EFFECTS OF APONEUROTOMY ON MECHANICS OF MUSCLE WITH INTACT NEIGHBORING MUSCULAR AND NONMUSCULAR STRUCTURES

by

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ABSTRACT

EFFECTS OF APONEUROTOMY ON MECHANICS OF MUSCLE WITH INTACT NEIGHBORING MUSCULAR AND NONMUSCULAR STRUCTURES

Aponeurotomy (AT) is a surgical technique used to lengthen spastic and/or short muscles. In previous studies, the biomechanical effects of AT were studied both experimentally and by finite element modeling in isolated muscle. In this study, the aim is to determine the effects of AT on mechanics of muscle with intact neighboring muscular and non-muscular structures. In order to achieve this goal AT was performed on the proximal aponeurosis of extensor digitorum longus (EDL) muscle of rat. Length–isometric force characteristics of EDL distally and proximally as well as the tibialis anterior (TA) and extensor hallucis longus (EHL) muscle complex distally were determined in (1) the intact condition, (2) the acute AT condition (after partial fasciotomy and proximal aponeurotomy), (3) the post AT condition (i.e. repeating the second step), (4) the fasciotomy condition and (5) TA+EHL removal condition. EDL distal and proximal length-force characteristics were altered significantly after all surgical interventions. EDL distal forces at optimum muscle length were decreased by 34.8 % in post AT, 41 % in fasciotomy and 52 % in TA+EHL removal conditions compared to intact condition. Also muscle optimum length shifted to higher lengths by 0.53 mm in post AT, 0.66 mm in fasciotomy and 0.28 mm in TA+EHL removal conditions. EDL proximal forces at optimum muscle length were decreased by 42.2 % in post AT, 43.4 % in fasciotomy and 48 % in TA+EHL removal conditions compared to intact condition. For short lengths drop of muscle force after AT was more pronounced and muscle force decreased by 73 % in post AT condition. TA+EHL forces decreased gradually as EDL was lengthened distally. Besides this after each intervention overall TA+EHL force decreased. It is concluded that the presence of epimuscular connections limits the effects of aponeurotomy and it should be noted that before planning a surgery to restore the motion of a joint, the possible effects on the other end of the muscle and the synergetic muscles should be taken into account.

Keywords: Aponeurotomy, epimuscular myofascial force transmission, rat EDL, fasciotomy.

ÖZET

İNTAKT DURUMDAKİ KOMŞU KASLAR VE KAS DIŞI YAPILARIN VARLIĞINDA APONÖROTOMİNİN KAS MEKANİĞİNE ETKİLERİ

Aponörotomi (AT) spastik ve/veya kısa kasların uzatılmasında kullanılan cerrahi bir tekniktir. Daha önceki çalışmalarda izole kasta AT'nin biyomekanik etkileri deneysel ve sonlu eleman modelleme ile incelenmiştir. Bu çalışmada ise AT'nin intakt kas ve kas dışı yapılara komşuluğu olan kasların mekaniğine etkisini saptamak hedeflenmektedir. Bu amaç için sıçanın Extensor digitorum longus (EDL) kasının proksimal aponevroz kısmında AT uygulanmıştır. EDL'nin distal ve proksimal; tibialis anterior (TA) ve extensor hallucis longus (EHL) kas kompleksinin distal boy-izometrik kuvvet karakteristiği; (1) intakt durumda (2) akut AT durumunda (kısmı fasyotomi ve proksimal aponevroz sonrası) (3) AT durumu sonrası (ör. ikinci adımın tekrarlanması) (4) fasyotomi durumunda ve (5) TA+EHL çıkarılması durumunda belirlenmiştir. Her cerrahi müdahaleden sonra EDL distal ve proksimal boy kuvvet karakteristiği önemli şekilde değişmiştir. Optimum kas boyunda EDL distal kuvvetleri intakt duruma gore AT sonrası % 34,8, fasyotomi durumunda % 41, TA+EHL çıkarılması durumunda % 52 azalmıştır. Bununla birlikte, optimum boy AT sonrası durumda 0,53 mm, fasyotomi durumunda 0,66 mm, TA+EHL çıkarılması durumunda 0,28 mm daha uzun boylara kaymıştır. Optimum kas boyundaki EDL proksimal kuvvetleri intakt duruma göre AT sonrası durumda 42.2 %, fasyotomi durumunda 43.4 %, TA+EHL çıkarılması durumunda 48% azalmıştır. Kasın kısa boyları için kuvvet düşüşü daha belirgindir ve kas kuvveti AT sonrası durumda 73 % düşmüştür. EDL distal olarak uzatıldıkça TA+EHL kuvvetleri düşmüştür. Bunun yanında her müdahaleden sonra TA+EHL kuvvetleri bütün boylarda düşmüştür. Sonuç olarak epimüsküler bağlantıların varlığının aponevrozun etkilerini sınırladığı ve eklem hareketini düzeltmeye yönelik AT planlamadan önce kasın diğer ucunda ve sinerjik kaslardaki olası etkileri hesaba katılmasının gerekliliği sonucuna varılmıştır.

Anahtar kelimeler: Aponöronotomi, epimüsküler miyobağdokusal kuvvet iletimi, sıçan EDL'i, fasyotomi

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α	Angle
℃	Degree Celsius
Δl_{m+t}	Deviation from Optimum Length
$(F_{distal} - F_{proximal})$	Proximo-distal Total Force Difference

LIST OF ABBREVIATIONS

n	Number
gr	Gram
SD	Standard Deviation
ml	Milliliter
FT	Force Transducer
TA	Tibialis Anterior
EDL	Extensor Digitorum Longus
EHL	Extensor Hallucis Longus
SIA	Anterior Intermuscular Septum
STMISOC	Biopac System Stimulator
ms	Millisecond
Hz	Hertz
mA	Milliamper
mm	Millimeter
SE	Standard Error
ANOVA	Analysis of Variance
µm	Micrometer
$F_m(N)$	Muscle force (Newton)
F_{moa}	Muscle force at optimum length
AT	Aponeurotomy
l_{opt}	Optimum muscle length

1. INTRODUCTION

Muscle is an activatable soft tissue which is responsible for generation and exertion of force via contractions to create motion yielding bodily locomotion. Contraction is the mechanism in which muscle shortens and produces force when stimulated. Muscle tissue is classified into three different types according to functional and morphological differences.

1) Skeletal muscle: They are striated muscles connected to the skeletal system and are responsible for locomotion by transmitting the force they generate to bones and joints through tendons. Skeletal muscles are highly organized and comprised of structural units decreasing in size.

2) Smooth muscle: They are non-striated muscles of the internal organs, blood vessels and hair follicles. Contractile elements of smooth muscles are elongated, usually spindle-shaped cells with centrally located nuclei. Although transverse striations are lacking, both thick and thin myofibrils occur: such fibers are bound together into sheets or bundles by reticular fibers, and frequently elastic fiber nets are also abundant.

3) Cardiac muscle: It is found in the walls of the heart. The cardiac muscle cell has one central nucleus, like smooth muscle, but it also is striated, like skeletal muscle. The cardiac muscle cell is rectangular in shape. The contraction of cardiac muscle is involuntary, strong, and rhythmical.

1.1 Skeletal muscle

Skeletal muscle can be considered as activatable functional units (muscle fibers) surrounded by a three-dimensional tunnel like network of connective tissue. The overall muscle is surrounded by a fascia and a connective tissue referred as *epimysium* which is composed of irregularly distributed collagen fibers, connective tissue cells and fat. Inside

the epimysium, the next level of structure is a *fascicle* which is a bundle that contains a number of muscle cells i.e., muscle fibers. Each fascicle is surrounded by a connective tissue called *perimysium*. A muscle fiber is comprised of *myofibrils* which are suspended in a matrix called sarcoplasm and the cell membrane of a muscle fiber is called *sarcolemma*. Each muscle fiber is covered with a connective tissue called *endomysium* (Fig 1.1).

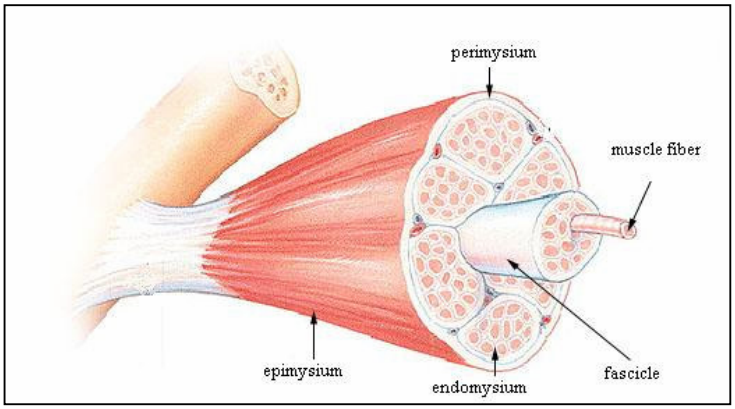

Figure 1.1 Structural organization of a skeletal muscle. Modified from [26]

In that respect, skeletal muscle can be considered as a three-dimensional system of endomysial tunnels within which muscle fibers operate (Fig. 1.2).

