Myofascial Force Transmission

Maas, H.

2003

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Intra-, inter- and extramuscular pathways

Huub Maas

The research presented in this thesis was carried out at the Institute for Fundamental and Clinical Human Movement Sciences, Faculty of Human Movement Sciences, Vrije Universiteit Amsterdam, The Netherlands.

ISBN: 90-9017232-7
NUR: 744

Printer: Digital Printing Partners Utrecht, Houten (www.dpp-utrecht.nl)

Cover photo: Cross-section of the anterior crural compartment of rat

VRIJE UNIVERSITEIT

Myofascial Force Transmission

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ACADEMISCH PROEFSCHRIFT

ter verkrijging van de graad van doctor aan
de Vrije Universiteit Amsterdam,
op gezag van de rector magnificus
prof.dr. T. Sminia,
in het openbaar te verdedigen
ten overstaan van de promotiecommissie
van de faculteit der Bewegingswetenschappen
op donderdag 16 oktober 2003 om 15.45 uur
in de aula van de universiteit,
De Boelelaan 1105

door

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geboren te Eindhoven

promotor: prof.dr. P.A.J.B.M. Huijing

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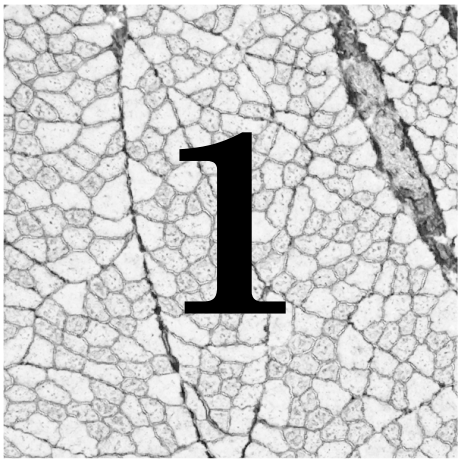
Introduction



INTRODUCTION

Humans are able to perform controlled movements of body segments due to the fact that muscle fiber force is transmitted to the bony skeleton, thereby creating a moment with respect to joints. Therefore, the process of force transmission from sarcomere to bone is essential for the understanding of how humans achieve movements.

The active force-generating units within a muscle fiber are the sarcomeres, in which cross-bridges between actin and myosin molecules cause the contraction (Huxley, 2000). Excitation of a muscle leads to shortening of sarcomeres until active force exerted by cross-bridges connecting the actin and myosin filaments together with passive force exerted by intra-sarcomeric passive elements (e.g. titin) is equal to opposing forces. Opposing forces may originate from several structures between sarcomere and bone. This process is defined as muscular force transmission and the chain of structures the pathway of force.

Within a muscle, the most recognized pathway of force transmission is via sarcomeres in series and the myotendinous junction (e.g. Tidball, 1991; Trotter, 2002). This process is defined as myotendinous force transmission. In addition, force from muscle fibers can be transmitted to the surrounding endomysium by shear, which was clearly demonstrated by Street (1983). This process is defined as myofascial force transmission (Huijing et al., 1998). Within muscle fibers (Fig. 1A), sarcomeres in series are linked to each other by Z-lines and adjacent sarcomeres are linked to each other by desmin at the Z-line (Lazarides, 1980) and by skelemin at the M-line (Price, 1987; Price and Gomer, 1993). Complexes of structural proteins located between the sarcomeres, the subsarcolemmal cytoskeleton and the extracellular matrix (Berthier and Blaineau, 1997) connect muscle fibers to the intramuscular connective tissue network (Fig. 1B). It has been shown that the endomysium is a continuous stroma of collagen fibrils, in which the muscle fibers are embedded (Purslow and Trotter, 1994; Swatland, 1975; Trotter and Purslow, 1992). In the same way, the perimysium is continuous with the endomysium (Moore, 1983; Rowe, 1981).

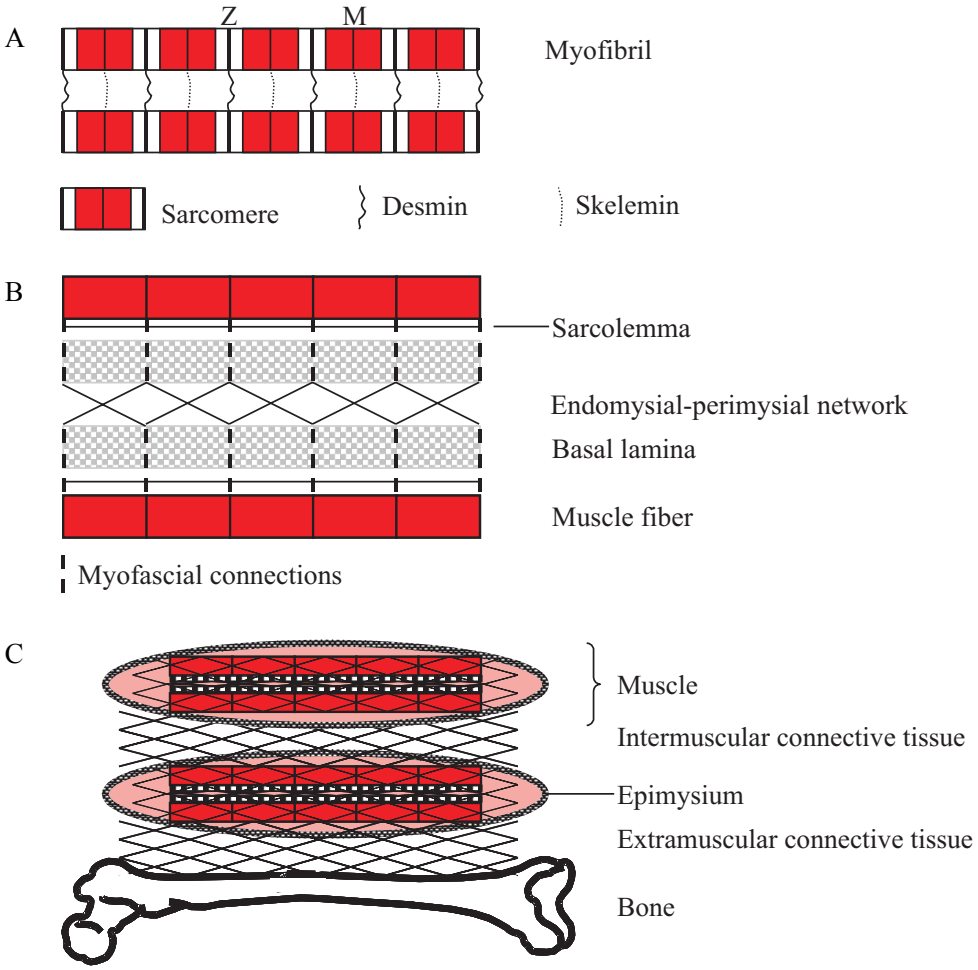


Fig. 1. A schematic representation of myofascial pathways. (A) Within muscle fibers. (B) Within a muscle. (C) Within a compartment.

Force exerted at the endomysium can in principle be transmitted onto the muscle's own tendon (a) via neighboring fibers and their myotendinous junction (Purslow and Trotter, 1994; Trotter, 1993; Trotter and Purslow, 1992) and/or (b) via the endomysial-perimysial network (Huijing et al., 1998; Jaspers et al., 2002; Jaspers et al., 1999). The latter means that force is transmitted onto the tendon without passing any myotendinous junction.

If a muscle is isolated from surrounding tissues, muscle fiber force can only be transmitted via myotendinous or intramuscular myofascial pathways to the proximal

and distal insertions to bone (e.g. tendons). *In vivo*, however, muscles are surrounded by synergists and embedded within connective tissues of a compartment (Fig. 1C). It has been noticed that the muscles within the limbs of horses are tightly attached to each other (Pond, 1982). Anatomical investigations have shown mechanical connections between muscles, between muscles and joint connective tissue, as well as between muscle and intermuscular septa (Van der Wal, 1988; Vleeming et al., 1995). In addition, muscles are connected to neurovascular tracts (i.e. nerves and blood vessels encapsulated by connective tissues) in the extramuscular space of the compartment (Huijing and Baan, 2001b). Therefore, the connective tissues of the compartment or even of the whole limb may be considered as a continuous network with tunnels of connective tissue in which muscles (epimysium, compartmental fascia), fascicles (perimysium) and muscle fibers (endomysium) operate.

On the basis of myofascial force transmission within a muscle and the view that the intramuscular connective tissue should be considered as a part of a total continuous connective tissue network of a compartment, it was hypothesized that *in vivo* muscle fiber force is also transmitted out of the muscle via pathways other than the tendons (Huijing, 1999b; Huijing et al., 1998). Possible pathways are: (1) connective tissue at the interface between the muscle bellies of adjacent muscles (i.e. intermuscular connective tissue) and (2) via extramuscular connective tissues (e.g. connective tissue that supports the nerves and blood vessels, compartmental fascia).

Several previous experiments have yielded implicit support for such inter- and extramuscular myofascial force transmission:

- Early work already indicated mechanical interactions via intermuscular connective tissue between soleus and gastrocnemius muscles, if these muscles were not dissected (Denny-Brown, 1929). In fact, dissection in that study was performed to remove those effects unwanted for the experiment.
- It was found that the cat hamstring muscles produced a torque at the ankle joint (Wicke and Zajac, 1981). The authors explained this result by force transmission via a fascial sheath between hamstrings and the calcaneous.
- In response to a target paper by Stein (1982), Pond (1982) noted she had observed effects of isolating guinea pig hind leg muscles from their surrounding tissues on forces exerted at the distal tendons of those muscles. However, the results of a systematic study have never been published.
- It has been observed that during the late stance phase of cat locomotion

force exerted at the distal tendon of soleus muscle was higher than force at the same shortening velocity of fully isolated soleus muscle (Gregor et al., 1988). This is remarkable because the muscle was stimulated submaximally *in vivo* and maximally *in situ*. *In vivo*, force may have been transmitted from adjacent muscles via inter- and or extramuscular myofascial pathways onto the soleus muscle.

- Investigating biomechanical effects of rectus femoris transfer surgery has also yielded some indirect evidence of myofascial force transmission (Riewald and Delp, 1997). In such a transfer surgery, the distal tendon of rectus femoris muscle is detached from the patella and reattached to a flexor site of the knee. It was found that the intramuscular stimulated rectus femoris muscle still generated a knee extension moment after this surgery, at a fixed knee angle of 90°. In a follow-up study, cine phase-contrast magnetic resonance imaging showed that also the length of the muscle belly of rectus femoris muscle decreased during active knee extension (Asakawa et al., 2002).
- Severing connective tissue at the interface between rat extensor digitorum longus (EDL) and tibialis anterior (TA) muscles as well as compartmental fascia caused major changes in length-force characteristics of EDL muscle (Huijing and Baan, 2001b): optimal force decreased and the length range between active slack length increased.

Until recently, no systematic experiments, designed to demonstrate the existence of inter- and extramuscular myofascial force transmission, were performed. If some force is transmitted out of a muscle bypassing its tendons, force exerted at its origin is not necessarily equal to force exerted at its insertion. The proximo-distal force difference is a direct measure of the magnitude of net inter- and extramuscular myofascial force transmission. Unequivocal evidence for such force transmission was shown first by Huijing (2001a). For several muscle-tendon complex lengths tested, it was found that force exerted at the proximal tendon was unequal to force exerted at the distal tendons of rat EDL muscle. However, several questions remain to be answered.

Therefore, the general aim of this thesis is to further analyze force transmission via inter- and extramuscular myofascial pathways and to determine its relevance for force transmission from muscle fiber to bone. Specific aims are: (1) to identify inter- and extramuscular myofascial force transmission for isometric and dynamic muscle conditions as well as (2) factors that determine its magnitude, (3) to identify

(connective tissue) structures other than the tendons that may transmit force out of the muscle, (4) to determine the effects of myofascial force transmission on the distribution of lengths of sarcomeres within the muscle, (5) to determine the effects of intramuscular myofascial force transmission, but also inter- and extramuscular myofascial force transmission for functioning of individual heads of multi-tendoned muscles, and (6) to study effects of prolonged length changes of a single head of multi-tendoned muscle on muscle tissue and myofascial force transmission.

OUTLINE OF THIS THESIS

The experiments were carried out by measuring forces of the muscles within the anterior crural compartment of rat: EDL, TA and extensor hallucis longus (EHL) muscles. It should be noted that rat EDL is a multi-tendoned muscle. This muscle consists of four heads of which the muscle fibers share a common aponeurosis and tendon proximally, but have individual aponeuroses and tendons distally (Balice-Gordon and Thompson, 1988). The heads are named after their insertions on the digits within the foot (II, III, IV, V). For the experiments described in chapters 2, 3 and 4, the distal EDL tendons were tied together. For the other experiments (chapter 5, 6 and 7), the distal tendon of head III of EDL muscle (EDL III) was connected to a force transducer while the other distal tendons of EDL muscle were left attached to their insertions on the digits within the foot.

EDL muscle is equipped with both proximal and distal tendons and these tendons extend beyond the anterior crural compartment. Because these tendons can be attached to force transducers after minimal surgical disruptions with the connective tissues of the compartment, EDL muscle is a very useful experimental model for the assessment of myofascial force transmission. The distal tendons of TA and EHL muscles are, for a substantial part of their length, quite close to one another. As it is difficult to measure force exerted at each tendon individually without friction between them, the distal tendons of TA and EHL were tied together. This complex of muscles will be referred to as TA+EHL.

The experiment of **chapter 2** investigates intermuscular myofascial force transmission by measuring effects of length changes of TA+EHL on forces exerted at the distal as well as at the proximal tendons of EDL muscle, kept at a constant muscle tendon complex length. Furthermore, connective tissue structures that could mediate inter- and extramuscular myofascial force transmission were identified.

In **chapter 3** a study is presented in which the effects of changes of the position of EDL muscle relative to adjacent synergists as well as relative to other surrounding structures within the anterior crural compartment were investigated. The muscle-tendon complex length of EDL and TA+EHL was kept constant and exclusively the relative position of EDL muscle was changed.

