EFFECTS OF PRECONDITIONING OVER HISTORY EFFECTS IN SKELETAL MUSCLES OF RAT

by

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ABSTRACT

EFFECTS OF PRECONDITIONING OVER HISTORY EFFECTS IN SKELETAL MUSCLES OF RAT

It has been already known that activity at high lengths, leads at least, to major decreases of active force at low lengths, whereas forces at high length are hardly changed [21]. This impact on muscle force is named as length-history effects. And it has been experienced that such effects can be minimized by a method called preconditioning in which alternating contractions are done at high and low lengths until no further decreases of active force at low lengths are seen. However, whether preconditioning does minimize the history effects or not has not been investigated systematically in any studies so far. One of the goals of this study is to be able to observe the effects of history effects in repeated measurements by taking control measurements. Another aim of this work is to assess the effects of preconditioning over history effects in rat muscles. In order to achieve this goal, length force graph was obtained on the extensor digitorium longus (EDL) as well as to that of its synergistic muscles i.e., TA+EHL complex. Then preconditioning was performed. After that, three more length force graphs were obtained again to quantify the changes to the forces produced by these muscles. In this study, it was found that preconditioning helps to minimize the history effects in EDL distal tendon. In contrast to EDL distal, control measurement shows that preconditioning performed by EDL lengthening distally is not a solution for force decreases in EDL proximal although after preconditioning EDL muscle seems history-free. On the basis of results obtained from TA+EHL complex, the measurements taken from neighboring muscle is reliable for analysis. As a result, it can be said that any studies involving control measurement should perform preconditioning to minimize history effects. Our results therefore provide a better way to minimize the history effects for the scientists designing muscular mechanics experiments involving rat muscles.

Keywords: Length-history effects, Preconditioning, Extensor Digitorum Longus, Rat

ÖZET

ÖNKOŞULLAMANIN SIÇAN İSKELET KASINDA UZAMA GEÇMİŞİ ETKİLERİ ÜZERİNDE ETKİLERİ

Kasın uzun boyda uyarılmasının uzun boyda kas kuvvetini etkilemezken, kısa kas boylarında kuvvet düşüşüne yol açtığı önceki çalışmalardan bilinmektedir. Kasın ürettiği kuvvet üzerindeki bu etki *uzama geçmişi etkileri* olarak adlandırılmaktadır. Bu etkinin *önkoşullama* ile, kasın sırasıyla uzun boyda ve kısa boyda uyarılması yoluyla minimum seviyeye indirildiği de gösterilmiştir. Fakat literatürde bu yöntemi ve etkilerini sistematik olarak araştıran bir çalışma henüz yapılmamıştır. Bu çalışmada *uzama geçmişi etkilerinin* varlığı kontrol ölçümleriyle gösterildikten sonra, *önkoşullama* yönteminin *uzama geçmişi etkileri* üzerinde nasıl bir etkisinin olduğu i) EDL distal ii) EDL proximal iii) TA+EHL'e bakılarak incelendi ve kontrol ölçümleriyle *uzama geçmişi etkilerinin* uzun boydan kısa boya geçildiğinde kuvvet düşüşüne neden olup olmadığı araştırıldı. Deney sonucunda *önkoşullama* ile bu kuvvet düşüşünün EDL distal tendonunda ortadan kalktığı gözlendi. EDL proximal tendonunda ise *önkoşullama* sonrası etki hemen ortadan kalkmamasına rağmen, sonraki ölçümlerde *önkoşullamanın* işe yaradığı gözlenmiştir. Uzatılmayan komşu kasta (TA+EHL) ise önceki çalışmaların sonuçlarına uygun olarak kuvvet düşüşü gözlenmedi. Sonuç olarak *önkoşullamanın* EDL kasında, en azından EDL distal kuvveti tarafında, *uzama geçmişi etkilerini* ortadan kaldırdığını gösteren bu çalışma kas mekaniği üzerine çalışan araştırmacıların deneylerini düzenlerken dikkate almaları gereken bulgular sunmuştur.

Anahtar Sözcükler: Uzama geçmişi etkileri, Önkoşullama

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Δl_{m+t}	Deviation from Optimum Length

LIST OF ABBREVIATIONS

n	Number
SD	Standart Deviation
gr	Gram
ml	Milliliter
FT	Force Transducer
TA	Tibialis Anterior
EDL	Extensor Digitorum Longus
EHL	Extensor Hallucis Longus
STMISOC	Biopac System Stimulator
ms	Millisecond
Hz	Hertz
mA	Milliamper
mm	Millimeter
ANOVA	Analysis of Variance
Fm(N)	Muscle force (Newton)
l-f	length-force

1. INTRODUCTION

Muscle is an activatable soft tissue composed of fibers 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 such as skeletal muscle, smooth muscle and cardiac muscle.

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 consists of irregularly distributed collagen fibers, connective tissue cells and fat. Number of muscle fibers composes a muscle bundle which is called as fascicle. And each fascicle is surrounded by a connective tissue structure 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 cell is surrounded by the endomysium that is a thin sheet of connective tissue.

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.

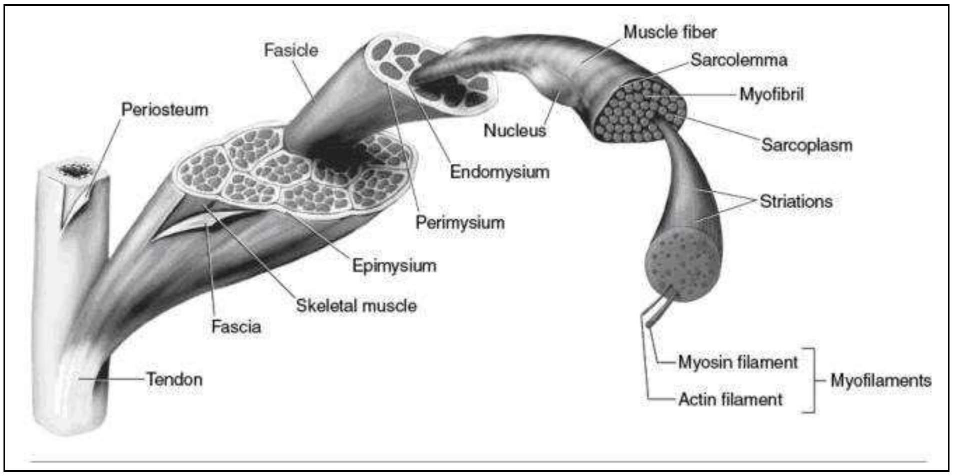

Figure 1.1 A classic length-tension curve taken from cat soleus muscle. Adapted from Keynes 2001 [1].

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The myosin filaments are connected to each other with a system of fixed transverse filaments called M-bridges, forming the M-bridges (Fig 1.2).