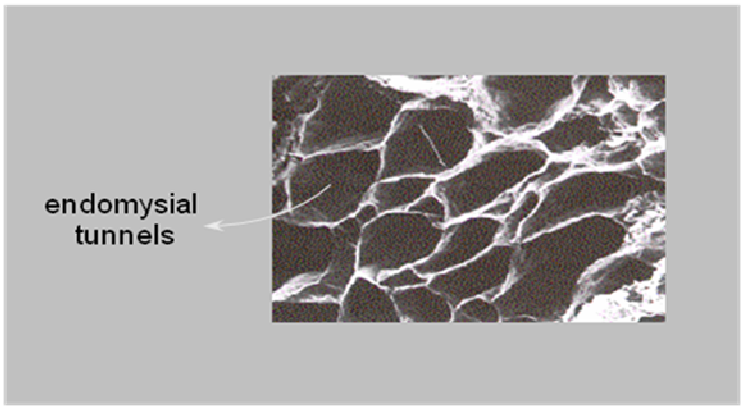

Figure 1.2 Picture illustrating the mental picture of skeletal muscle as a three-dimensional tunnel of endomysial tunnels. Modified from Trotter and Purslow (1992).

An individual muscle fiber has a striated pattern when viewed under the light microscope. These bands are comprised of *sarcomeres* which are the smallest functional

units of a muscle, mainly composed of thin (actin) and thick (myosin) myofilaments. Sarcomeres are bordered by the Z-discs which are structural membranes running through the all cross-section of a myofibril. Actin filaments are bisected by Z-disks where the myosin filaments are located in the center of a sarcomere. Myosin filaments are responsible for the dark areas within the striated pattern, so called A-bands whereas; actin filaments make up the light patterns of the striation which are called I-bands. The area within the A-band with a lower refractive index is called H-band. The myosin filaments are connected to each other with a system of fixed transverse filaments called M-bridges, forming the M-bridges (Fig 1.3).

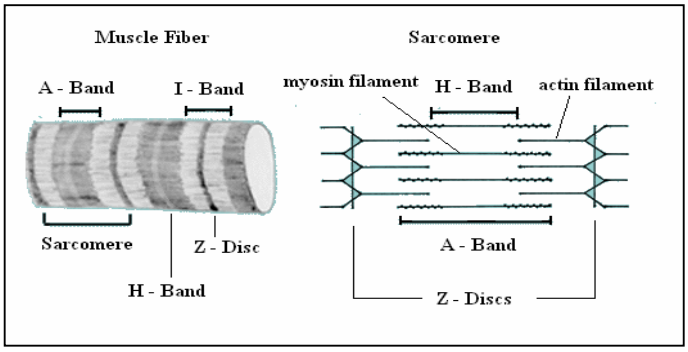

Figure 1.3 A schematic view of a muscle fiber and a sarcomere. Modified from [27]

1.2 Transmission of Muscular Force

1.2.1 Myotendinous Force Transmission

A major site for transmission of muscular force is the myotendinous junction. These junctions are localized between the muscle fibers and the aponeurosis which is a fibrous sheet or flat, expanded tendon. Aponeurosis gives attachment to muscular fibers and serves as means of origin or insertion of a muscle (Fig. 1.4). These structures sometimes also perform the functions of fascia for other muscles.

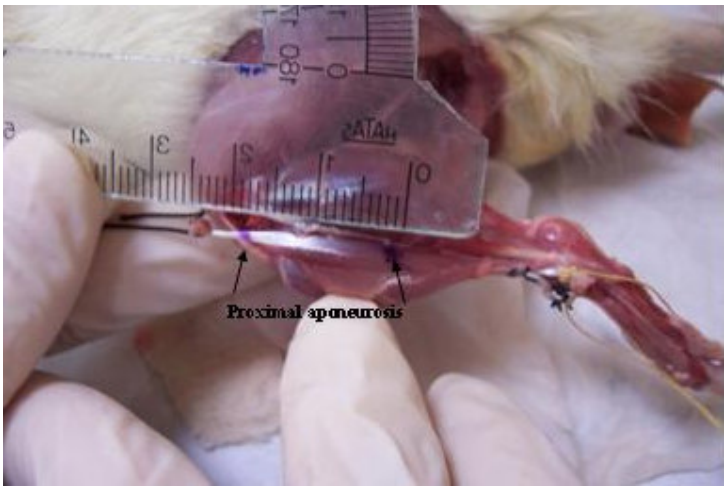

Figure 1.4 Proximal aponeurosis of rat EDL.

In the myotendinous junction, the diameter of muscle fibers decreases substantially towards their tendinous insertion while at the same location the sarcolemma folds extensively in the muscle fibers' longitudinal direction form (i) invaginations into the muscle fiber and (ii) finger-like processes protruding from the muscle fiber. Between these invaginations collagen fibers are located and these fibers are combined to form an intramuscular aponeurosis which later on turns into a tendon outside of the muscle belly. The invaginations effectively increase the surface area available for force transmission and provide a highly favorable site for force transmission.

In the classical approach, muscle force is accepted to be transmitted to the bone exclusively through the myotendinous junction due to their highly specialized morphology. If the myotendinous junctions were actually the exclusive sites for force transmission, one would expect to (1) measure equal forces at the two ends of a muscle and (2) length-force characteristics determined to be unique properties of the muscle studied while performing isometric contraction experiments. In the classical approach, such issues are considered widely to be valid implicitly. However, recent studies [e.g. 1, 2, 3] showed that other structures and therefore, pathways also have an important role in muscular force transmission (for a review see Yucesoy and Huijing 2007).

1.2.2 Myofascial Force Transmission

Muscle tissue can be considered as muscle fibers and extracellular matrix - a 3D tunnel network of connective tissue composed of collagen fibers (i.e. epimysium, perimysium, endomysium) - connecting these fibers. Peripheral myofibers have mechanical connections to the extracellular matrix provided by trans-sarcolemmal proteins through their sarcolemma. The internal myofibers which are lying parallel to each other are also connected with each other via proteins embedded in the subsarcolemmal cytoskeleton.

Recent studies have shown that all these mechanical relations play an important role in muscular force transmission and are proven to be an additional pathway to the well known myotendinous pathway. This kind of force transmission mechanism is referred as *myofascial force transmission* [4].

Myofascial force transmission pathways are classified according to the type of connections taking part:

Intramuscular myofascial force transmission: Muscle force is transmitted to the endomysium via the complex trans-sarcolemmal molecular connections of the muscle fiber to the extracellular matrix. The continuous structure of the intramuscular connective tissue leads to further transmission of force from the endomysium to the higher level intramuscular connective tissues, perimysium and epimysium [4, 5].

Intermuscular myofascial force transmission: Muscle is not an isolated entity in its natural environment. It is surrounded by synergist muscles extracellular matrices of which are in mechanical connection. Transmission of force from the extracellular matrix of a muscle onto the extracellular matrix of the adjacent muscle is referred to as *intermuscular myofascial force transmission* [6, 7].

Extramuscular myofascial force transmission: In addition to the above connections, structures supporting tissues like blood vessels and nerves (referred to as neurovascular tracts) and also the compartmental connective tissue surrounding groups of muscles provide means of muscular force transmission. This kind of force transmission is referred to as *extramuscular myofascial force transmission* [2, 8].

Epimuscular myofascial force transmission: Both the inter- and extramuscular connections within a muscle form an integral system referred as *epimuscular connections*. In cases where the specific role of inter- or extramuscular connections cannot be distinguished for force transmission, the term *epimuscular myofascial force transmission* is used instead [9].

The effects of epimuscular myofascial force transmission is observed as 1) proximo-distal force differences measured in biarticular muscles, 2) alteration of length-force characteristics of a muscle in relation to its position according to its synergist muscles and 3) altered sarcomere length distributions within a muscle.