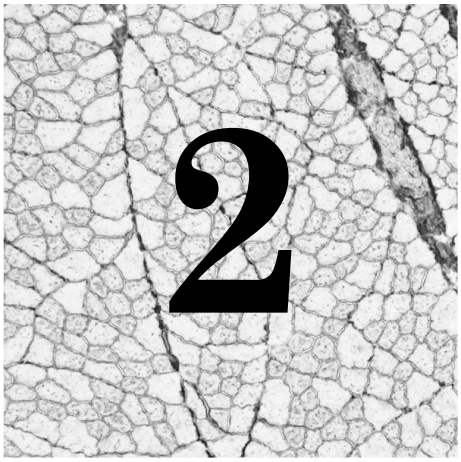
Subsequently, effects of the relative position of EDL muscle, which was dissected free from surrounding synergists, were studied (**chapter 4**). Thus, only extramuscular connective tissue that supports the nerves and blood vessels of EDL was left intact. The linked fiber-matrix mesh model was used to investigate effects of extramuscular myofascial force transmission on the distribution of lengths of sarcomeres within EDL muscle. A detailed description of this 3D finite element model as well as a more extensive analysis of effects of myofascial force transmission on the distribution of lengths of sarcomeres is reported in the thesis of Yucesoy (2003).

The following three chapters address experiments on distal length changes of EDL III. Effects of length changes of EDL III on myofascial force transmission between that head and adjacent tissues were studied for isometric muscle conditions in **chapter 5**, and for concentric muscle conditions in **chapter 6**. **Chapter 7** deals with the effects of prolonged intermittent concentric exercise of EDL III on (a) indices of early muscle damage, and on (b) force transmission between EDL III and adjacent tissues of the intact anterior crural compartment, were investigated.

In **chapter 8**, some previous experimental results are reviewed as well as new experimental data are presented that show effects of inter- and extramuscular myofascial force transmission. Furthermore, implications of myofascial force transmission for studies investigating muscle properties are discussed.

The thesis is concluded by an epilogue (**chapter 9**), which includes some remarks on issues not described in other chapters of this thesis as well as a more extensive discussion of some previous issues.

Intermuscular interaction via myofascial force transmission: effects of tibialis anterior and extensor digitorum longus length on force transmission from rat extensor digitorum longus muscle



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Journal of Biomechanics 34: 927-940, 2001

ABSTRACT

Force transmission in rat anterior crural compartment, which envelopes tibialis anterior (TA), extensor hallucis longus (EHL) and extensor digitorum longus (EDL) muscles, was investigated. These muscles together with the muscles of the peroneal compartment were excited maximally. Force was measured at both proximal and distal tendons of EDL muscle as well as at the tied distal tendons of TA and EHL muscles (the TA+EHL complex). Effects of TA+EHL complex length and force on proximally and distally measured forces of EDL muscle kept at constant muscle-tendon complex length were assessed. Length changes of EDL muscle were imposed by movement of the proximal force transducer to different positions.

Proximal EDL force was unequal to distal EDL force (active as well as passive) over a wide range of EDL muscle-tendon complex lengths. This is an indication that force is also transmitted out of EDL muscle via pathways other than the tendons (i.e. inter- and/or extramuscular myofascial force transmission). At constant low EDL length, distal lengthening of the TA+EHL complex increased proximal EDL force and decreased distal EDL force. At optimum EDL length, TA+EHL active force was linearly related to the difference between proximal and distal EDL active force. These results indicate intermuscular myofascial force transmission between EDL muscle and the TA+EHL complex. The most likely pathway for this transmission is via connections of the intact intermuscular connective tissue network. The length effects of the TA+EHL complex can be understood on the basis of changes in the configuration, and consequently the stiffness, of these connections. Damage to connective tissue of the compartment decreased the proximo-distal EDL force difference, which indicates the importance of an intact connective tissue network for force transmission from muscle fibers to bone.

INTRODUCTION

Individual muscles or muscle groups are frequently investigated *in vivo*. Properties of muscle groups have been assessed measuring joint angle–net joint moment characteristics (for a review see Kulig et al., 1984). Ultrasonography has been used to study individual muscles during voluntary *in vivo* human movements, e.g. by measuring movement and elongation of tendinous structures (Fukunaga et al., 1996; Kubo et al., 1999). In contrast to joint angle–net joint moment determinations, ultrasonography may be used to study intermuscular interactions morphologically. It should be noted that in these types of work, the degree of activation of the muscle is not usually controlled well, and neither are effects of antagonists, as co-activation cannot usually be controlled rigorously.

To gain a better understanding of joint movements, the contribution of several muscles to the net joint moment has been investigated. One approach is to implant force transducers onto tendons of synergistic cat muscles (m. gastrocnemius, m. soleus and m. plantaris) to measure individual muscle forces for different locomotor conditions (e.g. Herzog et al., 1993). The data of such studies will contain any effects of interaction between muscles. However, by attributing the forces exerted on each tendon to the individual muscles with which the tendons make a morphological unit, it is assumed implicitly that force measured at a particular tendon is also generated within the muscle belly corresponding to that tendon. In humans, forces produced by individual fingers during multi-finger tasks have been studied (e.g. Li et al., 2000; Zatsiorsky et al., 2000). One of the major findings is that force generation with fingertips of one, two or three human fingers is accompanied by force production of the other non-target fingers. Connections between tendons, extrinsic muscles that produce contraction forces in all four fingers as well as plastic changes within the central nervous system are thought to be responsible for this interdependent action of fingers.

Alternative pathways of force transmission (Street, 1983; Street and Ramsey, 1965) may also play a role in these interaction effects between fingers. Force from muscle fibers is transmitted via the endomysial–perimysial network to other neighboring fibers (e.g. Purslow and Trotter, 1994; Trotter, 1993; Trotter and Purslow, 1992) or, more likely, from the network directly onto the aponeurosis (intramuscular myofascial force transmission) (Huijing, 1999b; Huijing et al., 1998). Recent observations (Huijing, 1999a, 2000) indicated that muscle fiber force is also

transmitted out of the muscle via pathways other than the tendons: (1) via extramuscular connective tissue to other structures (extramuscular myofascial force transmission), simultaneously measured proximal and distal active forces of extensor digitorum longus muscle (EDL) within an intact anterior crural compartment were found not to be identical during a tetanus; (2) via intermuscular connective tissue to surrounding muscles (intermuscular myofascial force transmission). Explorative experiments have revealed effects of tibialis anterior (TA) muscle-tendon complex length on proximally measured EDL force kept at constant muscle-tendon complex length and vice versa, although it could not be concluded unequivocally that myofascial force transmission was responsible for it (Huijing, 1999a).

The aim of the present study was to identify intermuscular myofascial force transmission within the anterior crural compartment of rat by quantification of differences between forces exerted at proximal and distal tendons of EDL and to localize structures, which may play a role in such force transmission. The anterior crural compartment contains EDL, TA and EHL (extensor hallucis longus) muscles as well as intra-, inter- and extramuscular connective tissue. If intermuscular myofascial force transmission is present, it is expected that changes in characteristics of TA and EHL muscles will affect force transmission from EDL muscle. The purpose of this study is to investigate length effects of the TA+EHL complex on EDL forces.

MATERIALS AND METHODS

Surgical and experimental procedures were in strict agreement with the guidelines and regulations concerning animal welfare and experimentation set forth by Dutch law, and approved by the Committee on Ethics of Animal Experimentation at the Vrije Universiteit.

Surgical procedures

Male Wistar rats (n = 15) were anaesthetized using intraperitoneally injected urethane (initial dose: 0.15 g / 100 g body mass, extra doses if necessary: maximally 0.20 g). During surgery and data collection, the animals were placed on a heated water pad of approximately 37°C, to prevent hypothermia. Ambient temperature (22 ± 0.5 °C) and air humidity (80 ± 2%) were kept constant by a computer controlled air-conditioning system (Holland Heating). Muscle and tendon tissue was further

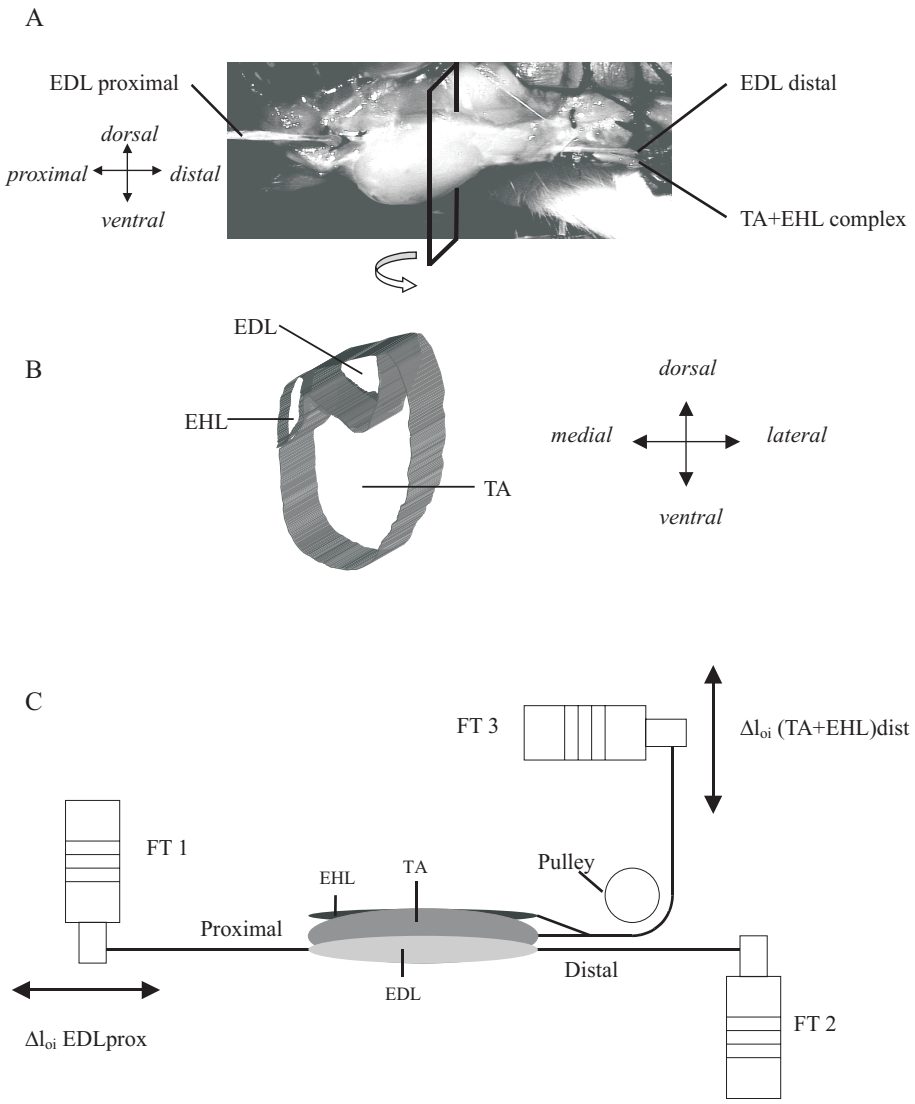


Fig. 1. The anterior crural compartment as in the experimental set-up. (A) Lateral view of the compartment in the experimental set–up. The Kevlar thread connecting the different tendons to force transducers and the anatomical orientation (cross of arrows) are indicated. (B) A schematic outline of the muscles within the compartment in proximal view. The curved arrow in A demonstrates how the cross–section was turned to obtain a proximal view. The rectangle in A indicates the approximate level of cross–section. Anatomical orientation is indicated (cross of arrows). (C) A schematic view of the experimental set-up, seen from above. FT 1 indicates the force transducer connected to the proximal tendon of EDL muscle, FT 2 indicates the force transducer connected to the distal tendons of EDL muscle, and FT 3 indicates the force transducer connected to the distal tendons of TA and EHL muscles. A pulley was used to guide the Kevlar thread from TA+EHL to FT 3. The double arrows demonstrate the direction of changes of muscle-tendon complex lengths to obtain several conditions imposed on the muscles.

prevented from dehydration by regularly irrigating the tissue with isotonic saline.

The anterior crural compartment, which envelopes TA, EDL and EHL muscles, was exposed by removing the skin and most of the biceps femoris muscle from the left hind limb. Connective tissue at the muscle bellies of TA, EHL and EDL was left intact (Fig. 1). However, the transverse crural ligament and the crural cruciate ligament were severed and limited distal fasciotomy was performed to dissect the distal tendons of EDL, TA and EHL. The four distal EDL tendons were tied together. The distal tendons of TA and EHL were also tied to each other. This complex will be referred to as the TA+EHL complex. The foot was attached to a plastic plate with tie wraps and was positioned in such a way that the ankle angle was 180°. Proximally, a small area of the femoral compartment had to be opened to detach the proximal tendon of EDL from the femur. The femoral compartment was opened further to secure the femur (at a knee angle of 90°) with a metal clamp when the rat was in the experimental apparatus. All tendons were cut and connected to force transducers (Hottinger Baldwin, maximal output error < 0.1 %, compliance of 0.0048 mm/N) with Kevlar thread (4% elongation at a break load of 800 N). For TA+EHL force measurement the Kevlar thread was connected to the force transducer via a pulley (Fig. 1C). Measurements of specified weights via this pulley revealed no effects of the system for force measurement: force measured by the force transducer (FT 3, Fig. 1C) was equal to the weight used. Note, however, that both EDL ends were connected directly to the force transducers, which were positioned in the line of pull.

The tibial nerve and the sureal branch of the sciatic nerve were cut as proximally as possible. The sciatic nerve with the peroneus communis nerve branch left intact was dissected, cut as proximally as possible and placed in a pair of silver electrodes.

Experimental conditions

The sciatic nerve was stimulated supramaximally with the electrodes connected to a constant current source (3 mA, pulse width 100 μs). Branches of the intact common peroneal nerve innervate EDL, TA and EHL muscles and stimulation will therefore activate all three muscles simultaneously. It should be noted that this nerve also activates the muscles in the peroneal compartment.

Isometric force was measured just before and during the tetanic contraction of the muscles. Simultaneously, images of the anterior crural compartment muscles in passive and active state were recorded using a digital camera (DVC, JAI CV-M10,

shutter speed 1/50 s). Stimulation of the nerve, A/D conversion (12-bit A/D converter, sampling frequency 1000 Hz, resolution of force 0.01 N), and photography were time-controlled by a microcomputer.