1.2 Skeletal Muscle Force Production

1.2.1 Skeletal Muscle Functioning

Most suggestions as to mechanism of muscular contraction involved the coiling and contraction of long protein molecules, rather like the shortening of a helical spring. In other words; in contrast to earlier beliefs, the muscle fiber is not becoming shorter during contraction. In 1954, the sliding filament theory was independently proposed by Hugh Huxley and Jean Hanson [3] and by Andrew Huxley and Rolf Niedergerke [4]. In each case the authors showed that the A band does not change in length when the muscle is stretched or when it shortens actively or passively. This observations suggest that contraction involves sliding of the I filaments between the A filaments. Actin filaments are only present in the I-band region of fiber; while the A-band corresponds to the position of the myosin filaments. With regard to this knowledge, it is known that the myosin heads are walking up the actin filaments, pulling them closer together;

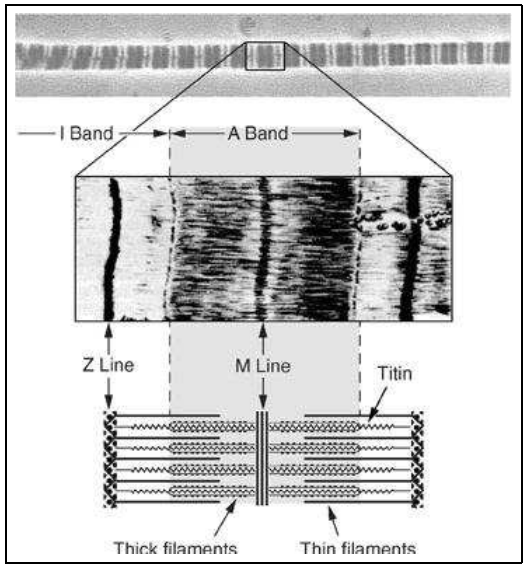

Figure 1.2 A schematic view of a muscle fiber and a sarcomere. Modified from Binder 2009 [2].

thus making the sarcomere shorter, but the length of the filaments themselves remain unchanged [5].

When the muscle shortens, actively or passively, the opposing actin filaments within each sarcomere slide along myosin filament. As the thin filament slides over thick filament, they cause the H-zone become narrower; similarly as more of each actin filament is drawn into the space between the myosin filaments, the I-band becomes shorter, as does the sarcomere. Since the myosin filaments do not alter their shape, the length of the A-band stays unchanged (Fig 1.2).

The investigators observed that, over a certain range, the tension developed is proportional to the degree of overlap between actin and myosin filaments. The sarcomere length corresponding to the greatest overlap and allowing the highest active tension in termed the optimum length. Moreover, when the muscle is stretched enough that there is no overlap between the myosin and actin filaments, no active tension could be developed [6].

The regulation of cross-bridge attachment to actin is commonly associated with

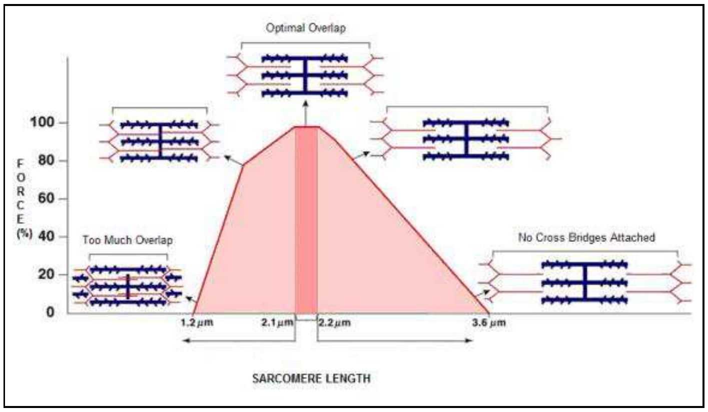

Figure 1.3 Active Length-Tension relationships during tetanic activation. Idealized length tension relationship and the sarcomere position believed to produce it. Adapted from Redmond 2009 [7].

the effects of Ca2+ on the thin filament [6]. When Ca2+ binds to the regulatory protein troponin C (TnC), it causes the displacement of tropomyosin allowing cross-bridge attachment to actin, forming a weakly bound myosin- actin-ATP complex. ATP is then hydrolyzed and phosphate is released, forming a strongly bound myosin-actin-ADP complex. The strongly bound complex causes conformational changes in the thin filament, increasing the probability of new cross-bridges to attach to actin. Therefore, activation of the thin filament is coordinated by Ca2+ binding to TnC and also strong binding of cross-bridges to actin filaments.

It was long known that the combination of ATP with the myosin head was necessary to break the bond between actin and myosin. In other words; hydrolysis of ATP is needed for the movement of the myosin head. When the actin site for myosin binding has been exposed by Ca2+ binding to troponin C, the following sequence occurs:

1. The head of a myosin molecule binds to actin.
2. Actin stimulates the complete hydrolysis of ATP to ADP, and subsequent release of ADP results in the release of Pi and transition to a strong-binding state.
3. The conformation change in myosin exposes a site where ATP can bind. When a new Mg-ATP molecule binds to the myosin head, the bond between the two

filaments is broken.

4. The myosin head releases from the actin; Hydrolysis of ATP to ADP causes the myosin to straighten out. The split of ATP (into ADP + phosphate) stores energy in the myosin head and releases some heat. Phosphate leaves the reaction site.

5. The cycle starts again if the myosin head finds a new actin binding site.

1.2.2 Muscle Force Production

When muscles contract they exert a force on whatever they are attached to (this force is equal to the tension in the muscle) and they shorten if they are permitted to do so. Hence we can measure two different variables during the contraction of a muscle: its length and its tension. Most often one of these two is maintained constant during the contraction. In isometric contractions the muscle is not allowed to shorten (its length is held constant) and the tension it produces is measured. In an isotonic contraction the load on the muscle (which is equal to the tension in the muscle) is maintained constant and its shortening is measured.

1.2.3 The Length Dependence of Force

When a muscle is maximally activated, the isometric force that is developed depends on the length at which muscle is held. At very short lengths, active force is small. The length-force relationship has an ascending limb, a plateau, and a descending limb (from short to long length). When the muscle is lengthened, the active force increases to a maximum, which corresponds with the sarcomere length at which optimal cross bridge occurs.

In vivo, muscles in the human body are thought to operate along the ascending limb and at the plateau of l-f relationship.