1.3 Aponeurotomy as a technique of remedial surgery

In spastic movement disorders an excessive reflex activity is observed in the affected muscle which in turn results in a permanent shortness and the range of joint motion is altered as a consequence [1]. Such a case is the *equinus* deformation of ankle which is common in cerebral palsy. It is defined as dorsiflexion of ankle less than 5 degrees with the knee extended or dorsiflexion less than 10 degrees with the knee flexed [10]. It is generally due to contractures in gastrocnemius muscle and surgical interventions are required in order to lengthen it.

Aponeurotomy is a general term that covers surgical techniques in which the aponeurosis is cut transversely in order to lengthen a short muscle due to spasticity (Fig. 1.5). In case of equinus, aponeurosis of gastrocnemius is lengthened. After the aponeurosis is cut the foot is forced to dorsi flexion by the surgeon so that a more normal range of motion is attained and the foot is placed in the cast during the healing period. Although this procedure is effective in restoring normal patterns of gait in spastic children little was known about the mechanisms and its effect on muscle mechanics.

Recent studies including both experimental studies [i.e. 11, 12, 13] and finite element modeling of aponeurotomy [i.e. 14, 15] were reported explaining the effects and the mechanisms taking part in aponeurotomy. However, these studies were performed on isolated muscle and as it was mentioned before muscle mechanics is strongly dependent to the surrounding tissues. In order to determine the acute effects of aponeurotomy with both

contact neighboring muscular and non-muscular tissues on muscle mechanics this study was performed.

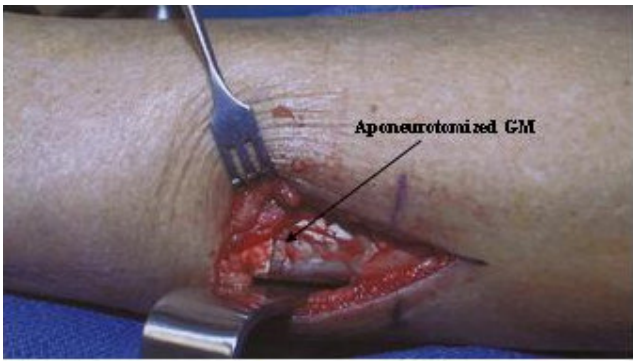

Figure 1.5 An example of incission made in GM recession technique (adapted from Pinney 2004).

1.4 Goal of study

The specific goal of this study is to assess the effects of aponeurotomy on mechanics of muscle with intact neighboring muscular and non muscular structures (i.e, epimuscular myofascial connections). In order to achieve this goal proximal aponeurotomy is performed on the extensor digitorium longus (EDL) muscle of the rat and length-isometric force characteristics of aponeurotomized EDL are determined under several experimental conditions.

2. METHODS

Surgical and experimental procedures were in strict agreement with the guidelines and regulations concerning animal welfare and experimentation set forth by Turkish law, and approved by the Committee on Ethics of Animal Experimentation at Boğaziçi University. Immediately after all experiments, animals were sacrified using an overdose of urethane solution.

2.1 Surgical Procedures

Male Wistar rats (n = 6, mean body mass = 285.5 (S.D. 13.7g) were anaesthetized with intraperitoneally injected urethane solution (1.2mg of 12.5 % urethane solution /100 g body mass). Extra doses (up to 0.5ml) were given if necessary. During surgery and data collection, the animals were placed on a heated pad (Harvard Apparatus, Homoeothermic Blanket Control Unit) of approximately 37 ^{o}C to prevent hypothermia. The body temperature of the animals were monitored using an integrated rectal thermometer and kept at approximately 37 ℃

The skin and the biceps femoris muscle of the left hind limb were removed in order to expose the anterior crural compartment which encloses “extensor digitorum longus” (EDL), “extensor hallucis longus” (EHL) and “tibialis anterior” (TA) muscles. The retinaculae was severed to set free the distal tendons of EDL and TA+EHL complex. Only a small amount of fascia was removed to reach the retinaculae and the rest was left intact. Following this, four distal tendons of EDL and two distal tendons of TA+EHL complex were dissected and and each group of tendons were tied together using a silk yarn. A piece of bone was left on the distal TA+EHL tendons. Connective tissue at the muscle bellies within the anterior crural compartment was left intact to maintain the physiological relations of intra-, inter- and extramuscular connections (Fig. 2.1).

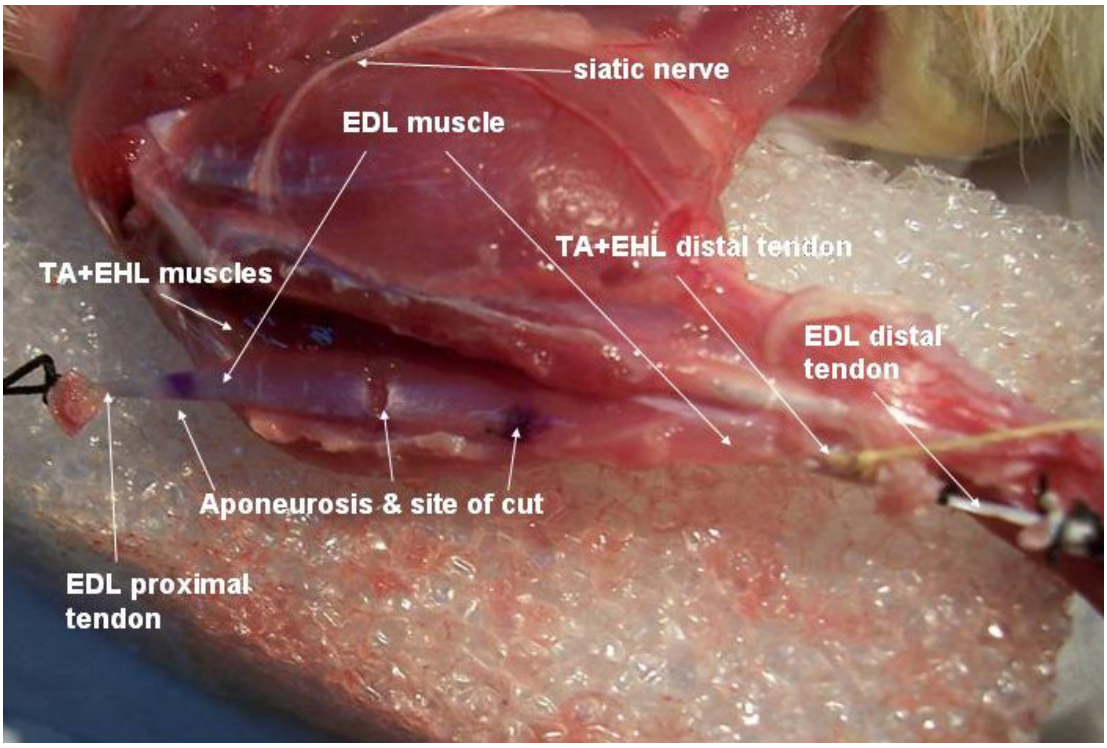

Figure 2.1 A view of anterior crural compartment after the compartment was fully fasciotomized. EDL is completely visible with its proximal aponeurosis on the top. TA+EHL complex is behind the EDL muscle and only its distal tendon is visible.

The knee and the ankle angels were set to 120^{o} and 100^{o}, respectively. These angles were selected as the reference condition which are also present in *in vivo* conditions. These angels are attained in the stance phase of the rats' gait [16]. This fact lets the *in situ* experiments carried on the anterior crural compartment muscles to be performed closer to *in vivo* conditions.

Matching markers were placed on the distal tendons of both EDL and TA+EHL complex and on a fixed point on the lower limb with silk yarn. The proximal tendon of the EDL was exposed by cutting a piece of bone, bone was used to secure the knot since tendon is a lubricious material. A marker was placed on the proximal tendon as well with respect to the position where the bone was removed. Kevlar threads were tied to all three tendons.

The sciatic nerve was dissected free from the upper limb muscles and severed as proximally as possible. The dehydration of the whole opened lower leg and the siatic nerve was prevented by applying isotonic saline solution regularly.

2.2 Experimental set-up and conditions

The rat was positioned on the experimental set up in such a way that ankle angle was in maximal plantar flexion (180^{o}) and the knee was at 120^{o}. The foot of the rat was fixed firmly into a rigid frame. All tendons were connected to force transducers (BLH Electronics Inc., Canton MA) by Kevlar threads, which were aligned carrefully with the muscles' line of pull (Fig 2.2). The sciatic nerve was placed on a bipolar silver electrode and was covered with a piece of latex to avoid drying. Temperature of the room was kept at 22°C. Muscle and tendon tissue was irrigated regularly by isotonic saline against dehydration during the experiment.