Before each contraction the target muscle was brought to the desired length passively by moving a particular force transducer. Two twitches were evoked to allow the muscles to adapt to the new condition, followed by a tetanic contraction after 300 ms (pulse train 400 ms, frequency 100 Hz). After each contraction the muscles were allowed to recover below active slack length for 2 minutes. The force transducer connected to the distal EDL tendons was kept at a constant position in all experimental protocols.

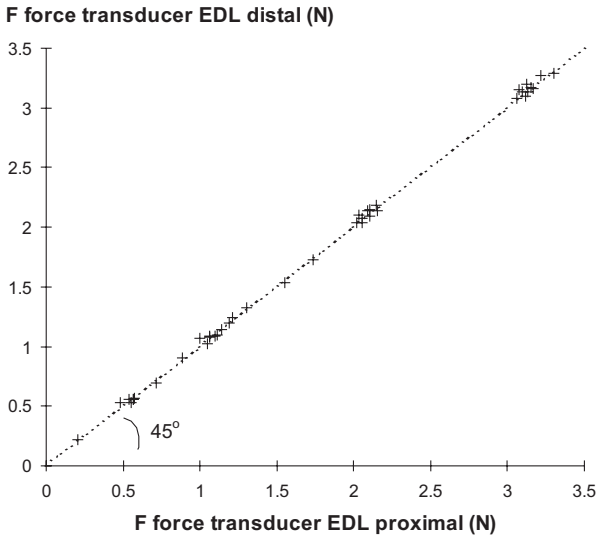


Fig. 2. An example of a comparison of isometric characteristics of the force measurement system. In the animal experiment, the proximal and distal tendons of EDL muscle were connected to the system (force transducers, amplifiers, A/D converter). The two force transducers were connected to each other by a compliant spring (Fig.1) and different force levels were obtained by changing the distance between the transducers. The dotted 45° line indicates identical force measurement systems.

In order to make sure that any differences in force transducers and their calibration prior to the experiment introduced no artifact, the two force transducers to be used for measurement of EDL forces (Fig. 1) were directly connected to each other using Kevlar thread attached to a compliant spring. The output was recorded with the identical measurement system as used in the animal experiment. The slope

of the regression line (r^2 = 0.999) of the simultaneously measured forces deviated 0.7% from the expected 45°. It is concluded that differences in force between these transducers greater than 0.7% cannot be ascribed to the measurement system used (Fig. 2).

Length-force characteristics of EDL muscle

EDL isometric force was measured at various muscle-tendon complex lengths (n = 8, body mass = 304.8 ± 23.1 g). To exclude myotendinous- and intramuscular myofascial pathways to the distal TA+EHL tendons, these tendons were released in such a way that no force was exerted at that location. EDL muscle was lengthened proximally with 1 mm increments from the most distal position of the proximal EDL tendon to approximately 3 mm over optimum length.

Length- force measurements of the TA+EHL complex

Directly following determination of length-force characteristics of EDL, EDL muscle was kept at a constant length corresponding to a force of approximately 20% of the previous assessed optimal active force of proximally measured EDL. Isometric length-force characteristics of the TA+EHL complex were measured (n = 7, body mass = 307.3 ± 23.7 g). The TA+EHL complex was lengthened distally with increments of 1 mm from active slack length to approximately 3 mm over optimum length.

EDL force at different forces of TA+EHL

For a separate group of animals (n = 7, body mass = 319.4 ± 11.6 g), isometric force in proximal and distal tendons of EDL was measured at various muscle-tendon complex lengths to determine optimum length of proximal EDL force with the fitting process (see below). In contrast to the other protocols, EDL muscle was lengthened proximally with 2 mm increments. Each length-force determination of EDL muscle was performed at a different TA+EHL complex length in such a way that active TA+EHL force ranged between 0 and 2.63 N.

Post-experimental treatment of data

The individual relationships between passive muscle force (F_{mp}) and muscle-tendon complex length (l_{oi}) were fitted with an exponential curve (Eq. 1), using a least-squares criterion:

$$y = e^{ax+b} \quad (1)$$

where y represents F_{mp}, x represents l_{oi}, and a and b are coefficients determined in the fitting process. Active muscle force (F_{ma}) was assessed by subtracting fitted F_{mp} from total muscle force (F_m) at equal muscle-tendon complex length. The relationship of F_{ma} with l_{oi} was fitted by a polynomial:

$$y = b_0 + b_1x + b_2x^2 + b_3x^3 + b_4x^4 + \ldots + b_nx^n, \quad (2)$$

where y and x represent F_{ma} and l_{oi} respectively, and b_0 through b_n are coefficients determined in the fitting process. The order of the polynomial most adequately describing the relationship was selected (see statistics). Fitted curves were used to calculate mean data and standard deviations and to determine optimal active force (F_{mao}) and optimum muscle-tendon complex length.

All force data were normalized for optimal force of the individual muscle, and l_{oi} was expressed as the deviation from optimum muscle-tendon complex length (Δl_{oi}).

Morphology of the anterior crural compartment

Two additional animals were used for anatomical survey of the anterior crural compartment. Images (digital camera) were made to identify inter- and extramuscular connective tissue of EDL, EHL and TA muscles. For cross-sections, the muscles of the compartment were isolated from the leg and altogether frozen in isopentane (at −160° C). Frozen sections (10 μm) were cut approximately perpendicular to the muscles, using a cryo-microtome. Sections were fixed in Bouin fixative for 30 min, subsequently rinsed with water, and stained with a Sirius Red (FB3, Brunswig Chemie, Amsterdam) staining solution. The sections were dipped three times for approximately 2 seconds in absolute ethanol and then put in Xylene for 5-15 minutes, and finally embedded in Entallan (Merck, Für Mikroskopie).

Statistics

The fitting procedure for the length-active force data started with a first order polynomial and the power was increased up to a sixth order. One-way analysis of variance (ANOVA) was used to select the lowest order of the polynomials that yielded a significant improvement to the description of the length active force data. Two-way ANOVA for repeated measures (factors: muscle-tendon complex length and location of EDL force measurement) was performed to test for differences between the force in proximal and distal tendons of EDL. If significant, Bonferroni post-hoc tests were executed to identify at which l_{oi} the proximo-distal difference was significant (Neter et al., 1996). A regression analysis was used to describe the data from individual muscles relating active TA+EHL force to the difference between proximally and distally measured EDL active force at proximally determined optimum length. The significance of the slope of the regression line was tested using a t-test. Differences were considered significant at $p<0.05$.

RESULTS

Effects of changing length of the TA+EHL complex distally

Length-force characteristics of the TA+EHL muscle-tendon complex as well as the results of the simultaneously measured forces at proximal and distal tendons of EDL are shown in Fig. 3. Optimal active force (F_{mao}) of the TA+EHL complex was 8.55 ± 1.01 N (mean ± SD) and proximally measured optimal active EDL force was 2.60 ± 0.40 N (mean ± SD) (see also Fig. 5A).

Distal length changes of TA+EHL affected proximal as well as distal EDL active force (Fig. 3B), despite the fact that EDL muscle-tendon complex length was left unchanged. For reference only, this constant length is indicated in Fig. 7A (left arrow). It should be noted that at that particular EDL length passive forces were negligible. ANOVA indicated significant effects for length of TA+EHL muscle-tendon complex as well as for locations of EDL force measurement (proximal-distal), and for interaction between these factors. Distal EDL forces were significantly higher than proximal EDL forces at the lower TA+EHL lengths (Fig. 3B). Furthermore, distal TA+EHL lengthening decreased the proximo-distal difference in active EDL force (from ≈ –18% to 0%) (Fig. 3C). This is brought about by differential effects of increasing TA+EHL length on proximally and distally measured EDL force (indicated by a significant interaction): diminishing distal force but increasing

proximal force significantly. Therefore, EDL proximal and distal forces did attain similar values that were persistent at several higher TA+EHL lengths ($\Delta l_{oi} \approx -4$ through +2 mm). Expressing the difference between proximal and distal EDL active force as a function of distal TA+EHL active force yields a rather linear relationship (Fig. 3C).

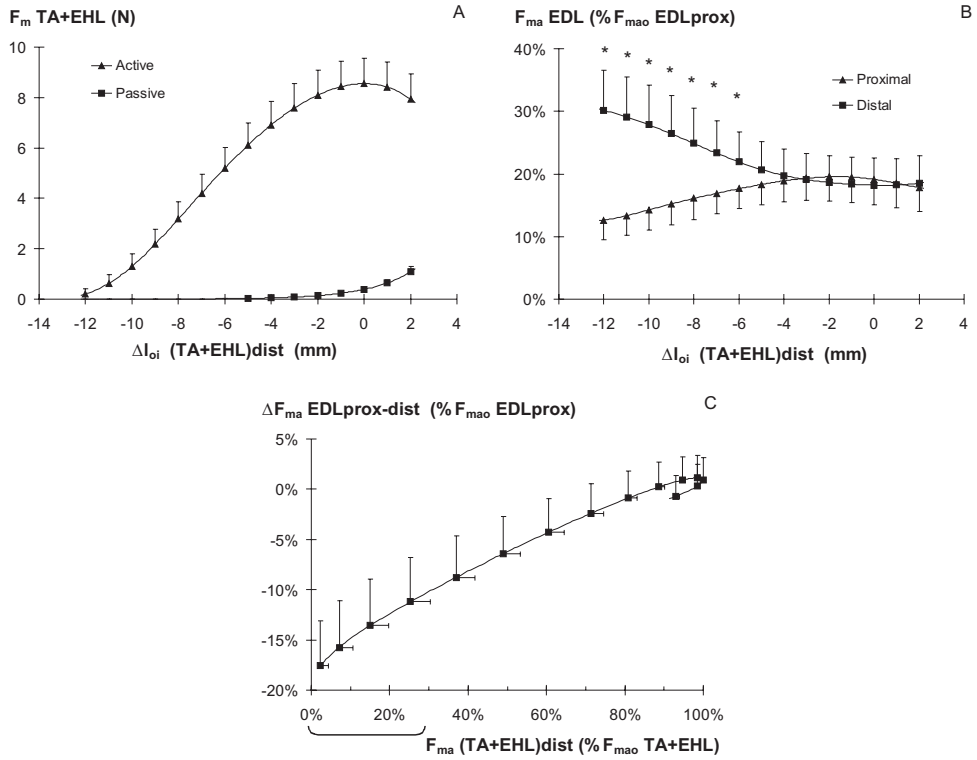


Fig. 3. Length-distal force characteristics of the TA+EHL complex and simultaneously measured EDL forces at constant muscle-tendon complex length. (A) Absolute values of active and passive TA+EHL forces as a function of TA+EHL muscle-tendon complex length (Δl_{oi}). (B) Proximal and distal active force (F_{ma}) of EDL at a constant low length as a function of TA+EHL muscle-tendon complex length. Significant differences between proximally and distally measured EDL forces are indicated (*). (C) The proximo-distal difference of EDL active force (ΔF_{ma}) as a function of active TA+EHL force. The accolade represents the range of forces of TA+EHL that is to be studied also in Fig. 4A. Values are shown as mean either + or - SD (n = 7). Forces in B and ΔF in C are normalized with respect to optimal active force (F_{mao}) of proximal EDL (2.60 ± 0.40 N, mean ± SD). Muscle-tendon complex length is expressed as deviation (Δl_{oi}) from optimum length of the TA+EHL complex. Forces of the TA+EHL complex in C are normalized with respect to optimal active force of the TA+EHL complex (8.55 ± 1.01 N, mean ± SD).

The interaction between properties of the TA+EHL complex and EDL muscle indicates intermuscular myofascial force transmission. The most likely pathway for this transmission is via connections of the intact intra-, inter- and extramuscular connective tissue. Changes in the properties of these connections by increasing TA+EHL length distally decreased the magnitude of net inter- and extramuscular myofascial force transmission (ΔF_{ma} EDLprox–dist). This indicates that connections between the TA+EHL complex and EDL muscle play a role in force transmission from EDL.

Effects of varying TA+EHL force on EDL muscle at optimum length

For a separate group of animals, active force at proximal and distal tendons of EDL at optimum length (as determined proximally) was measured for different active forces of the TA+EHL complex (between 0 and 2.63 N), obtained by lengthening the distal tendons. Proximal optimal active force was variable per individual EDL muscle (between 2.31 and 3.02 N).

A linear relationship between TA+EHL force and normalized proximo-distal difference in EDL force was found for individual muscles (Fig. 4A). Regression analysis yielded a significant correlation ($r^2 = 0.95$) with a significant positive slope. In agreement with Fig. 3C increments of TA+EHL force resulted in higher proximal EDL force compared to distal EDL force. The opposite signs of the proximo-distal difference, at zero TA+EHL force, should be attributed to the experimental conditions of EDL muscle: $\Delta l_{oi} = -6$ mm in Fig. 3 and $\Delta l_{oi} = 0$ mm in Fig. 4A. This is confirmed by the EDL proximal and distal force curves in Fig. 5A that show a crossover point at $\Delta l_{oi} \approx -4$ mm. These observations indicate that lengthening EDL muscle proximally has changed the configuration of its surrounding connective tissue network in such a way that net force is transmitted via inter- and extramuscular myofascial pathways to the proximal tendon of EDL compared to the distal tendons at lower EDL lengths.

The present results show that the magnitude of net inter- and extramuscular myofascial force transmission from EDL varies with changing TA+EHL complex force. Force is transmitted via different pathways in such a way that work performed is minimized. This means that force is transmitted via those pathways that result in the minimum possible deformation. The stress-strain characteristics of biological structures such as tendons and aponeuroses are non-linear and its stiffness is length

dependent (Ettema and Huijing, 1989; Huijing and Ettema, 1988-89; Rack and Westbury, 1984; Scott and Loeb, 1995; Woo et al., 1980). Thus within the compartment the pathway with the stiffest properties will transmit the greatest fraction of force. Enhancement of the difference between force in proximal and distal EDL tendons, therefore, indicates an increasing stiffness of the inter- and extramuscular myofascial pathways relative to intramuscular pathways at higher TA+EHL force.