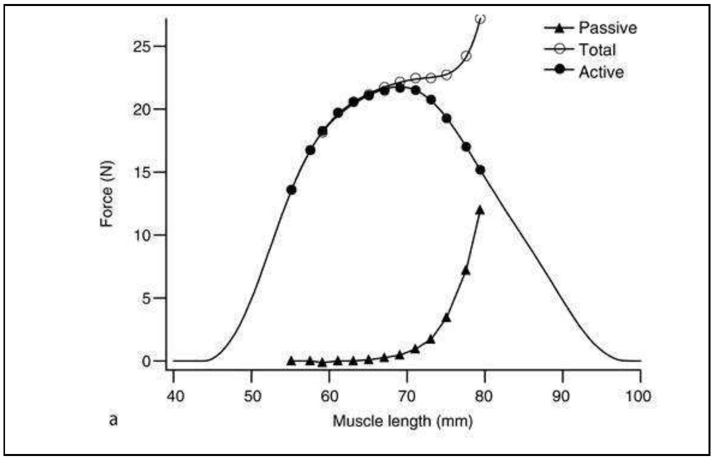

Figure 1.4 A typical length-tension curve measured from cat soleus muscle. Adapted from Keynes 2001 [1].

1.2.4 History Dependence of Force Production

Well established knowledge on muscle contraction states that force production depends only on muscle length, velocity and activation. However, e.g., Abbott and Aubert showed almost half a century ago that muscle force production is also history dependent [8]. History dependence is typically assessed by the increase or decrease of an isometric steady-state force at a given level of activation that is caused by prior shortening, lengthening or a combination of shortening/lengthening of ”muscle’ [9]. Typically, force depression is produced by shortening of an activated ”muscle”, and force enhancement by stretching of an activated ”muscle” [10, 11].

Although the existence of such history dependence of force production has been accepted for more than 50 years [8], its mechanism still remains obscure, which plausibly represents a significant gap in our understanding of muscle contraction. It is already known that history-dependent properties cannot be explained by existing phenomenon such as the cross-bridge model of contraction [12] or the sliding filament theory [3, 4] alone. A hypothesis that receives much attention in the literature proposes that force enhancement and force depression are caused by sarcomere length non-uniformity and instability along the descending limb of the force length relationship [13]. Accordingly, sarcomeres are assumed to shorten by different amounts because of instability.

However, there is mounting evidence suggesting that sarcomere length non-uniformity and instability cannot solely explain the history-dependent properties of force production [14, 15]. An alternative mechanism to explain force depression proposed many years ago has not yet been rejected: force depression would be caused by a stress induced inhibition of cross-bridge attachments in the miyofilament overlap zone that is newly formed during shortening [16], which may result from actin filament deformation. With this respect, another study suggested that when myofibrils were activated with MgADP, which potentially decreases the inhibition of cross-bridgeattachment in the overlap zone newly formed during shortening, they produced a dynamic FL relation that was left-shifted when compared to that induced by Ca2+ activation [17].

Most studies showed that history effect was time and velocity related [14, 15, 17, 10], but in recent studies its effects have also been shown in isometric muscle activities. In one of these studies, it has been shown that isometric muscle activity at higher length substantially altered subsequent conditions of measurements at lower length, without affecting the high length properties themselves [18]. It has also been experienced that systematic and major decreases in force for muscle at low reference length minutes after it has been active at high lengths [19]. Ateş et al, 2009 also found similar results showing that history effects occurred only for muscle that has been active at lengths near optimum length. After being exposed to activity at high length, the effects on active force are found particularly at lower lengths; for lengthening of EDL exclusively [20].

These results showed that length-history effects typically causing active force reductions should be considered as a fundamental phenomenon for muscle physiology and pathology. Any method that could limit the history effect has to be taken into account in designing muscular mechanics experiments. One of the candidates for this method is preconditioning in which the muscle is lengthened and shortened between two different muscle lengths (a lower length and a higher length) in succession until no further decreases of active force at low lengths are seen [19]. Preconditioning was also tested in Mass et al.'s experiments [21], however whether it does minimize the history effects or not has not been investigated systematically in any studies so far.

1.3 Goal of the Study

The goals of this study are:

1. To be able to observe the effects of history effect in repeated measurements. How the contractions at low lengths are affected from the activation in high lengths, is investigated by taking control measurements.

2. To assess the effects of preconditioning over history effects in rat muscles. In order to achieve this goal, standard length force graph was obtained on the extensor digitorium longus (EDL). After preconditioning was performed, length force graphs were obtained again to quantify the changes to the forces produced by EDL muscle as well as to that of its synergistic muscles i.e., TA+EHL complex.

2. METHODS

2.1 Surgical Procedures

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 sacrificed using an overdose of urethane solution.

Male Wistar rats (n = 8, mean body mass = 325.5 (S.D. 13.7g) were anaesthetized with intraperitoneally injected urethane solution (1.2mg of 12.5% urethane solution /100g body mass). Whole solution was administered in two times with 10 minutes intervals. Extra doses (up to 0.5 ml) 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 °C to prevent hypothermia. The body temperatures of the animals were monitored using an integrated rectal thermometer and kept at approximately 37 °C.

After an appropriate time following anesthesia, 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 that attaches the tendons tightly to the extramuscular connections was severed to release 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, the four distal tendons of EDL was dissected from the end positions as far as possible. The tendons were folded and tied together. In order to tie distal tendons of TA and EHL complex, the tendons were dissected from the bone in a way that a piece of bone was left on the tendons. Then these tendons were tied together with Kevlar thread too. 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.

The knee and the ankle angels were set to 120° and 100°, respectively. These angels which are also present in vivo conditions are attained in the stance phase of the rats' gait [22]. This fact lets the in situ experiments carried on the anterior crural compartment muscles to be performed closer to in vivo conditions.

After the distal tendons of target muscle were prepared ready for experiment, the proximal tendon of the EDL was exposed by cutting a piece of bone. This procedure is applied to secure the knot that keeps the tendon tightly to Kevlar. After completing detachment of tendons from bone, the sciatic nerve was dissected from upper limb as proximally as possible.

The dehydration of the muscles and the sciatic nerve was prevented by applying isotonic saline solution. And this application was repeated regularly during whole surgery.

2.2 Experimental Set-up

The rat was positioned on the experimental set up in such a way that ankle angle was in maximal plantar flexion (180°) and the knee was at 120°. 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 carefully with the muscles' line of pull (Fig 2.1).

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.

Sciatic 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. 400 ms 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 100 ms after the second twitch. EDL distal and proximal forces as well as TA+EHL distal measured simultaneously were recorded. After each application of this stimulation protocol, the muscles were allowed to recover at low muscle length, for 2 minutes.

Proximal and distal EDL isometric forces, as well as TA+EHL distal isometric forces were measured simultaneously. During the tetanic plateau, muscle total forces were determined (as the mean force for an interval of 150 ms subsequent to 25 ms after evoking tetanic stimulation).

After that, the following conditions were tested. Note that all measurements were performed while the muscle is in intact condition where the antreior crural compartment is not severed and the muscles function in their normal fashion.

