address the latter, by keeping the EDL length constant and changing its position, the changes in proximal and distal EDL forces were assessed at short and long lengths. In addition, by keeping muscle-tendon complex length of the TA constant, changes in distal TA forces as a function of different EDL muscle lengths were studied. Previous research applied BTX doses on TA muscles of rats ranging from 0.02 U to 20.0 U in which even the small doses were shown to cause cross-sectional area to be significantly paralyzed after 24 hours of injection [28]. In our research, we applied an intermediate BTX dose of 0.1 U with single injection from the superficial half of TA muscle to assess short term effects of BTX. Previous literature demonstrated that BTX caused greatest force decrease after 4 days of injection in the rat lower hind limb flexors with maximum effects at approximately 5 to 7 days, afterwards. It is important to note that, BTX injection was shown to lead to sprouting of motor nerve fibres in the gastrocnemius after the first week [29]. This indicates that in the longer term, although the paralysis via the original neuromuscular junctions imposed will be valid, new nerve branches developing can allow certain added muscle stimulation. Hence, we studied BTX effects 5 days after injection in order to avoid new neuromuscular junctions occurring via nerve sprouting and to have a steady-state force decrease in the muscles studied. Our protocol differs from the previous clinical practice particularly in two ways: (1) Doses used in children with cerebral palsy ranged from 2 and 6 U/kg [30] and even dose of 8 U/kg were used. The dose we used was much smaller than that used in those clinical studies, but we found substantial reductions in muscle forces which show that the dose we used was quite effective for the muscles studied. Studies also demonstrated variable data on the effects of BTX doses in different species. Although doses of 8.3 U/kg in the

mouse [31] and 3.2-.3.5 U/kg in the cat [12] were previously reported as appropriate for experimentation, such different quantities of doses injected indicate that it is hard to predict the clinical relevance towards human subjects of the doses injected in animal studies. This issue is similarly important for any animal studies hence is a limitation in our study. More and dedicated studies are necessary to find out the specific doses that can be relevant for clinical implications by taking into account the muscle size and body mass index. However, the dose used presently did allow testing of new aspects of BTX successfully. (2) We applied a single injection, which is known to have a great impact on saturation of the local binding sites within the injected muscle. Hence, BTX may spread through to the adjacent muscles (e.g., from the injected TA muscle to the neighboring EDL muscle in the compartment). As a result, diffusion of BTX might be considered as side effect [32] due to the possibility that it impairs the effect of treatment as additional and unintended paralysis in the surrounding musculature is plausible. Although, in animal studies, possible outcome of BTX spread to antagonistic muscles has not been sufficiently evaluated yet, previous literature points out local diffusion [28] and even vascular diffusion via circulation might play a role in such even wider scale spread [33]. More studies are required to investigate such mechanisms as well as the outcome of different injection protocols as not only injected dose but also the volume of normal saline within which BTX is injected can affect leakage of BTX into sites within and outside the injected muscles.

4.1 BTX Effects on Mechanics of a Non-injected Muscle in the Same Compartment

The effects BTX on muscle forces were studied for a wide range of muscle lengths in order to characterize its effects on the muscular mechanics comprehensively. In parallel to our hypothesis, injection of BTX on TA altered total and passive forces exerted proximally and distally in the EDL muscle. The data indicated that EDL tendon relocated to impose different muscle lengths demonstrated decreased total forces in both proximal and distal lengthening conditions (see figure 3.1 and 3.2). Shifting of the active slack length of muscle (the shortest length that muscle exerts non-zero