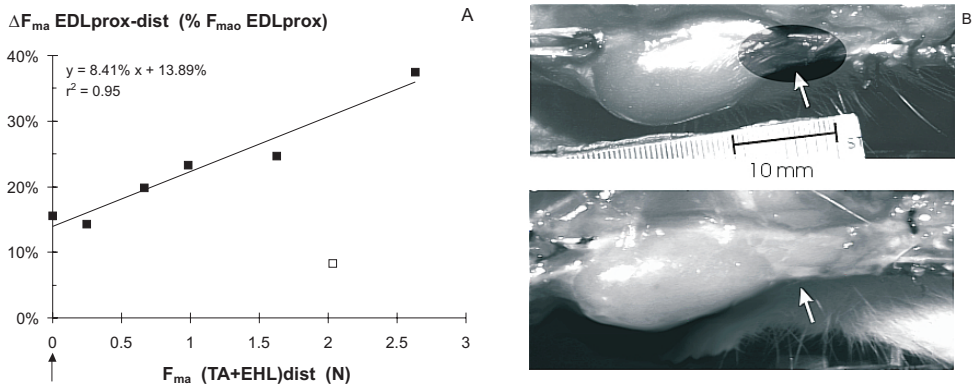


Fig. 4. (A) The relationship between active force of the TA+EHL complex and the proximo-distal EDL active force difference. EDL forces were measured at proximally determined optimum length and normalized for proximal optimal active EDL force (F_{mao} = 2.60 ± 0.24 N, mean ± SD). Individual data of a separate series of experiments (n = 7), the least-squares regression line, its equation and the value of r^2 are shown. One data point (open square), not included in the regression analysis, indicates effects of connective tissue damage. TA+EHL force at which the results of Fig. 5 were obtained is indicated (arrow). (B) Comparison of morphology of an intact and a damaged compartment. The individual compartment yielding the deviant result in the regression analysis (open square in A) due to connective tissue damage in the distal region of the compartment (upper panel) and an intact compartment (lower panel). Within the shaded region of the damaged compartment, irregularities are present on the surface, whereas the surface of the intact compartment is smooth. These irregularities are damaged muscle fibers, as compartmental fascia is missing within this region. Arrows indicate region of interest, bar = 10 mm.

One additional measurement is of special interest because of substantial damage of connective tissue in the distal region of the anterior crural compartment, which was confirmed by images taken during the experiment (Fig. 4B). Therefore, this measurement was excluded from the regression analysis. It is important to note that this particular data point did not fit in the linear relationship between TA+EHL force and the proximo-distal EDL force difference (Fig. 4A, open square). This finding suggests that interference with the compartmental connective tissue network will affect the magnitude of inter- and extramuscular myofascial force transmission.

Effects of proximal lengthening of EDL muscle

Fig. 5 shows results regarding EDL length-force characteristics. Length changes of EDL were obtained by movement of the proximal force transducer to different lengths. This was accomplished with the connection between the TA+EHL complex and its force transducer kept slack. At proximally determined optimum length (l_{oi}), proximal EDL active force was 2.60 ± 0.40 N (mean ± SD) and distal EDL active force was 2.28 ± 0.36 N (mean ± SD).

ANOVA revealed significant effects of EDL length as well as location of EDL force measurement (proximal-distal), and interaction between these factors. The significant interaction indicates that changing EDL length at the proximal tendon has different effects on distally measured EDL force than on proximally measured EDL force. Accordingly, increasing EDL muscle-tendon complex length resulted in a change of active force difference (from ≈ –4% to ≈ +12% of proximal optimal active force) (Fig. 5C).

For almost all lengths tested, significant differences were identified for active forces measured at proximal and distal tendons of EDL (Fig. 5A): proximal force being higher than distal force. Furthermore, the present data suggest a crossover point of EDL proximal and distal force curves (Fig. 5A, at $\Delta l_{oi} \approx -4$ mm): distal force becoming higher than proximal force at lower lengths. This is confirmed by the proximal and distal forces of EDL kept at low constant length ($\Delta l_{oi} = -6$ mm) in Fig. 3B at the lower TA+EHL lengths.

Furthermore, significant proximo-distal differences were found for passive EDL forces, particularly at higher EDL lengths (Fig. 5B). Also for passive forces the magnitude of the difference is length dependent. With proximal lengthening of EDL the difference increased from 0 to ≈ 5% of optimal proximal active force (Fig. 5C).

These results indicate inter- and/or extramuscular myofascial force transmission of passive as well as active force over a wide range of EDL muscle-tendon complex lengths. It should be noted that the distal tendons of TA+EHL were released in such a way that force exerted at that location equaled zero. This implies only that the myotendinous- and intramuscular myofascial pathways to the distal TA+EHL tendons were excluded. It is likely that surrounding connective tissue structures keep the TA+EHL muscle fibers at such length that active force can still be generated.

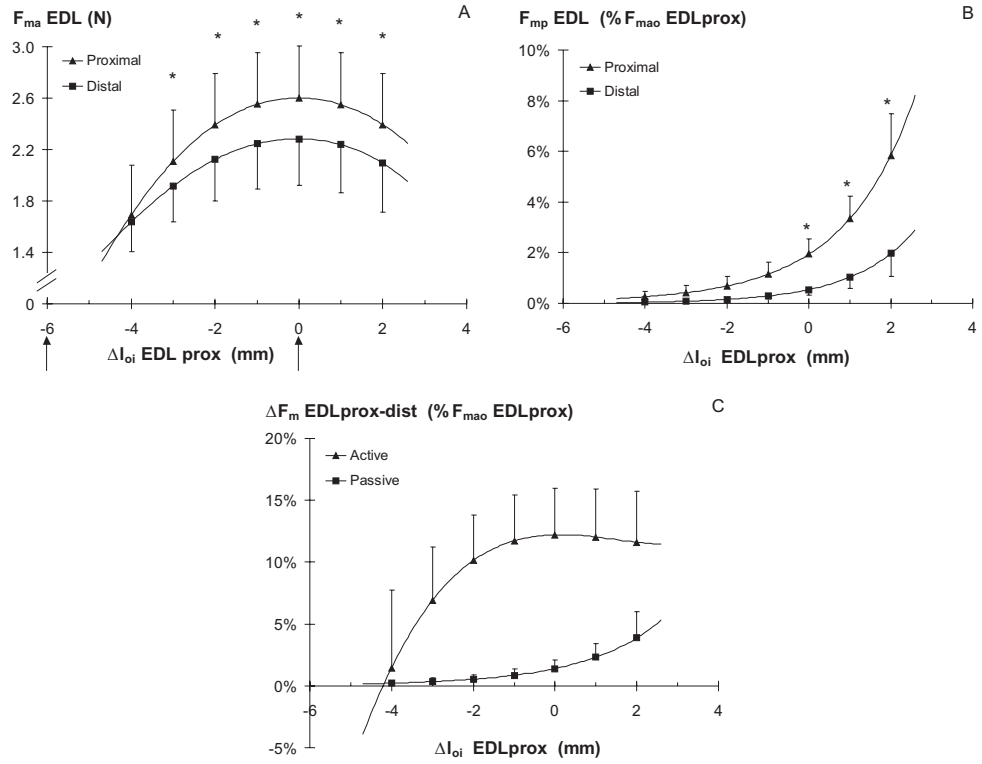


Fig. 5. Length-force characteristics of EDL as measured simultaneously at proximal and distal tendons. (A) Absolute values of active forces (F_{ma}) at proximal and distal tendons of EDL muscle-tendon complex as a function of its length (Δl_{oi}). (B) Passive forces (F_{mp}) at proximal and distal tendons of EDL muscle-tendon complex as a function of its length. (C) The difference in force (ΔF_m) exerted at proximal and distal EDL tendons (active and passive) with proximal EDL length changes. Length changes were brought about by moving the proximal force transducer to different positions, while keeping the TA+EHL complex at such length that no force was exerted at its distal tendons. Values are shown as mean either + or – SD (n = 8). EDL muscle-tendon complex length is expressed as the deviation from optimum length (Δl_{oi}) as determined proximally, and forces in B and C are normalized for proximal optimal active EDL force (Fmao = 2.60 ± 0.40 N, mean ± SD). Significant differences between proximal and distal EDL force are indicated (*). Arrows in A represent the length of EDL at which the results in Fig. 3 (left arrow) and Fig. 4 (right arrow) were obtained.

Intermuscular and extramuscular connections

Images of intra-, inter-, and extramuscular connective tissue within the anterior crural compartment are shown (Fig. 6 and 7). Fig. 6 demonstrates compartmental fascia (connective tissue that encloses the muscles within the compartment) and extramuscular connective tissue that supports the neuro-vascular tract. At a more proximal level this tract for EDL, TA and EHL muscles is also shown in Fig. 7A. In contrast to the experiments, these muscles were isolated from their surrounding

tissue: only the blood supply and innervation was left intact. A downward force was exerted on the distal tendons. The above-described images indicate that EDL, EHL and TA muscles are indirectly connected via extramuscular connective tissue that supports the nerves and blood vessels of the tract.

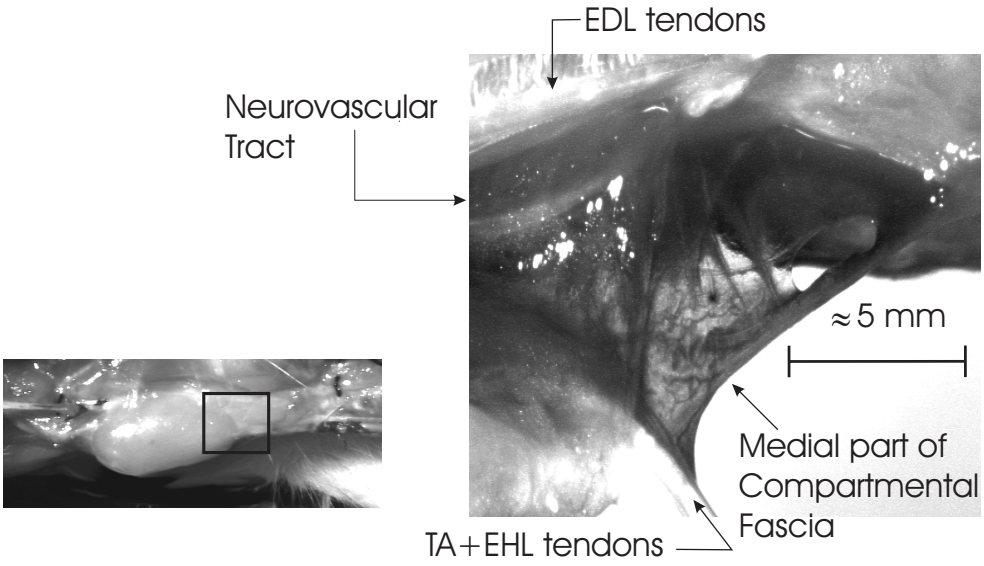


Fig. 6. Extramuscular connective tissue of the anterior crural compartment that supports the neuro-vascular tract and compartmental fascia (latero-distal-ventral view of the lower rat leg). This fascia is connective tissue that encloses the muscles within the compartment. The overview image (left) is shown as a reference for the region in the whole compartment of the detailed image. Bar ≈ 5 mm.

A cross-section of EDL and TA muscles shows the organization of connective tissue within the compartment at the level of the proximal third of the tibia (Fig. 7B). The neuro-vascular tract is continuous with epimysia and thick perimysia of TA and EDL muscles. The intramuscular connective tissue networks of EDL and TA muscles are also connected to each other (intermuscular connections). This indicates that the anterior crural compartment may be considered as one connective tissue network that embeds muscle fibers as well as nerves and blood vessels.

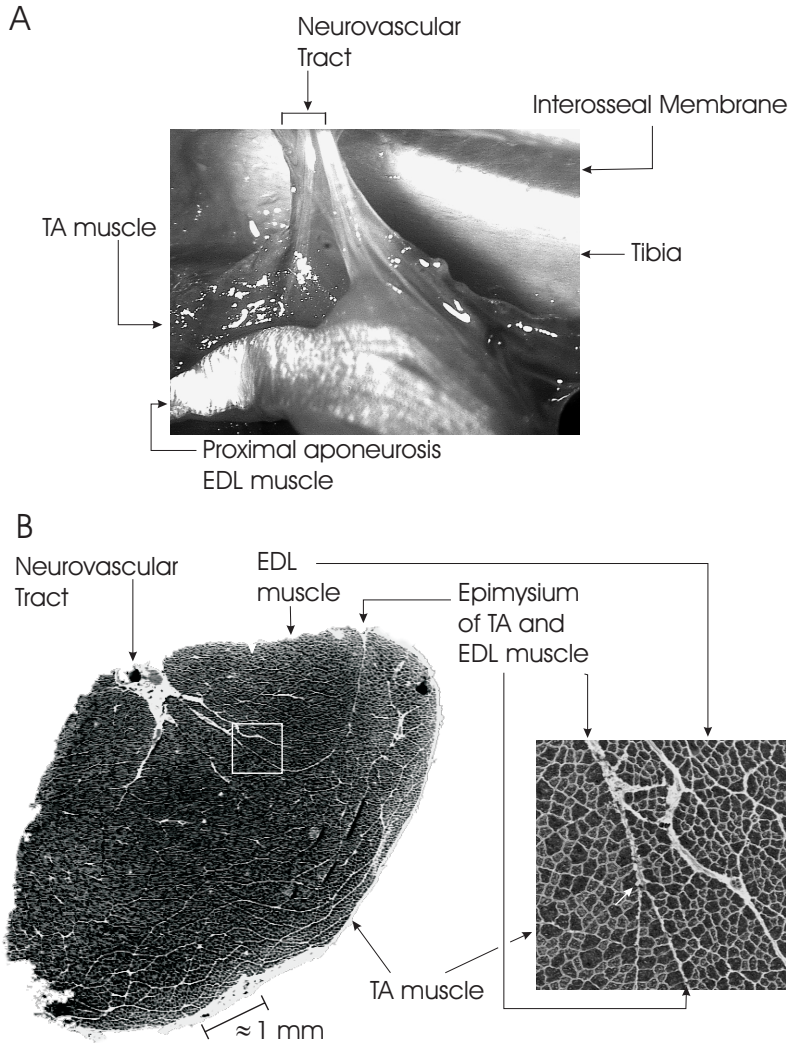


Fig. 7. Images of extramuscular connective tissue and of a cross-section of the anterior crural compartment of the rat. (A) Proximal view of the neuro-vascular tract of EDL, TA and EHL muscles. TA, EHL and EDL muscles are isolated from their surrounding tissue, only the blood supply and innervation was left intact, and a downward force is exerted on the distal tendons (not shown). EDL and TA muscles are indirectly connected via extramuscular connective tissue that also supports the nerves and blood–vessels within the tract. (B) Cross-section of the anterior crural compartment at the proximal third of the tibia showing the organization of connective tissue. The section (thickness = 10 µm) was fixed and stained with a Sirius red solution. The digital images were inverted to create the appearance of a photographic negative. The neuro-vascular tract is continuous with epimysia and thick perimysia of TA and EDL muscles. Note that the ventro-lateral epimysium of TA is thick and that its arrangement indicates that it may help the fascia to resist intracompartmental pressures. The inset indicates the area shown enlarged to the right. The enlarged image illustrates the continuity of the intramuscular connective tissue networks of EDL and TA muscles (intermuscular connections). Bar ≈ 1 mm.