2.3 Experimental Protocol

1. **l-f 1: Distal lengthening of EDL before preconditioning**

   - *l-f data collection referred to as l-f 1:* Isometric muscle forces were measured from EDL proximal, distal and TA+EHL distal tendons at various lengths of EDL: starting at active slack length of EDL, length was increased by moving its distal force transducer in steps of 1 mm, until 2 mm over EDL distal optimum length. Note that, the distal tendon of the TA+EHL complex and the proximal tendon of EDL muscle were kept at the reference position at all times during the experiment.

- *Control measurement:* After the all measurements in l-f data collection were completed, two more contractions were done at reference point and optimum length as control measurements.

2. **Preconditioning:**

   - Determination of preconditioning points: 3 mm over active slack length (lref2) and 2 mm over optimum length (lopt+2).
   - Preconditioning procedure was performed at these determined lengths: l-f data was taken at lref2 and lopt+2, respectively. Then these measurements were repeated until the force produced by muscle at these lengths was not 3% higher than previous measurement. And then, it was assumed that the target muscle is preconditioned and the contribution of history effect to force-length characteristic is maximally minimized, at least in the region between preconditioning points.

3. **l-f 2: Distal lengthening of EDL after preconditioning to be able to assess the effects of preconditioning over history effect**

   - After preconditioning, l-f data collection referred to as l-f 2 was done by same method used in first l-f data collection
   - Control measurement at reference length and optimum length.

4. **l-f 3: Distal lengthening of EDL**

   - Second L-F data measurement after preconditioning referred to as l-f 3 was done by same method used in first l-f data collection.
   - Control measurement at reference length and optimum length.

5. **l-f 4: Distal lengthening of EDL**

   - Third L-F data measurement after preconditioning referred to as l-f 4 was done by same method used in first l-f data collection.
   - Control measurement at reference length and optimum length.

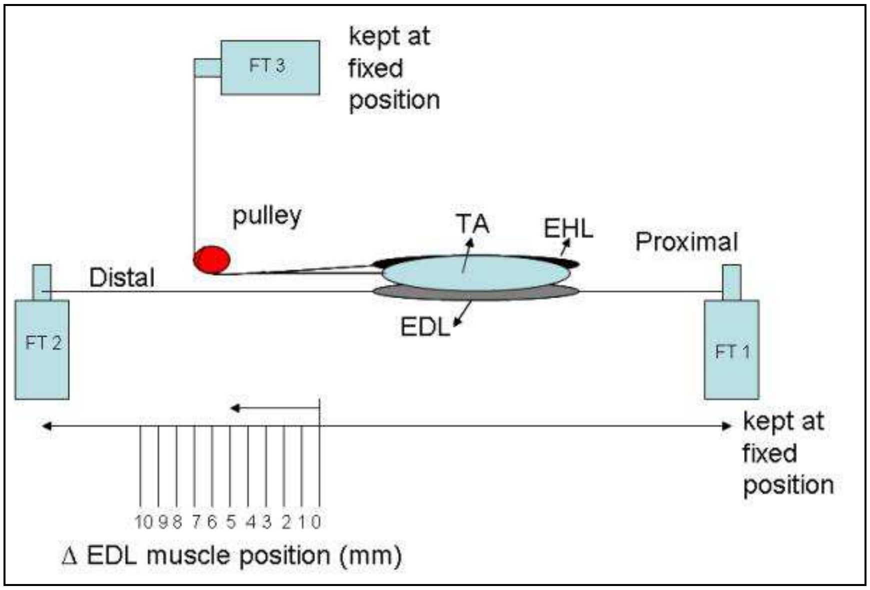

Figure 2.1 A schematic view of the experimental set-up and experimental protocol. FT 1 indicates the force transducer connected to the proximal tendon of EDL muscle, FT 2 indicates the force transducer connected to the tied distal tendons of EDL, and FT 3 indicates the force transducer connected to th tied distal tendons of TA and EHL muscles.

2.4 Treatement of Data and Statistics

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

$$y = e^{ax+b} \tag{2.1}$$

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.

$$y = b_0 + b_1x + b_2x^2 + b_3x^3 + b_4x^4 + ... + b_nx^n \tag{2.2}$$

where y represents active muscle force, x represents active muscle force length and b0 through bn 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 two-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. Two-way ANOVA was used to test for the effects of altered muscle length and experimental conditions on i) distal EDL forces, ii) proximal EDL forces iii) TA+EHL distal forces. There were four conditions in this study; first is l-f 1 which is l-f data collection before preconditioning second is l-f 2 which is l-f data collection just after preconditioning. Then third and fourth are l-f 3 and l-f 4 respectively which are subsequent measurements after l-f 2. 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 $-8 \leq \Delta l_{m+t} \leq 2$). Optimum muscle length is accepted as zero point and then force values are determined by moving 8 mm before optimum length and 2 mm over optimum length

Moreover two-way ANOVA was also performed to test the effects of control measurements on reference length and optimum length of the EDL muscle, which are composed of unfitted data. Differences were considered significant at $P < 0.05$. If significant main effects were found, Bonferroni post-hoc tests were performed to locate significant differences.

3. RESULTS

3.1 History Effects on l-f measurements

3.1.1 EDL Distal

To be able to quantify the force drop between before (l-f 1) and after preconditioning (l-f 2) in EDL distal tendon, the force produced by the muscle was also compared in different lengths. Two-way Anova showed that the differences in experimental conditions were significant, and post-hoc test located where these significant differences were present.

Table 3.1
The relation between the conditions in the experiment are shown below: The percentages of force change [decrease (-) or increase (+)] in EDL distal tendon before and after preconditioning and the data showing whether these drops are significant or not. ($P<0.05$: significant statistically. NS: non-significant).

#of	l-f 1 vs l-f 2		l-f 1 vs l-f 3		l-f 1 vs l-f 4	
measurements	% force change	p-value	% force change	p-value	% force change	p-value
1	-68.71	P<0.05	-82.89	P<0.05	-73.22	P<0.05
2	-50.79	P<0.05	-56.74	P<0.05	-56.25	P<0.05
3	-38.39	P<0.05	-38.80	P<0.05	-38.23	P<0.05
4	-21.17	NS	-20.85	P<0.05	-18.94	P<0.05
5	-8.11	NS	-9.08	NS	-8.42	P<0.05
7	-0.73	NS	-3.00	NS	-4.51	P<0.05
7	+2.34	NS	-0.89	NS	-3.71	P<0.05
8	+2.90	NS	-0.97	NS	-3.80	NS
9	+1.22	NS	-2.60	NS	-8.01	NS
10	-2.56	NS	-4.24	NS	-13.63	NS

In low length of EDL muscle (first three lengths of EDL), the force drop measured from EDL Distal between l-f 1 and l-f 2 is too high (from 38% to 68%) as expected (Fig 3.1). Moreover in this region of the force-length curve, the force significantly decreases (Table 3.1). However when moved to higher lengths, it is obviously seen that the force drop is becoming smaller, even it is enhanced in higher lengths.