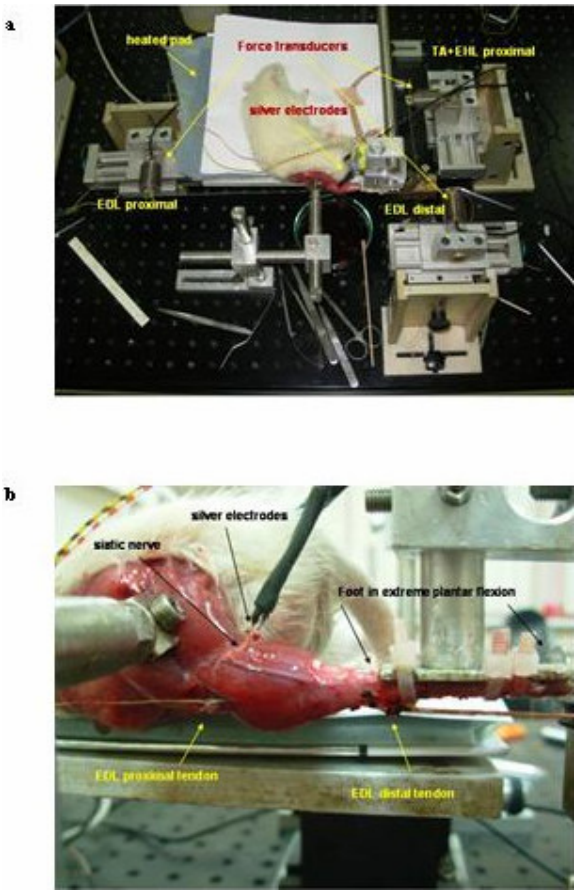

Figure 2.2 The experimental set up:

a) The animal is placed on the heated pad. The tendons of three muscles are tied to the transducers.

b) A closer view. The foot is in maximum plantar flexion and the knee angle is 120^{o}. Both the knee and the foot are firmly attached to the metal rods. The siatic nerve is placed on the silver electrodes.

Siatic nerve was stimulated with a constant current of 2mA (square pulse with 0.1 ms, pulse train 200 ms, stimulation frequency 100 Hz) which activated all the muscles studied supramaximally. Timing of stimulation of the nerve and A/D conversion (Biopac Systems, STMISOC) were controlled by a special purpose microcomputer. Two twitches were evoked and 500ms after the second twitch the muscles were tetanized. 400ms after the tetanized contraction a final twitch was evoked. Muscle total force was measured during the tetanic plateau and the muscle passive force was measured 100ms after the second twitch. EDL distal and proximal forces as well as TA+EHL distal forces measured simultaneously were recorded. After each stimulation the muscles were allowed to recover at low muscle length, for 2 minutes.

As a preconditioning procedure in order to limit the effects of previous former activity at higher muscle length, all tendons were set at the reference position. EDL muscle was shortened by 4 mm distally and stimulated. Subsequently, it was lengthened to the reference position and stimulated again. Such preconditioning was repeated until the forces measured at reference position showed minor differences. This process was called repetition measurement and was done at the begining of each experiment.

After the completion of repetition measurements, the following five conditions were tested:

1. Muscles were first studied in *intact condition* where the antreior crural compartment is not severed and the muscles function in their normal fashion. Contractions were started at low lengths (i.e. the reference point – 5mm) and continued by 1mm increments until 2mm over EDL distal optimum length. After the all measurements were completed, two more contractions were done at reference point and optimum length, respectively. These recordings were used to test the history effect.

2. After the measurement in intact condition, fascia of the EDL was opened (partial fasciotomy) proximally about 10mm. A transverse cut was made on the half way of the proximal aponeurosis (aponeurotomy abbriviated as AT) with the scalpel. The muscle was contracted at its optimum length first in order to tear the aponeurosis as much as possible. This condition was referred to as *acute AT*. The rest of the measurements were done as described before (Fig. 2.3).

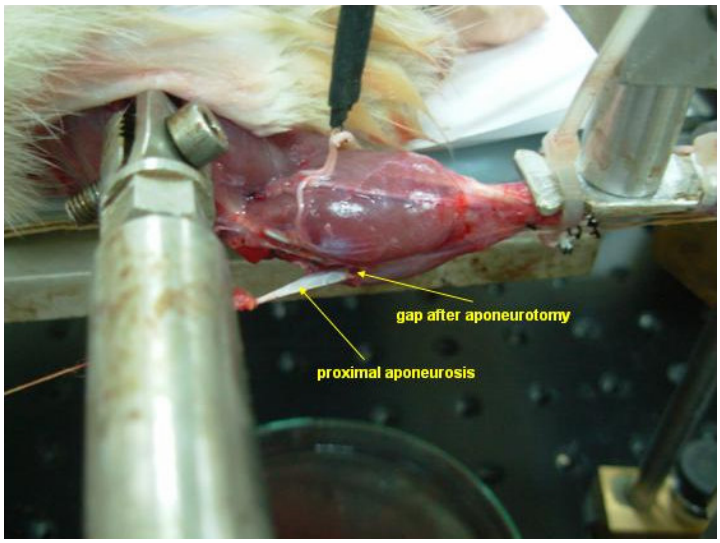

Figure 2.3 EDL muscle after partial fasciotomy and aponeurotomy. The EDL muscle is pulled sligthly on purpose, in order to make the proximal aponeurosis visible. This image was taken out of the routine experiment session.

3. In order to avoid lack of stabilization of the tear on the aponeurosis and muscle geometry after the intervention a second set of measurements were done in identical conditions. This third step was called *post AT*.

4. Following the post AT, the remaining fascia was dissected fully (full fascitomy) and the same measurement procedure was done. This condition was referred to as fasciotomy (Fig. 2.4)

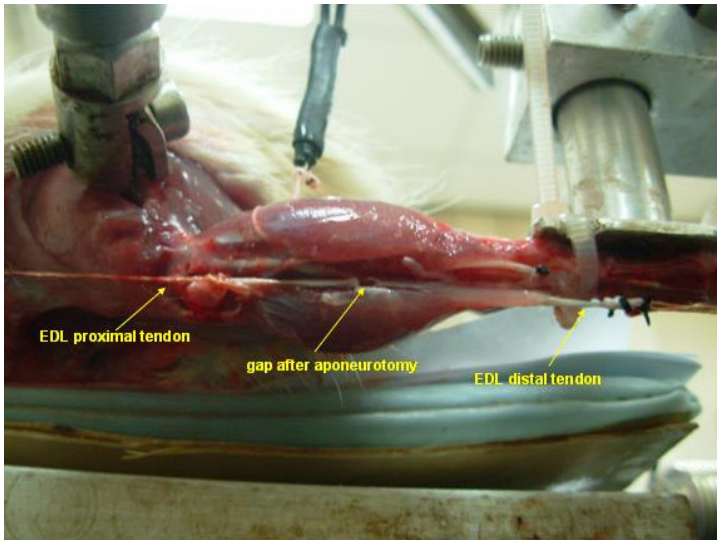

Figure 2.4 Muscle at optimum length after full fascitomy.

5. Finally, TA+EHL complex was removed as much as possible without damaging the neurovascular tract to the EDL muscle. This step referred to as *TA+EHL removal*. The measurements were done as described before (Fig 2.5).

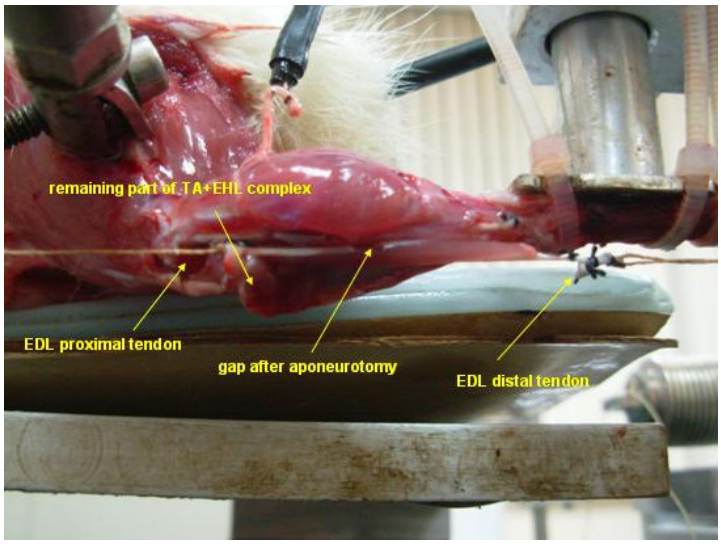

Figure 2.5 Muscle at optimum length after TA+EHL removal

Muscle force measurements were performed at reference position and at the optimum length of EDL muscle distally the collection of each length-force data (*control contractions*) to test if history effects play a major role. Corresponding forces measured after control contractions and during the collection of length-force data (referred to as the *actual data*) were compared.