In summary, the difference between proximally and distally measured EDL forces indicates net inter- and/or extramuscular myofascial force transmission from EDL muscle. Proximal length changes of EDL as well as changes in length and force of the TA+EHL complex influence these myofascial pathways. These results indicate that EDL muscle in the lower rat leg is mechanically connected to its surrounding tissues via the intra-, inter- and extramuscular connective tissue network. Length changes of EDL and TA+EHL influence the stiffness of these connections and consequently the relative importance of the inter- and extramuscular myofascial pathways in force transmission from muscle fibers to the bony skeleton.

DISCUSSION

Anatomical investigations revealed intermuscular mechanical connections between human gluteus maximus and latissimus dorsi muscles via the thoracolumbar fascia (Vleeming et al., 1995) and within the forelimb of the rat via intermuscular septa (Van der Wal, 1988). Recent experiments have shown that force generation with fingertips of one, two or three human fingers is accompanied by force production of the other non-intended fingers (e.g. Li et al., 2000; Zatsiorsky et al., 2000). Connections between tendons, extrinsic muscles that produce contraction forces in all four fingers as well as plastic changes within the central nervous system are thought to be responsible for this interdependent action of fingers. If the results of the present study may also be extrapolated to the forearm of humans, interactions at the level of the muscle belly between muscles moving the different fingers may also play a role in this phenomenon (e.g. intermuscular myofascial force transmission between m. flexor digitorum profundus and m. flexor digitorum superficialis). Other evidence, indirectly indicating intermuscular force transmission, is also encountered in the literature (see Huijing, 1999b). It has been found that the intramuscular stimulated rectus femoris muscle still generated a knee extension moment after a transfer surgery, where the muscle was detached from the patella and reattached to a flexor site of the knee (Riewald and Delp, 1997). Gregor et al. (1988) compared *in vivo* and *in situ* treadmill locomotion of the soleus muscle of cats. They observed that, during late stance, higher force and power were generated *in vivo* than at the same shortening velocity by the same muscle, after it had been isolated *in situ*. This is remarkable because the muscle was excited submaximally *in vivo* but maximally *in situ*. *In vivo*, force may have been transmitted from other plantar flexors onto the

soleus muscle. Furthermore, it was found that the cat hamstring muscles produced a torque about the ankle, reportedly via a fascial sheath between hamstrings and the calcaneous (Wicke and Zajac, 1981).

Recently, simultaneously measured proximal and distal active forces of EDL muscle within an intact anterior crural compartment were found not to be identical during a tetanus (Huijing, 1999a, 2000). These observations proved that muscle fiber force is also transmitted out of the muscle via pathways other than the tendons: (1) Via extramuscular connective tissue to other structures (extramuscular myofascial force transmission). (2) Via intermuscular connective tissue to surrounding muscles (intermuscular myofascial force transmission). The difference between proximal and distal EDL force is a measure of the magnitude of net extra- and intermuscular myofascial force transmission. Also in the present study, proximally measured EDL force was found to be unequal to distally measured EDL force in several conditions. The proximo-distal EDL force difference was decreased if the connective tissue of the compartment was damaged. The latter demonstrates the importance of an intact compartmental connective tissue network for force transmission from muscle fibers to bone. In addition, lengthening TA+EHL complex affected proximal as well as distal EDL active force, indicating intermuscular myofascial force transmission.

The maximal proximo-distal difference observed (37%, Fig. 4A), as well as the fact that such differences were measured in several combinations of EDL and TA+EHL muscle-tendon complex lengths, indicate the potential functional importance of these myofascial pathways under normal movement conditions. However, in all protocols, the length of only one muscle-tendon complex was changed while the other muscles were kept at a constant muscle-tendon complex length. If for example the ankle is flexed *in vivo*, lengths of all synergists will be altered simultaneously. However, differences in moment arm will cause relative motions of muscle bellies. It is concluded that the functional relevance of inter- and extramuscular myofascial force transmission still has to be further elucidated, but that the principle is clearly indicated.

Implications for biomechanical modeling

Up to now, it was customary to experiment on "isolated" *in situ* muscles with the extramuscular myofascial pathways almost completely removed and to measure muscle force exclusively at one tendon (Bobbert et al., 1990; Gareis et al., 1992;

Huijing et al., 1998; Jaspers et al., 1999; Rack and Westbury, 1969). For validation, Hill-type models to simulate the behavior of single skeletal muscles have been compared with such experimental results (Jewell and Wilkie, 1958; Van Ingen Schenau et al., 1988; Van Soest et al., 1995; Zajac, 1989). Our present results indicate that *in vivo* muscle properties may be different, as muscles operate within an intact connective tissue environment. Methods to assess the biomechanical properties of intact muscle synergists in rat and mouse have been developed recently (Ashton-Miller et al., 1992; Gorselink et al., 2000; Willems and Stauber, 1999).

Hill-type muscle models are used also to study human limb movements such as jumping (e.g. Bobbert et al., 1986; Hatze, 1981; Hoy et al., 1990; Pandy and Zajac, 1991; Van Soest and Bobbert, 1993). These models consider muscles in functional groups (hamstrings, mm. vasti) as well as individual muscles (m. gastrocnemius, m. soleus, m. rectus femoris). Furthermore, several parameters of the models have been obtained from experiments on isolated muscles or muscle fibers. The present study indicates that neighboring muscles are not functioning fully independently with regard to force transmission. This also suggests that length-force characteristics of "isolated" *in situ* muscles are different from length-force characteristics of the same muscles *in vivo*. Models of human movement should acknowledge these new insights of muscle functioning and where possible incorporate them.

Myofascial force transmission between the TA+EHL complex and EDL muscle

The present study has demonstrated intermuscular myofascial force transmission between EDL muscle and the TA+EHL complex. This is direct force transmission from muscle to muscle, unless the effect on EDL is mediated solely via extramuscular connections. Within the low TA+EHL length range, distally lengthening of TA+EHL resulted in an increase of proximally measured EDL force and a decrease of distally measured EDL force, while EDL was kept at constant low muscle-tendon complex length (Fig. 3B). Also at optimum length of EDL, higher TA+EHL complex forces resulted in higher proximal EDL force compared to distal EDL force (Fig. 4A). These intermuscular effects can be understood on the basis of changes in the configuration of extra- and intermuscular connective tissue of EDL and the TA+EHL complex. Such a change of configuration, yielding results comparable to those of Fig. 3, is illustrated using representations of a physical model (Fig. 8B). This model consists of EDL muscle, the TA+EHL complex as well as

intra-, inter- and extramuscular connective tissue, all modeled as springs. Different lengths of the inter- and extramuscular connections imply a change of the stiffness of these structures. Stiffer pathways will transmit relatively more force than less stiff pathways, because this is the most efficient route of force transmission (minimum of work performed). We acknowledge that this physical model is a very much simplified representation of the *in vivo* condition, but it contributes to the insight of inter- and extramuscular myofascial force transmission within the anterior crural compartment.

The present study shows also that proximal EDL force can be higher (Fig. 4) as well as lower (Fig. 3) than distal EDL force. The sign of the proximo-distal EDL force difference is dependent on the actual length of EDL muscle as varied by proximal length changes (Fig. 5). Representations of the physical model show changes of configuration of inter- and extramuscular connections yielding results comparable to Fig. 5 (Fig. 8C). Keeping the surrounding muscles at constant length, lengthening of EDL muscle within a low length range changes the configuration of its surrounding connective tissue network. This leads to a shift of net inter- and extramuscular myofascial force transmission from the distal to the proximal tendon of EDL. It may seem paradoxical that intermuscular connections between EDL muscle and the TA+EHL complex transmit considerable force if force exerted by the TA+EHL complex at the distal tendons was zero (e.g. Fig. 5). It should be noted, however, that this condition does not imply that the TA+EHL muscle fibers are at such a length that they cannot exert force. These fibers are kept at a considerable length by their surrounding connective tissue. In those conditions at which force in the distal tendons of EDL is higher than in the proximal tendon, force is expected to be transmitted between EDL and the proximal attachments of EHL and TA muscles (represented by the proximal spring) to bone. The proximal spring of the TA+EHL complex in Fig. 8C (gray lines) is extended, which illustrates that force is exerted at the proximal site of TA+EHL.

At high TA+EHL lengths, proximal and distal forces of EDL, which itself was kept at constant low length, were found to be equal (Fig. 3B). Force exerted on both tendons may be equal if: (1) muscle fiber force is only transmitted to its tendons via myotendinous or intramuscular myofascial pathways, as in *in situ* "isolated" muscle. (2) Effects of inter- and/or extramuscular myofascial force transmission cancel each other.

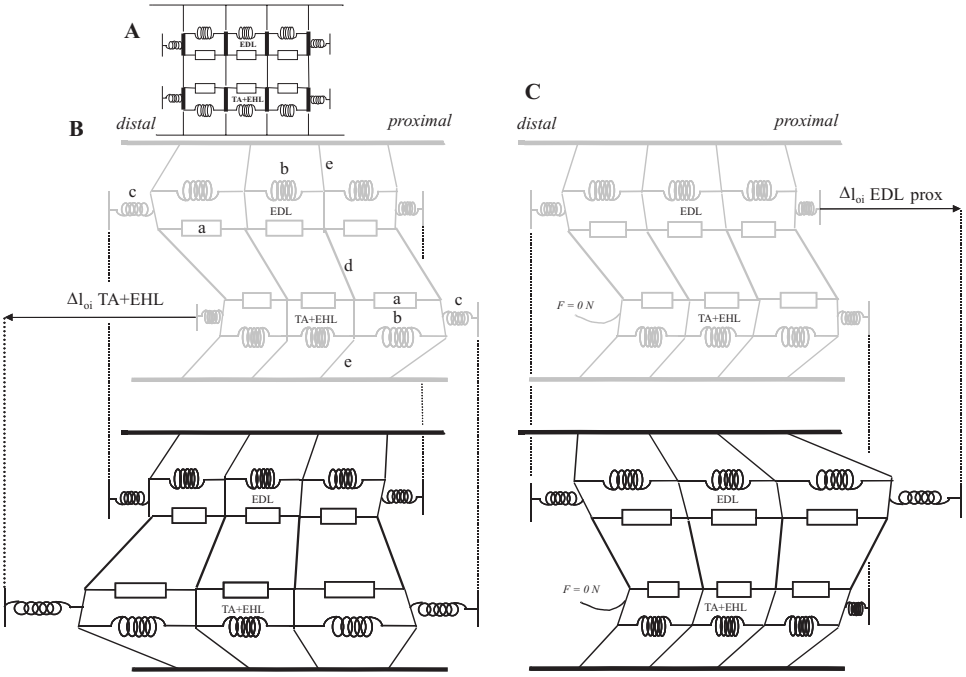


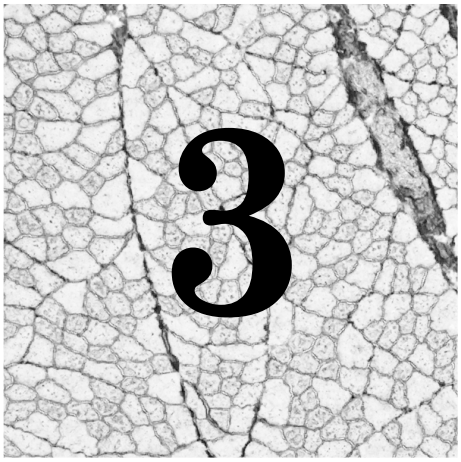
Fig. 8. Representation of a physical model illustrating effects of extra- and intermuscular connections of EDL muscle and the TA+EHL complex. (A) General structure of the model. The muscles are represented as to consist of (a) contractile elements (represented as rectangles), (b) intramuscular connective tissue and (c) proximal and distal tendinous connections to bone (both represented as springs). (d) Inter- and (e) extramuscular connections of EDL and TA+EHL are represented as straight lines. It should be noted that in the physical model all components are springs of different stiffness; in order of stiffness: (c) > (a) >(b) = (d) = (e). The springs are connected to inextensible linking elements (represented as bold lines in A) in such a way that rotation of these elements is possible. (B) Effects of lengthening TA+EHL: Conditions resembling those of the experimental results shown in Fig.3. EDL is kept at constant low length, whereas TA+EHL length is increased distally (compare gray and black representation of the model). In the initial condition (gray lines), the arrangement of the intermuscular connections indicates net intermuscular force transmission, leading to a higher distal EDL force than proximal EDL force. The proximo-distal difference is also evident from different spring lengths at proximal and distal tendinous ends of EDL muscle. Also extramuscular myofascial force transmission from EDL muscle is evident locally, although the arrangement of extramuscular connections indicates that no net force is transmitted if the whole EDL muscle is considered. In the final condition (black lines) the TA+EHL complex is at high length. The arrangement of intermuscular connections and extramuscular connections of EDL now indicates no large net inter- and extramuscular myofascial force transmission. Force exerted at the distal EDL tendon is similar to force exerted at the proximal EDL tendon as indicated by similar spring lengths. This is in agreement with the results shown in Fig. 3B. Note that the inextensible linking components between springs of EDL and TA+EHL are rotated compared to their initial position for TA+EHL at low length (gray lines). (C) Effects of lengthening EDL proximally: Conditions resembling those of the experimental results shown in Fig.5. The distal tendons of TA+EHL were released in such a way that force exerted at that location equals zero. TA+EHL is kept at constant low length, whereas length of EDL is increased proximally (compare gray and black representation of the model). In the initial condition (gray lines), the qualitative effects of the configuration of the model are similar to those of the initial condition of B. In the final condition (black lines), EDL muscle is at high length. The lengths of springs representing proximal and distal tendons of EDL indicate a higher proximal force than

distal force. The length of the spring representing proximal tendinous connection of the TA+EHL complex to bone is decreased compared to the initial condition. The extramuscular connections of EDL indicate net force transmission to and from the proximal end. The configuration of intermuscular connections has also changed. Initially they favored the distal tendinous end of EDL, but after lengthening EDL there is no over all favored direction. This is accompanied by the following changes: (1) a shift of whole TA+EHL muscle belly in proximal direction, (2) rotations of the linking components between springs and (3) a length increase of extramuscular connections of the TA+EHL complex.