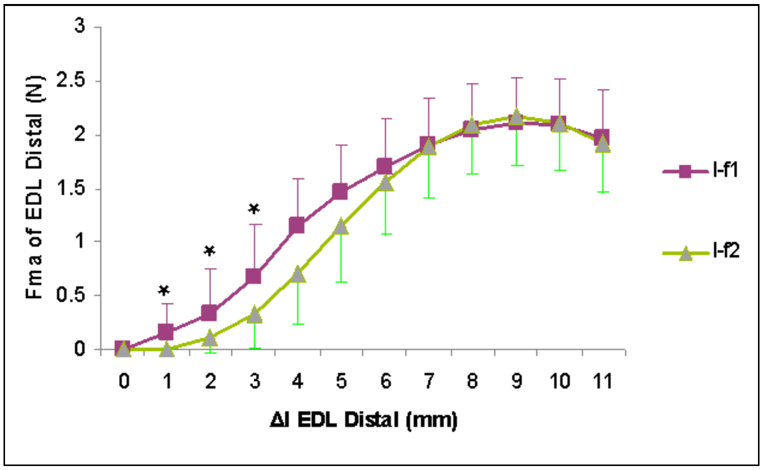

Figure 3.1 This figure represents the data including forces produced by EDL Distal in l-f 1 and l-f 4 data sets when EDL muscle is lengthened distally. * shows where the decrease is statistically significant.

When we compare l-f 1 with l-f 3, it is easily seen that force drop is getting larger with respect to previous comparison (l-f 1 and l-f 2) in lower lengths of EDL muscle. And the force drop is statistically significant in the first region of ascending limb of the force length curve.

Moreover when l-f 2 vs. l-f 3 and l-f 3 vs. l-f 4 are compared, no significant force drop was observed, thus implying that preconditioning might make the EDL muscle history free.

After preconditioning, length-force characteristic of the muscle seems unchanged among l-f 2, l-f 3 and l-f 4 (Fig 3.2). Only there is slight difference around optimum length in these data sets, but this difference is not statistically significant. However when we compare l-f 2 and l-f 4, it is significant to note that around and just before optimum length of EDL muscle, the force drops between these data sets are statistically significant.

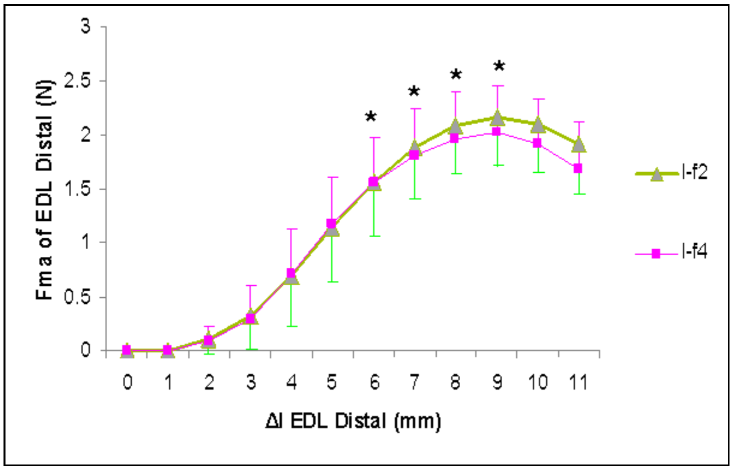

Figure 3.2 This figure represents the data including forces produced by EDL Distal in l-f 2 and l-f 4 data sets when EDL muscle is lengthened distally. * shows where the decrease is statistically significant.

3.1.2 EDL Proximal

In this experiment, the force change in EDL proximal is also observed while EDL muscle is lengthened distally. It can be clearly seen from the figure 3.3 that there is a significant force differences in EDL proximal before (l-f 1) and after (l-f 2) preconditioning.

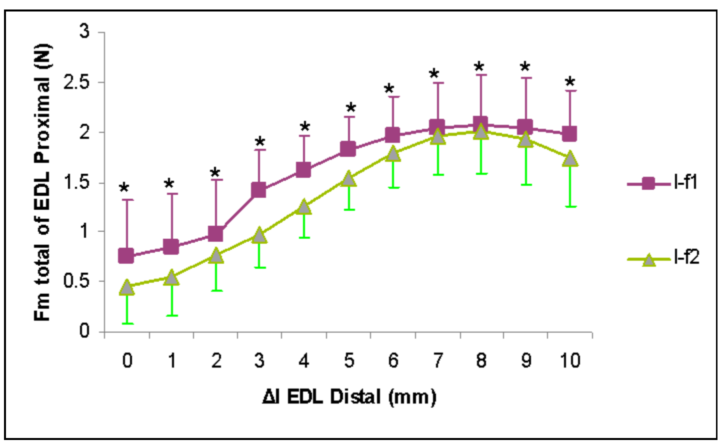

Figure 3.3 This figure represents the data including forces produced by EDL Proximal in l-f 1 and l-f 2 when EDL muscle is lengthened distally. * shows where the decrease is statistically significant.

In shorter lengths, the force difference is more noticeable than in higher lengths of EDL muscle. In second point of ascending limb of the graph, this force difference reaches to 34% and this force drop is statistically significant. Furthermore, despite the fact that the decrease is getting narrower in ascending limb of the graph (for example in 8th point: by 3%), they are still statistically significant (Fig 3.3).

In addition to differences between data taken from l-f 1 and l-f 2 set, the force differences are getting more obvious in successive force measurements. The gap in force measured from proximal end of the EDL muscle is enlarged in these data sets (Table 3.2).

Table 3.2
The percentages of the force drops in EDL Proximal between the data sets and whether these force drops are significant or not, are shown below ($P<0.05$: significant statistically. NS: non-significant).

#of	l-f 1 vs l-f 2		l-f 1 vs l-f 3		l-f 1 vs l-f 4	
measurements	% force decrease	p-value	% force decrease	p-value	% force decrease	p-value
1	39.37	P<0.05	53.88	P<0.05	44.63	P<0.05
2	34.91	P<0.05	56.16	P<0.05	33.01	P<0.05
3	22.03	P<0.05	24.39	P<0.05	26.42	P<0.05
4	30.59	P<0.05	31.99	P<0.05	36.97	P<0.05
5	22.82	P<0.05	25.93	P<0.05	29.61	P<0.05
7	15.22	P<0.05	20.05	P<0.05	21.73	P<0.05
7	8.55	P<0.05	14.10	P<0.05	15.07	P<0.05
8	3.93	P<0.05	9.13	P<0.05	10.94	P<0.05
9	2.64	P<0.05	6.91	P<0.05	10.15	P<0.05
10	5.57	P<0.05	8.98	P<0.05	12.57	P<0.05
11	12.04	P<0.05	14.53	P<0.05	16.00	P<0.05

In addition to comparison between l-f 1 and l-f 2, the behavior of force production in EDL proximal after preconditioning is also observed (Figure 3.4). To do this, three data sets (l-f 2, 3, 4) that are formed by force measurement after preconditioning are put together into a graph. As seen from this graph, the force-length characteristics of these graphs are similar. Between l-f2 and l-f 3 almost no force change was observed in higher lengths of EDL muscle. Furthermore, when we compare the next data set (l-f 4) with previous one (l-f 3) we obtain similar results. In lower lengths force decrease is becoming larger; on the other hand in higher lengths the drop is getting smaller. But in both situations, they are not statistically significant.