2.3 Treatement of Data and Statistics

Passive muscle length - force data were fitted using an exponential curve

$y = e^{ax+b}$,

where y represents passive muscle force, x represents muscle-tendon complex length and 'a' and 'b' are fitting constants. Active EDL muscle force (Fma) was estimated by subtracting the calculated passive force (Fmp) using the fitted function, from total force (Fm) for the appropriate muscle length. Active EDL length–force data were then fitted with a stepwise polynomial regression procedure.

$$y = b_0 + b_1 x + b_2 x^2 + b_3 x^3 + b_4 x^4 + \ldots + b_n x^n ,$$

where y represents active muscle force, x represents active muscle force length and b_0 through b_n are fitting constants. Using the polynomials selected, mean and standard errors (SE) of active muscle force were calculated for given EDL lengths. Optimum muscle length was determined for each individual curve as the active muscle length at which the fitted active force curve showed maximum force (Fmoa). The curved fitted data of all muscle forces of each measurement was rearranged according to Fmoa of EDL distal and were represented as such.

In the muscle force fitting procedure, the order of polynomials used was determined by one-way analysis of variance (ANOVA): the power was increased from one to maximally six until no significant improvement to the description of changes of muscle length and force data was added. One-way ANOVA was also performed to test length effect on EDL and TA+EHL muscle forces. Two-way ANOVA was used to test for the effects of altered muscle length and lengthening condition on i) distal and proximal EDL forces, ii) the proximo-distal EDL force differences and iii) TA+EHL distal forces. Differences were considered significant at $P < 0.05$. If significant main effects were found, Bonferroni post-hoc tests were performed to locate significant differences. Force values were plotted (mean + SE), and muscle length is expressed as a deviation of distal EDL optimum muscle length (for the interval $-9 \leq \Delta l_{m+t} \leq 2$).

3. RESULTS

3.1 Control Measurements

In figure 3.1 EDL distal control measurements – lsc, loc - are shown. Muscle forces in the control measurements are less than those in the actual measurements. The force drop is more pronounced in lower muscle lengths than the higher ones. The drop of muscle force between ls and lsc is the highest (by 61%) in intact condition. In acute AT, the drop in muscle force between ls and lsc is 38% whereas it is 42% in post AT. The force difference between ls and lsc after full fasciotomy is 43%. Finally, after the TA&EHL muscles are removed the difference between the ls and lsc is 38%. The drop of muscle force in controls is less at high muscle lengths with 4 – 5% in each step with the exception of TA+EHL removal (the drop is 11% for this step).

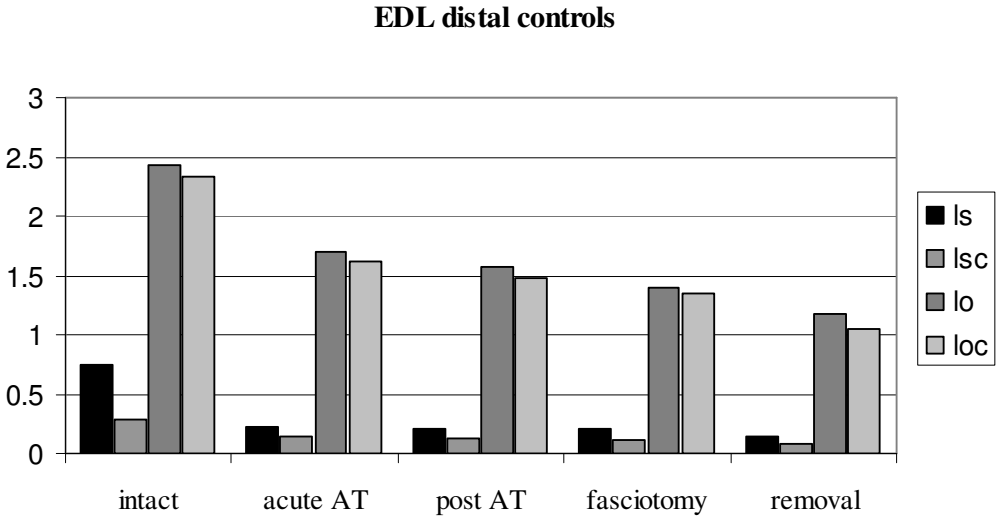

Figure 3.1 EDL distal control data and actual data are compared. The difference between two sets of data is higher for short muscle lengths. However, the decrease of muscle force is not pronounced that much at optimum length. Note that the amount of difference is similiar for each condition.

Figure 3.2 shows the control measurements of EDL muscle taken proximally. The drop of muscle force between the first measurements and the control measurements is less in EDL proximal when compared to EDL distal. Again, the most pronıunced difference is between ls and lsc in intact condition. Differences in higher muscle lengths are again very small, as it was in EDL distal controls. The difference between ls and lsc in intact condition is 42%. The difference is smaller in the other steps. In acute AT and post AT, the difference between the lsc and ls is 12%. In fasciotomy the difference is 20%. Finally, as the TA & EHL muscles are removed the difference between the lsc and the ls is 18.8%. There is also force drop between lo and loc but it is not that much pronounced as the drop between lsc and ls. The amount of difference between lo and loc is around 1% in intact, acute AT and fasciotomy where as 4.6% and 7.8% in post AT and TA+EHL removal, respectively.

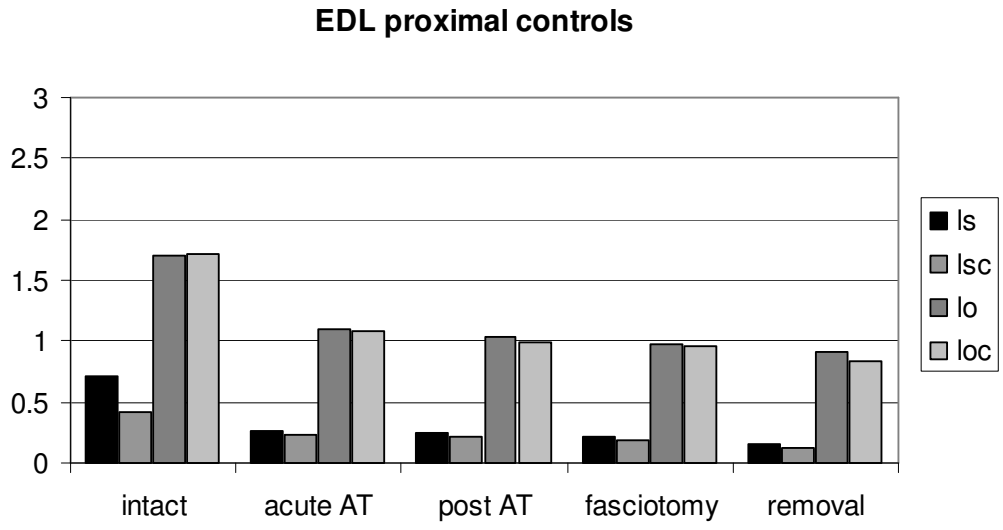

Figure 3.2 EDL proximal control data and actual data compared.

Figure 3.3 represents the control measurements of TA+EHL complex. Very minor differences are present since these muscles are not lengthened or shortened. It is only 4.7% and 1.6% between ls and lsc of intact and acute AT conditions, respectively. One different finding is that very small increases are measured for post AT and fasciotomy, which are by

0.7% and 2.3%, respectively. Differences between lo and loc are even smaller with around 0.5%. Increases are measured for acute AT and fasciotomy for loc which are around 1 %.