The physical muscle model illustrates that inter- and/or extramuscular connections can be arranged such that they exert forces on the muscles in opposite directions (Fig. 8B). Furthermore, effects of extramuscular connections may counter effects of intermuscular connections. The latter point may explain the equality of proximal and distal EDL forces at several high TA+EHL lengths (Fig. 3B). It is shown that at high TA+EHL complex length (Fig. 8B, black lines), the intermuscular connections and the extramuscular connections of EDL distally have an opposite orientation. This indicates that these connections will exert forces at the stiff component attached to the distal EDL tendon in opposite directions. It is likely that extramuscular effects changed as much as intermuscular effects with each increment of TA+EHL complex length from Δl_{oi} = –4 mm. Consequently proximal and distal EDL forces remained fairly constant.

In conclusion, length and force changes of the TA+EHL complex influence force transmission from EDL muscle. Length–force characteristics of EDL are, therefore, not only dependent on the properties of muscle fibers and connective tissue within the muscle but also on the properties of muscles and connective tissue structures in its direct environment. It is likely that this intermuscular interaction is mediated by collagen structures within the intra-, inter- and extramuscular connective tissues. Further experiments are necessary to elucidate the role of these connective tissue networks in force transmission from muscle fibers to the bony skeleton. The present findings emphasize that neighboring muscles within the anterior crural compartment are one functional unit consisting of a connective tissue tunnel-like network and muscle fibers that are able to shorten. Images of the cross-section of EDL and TA muscles (Fig. 7B) reinforce this view of compartmental organization. Despite the fact that each muscle is innervated independently, it is concluded that the concept of morphologically defined muscles acting as independent components is not appropriate to describe force transmission from muscle to bone.

Muscle force is determined also by muscle relative position: isolated effects



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Journal of Biomechanics, **In press**

ABSTRACT

Effects on force of changes of the position of extensor digitorum longus muscle (EDL) relative to surrounding tissues were investigated in rat. Connective tissue at the muscle bellies of tibialis anterior (TA), extensor hallucis longus (EHL) and EDL was left intact, to allow myofascial force transmission. The position of EDL muscle was altered, without changing EDL muscle-tendon complex length, and force exerted at proximal and distal tendons of EDL as well as summed force exerted at the distal tendons of TA and EHL muscles (TA+EHL) were measured. Proximal and distal EDL forces as well as distal TA+EHL force changed significantly on repositioning EDL muscle.

These muscle position-force characteristics were assessed at two EDL lengths and two TA+EHL lengths. It was shown that changes of muscle force with length changes of a muscle is the result of the length changes per se, as well as of changes of relative position of parts of the muscle. It is concluded that in addition to length, muscle position relative to its surroundings co-determines isometric muscle force.

INTRODUCTION

It is commonly known that skeletal muscle force is dependent on the degree of activation, muscle-tendon complex length, shortening velocity, fatigue, and contraction history. However these muscle properties have been assessed predominantly using dissected muscles (e.g. Burke et al., 1976; Close, 1964; De Haan et al., 1989; Ettema et al., 1990; Meijer et al., 1998; Rack and Westbury, 1969), assuming implicitly that all muscle force is transmitted via its tendons to bone. In such a case, force at the proximal tendon should be equal to force at the distal tendon of that muscle. Therefore, in such experiments muscle force is measured exclusively at one tendon. Recent experiments simultaneously measuring isometric force exerted at both proximal and distal tendons of extensor digitorum longus muscle (EDL), that was not dissected free from its surrounding tissues, proved that force is transmitted also out of muscle via other pathways than its tendons (Huijing and Baan, 2001a; Maas et al., 2001). Force may be transmitted via intermuscular connective tissue to adjacent muscles (intermuscular myofascial force transmission). Force may be transmitted also via extramuscular connective tissue to bone (extramuscular myofascial force transmission). Therefore, connective tissues embedding muscles play an important role for force transmission from muscle fibers. Severing this connective tissue network caused major changes in length-force characteristics of EDL muscle as well as in inter- and extramuscular myofascial force transmission (Huijing and Baan, 2001a, 2001b). Furthermore, dissection of rat flexor carpi ulnaris muscle to simulate surgical procedures for tendon transpositions in human caused substantial changes of its length-force characteristics (Smeulders et al., 2002).

In vivo, joint movements involve simultaneous but opposite length changes of synergistic and antagonistic muscles. The magnitude of length changes as a function of joint angle depends on its moment arm, defined as the shortest distance between the line of action and the joint axes of rotation. Due to the fact that differences in moment arm between synergists exist, joint movements will also cause changes in relative position of different adjacent muscle bellies. Another major cause for such a change of muscle relative position is the fact that some muscles span only one joint (mono-articular muscles) and other muscles span more than one joint (bi or poly-articular muscles). Based on previous results regarding inter- and extramuscular myofascial force transmission, it seems likely that these relative motions of different muscle bellies will affect force transmission of the different muscles involved.

However, in those studies effects of changing length were always combined with effects of changing relative position, but were ascribed in terms of effects of length.

The aim of the present study was to investigate effects of changes of relative position of muscle within the anterior crural compartment. This compartment envelops the poly-articular EDL, tibialis anterior (TA) and extensor hallucis longus (EHL) muscles. The position of EDL muscle relative to surrounding muscles and other connective tissues was altered without changing its muscle-tendon complex length, and isometric forces exerted at proximal and distal tendons of EDL muscle as well as at distal tendons of TA and EHL muscles were measured. EDL muscle position–force characteristics were assessed at several constant lengths of EDL muscle and the TA+EHL complex. We tested the null hypothesis that proximally and distally measured EDL forces are affected solely by length changes and not by changes of the position of EDL muscle.

MATERIALS AND METHODS

Surgical and experimental procedures were in strict agreement with the guidelines and regulations concerning animal welfare and experimentation set forth by Dutch law, and approved by the Committee on Ethics of Animal Experimentation at the Vrije Universiteit.

Surgical procedures

Male Wistar rats (n = 7, body mass = 303.9 ± 24.7 g) were anaesthetized using intraperitoneally injected urethane (1.2 ml/ 100 g body mass 12.5 % urethane solution, extra doses were given if necessary: maximally 1.5 ml). During surgery and data collection, the animals were placed on a heated water pad of approximately 37°C, to prevent hypothermia. Ambient temperature (22 ± 0.5 °C) and air humidity (80 ± 2 %) were kept constant by a computer controlled air-conditioning system (Holland Heating). Muscle and tendon tissue was further prevented from dehydration by regularly irrigating the tissue with isotonic saline.

The anterior crural compartment, which envelops tibialis anterior (TA), extensor digitorum longus (EDL) and extensor hallucis longus (EHL) muscles, was exposed by removing the skin and most of the biceps femoris muscle from the left hind limb. Connective tissue at the muscle bellies of TA, EHL and EDL was left intact.

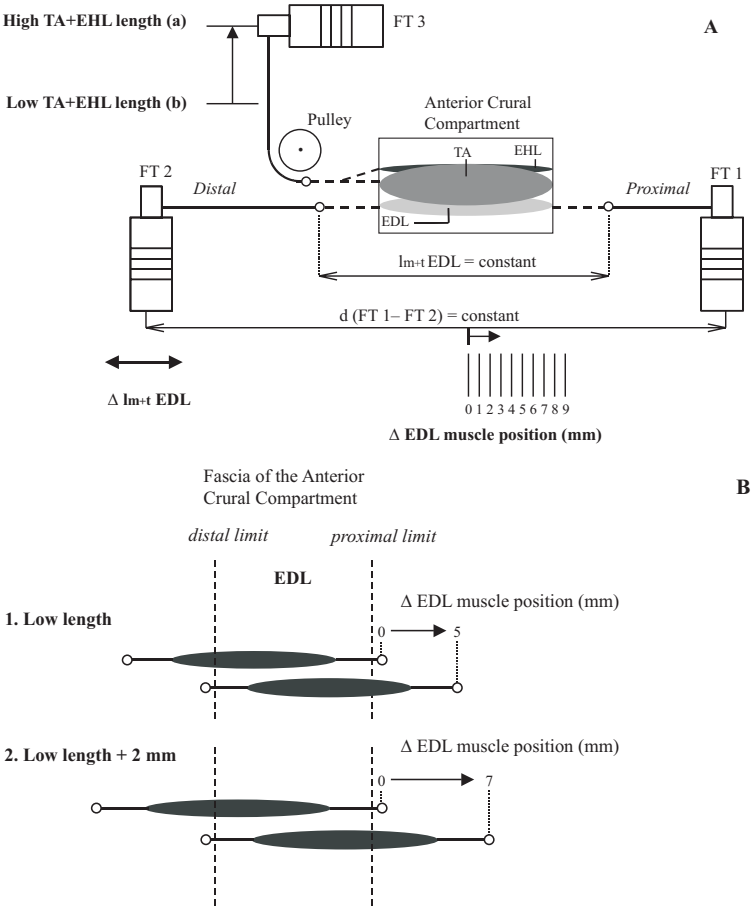


Fig. 1. A schematic view of the experimental set-up and experimental protocol. (A) Experimental set-up, seen from above. FT 1 indicates the force transducer connected to the proximal tendon of EDL muscle, FT 2 indicates the force transducer connected to the tied distal tendons of EDL, and FT 3 indicates the force transducer connected to the tied distal tendons of TA and EHL muscles. A pulley guided the Kevlar thread from TA+EHL to FT 3. The origin of TA and EHL muscles was not manipulated. Dashed lines indicate tendons and solid lines indicate Kevlar thread that was used to connect the muscles to force transducers. Each relative position of EDL muscle (Δ EDL muscle position) was obtained by repositioning FT 1 as well as FT 2, in a random order, one mm in proximal direction. Δ EDL muscle position = 0 refers to the most distal position of EDL muscle. The distance between FT 1 and FT 2, i.e. d(FT1-FT2) and, thus also the muscle-tendon complex length of EDL muscle (l_{m+t} EDL) remained constant. The TA+EHL lengths at which these EDL muscle positions were measured are also indicated (a: high = 2 mm over optimum length; b: low = 9 mm below optimum length). The double arrow below FT 2 indicates that length changes of EDL muscle were obtained by changing the distal force transducer to different positions. (B) Experimental protocol. EDL muscle relative position–force characteristics were assessed for two lengths of EDL muscle (1: low length = optimum length–7 mm; 2: low length+2 mm = optimum length–5 mm), each tested at two lengths (illustrated in A) of the TA+EHL complex (not shown). The most distal and the most proximal EDL muscle relative positions are shown. Note that at low EDL length 6 positions of EDL muscle and at low length + 2 mm 8 EDL muscle positions could be imposed.

However, the transverse crural ligament and the crural cruciate ligament (retinaculae near the ankle joint) were severed and a limited distal fasciotomy was performed to reach the distal tendons of EDL, TA and EHL. With knee and ankle joint at 90° (referred to as reference position) the four distal EDL tendons as well as the distal tendons of TA and EHL muscles were tied together, respectively. The latter complex will be referred to as the TA+EHL complex. Proximally, the femoral compartment had to be opened (1) to detach the proximal tendon of EDL from the femur, and (2) to secure the femur (at a knee angle of 90°) with a metal clamp when the rat was in the experimental apparatus. The tendons of EDL muscle and the TA+EHL complex were cut and connected to force transducers (Hottinger Baldwin, maximal output error < 0.1 %, compliance of 0.0048 mm/N) with Kevlar thread (diameter 0.5 mm, 4% elongation at a break load of 800 N). The foot was attached to a plastic plate with tie wraps. To create a resistance free passage for this thread between tendon and force transducer, this plate was positioned in maximal plantar flexion of the ankle and approximately 40° pronation of the foot. For TA+EHL force measurements, the Kevlar thread was connected to the force transducer via a pulley. Both EDL ends were connected directly to the force transducers, which were positioned in the line of pull (Fig. 1). Experimental data indicating that differences in force between the two force transducers greater than 0.7 % cannot be ascribed to this measurement system have been reported previously (Huijing and Baan, 2001a; Maas et al., 2001).

Within the femoral compartment, the sciatic nerve branches into the tibial nerve, the sureal branch and the common peroneal nerve. The common peroneal nerve enters the anterior crural compartment from the peroneal compartment through a fenestration within the anterior intermuscular septum (Huijing and Baan, 2001b). Branches of the intact common peroneal nerve innervate EDL muscle as well as the muscles in the peroneal compartment. The tibial nerve, the sureal branch as well as all other more proximal branches of the sciatic nerve were cut. The sciatic nerve, with only the common peroneal nerve branch left intact, was dissected, cut as proximally as possible and placed in a pair of silver electrodes.

Experimental conditions

The sciatic nerve was stimulated supramaximally using electrodes connected to a constant current source (3 mA, pulse width 100 μs). Branches of the intact common

peroneal nerve innervate EDL, TA and EHL muscles and stimulation will therefore activate all three muscles simultaneously. It should be noted that this nerve also innervates the muscles in the peroneal compartment.

For each condition, two twitches were evoked followed by a tetanic contraction after 300 ms (pulse train 400 ms, frequency 100 Hz). After each tetanic contraction the muscles were allowed to recover near active slack length for 2 minutes. Isometric force was measured just before and during the tetanic contraction of the muscles. Simultaneously, images of the anterior crural compartment muscles in passive and active state were recorded using a digital camera (DVC, JAI CV-M10, shutter speed 1/50 s). Stimulation of the nerve, A/D conversion (12-bit A/D converter, sampling frequency 1000 Hz, resolution of force 0.01 N), and photography were time-controlled by a computer. Movements of the different force transducers to bring the muscles at the desired length and position, were performed not simultaneously but in a random order.