force) to a longer length may imply more prominent muscle weakening for short muscle lengths. In addition, more prominent active force reductions might occur at other short lengths. However, after BTX administration, high total force decreases were observed in longer lengths. For example, for EDLproximal total forces, a major total force decrease was observed at $\Delta EDL = -1mm$ by 79.4% and $\Delta EDL = -2mm$ by 77.67% after proximal and distal lengthening conditions, respectively. For EDLdistal total forces, a substantial total force decrease was observed at $\Delta EDL = 0mm$ by 81.70% and $\Delta EDL = -1mm$ by 77.48% after proximal and distal lengthening conditions, respectively. These data may indicate that BTX affects total muscle forces at longer lengths via elevated ECM stiffness rather than solely by creating a muscle weakness. Indeed, it was previously shown for BTX treated muscle that muscle fibers interacts with the extracellular matrix and that this limits sarcomere shortening in the muscle microenvironment [34]. Therefore, increased stiffness of the ECM represents an important issue in increased muscle lengths. BTX may promote such events at longer muscle lengths via muscular interactions. One may speculate that reduced total force productions within the muscle compartment may result in diminished muscular interactions. Hence, BTX may not influence EMFT on muscular mechanics after discontinuation of treatment. Although how direct injection of BTX on the EDL muscle or diffusion mechanisms of the BTX from TA through EDL impacts on muscle mechanics remain unknown, the data may represent the condition in which EDL muscle may be affected through BTX leakage as explained before [12]. Furthermore, EDL passive forces were higher after BTX administration, which indicates that BTX changed passive characteristics of muscle. The slope of EDL passive force length curves of BTX groups were higher than control group (e.g., at $\Delta EDL = -2mm$ $EDL_{proximal}$ force was increased by about 13 folds after proximal lengthening). In addition, both of the EDLproximal and EDLdistal passive forces were shown to increase at longer lengths after proximal and distal lengthening conditions and no significant force increase was found at short lengths (see section 3.1). This also indicates increased stiffness of the ECM after BTX exposure. Specific tests of elevated collagen content are required to explain this effect. Note that, in the light of these findings, one cannot directly refer to a possible impact of BTX on increased stiffness in spastic muscles since normal and spastic muscle have different characteristics. The effect of BTX on muscle stiffness in spastic muscles

requires more specific experiments and studies in CP patients.

4.2 BTX Effects on EMFT

. We studied effects of BTX on EMFT through the following two assessments: (1) the changes in proximal and distal EDL forces as a function of different EDL muscle relative positions after the muscle was kept at a short and a long length and (2) the changes in distal TA forces as a function of different EDL muscle lengths. Previous literature suggest that EMFT leads to significant differences between the forces measured at the proximal and distal ends of a bi-articular muscle by influencing the length-force characteristics of sarcomere [8]. Such proximo-distal force difference is the characteristic effect of EMFT. Epimuscular connections have non-linear force deformation characteristics and they are heterogeneously distributed within muscle compartment [8]. Hence, the distribution of epimuscular myofascial loads on the muscle belly may change as a function of altering relative muscle position, as this will cause differential stretches on the epimuscular connections. In the present experiment, the control group did demonstrate the characteristic EMFT effect. Our results show that (a) EDL relative position changes towards the distal muscle positions caused EDL total forces to significantly decrease also in the BTX group, but proximo-distal force differences were different as well compared to control group. In other words, EDL force measured at most proximal position did not alter significantly with $\Delta EDL_{position}$ change (i.e., EDL total proximal force significantly dropped at $4mm \leq \Delta EDL_{position} \leq 8mm$) and (b) Force reductions dependent on relative muscle positions in BTX group. Henceforth, these results indicated that BTX administration reduces EMFT. However, histological assessments are required to analyze epimuscular connective tissue content after BTX administration to confirm these findings. In addition, $\Delta EDL_{position}$ did not affect distal EDL forces in the long length condition. Previous literature suggest that at more distal muscle positions, BTX-free animal group shows an increased EDL distal active forces [35]. In comparison to this study, we did not find such effect. Since epimuscular myofascial loads can result in complex sarcomere length distributions, such loads may change sarcomere lengths that leading to significant change in EDL proximal

forces but not the EDL distal forces. Therefore, EMFT do not seem to be always effective on long length condition. It should also be noted that, BTX injection on the TA muscle was not affected by changes in EDL length. Earlier BTX-free experiment showed that lengthening the distal TA tendon also affected by $\Delta LEDL$ [36]. Our results indicated that BTX diminished the interaction of muscle with its surrounding tissues in which the muscle paralysis through BTX exposure within the compartment may explain reduced force decreases and muscular interactions. This finding showed that EMFT was diminished after BTX injection in which connective tissues including EMFT pathways may be also diminished. This study has some clinical meaning as well. Spastic muscle in CP patients demonstrate low length range and low capacity for active force exertion. Ates et al. [37] studied the peak force exertion of limb muscle force which was observed maximally 79% and minimally 22% indicating that spastic muscles exert only a portion of their peak force. In addition, the role of EMFT was previously shown in spastic paresis [38]. According to Ates et al. [37], sarcomere length heterogeneity within muscle fibers may cause alteration in muscle length force characteristics. Enhanced myofascial loads acted on spastic muscle may lead to altered of sarcomere lengths, which favors both the force exerted at the joint and narrowing of the joint range motion. Therefore, an unknown positive implication of BTX on spastic muscles may be that it avoids an undesired change in the muscle length force characteristics via reduced EMFT. In conclusion, this study shows that BTX have impact on muscular mechanics and EMFT mechanism. Clinical implications of those findings should be tested in CP patients.

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