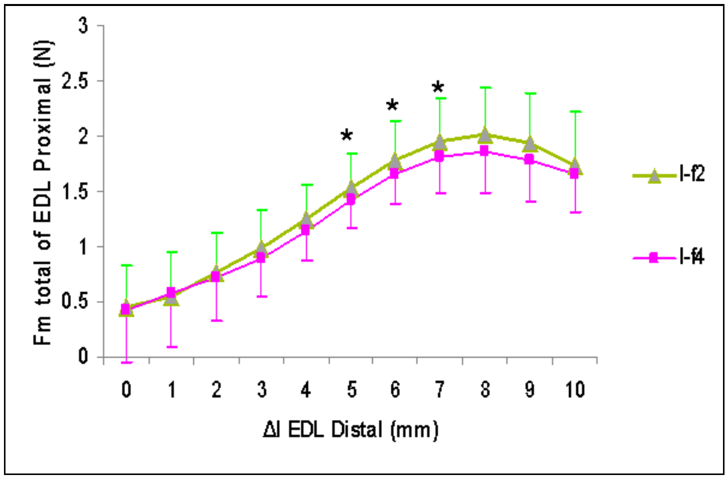

Figure 3.4 This figure represents the data including forces produced by EDL proximal in l-f 3 and l-f 4 data sets when EDL muscle is lengthened distally. * shows where the decrease is statistically significant.

However when we compare l-f 2 and l-f 4, it is significant to note that around and just before optimum length of EDL muscle, the force drops between these data sets are statistically significant.

3.1.3 TA+EHL Complex

Figure 3.5 shows the force differences in TA+ EHL complex between two data sets (l-f 1 and l-f 2). This graph also compares the force differences in neighbor muscle before (l-f 1) and after (l-f 2) preconditioning. In shorter lengths of EDL muscle the force difference is more obvious than in higher lengths of EDL muscle. In first point of the graph, the force drop is about 11% and interestingly it is statistically significant. But except this point, no significant force decrease was observed in TA+EHL complex while EDL is lengthened distally.

Moreover, Figure 3.6 also gives an idea to us what happens to force production of TA+EHL complex after preconditioning when its neighbor muscle is lengthened distally. And no significant force drop observed among these subsequent measurements.

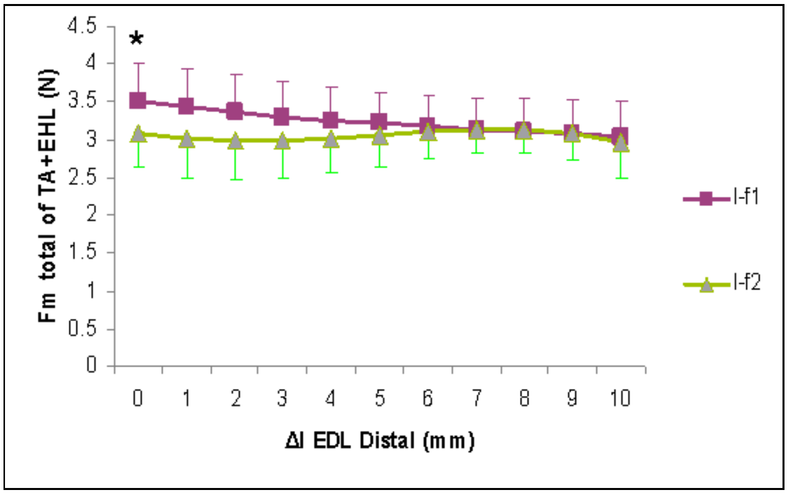

Figure 3.5 This figure represents the data including forces produced by TA+EHL complex in l-f1 and l-f 2 when EDL muscle is lengthened distally. * shows where the decrease is statistically significant.

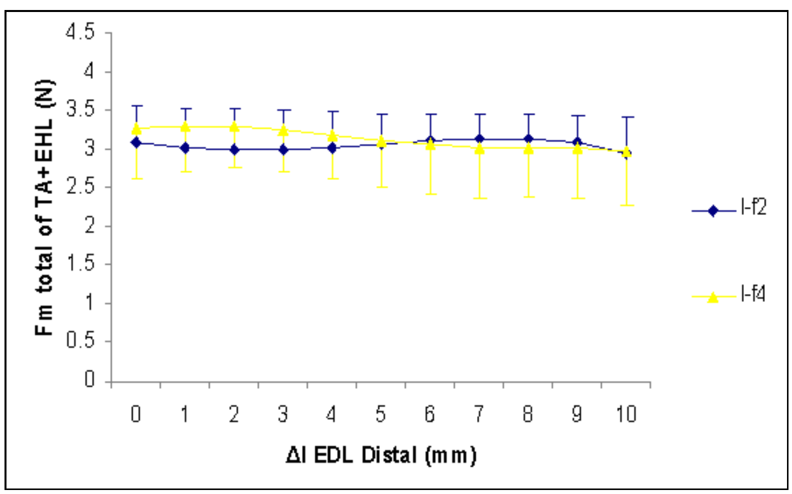

Figure 3.6 This figure represents the data including forces produced by TA+EHL complex in l-f2, l-f 3 and l-f 4 when EDL muscle is lengthened distally.

3.2 Control Measurements

3.2.1 EDL Distal

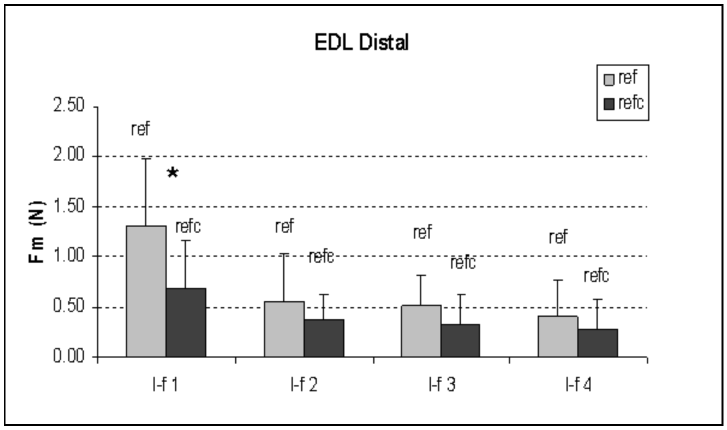

Figure 3.7 This figure compares the forces measured at reference positions (ref) and their controls (refc) in EDL Distal. * shows where the decrease is statistically significant.