Optimum length of EDL muscle and the TA+EHL complex

Optimum length (l_o) and optimal force of EDL muscle were assessed by lengthening of the proximal tendon. This will be referred to as proximally determined optimum length. The tied four distal tendons of EDL muscle were kept at reference position – 2 mm. The TA+EHL complex was kept at such a length that no force was exerted at its tied distal tendons.

Optimum length and optimal force of the TA+EHL complex were assessed by lengthening of the tied distal tendons (referred to as distally determined optimum length). The tied four distal tendons of EDL muscle were kept at reference position – 2 mm and the proximal tendon was kept at optimum length –7 mm. This low EDL length corresponds to a force of approximately 20% of optimal active EDL force exerted proximally.

EDL muscle relative position – force characteristics

Isometric force exerted at proximal and distal tendons of EDL as well as at the tied distal tendons of the TA+EHL complex was measured at various positions of EDL muscle relative to its surroundings. While EDL muscle as well as the TA+EHL complex were kept at constant muscle-tendon complex length, the position of only EDL muscle was changed (Δ EDL muscle position), from a distal one to a proximal

one, in steps of 1 mm. This is done by moving both proximal and distal tendons 1 mm in proximal direction (Fig. 1). The range of EDL muscle relative positions that could be imposed is dependent on the length of EDL muscle (Fig. 1B). The maximum EDL tendon positions were limited by the position at which the knot of the Kevlar thread on the tendon was just prevented from touching the compartmental fascia on activation.

EDL muscle relative position–force characteristics were assessed at several lengths of EDL muscle (7 and 5 mm below proximally determined optimum length) and the TA+EHL complex (9 mm below and 2 mm over distally determined optimum length). Length changes of EDL muscle were obtained by movement of the distal force transducer to different positions, while the position of the proximal force transducer was unchanged (Fig. 1B). Δ EDL muscle position = 0 mm refers to the most distal EDL muscle relative position of the experiment, which implies that the proximal tendon end is at its most distal position. Length changes of the TA+EHL complex were obtained by movement of the distal force transducer to different positions. EDL muscle relative position–force characteristics were assessed in the following order (Fig. 1):

1. low EDL length = lo–7 mm:	(a) low TA+EHL length = lo–9 mm,
	(b) high TA+EHL length = lo+2 mm
2. low EDL length + 2 mm = lo–5 mm:	(a) low TA+EHL length = lo–9 mm
	(b) high TA+EHL length = lo+2 mm

Treatment of data and statistics

Active muscle force (F_{ma}) was estimated by subtracting initial passive force (F_{mp}) from total force (F_m) during isometric contraction. For all data mean ± standard error of the mean (SE) were calculated.

To test for the effects of EDL muscle relative position on proximal and distal EDL forces as well as on distal TA+EHL force one-way ANOVA's for repeated measures (factor: EDL muscle relative position) were performed (Neter et al., 1996). To test for effects of lengthening EDL muscle and the TA+EHL complex two-way ANOVAs for repeated measures (factors: EDL muscle relative position and muscle-tendon complex length) were performed. P values < 0.05 were considered statistically significant.

RESULTS

EDL muscle relative position-force characteristics were assessed for different length conditions of EDL muscle (i.e. of EDL muscle kept at low length or low length + 2 mm) and the TA+EHL complex (i.e. the TA+EHL complex kept at low or high length, i.e. low length + 11 mm).

Effects of moving EDL muscle in proximal direction at constant muscle-tendon complex length

Effects of relative position of EDL muscle on proximally and distally measured EDL forces as well as on distally measured TA+EHL force are shown in Fig. 2. Note that force is expressed as a function of EDL muscle relative position rather than muscle length, as EDL muscle-tendon complex length was kept constant (i.e. 5 mm below proximally determined optimum length = low length + 2 mm). The TA+EHL complex was also kept at constant length (i.e. 9 mm below distally determined optimum length = low length).

ANOVA indicated significant effects of EDL muscle relative position for proximal and distal EDL passive and active forces (Fig. 2A). Passive EDL force exerted at the proximal tendon was zero at the more distal EDL muscle positions and increased exponentially with EDL muscle moving in proximal direction (i.e. from Δ EDL muscle position 0 to 7 mm). A similar pattern, but in inverse direction, was observed for distally exerted EDL passive force. The most likely pathway for these, muscle-tendon complex length independent, effects of EDL muscle relative position on EDL passive forces are the inter- and/or extramuscular connective tissues that are linked with the intramuscular connective tissue of EDL muscle.

Changing the relative position of EDL muscle from distal to more proximal locations, decreased distal EDL active force and increased proximal EDL active force significantly. Consequently, the difference between EDL proximal and distal active forces, a direct measure of the magnitude of net inter- and extramuscular myofascial force transmission, changed from –0.87 ± 0.26 N to 0.78 ± 0.14 N (i.e. –32.7 ± 9.5 % to 29.1 ± 4.1 % of proximally determined optimal active force). These observations indicate that positioning EDL muscle more proximally changed the configuration of inter- and extramuscular connective tissue. At more distal relative positions, net force is transmitted via inter- and extramuscular myofascial pathways to the distal tendon of EDL muscle (i.e. $F_{EDLprox} < F_{EDLdist}$). In contrast, at more

proximal EDL muscle relative positions such transmission is to the proximal tendon (i.e. $F_{EDLprox} > F_{EDLdist}$). Such a change of configuration, yielding results comparable to those of Fig. 2A, is illustrated schematically (Fig. 3B).

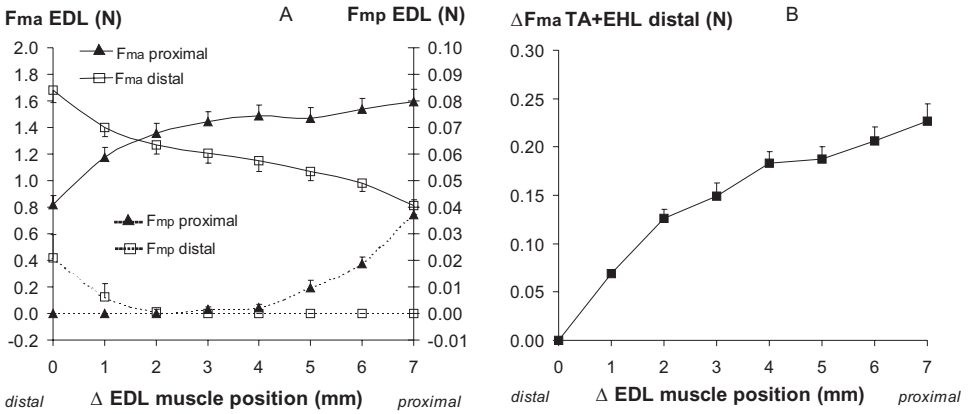


Fig. 2. Isometric forces of EDL muscle and the TA+EHL complex, kept at constant length, with varying EDL muscle relative positions. (A) Proximally and distally measured active (F_{ma}) and passive (F_{mp}) forces of EDL muscle kept at constant length (low length + 2 mm) as a function of EDL muscle relative position (Δ EDL muscle position). (B) Distally measured forces of the TA+EHL complex, kept at constant low length, as a function of EDL muscle relative position. Note that TA+EHL force is expressed as the deviation (ΔF_{ma}) from the value (0.36 ± 0.19 N) measured at the most distal position of EDL muscle (Δ EDL muscle position = 0 mm). EDL muscle relative position is expressed as the deviation from the most distal position. Low EDL length corresponds to 7 mm below proximally determined optimum length. Low TA+EHL length corresponds to 9 mm below distally determined optimum length. Note that in A different y-axes with different scaling factors are shown for active (left axis) and passive forces (right axis). Note also that the x-axis does not cross the y-axes at zero. Values are shown as mean ± SE (n = 7).

Active force of the TA+EHL complex, kept at constant low length, increased significantly if only EDL muscle was positioned more proximally (Fig. 2B). As the TA+EHL complex was kept at low length, passive force exerted by this complex remained negligible. Note that TA+EHL force is expressed as the deviation from the value (0.36 ± 0.19 N) measured at the most distal position of EDL muscle (i.e. Δ EDL muscle position = 0 mm). These results indicate mechanical interaction between EDL, TA and EHL muscles.

These results show that the relative position of whole EDL muscle-tendon complex with regard to TA and EHL muscles and other connective tissue structures within the anterior crural compartment is a major determinant of passive and active EDL force as well as of active TA+EHL force. These muscle length-independent

effects indicate mechanical interactions between EDL muscle and its surroundings, via inter- and/or extramuscular connective tissue (i.e. inter- and extramuscular myofascial force transmission). Thus, the view that muscle-tendon complex length is the only determinant of passive and active isometric muscle force should be discarded for muscles operating within an intact compartment.

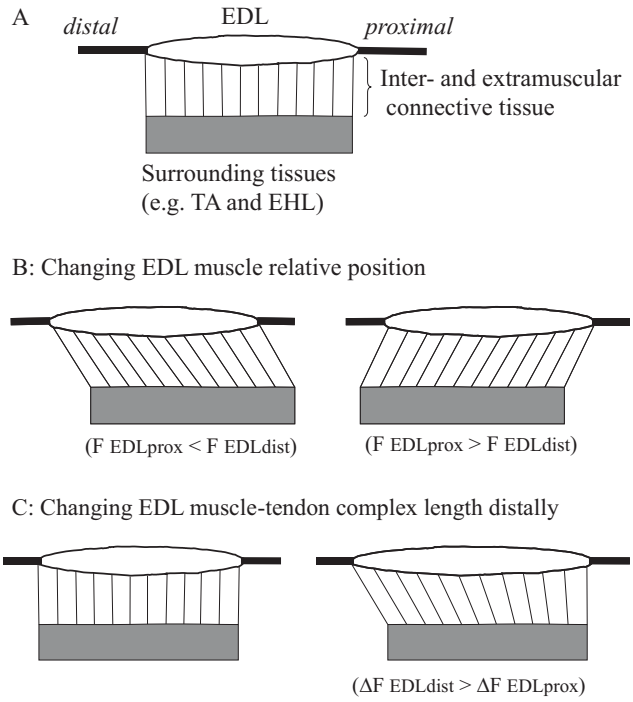


Fig. 3. Schematic drawings to illustrate configuration changes of inter- and extramuscular connective tissue. (A) General structure. EDL muscle, with proximal and distal tendons, is connected to surrounding tissues within the anterior crural compartment (represented as a rectangle), via inter- and extramuscular connective tissue (represented as straight connector lines). (B) Effects of moving EDL muscle from distal (left drawing) to proximal (right drawing): conditions resembling those of the experimental results shown in Fig. 2. The configuration of inter- and extramuscular connective tissue changed in such a way that net force is transmitted via inter- and extramuscular myofascial pathways to the proximal tendon ($F_{EDLprox} > F_{EDLdist}$) of EDL muscle compared to the distal tendons ($F_{EDLprox} < F_{EDLdist}$) at more distal EDL muscle relative positions. (C) Effects of lengthening EDL muscle distally: conditions resembling those of the experimental results shown in Fig. 4. It is illustrated that lengthening EDL muscle distally affects not solely EDL length but involves also a position change of distal parts of EDL muscle. Consequently, the increase of distal EDL active force was significantly higher than the increase of proximal EDL active force ($\Delta F_{EDLdist} > \Delta F_{EDLprox}$).

Length effects of EDL muscle on EDL forces are mediated partially by changes of relative position

A major determinant of isometric force exerted by a muscle is certainly the length of its muscle fibers. However, possible effects of muscle relative position should be considered as well. Effects of EDL muscle relative position on EDL proximal and distal active forces are shown in Fig. 4 for two EDL lengths (i.e. low length and low length + 2 mm). Length changes of EDL muscle were obtained by repositioning of the distal force transducer to different positions, while the position of the proximal force transducer was unchanged (illustrated schematically in Fig. 1B). Thus, a comparison between EDL lengths at each Δ EDL muscle position yields a comparison at an identical position of the proximal tendon and most proximal part of the muscle belly but at a different position of the distal tendons and distal parts of EDL muscle bellies. The TA+EHL complex was kept at constant low length (i.e. l_o–9 mm).

EDL muscle relative position-active force characteristics, as assessed at proximal and distal tendons, are shown for two EDL lengths (Fig. 4AB). Lengthening EDL muscle distally increased proximal (Fig. 4A) and distal (Fig. 4B) EDL active forces significantly at all EDL muscle relative positions. If this force increment was merely an effect of increased length, it would have been equal for EDL proximal and distal active force. However, at each EDL muscle relative position, the increase in distally measured EDL force is significantly higher than the increase in proximally measured EDL force. For the most distal EDL muscle relative position (i.e. Δ EDL muscle position = 0 mm), this discrepancy is illustrated in Fig. 4C. The higher increment of distal EDL force compared to proximal EDL force on lengthening EDL muscle distally is explained by additional force transmission to the distal tendon via inter- and/or extramuscular myofascial pathways. These results indicate that distal lengthening of EDL muscle also involves a change of relative position of distal parts of EDL muscle. The effect of such a change of relative position is mediated by changes of the configuration of the inter- and extramuscular connected tissues (illustrated schematically in Fig. 3C).

It is concluded that if the length of EDL muscle is increased, the effects on proximal and distal EDL forces are mediated by changes of EDL muscle relative position as well as length per se. As the increment of proximal EDL force is predominantly caused by the change of EDL muscle length, at Δ EDL muscle

position = 0 mm the relative contribution of relative position change of distal parts of EDL muscle in the increase of distal EDL force must be approximately 50 %.