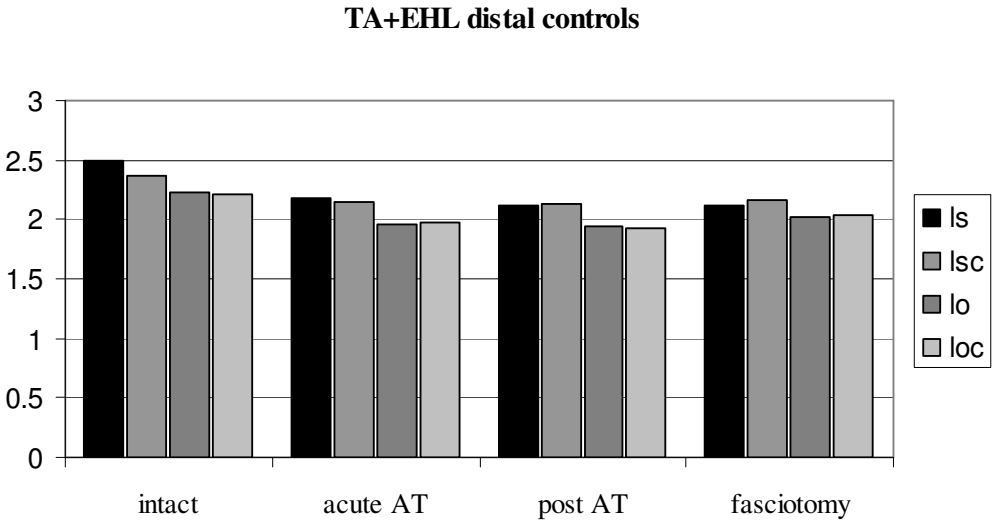

Figure 3.3 Comparison of control data and actual data for TA+EHL complex. Not that the differences between two data set in all conditions are very minor.

3.2 Effects of interventions on muscle length – force characteristics

3.2.1 Changes in EDL distal length-force characteristics:

Figure 3.4.a shows length–force characteristics in all conditions for EDL muscle distally. The drop in F_{moa} was 34.8 % in post AT state when compared to the intact condition. F_{moa} drops 9.5 % more when the fascia is completely dissected. Finally, after the TA+EHL removal, F_{moa} drops 18.7 % to that of measured in fasciotomy step.

Maximum passive muscle force also drops after the interventions. The maximum passive force during post AT is 30 % lower than that of intact condition. After full fasciotomy, it drops 20 % more. However, remarkably the measured passive forces after TA+EHL removal was 9 % higher than the passive forces measured after fasciotomy.

In addition to F_{moa} drop, optimum length (l_{opt}) shifted to higer muscle lengths. The amount of shift is 0.53mm in post AT, 0.66mm after complete fasciotomy and 0.18mm after TA+EHL removal when compared to the lopt in intact condition.

3.2.2 Changes in EDL proximal length-force characteristics

Figure 3.4.b shows length–force characteristics in all conditions for EDL proximal part. The F_{moa} drops by 42.2 % in post AT condition. The drop in F_{moa} between post AT and fasciotomy is very minor (by 2.2%) and not statistically significant. However after the TA+EHL complex was removed, F_{moa} drops further by 8.1%.

For low muscle lengths there is a high a force drop as a result of aponeurotomy. Muscle force drop almost by 73% in post AT condition.

The characteristics of muscle passive forces differ from passive forces of EDL distal. The maximum passive muscle forces of EDL proximal does not decrease after the interventions. The maximum passive muscle force is the highest after TA+EHL removal, passive forces of intact condition and after full fasciotomy almost identical and only 15 % less than the maximum passive force of previous step. Finally, in post AT maximum passive force drops 13.8 % more.

3.3.3 Changes in TA+EHL distal length-force characteristics

Figure 3.5 shows the length-force characteristics of TA+EHL muscles. The active muscle forces droped gradually as EDL is lengthened distally after aponeurotomy, as well as in each step. However, after full fasciotomy, the measured active forces were significantly higher than that of post AT. One-way ANOVA test shows no significant change for any length during fasciotomy step.

3.3.4 EDL proximo-distal total force differences

Differences between EDL distal and EDL proximal forces were observed in all experimental conditions. Figure 3.6.a shows that proximal total forces were higer than the distal total forces for lower muscle lengths. However as the EDL is lenghtened distally, distal forces dominated the proximal forces in all conditions. Proximo-distal force

differences are also present for passive forces (fig 3.6.b). On the contrary to the total forces, EDL proximal passive forces were higher than the EDL distal forces for almost all lengths in all conditions with the exception that in intact and post AT conditions EDL passive forces were higher for muscle lengths over optimum.

Although the proximo-distal total for difference was expexted to increase as the muscle length is increased in all conditions, it was decreased in intact and TA+EHL removal conditions at maximum muscle length.

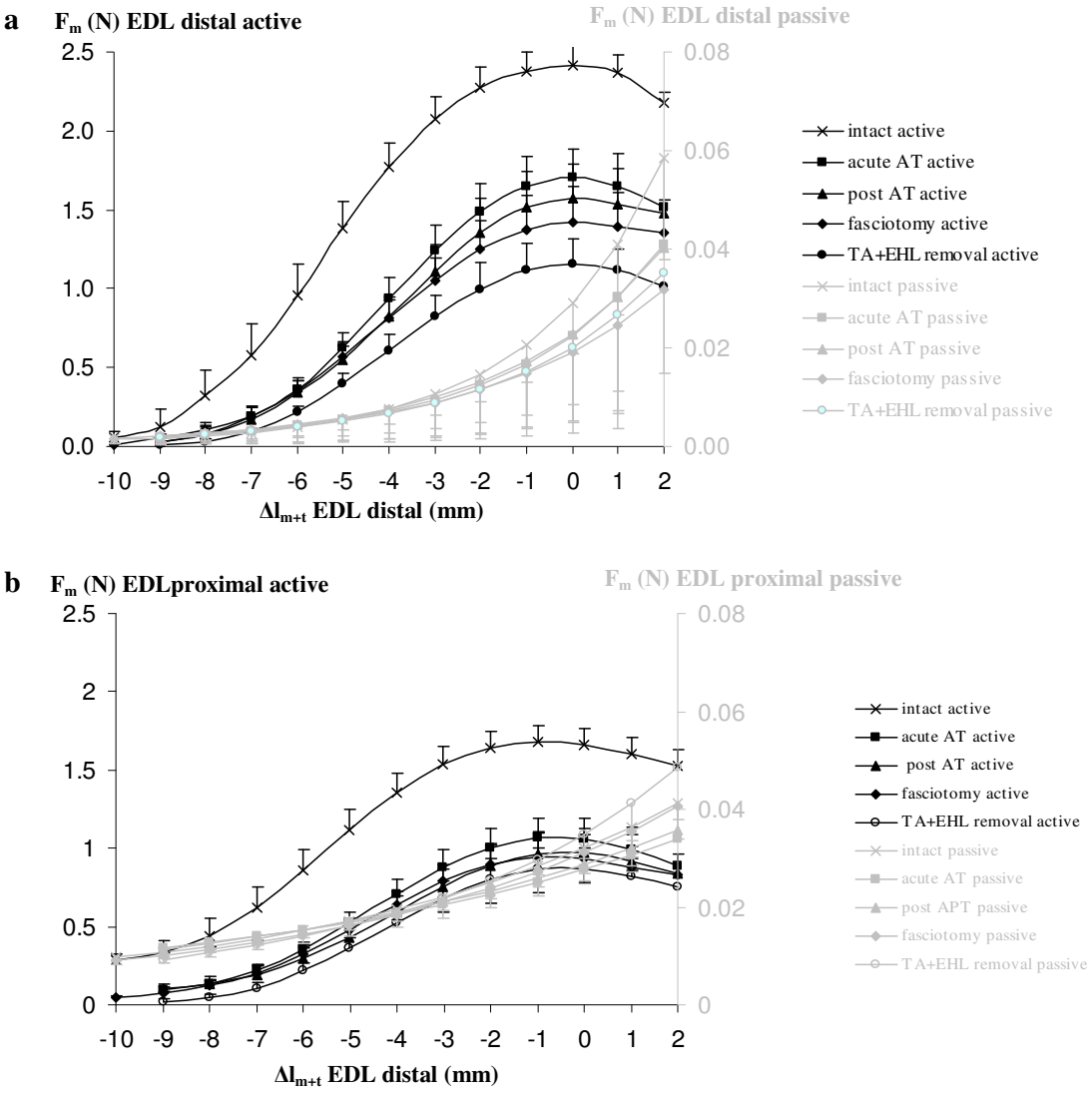

Figure 3.4 a) EDL distal active and passive forces measured in each step are given together.
b) EDL proximal active and passive forces in each step are given. Note that at short lengths active force drops substantially.