Muscle forces in the control measurements are less than those in the actual measurements. The force drop is relativley more pronounced in lower muscle lengths than the higher ones. The drop of muscle force between lref and lrefc is the highest (by 48%) in intact condition before preconditioning and this force drop is statistically significant (Fig 3.7). After preconditioning procedure was performed, this force drop becomes 33%. And after subsequent measurements were performed, the force drop was decreased to 30%. However these decreases are not statistically significant.

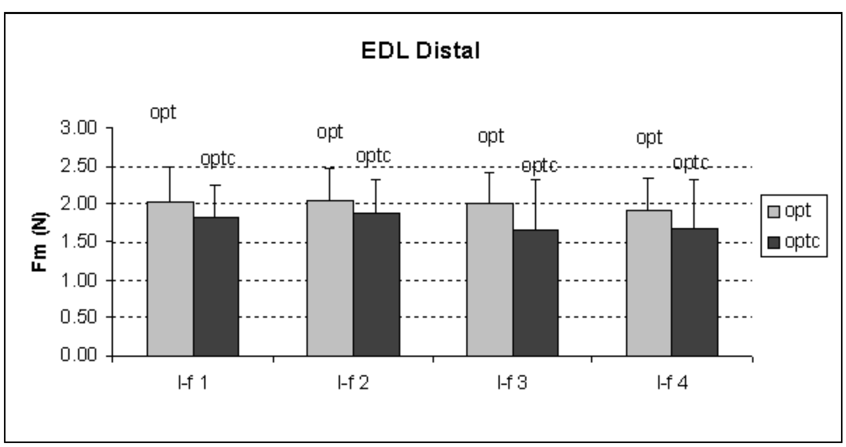

Figure 3.8 This figure compares the forces measured at optimum length (opt) and their controls (optc) in EDL Distal.

On the other hand, in the higher lengths the force drop is less than those in the lower lengths, and they are non-significant (Fig 3.8).

3.2.2 EDL Proximal

At Ref Length (Fig 3.9), the force drop between ref and ref c in l-f 1 is about 33% and statistically significant. And in l-f 2 this force decrease becomes 5%, but it is still significant. The force drop between reference position and its control is also observed in l-f 3 and l-f 4; but they are not statistically significant. On the other hand, the force drop between the measurements taken from optimum length and its control is also non-significant as in the previous studies.

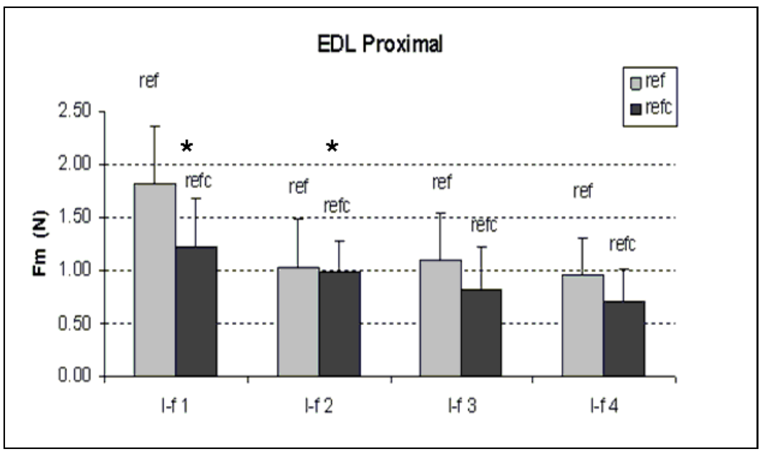

Figure 3.9 This figure compares the forces measured at reference positions (ref) and their controls (refc) in EDL Proximal. * shows where the decrease is statistically significant.

3.2.3 TA+EHL

No significant results were obtained from control measurements (at reference and at optimum length) in l-f data sets taken from TA+EHL complex.

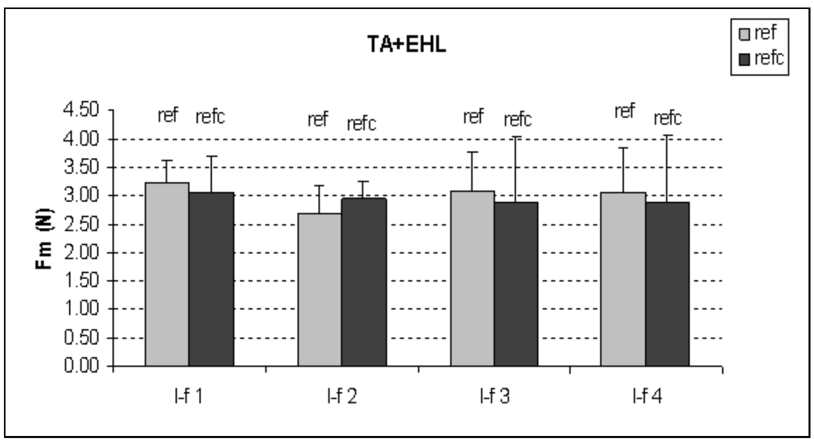

Figure 3.10 This figure compares the forces measured at reference positions (ref) and their controls (refc) in TA+EHL.

4. DISCUSSION

4.1 The Effects of preconditioning over history effects

4.1.1 EDL Distal

Previous studies have already showed that isometric muscle activity at higher length substantially altered subsequent conditions of measurements at lower length, without affecting the high length properties themselves [18]. In this study it was also observed that force drop in high length is not as much as occurred in low length. The results obtained in this experiment also imply that our preconditioning procedure worked well to restore the forces produced by EDL muscles in first length-force measurement.

With regard to the distal tendon of EDL muscle, force depression in high lengths is not statistically significant. In addition to these non-significant results, it was observed that there are force enhancements in very high lengths after preconditioning was performed. This situation can be attributed to the alteration of the length-force characteristics after preconditioning. When preconditioning was performed, the length range of force exertion by EDL muscle is changed, the muscle starts to produce high forces in higher lengths. These implications says that in any study involving multiple determinations of length-force characteristics, the higher lengths are reliable to determine force changes in different conditions.

Moreover after preconditioning, the force decreases non-significantly in whole lengths among subsequent measurements, thus also implying that our preconditioning procedure is a good candidate to discard the effects of history effects and after preconditioning either low lengths or high lengths become reliable to determine force changes in following experiments.