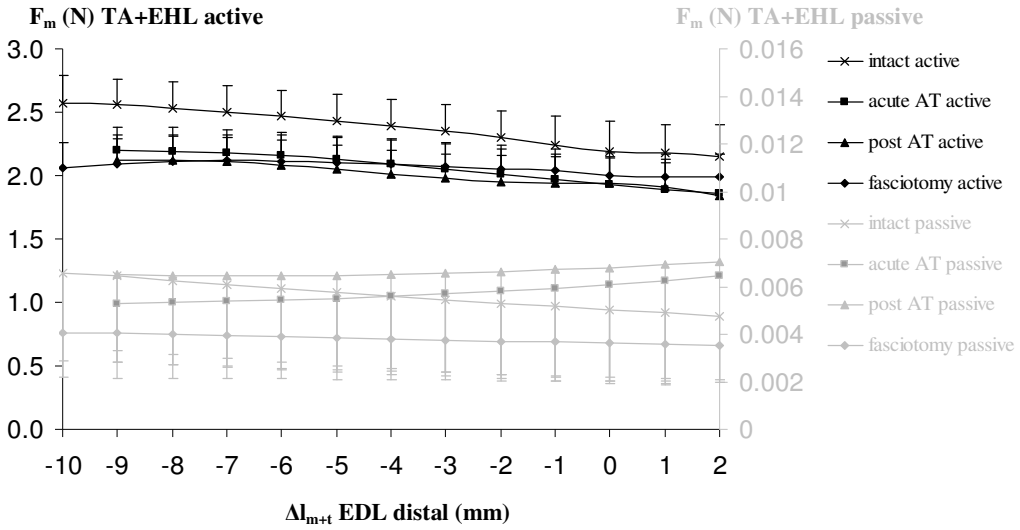

Figure 3.5 TA+EHL forces decreases gradually as the EDL is lengthened distally. Also after aponeurotomy is performed TA+EHL forces decreases for all lengths.

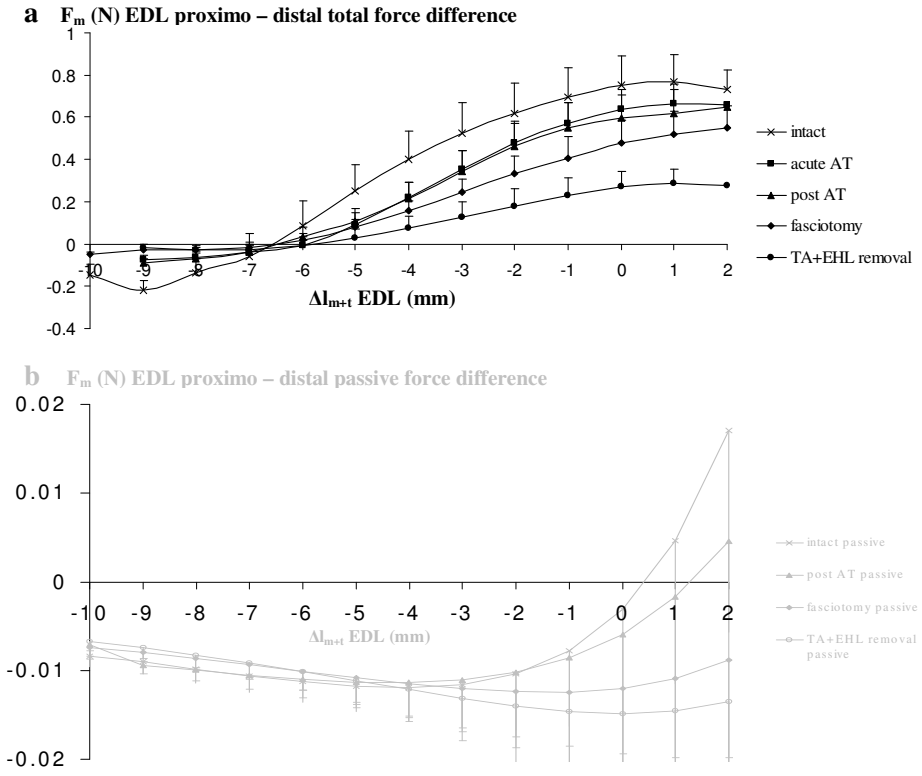

Figure 3.6 a) As the muscle is lengthened distal forces prevail the proximal forces
b) Proximal passive forces are higher than distal forces for all lengths except l_{opt} and beyond.

4. DISCUSSION

4.1 Control Measurements

It is important to design an experimental set up which has reproducable results under predetermined conditions. However, errors can ocur during measurements which should be minimized and quantified in order to ensure that the results are reliable. Predetermined controlled conditions are the main precautions for the minimization of errors. While working on muscle even under strickly controlled conditions, it is observed that the produced muscle force alters as a result of a previous activity (referred to as history effects). In order to quantify such effects, two control measurements were taken after each experimental step: i) at reference length (lower muscle length) and ii) optimum length (higher muscle length), respectively.

At high muscle lengths, the difference between the measured control force and the actual data was very minor for all conditions. In a previous study performed by *Smulders et al* [17] on Flexor Carpi Ulnaris muscle of the rat, a similiar result was reported for control measurements at muscle optimum length. Hence, for the actual data alterations in measured muscle forces at optimum length in each condition can be ascribed almost fully to the intervention performed in that step (therefore, to mechanisms related to myofascial force transmission.

However, at lower muscle lengths (EDL distal tendon located at its reference length) considerable differences between control forces and the actual forces were measured which showed different characteristics for the two sides of EDL and TA+EHL complex: (1) the force differences were more pronounced on the distal end of the EDL than the proximal end and (2) there were very minor differences on the TA+EHL complex length of which was kept constant all the time. When EDL distal forces are taken into

account, there was 61 % force decrease for intact condition whereas, a 42 % decrease was found for EDL proximal forces. Therefore, alterations in muscle forces at low lengths may not be ascribable to aponeurotomy alone. Note however that the amount of force decrease became less pronounced for both distal and proximal EDL forces as the surgical procedures were performed, such that difference between control force and actual data for EDL distal was found to be around 40 % and for proximal forces around 16 % in post AT, fasciotomy and TA+EHL removal conditions. This indicates that history effects themselves are affected by epimuscular myofascial force transmission.

The underlying mechanisms of history effects are not clearly understood. One possible source for such effects may be the viscoelastic properties of muscles which include stress relaxation and hysteresis. Minor differences due to history effects in TA+EHL complex which was activated at a constant length in all conditions suggest that stress relaxation may not be the dominant mechanism to explain this phenomenon. On the other hand, hysteresis may play an important role for such effects. The muscle was lengthened distally with 1mm increments from active slack length until 2mm above optimum length *step by step* while collecting the actual data. However, for measuring control data the muscle was shortened to its reference position in a *single step*. This creates a difference in the loading and unloading cycle and may cause history effects. In two previous studies performed by *Huijing et al,* it was reported that previous activity at high muscle lengths was shown to cause decreased active force exerted at low lengths [18] and repeated activity at low length following that of high length was shown to minimize such reduction of active force [19]. The nature of such procedure involves a loading and unloading cycle for the muscle tissue indicating that hysteresis may be responsible with the history effects. This may also explain the fact that differences between the actual and the control data were minor at high muscle lengths because the control data at optimum length was taken just after one more contraction having a similar loading and unloading cycle.

When the overall picture is taken into account, it can be suggested that comparisons between the actual data of each experimental conditions are consistent. This is because the same loading and unloading path is followed in all steps.

4.2 Effects of aponeurotomy on muscles length-forces characteristics

In spastic movement disorders, the affected muscles are overly active and in time they structurally become shorter (i.e., develop contracture) with respect to their antagonists. Aponeurotomy is one of the techniques used to reduce the force produced and lengthen such muscles. By doing so, the joint range of motion is restored. Although this operation yields desired results in the short-term, recurrence in the long-term in some patients were reported [e.g. 25]. In order to understand the effects of aponeurotomy on muscle mechanics several studies were done both experimentally [i.e. 11, 13] and by finite element modeling [i.e. 14, 15]. However, such experimental studies were performed on isolated muscles exclusively. Nevertheless, it was shown previously on intact muscle that epimuscular myofascial pathways have a dominant role in force transmission and muscle mechanics is highly affected by the surrounding tissues [20]. The present study was performed to determine the effects of aponeurotomy under such conditions which may be conceived to show much greater similarity to those conditions in vivo.