When l-f 1 and l-f 2 are compared, it is important to note that the significant force drops are observed in first three points which correspond to the points where preconditioning was not applied.

4.1.2 EDL Proximal

When the proximal tendon of the EDL muscle is observed, it can easily be seen that there is a slight difference among both ends of EDL muscles. At this point it should be noted that in classic approach, the muscle studied in situ is considered as "fully isolated" from its surroundings [23]. With regard to this approach, it has been idealized that the muscle force exerted at the tendon from which measurements are taken was considered to be equal to the force exerted at the other tendon. However, recent studies have shown that, due to myofascial force transmission, such functional independence and unique muscle length-force characteristics are not representative, if the muscle is considered within the context of its intact surroundings (the condition in vivo) [18, 24]. Due to this fact, proximal and distal ends of EDL muscle are investigated separately in this experiment too.

EDL muscle is lengthened distally, but it was observed that the impacts of history effects are also observed in the proximal tendon of the muscle. Between before (l-f 1) and after (l-f 2) preconditioning, there are significant force drops in all lengths, thus implying that our preconditioning procedure has no significant effect on EDL proximal. Despite the fact that preconditioning has no contribution to discard the history effect in EDL proximal, after preconditioning muscle produced same amount of forces in successive measurements. Although there are again force depressions in certain amounts between following measurements, they are not statistically significant. On the basis of these non-significant force drops, it can be deduced that although our preconditioning is not enough to minimize the effects of history dependence force depression in EDL proximal, it has a significant effects in force production of EDL muscle. After preconditioning, no significant force depression was observed between successive obtained force-length curves.

The reason why EDL proximal was less affected than EDL distal seems a little tricky. This result can be attributed to that since preconditioning is also performed by lengthening EDL distally; the myofibrils present in EDL proximal have not been affected from this procedure. And so, history effect caused the significant decreases in EDL proximal while preconditioning was well to restore the forces produced by EDL muscles in first length-force measurement. With regard to this information, our result showed that preconditioning performed by lengthening distally cannot be a solution to minimize the effects of history effects on EDL proximal.

4.1.3 TA+EHL

This study showed that the force produced by TA+AHL complex whose length is kept fixed, has not changed during EDL lengthened distally. And this result is consistent with previous studies saying that since this muscle complex is not shortened or lengthened, the effects of history effects in this muscle are not observed [18, 20]. With regard to this information, it can be said that the history has no effect on neighboring muscles which are not restrained, so the measurements taken from neighbor muscle is reliable for analysis.

4.2 Control measurements

4.2.1 EDL Distal

In this experiment after measurements are performed consecutively, two more contractions are done at reference point and optimum length as control measurements. And by two-way ANOVA the effect of preconditioning over control measurement is also observed. Before preconditioning at reference position, the force drop between control measurement and actual measurement during the experiment is 48% and statistically significant. After preconditioning was performed, in l-f 2 this force drop becomes 33% and in last one (l-f 4) the decrease becomes 30%, but after preconditioning whole force drops in control measurement at reference position is statistically non-significant. This result shows that preconditioning has positive effect to minimize the history effects at reference position. The classic control measurements can be a good candidate to check the force decreases in low lengths if only preconditioning was performed; otherwise its contributions might be misleading.

In addition to control measurements at reference position, at optimum length control measurements were also taken. Although there are also differences between control measurements and actual measurements, in whole data sets these force drops are not statistically significant.

4.2.2 EDL Proximal

The control measurements taken from EDL proximal tendon showed that after preconditioning, in l-f 2 there was still significant force drop at reference length. These results imply that preconditioning is not a solution in EDL Proximal tendon to restore the muscle forces produced before preconditioning.

4.3 Length-Force Characteristics of EDL Distal

Length-force characteristics of EDL muscle are changed after preconditioning. The shape of curve and the force measured from EDL muscle in distal end was modified after preconditioning, but it becomes stable after preconditioning even though successive measurements were performed.

Figure 4.1 also shows that there is a clear difference between produced forces by EDL in short lengths corresponding to the ascending limb of the l-f graph. But this force differences cannot be observed in high lengths which is already present in the ascending limb of the length-force graph.

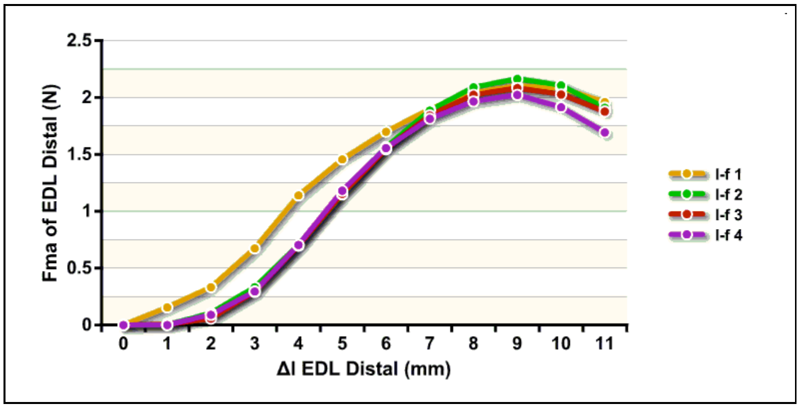

Figure 4.1 This figure shows the differences between different data sets taken from EDL distally.

As seen from the figure 4.1, although there is force depression in lower lengths of EDL muscle between before and after preconditioning, the forces produced by EDL are increased after preconditioning in very high lengths. And this force enhancement makes the muscle to produce near amount of forces in higher lengths as much as it produced before preconditioning.

The optimum force after preconditioning is increased by almost 3%. But this decrease is not regarded as statistically significant. After successive length-force measurements, the optimum force dropped by 4% between second and third measurements, but again this drop is not significant. In following measurements: between third and fourth, the force dropped not significantly by approximately 3% and between second and fourth, the drop in optimum force reaches to 6.5% and this decrease is statistically significant.

5. CONCLUSION

In this study, it was found that after preconditioning, EDL distal length-force curve are reliable to investigate force differences. Moreover in subsequent measurements, the contribution of preconditioning becomes more effective and whole l-f data can be considered as dependable. In contrast to EDL distal, control measurement shows that preconditioning performed by EDL lengthening distally is not a solution for force decreases in EDL proximal tendon although after preconditioning EDL muscle seemed history-free. On the basis of results obtained from TA+EHL complex, it can be said that history has no effects on neighboring muscles which are restrained, so the measurements taken from neighboring muscle is reliable for analysis. With regard to control measurements taken from EDL Distal, after preconditioning control measurements are not affected from history effects as they do before preconditioning. Any studies involving control measurement should perform preconditioning to minimize history effects. Our results therefore provide a better way to minimize the history effects for the scientists designing muscular mechanics experiment involving lengthening EDL muscle distally.

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