4.2.1 EDL distal forces

The present study shows that muscle active force exerted by the distal end of EDL decreases at all muscle lengths as a result of proximal aponeurotomy (e.g. F_{moa} measured in post AT was 34.8% less than, F_{moa} measured in intact condition). Two mechanisms are expected to affect this decrease:

(1) Altered sarcomere length distributions (mainly due to aponeurotomy). After the proximal aponeurosis is cut transversely, the distal muscle fibers lose their direct connections to the muscles' origin. The connective tissue below the cut is ruptured and the muscle fibers are divided in to two separate populations of distal and proximal fibers. Experimental study on rat gastrocnemius muscle (GM) [11] and finite element models on rat EDL muscle [14] in which aponeurotomy was performed showed that mean sarcomere length of distal fibers were significantly shorter than that of sarcomeres within the intact muscle. Since the sarcomeres located in the muscle fiber population distal to the location of aponeurotomy cannot attain higher lengths, the force produced decreases. The model

results reported by Yucesoy et al. suggests that sarcomeres which are near to the distal part of the cut have the shortest length and show minor length changes as the muscle is lengthened. However, more distally located sarcomeres where shown to attain higher lengths than the sarcomeres which are closer to the cut. This is explained with the role of intramuscular myofascial force transmission where the extracellular matrix prevents the shortening of the sarcomeres in more distal population to their active slack length.

(2) Decrease in active force transmitted from the TA+EHL muscle complex to the EDL muscle via the epimuscular connections (due to partial fasciotomy that precedes aponeurotomy). In intact condition, it was shown previously that some of the active force produced by TA+EHL complex was exerted on the EDL distal tendon [7, 21]. In our present study, partial fasciotomy is expected to limit such force transmission and therefore yield a decrease in EDL distal force. Note that further sizable decreases shown presently in muscle force as the remaining compartmental fascia was cut (full fasciotomy) resulted due to the elimination of the most of the epimuscular connections and as the TA+EHL removal due to the elimination of the direct source of intermuscular myofascial force.

4.2.2 EDL proximal forces

In intact condition, due to the position of proximal end, non-zero EDL active forces were measured presently even for the lowest muscle length studied. However, as partial fasciotomy and aponeurotomy were performed, muscle active force decreased for all muscle lengths. Such decrease was more pronounced at short lengths and the muscle active force dropped almost to zero. Note that, the effect of full fasciotomy on proximal active forces was found to be minor and statistically insignificant. Nevertheless, removal of TA+EHL complex caused a substantial significant effect on the proximal forces. Similar to distal forces, (1) changes in the distribution of sarcomere lengths and (2) the effects of epimuscular myofascial force transmission are conceivable to cause the decrease in the proximal forces. In a recent study on finite element modeling of aponeurotomy [15], it was reported that extramuscular myofascial force transmission limits the serial distributions of

sarcomere lengths in the proximal population. Unlike the distal muscle fibers, proximal fibers attain higher lengths which also play a role in the reduction of the muscle force.

Passive forces: Change in EDL distal and proximal passive forces after aponeurotomy has different characteristics. Distal passive forces of post AT and fasciotomy conditions measured at Δl_{opt} + 2mm (i.e. maximum passive force) are lower than the intact condition. As the TA+EHL complex is removed the distally measured maximum passive force is 9 % higher than that of measured in fasciotomy step. On the other hand, the proximally measured maximum passive force is highest after TA+EHL removal and lowest in post AT condition when compared to intact condition. There isn't any major difference between the passive forces measured after full fasciotomy and the intact condition.

Finite element modeling study of aponeurotomy by *Yucesoy et al* (14) allows mechanical explanation for these force decreases. According to the authors, aponeurosis of the intact passive muscle is strained more in the direction of the muscles' line of pull than those of the aponeurotomized muscle. Two main effects are suggested to be responsible for such a decrease in muscle passive force and change in muscle geometry: (1) Difference in shear strains between the cross-fiber and fiber directions of intact and aponeurotomized muscle and (2) the alteration of sarcomere lengths in distal and proximal populations of aponeurotomized muscle with respect to intact condition. These effects reduce the stress in the matrix mesh and hence result in lower passive forces after aponeurotomy. However, presently it is also observed that after removal of TA+EHL muscles passive forces increase. Such effect of TA+EHL removal is more pronounced especially proximally. A possible explanation may be that the synergistic muscles act on the proximal part in way to reduce the stress in the opposite direction and removing them diminishes the counter acting forces.

4.2.3 Shift in muscles optimum length

Besides the reductions in active muscle forces also a shift in muscle optimum length to higher muscle lengths was observed distally in the present study after surgical interventions, which is a desired effect of aponeurotomy in order to restore the limited range of joint motion due to contractions in overly short spastic muscles. An increased heterogeneity of mean sarcomere lengths of different fibers (a parallel distribution of sarcomere lengths) is suggested to occur due to disruption of epimuscular connections as a result of the interventions. In previous finite element models of EDL muscle [7, 8, 22] in conjunction with experimental studies [6] dealing with different levels of epimuscular connections it was reported that different components of epimuscular myofascial force transmission alter substantially the distribution of sarcomere lengths.

As a results of proximal aponeurotomy performed on isolated EDL and GM muscles of the rat both reduction of active muscle force exerted and the shift of optimum length to higher muscle lengths were observed in previous studies [11, 12]. The amount of shift was 2 mm for aponeurotomized GM and 3.5 mm for EDL muscles, respectively. In contrast to such substantial shifts, the shift in muscle optimum length was found to be only 0.55 mm presently for post AT condition. It should be noted that in this study, the neighboring muscles were kept intact. A similar result was reported previously in finite element study of aponeurotomy by Yucesoy et al [14], in which effects of aponeurotomy performed on isolated EDL and on EDL with intact extracellular matrix (ECM) were compared. It was reported that there were only minor differences in the optimum length between intact EDL and aponeurotomized EDL with intact ECM. However, the amount of shift was as high as 2 mm in isolated aponeurotomized EDL compared to intact muscle. We conclude that in agreement with the results of finite element modeling, the existence of epimuscular connections may limit the heterogeneity in sarcomere length distribution in aponeurotomized muscle. Hence the amount of shift in muscle optimum length to higher muscle lengths becomes limited.

The difference between the results of aponeurotomy performed on isolated muscle and muscle with intact neighbors is not limited to a shift in optimum length. The reduction

of active muscle forces is also different in both cases. Jaspers et al reported for isolated EDL muscle that proximally F_{moa} was reduced to 52.9 % of F_{moa} of intact condition in post AT [12]. The amount of reduction in post AT condition was less pronounced in the present study where distal F_{moa} was found to be 65 % and proximal F_{moa} being 57.7% of F_{moa} measured in intact muscle, respectively. However, removal of the synergistic muscles caused a substantial additional force reduction: distal F_{moa} measured after removal of TA+EHL muscles was 47.9 % and proximal F_{moa} was 51.9 % respectively of F_{moa} measured in intact condition. EDL forces decrease substantially as the force transmitted via epimuscular pathways are diminished after the removal of TA+EHL complex. However, with the presence of intact extramuscular connections the decrease in EDL muscle forces is limited because the sarcomere lengths are controlled with extramuscular loads and kept at more favorable lengths for force production.

4.2.4 TA+EHL distal forces:

In all conditions studied presently, the length of TA+EHL muscle complex was held constant. However, as EDL was lengthened distally, the active force produced by TA+EHL was decreased gradually. Recent studies report similar effects of synergistic muscle lengthening to the force of neighboring muscle [7, 23, 24] and such force reduction independent of muscle length is a direct evidence for epimuscular myofascial force transmission.

After performing partial fasciotomy and aponeurotomy, the measured active forces of TA+EHL complex showed an additional decrease for all muscle lengths. As discussed in detail above, active forces of EDL muscle were altered after aponeurotomy. Such reduction of EDL forces therefore, is evident also to alter the active forces of its synergist, since EDL forces are conceivable to be exerted onto TA+EHL complex through the intermuscular connections [7].

Note that, after full fasciotomy was performed presently, the measured TA+EHL forces were higher than those measured in the preceding condition (i.e., post AT). This may suggest that removal of intermuscular connections via fasciotomy removed the distal

load on TA+EHL complex and resulted in force increase. Therefore, a remarkable present finding is that surgery aiming at the agonist muscle exclusively has substantial effects also on the synergistic muscles that are not interfered with in any way. The mechanism for such complex effect is epimuscular myofascial force transmission.

In conclusion, although reduction in active force at optimum length and shift in optimum muscle length were found in this study, these reductions and shift are less pronounced when compared to those found in experiments with isolated muscle. In the light of previous FEM studies of aponeurotomized EDL muscle and studies concerning myofascial force transmission, it may be suggested that the acute effects of aponeurotomy is altered with the presence of epimuscular connections. Also it should be noted that although the intervention is done on the proximal aponeurosis, force production on both sides of the EDL muscle was reduced and after aponeurotomy the measured forces of the synergist muscles (TA+EHL complex) were decreased as well. Based on this fact, it may be suggested that before planning to perform aponeurotomy in order to restore the function of one joint, the effect of the operation on the other joint and on the function of the synergetic muscles should be taken into account.

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