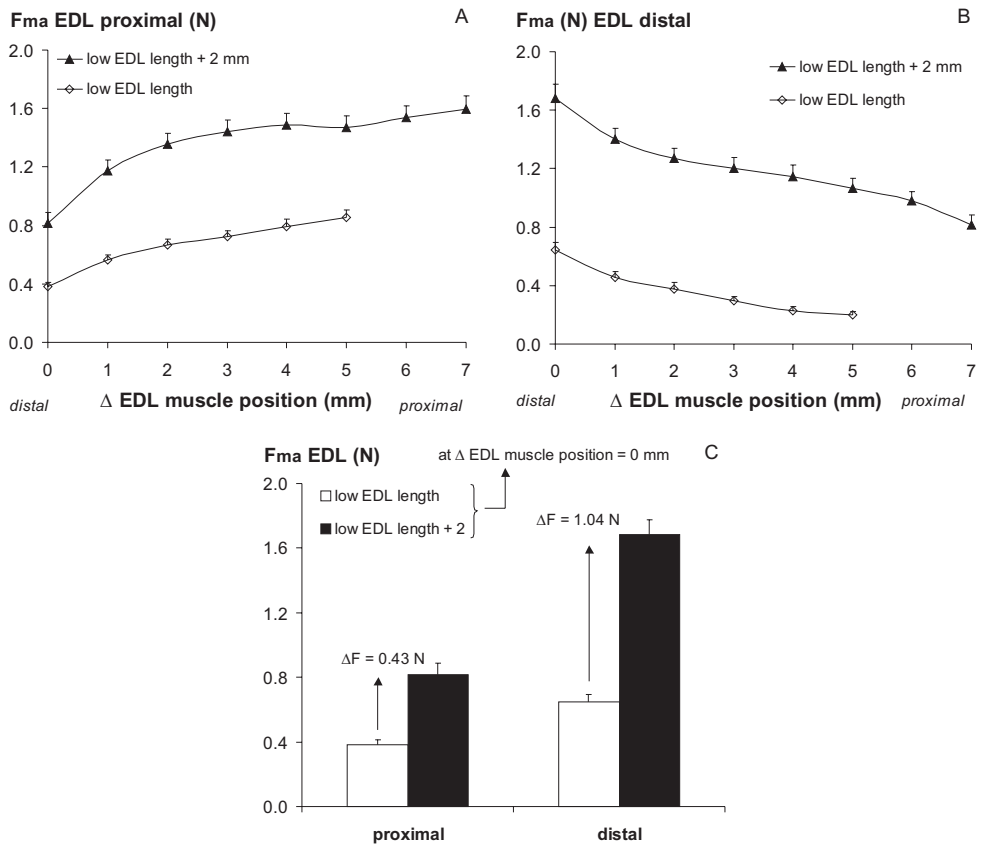


Fig. 4. Proximally and distally Δ EDL muscle position-active force curves at different lengths of EDL muscle (i.e. at low length and at low length + 2 mm). The TA+EHL complex was kept at constant low length (i.e. 9 mm below distally determined optimum length). (A) Proximal EDL active forces as a function of EDL muscle relative position (Δ EDL muscle position). (B) Distal EDL active forces as a function of EDL muscle relative position (Δ EDL muscle position). (C) Proximal and distal active forces of EDL muscle kept at low length and of EDL muscle kept at low length + 2 mm for the most distal EDL muscle relative position (Δ EDL muscle position = 0 mm). The effect of distally lengthening EDL muscle on proximal and distal forces is indicated (ΔF). EDL muscle relative position is expressed as the deviation from the most distal position. Low EDL length corresponds to 7 mm below proximally determined optimum length. Due to physical constraints, the range of EDL muscle relative positions was 5 mm at low length and 7 mm at low length + 2 mm. The F_{ma} represents active force. Values are shown as mean ± SE (n = 7).

Length effects of the TA+EHL complex on EDL forces

Fig. 5 compares proximally and distally measured EDL muscle relative position-force curves at constant low and high length (i.e. low length + 11 mm) of the TA+EHL complex. The TA+EHL length increase was obtained by repositioning of the distal tendons. EDL muscle-tendon complex length was kept constant (i.e. low length + 2 mm).

ANOVA indicated significant effects of TA+EHL length on proximal and distal EDL passive and active forces. Distal TA+EHL lengthening increased proximally measured EDL passive force significantly at EDL muscle relative position 6 and 7 mm (Fig. 5A). Distal EDL passive force (Fig. 5B) increased also (at position 0 and 1 mm), but this increase is small if compared to the increase of proximal EDL passive force. If passive force is exerted at one tendon of EDL muscle exclusively, this indicates myofascial force transmission between EDL muscle and its surrounding structures. Therefore, it is concluded that mechanical interactions between the TA+EHL complex and EDL muscle affect the amount of passive force that is transmitted via inter- and/or extramuscular myofascial pathways.

Effects of lengthening the TA+EHL complex distally on proximal and distal EDL active forces varied with EDL muscle relative position. For the most distal EDL muscle relative position (i.e. Δ EDL muscle position = 0 mm), changing TA+EHL length caused active force, exerted at the proximal tendon of EDL muscle, to increase significantly (Fig. 5A). In contrast, at more proximal EDL muscle relative positions higher TA+EHL lengths decreased proximal EDL active force.

Note that the pattern of the change of distal EDL active force as a function of EDL muscle relative position was different on TA+EHL lengthening: it never reversed sign (Fig. 5B). Distal EDL active force decreased significantly at some muscle positions (i.e. Δ EDL muscle position = 0, 1, 6, and 7 mm), but was unchanged at other positions (i.e. Δ EDL muscle position = 2 to 5 mm).

The interaction between the length of the TA+EHL complex and proximal as well as distal forces of EDL muscle shows that isometric muscle force is dependent also on the length of adjacent muscles. This must be mediated by connective tissue linking EDL muscle and the TA+EHL complex. The differential effects on proximal and distal EDL forces indicate that the length increment of the TA+EHL complex has also changed the relative position of EDL muscle with respect to these muscles.

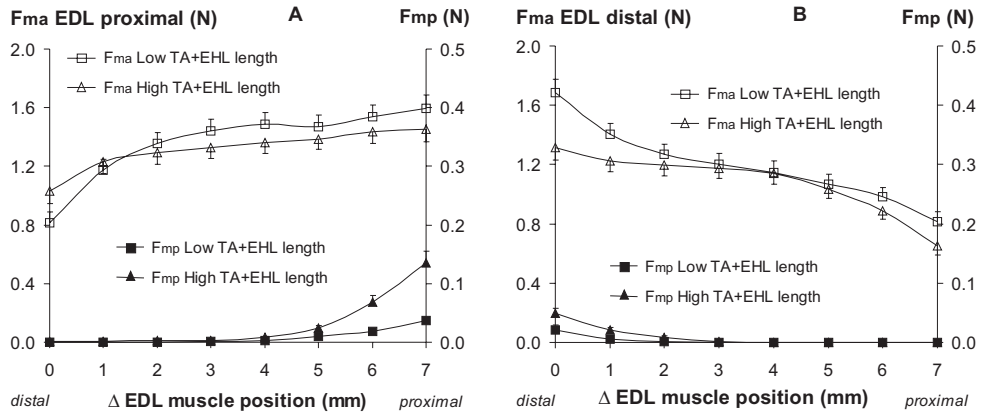


Fig. 5. Effects of distally lengthening the TA+EHL complex on proximal and distal Δ EDL muscle position–active and passive force curves of EDL muscle kept at constant low length + 2 mm. (A) Proximal active and passive forces of EDL muscle kept at low length + 2 mm as a function of Δ EDL muscle position at low and high length of the TA+EHL complex. (B) Distal active and passive EDL forces of EDL muscle kept at low length + 2 mm as a function of Δ EDL muscle position at low and high length of the TA+EHL complex. EDL muscle relative position is expressed as the deviation from the most distal position. Low EDL length corresponds to 7 mm below proximally determined optimum length. Low TA+EHL length corresponds to 9 mm below distally determined optimum length. High TA+EHL length corresponds to 2 mm over distally determined optimum length. The F_{ma} and F_{mp} represent active and passive force, respectively. Note that different y-axes with different scaling factors are shown for active (left axis) and passive forces (right axis). Values are shown as mean ± SE (n = 7).

In summary, the position of EDL muscle relative to other muscles and to extramuscular structures is a major determinant of isometric muscle force. These effects are likely to be mediated by both inter- and extramuscular myofascial force transmission. Length changes of EDL muscle and of the TA+EHL complex involve also position changes of parts of these muscles with regard to surrounding tissues. It is concluded that relative positions of (parts of) EDL, EHL and TA muscles play an important role for force transmission within an intact anterior crural compartment.

DISCUSSION

Isometric length-force curves have been predominantly assessed for dissected *in situ* muscles (e.g. Close, 1964; Gareis et al., 1992; Rack and Westbury, 1969; Roszek et al., 1994). In such experiments, the muscle is dissected free of its surrounding tissues, leaving its origin as well as its nerve and blood supply intact. However, early work already indicated mechanical interactions via intermuscular connective tissue between soleus and gastrocnemius muscles, if these muscles were not dissected

(Denny-Brown, 1929). In fact, dissection in that study was performed to remove those effects unwanted for the experiment. Several other studies also pointed towards a role of connective tissue that surrounds the muscle in force transmission from muscle fiber to the bony skeleton (Huijing et al., 1998; Pond, 1982). Implicit support for such muscle-connective tissue interactions can also be found in the literature (e.g. Garfin et al., 1981; Gerlach and Lierse, 1990; Riewald and Delp, 1997; Van der Wal, 1988).

Recent experiments yielded unequivocal evidence for force transmission between EDL muscle and other structures within its direct environment (Huijing, 1999a; Huijing and Baan, 2001a; Maas et al., 2001). For most lengths tested, it was found that force exerted at the proximal tendon was unequal to force exerted at the distal tendons of EDL muscle. Such a proximo-distal force difference is direct evidence for non-myotendinous force transmission between locations within EDL muscle and surrounding structures. Muscle fiber force may be transmitted via intermuscular connective tissue to surrounding muscles (i.e. intermuscular myofascial force transmission) and/or via extramuscular connective tissue to other surrounding tissues (i.e. extramuscular myofascial force transmission). The sum of forces transmitted at several locations via those myofascial pathways from or onto EDL muscle is measured as the difference between EDL proximal and distal forces. The proximo-distal force difference is, therefore, a direct measure of the magnitude of net inter- and extramuscular myofascial force transmission. It should be noted that on the basis of those experiments as well as on the basis of the present work, we cannot distinguish the individual contribution from each pathway.

Recent results indicated effects of muscle relative position on isometric muscle force (Huijing and Baan, 2003). However, effects of muscle length and relative position were not studied independently. In the present study, exclusive effects of EDL muscle position relative to surrounding tissues within the anterior crural compartment on force transmission of EDL muscle were investigated, as EDL muscle position was changed without imposing length changes on that muscle. Thus, effects of inter- and extramuscular connective tissue connections were assessed exclusively. The most striking result is that isometric force of EDL muscle as well as of the TA+EHL complex are affected significantly by the position of whole EDL muscle-tendon complex relative to neighboring muscles and extramuscular connective tissue structures within the anterior crural compartment. It is concluded

that in addition to length, muscle position relative to its surroundings determines isometric force of a muscle.

Furthermore, it was found that isometric muscle force is dependent also on the length of adjacent muscles. This intermuscular interaction suggests intermuscular myofascial force transmission (Maas et al., 2001).

The present results show also that any change of active force with length changes of a muscle that is not dissected free from its surrounding tissues, is not solely determined by the global length changes per se but is affected also by changes of relative position of most parts of the muscle.

Effects of changing the relative position of (parts of) EDL muscle on passive and active components within the muscle

A difference between isometric force exerted at the proximal and at the distal tendons of a muscle, as found in the present study, indicates that an additional force must act on the muscle. As a result of force equilibrium, this net force is equal to the proximo-distal force difference. Inter- and extramuscular connective tissues appear to have sufficiently stiff connections to intramuscular connective tissue (Huijing and Baan, 2001b; Huijing et al., 2003; Maas et al., 2001). Muscle fibers are linked to this connective tissue network via trans-sarcolemmal linkages between cytoskeleton, extracellular matrix and endomysium (for review see Berthier and Blaineau, 1997; Patel and Lieber, 1997). It has been indicated that that these myofascial connections are capable of bearing force (Goldberg et al., 1997; Huijing, 1999b; Huijing et al., 1998; Jaspers et al., 2002; Jaspers et al., 1999; Monti et al., 2001; Monti et al., 1999; Street, 1983; Street and Ramsey, 1965). Therefore, this additional force is expected to affect distributions of length of active (i.e. sarcomeres) and/or passive components (i.e. epimysium, perimysium and endomysium, trans-sarcolemmal linkages, non-contractile proteins within the sarcomeric cytoskeleton, such as titin) within the muscle. A higher proximal EDL force than distal EDL force (Fig. 2A) is most easily explained if sarcomeres located proximally within muscle fibers attain higher lengths than sarcomeres located distally within the same muscle fibers. As the proximo-distal EDL force difference changes as a function of EDL muscle relative position, changes of the distribution of lengths of sarcomeres are to be expected.

In a most simple approach of such distributions, proximal and distal segments of EDL muscle fibers may be distinguished. Using this concept, a higher proximal

compared to distal EDL passive force (Fig. 2A) is associated with a higher mean length of passive components within the proximal segment compared to these components within the distal segment of EDL muscle. Changes of proximally as well as distally measured passive force as a function of EDL muscle relative position indicates a redistribution of lengths of these passive structures within muscle fibers. The final distribution is dependent on the points of application of the inter- or extramuscular myofascial forces imposed on the muscle, their magnitude as well as their direction (a more proximal or more distal orientation). Furthermore, these effects of EDL muscle relative position on passive forces indicate that inter- and/or extramuscular connective tissue act as series elastic components for EDL muscle.

If the length of a muscle is increased, passive force rises monotonically while active force may increase as well as decrease depending on the specific length range. This may explain the observation of the present study that proximal EDL active force is decreased at the more proximal EDL muscle relative positions on lengthening the TA+EHL complex distally (Fig. 5A). Sarcomeres within the proximal segment of muscle fibers could be pulled beyond their optimum length. This view is supported by the fact that, at the most proximal EDL muscle relative positions (Δ EDL muscle position = 6 and 7 mm), passive forces exerted proximally reach high levels. These forces are comparable to passive forces measured at over optimum length of EDL muscle (Huijing and Baan, 2001a, 2001b; Maas et al., 2001).

Further research is necessary to elucidate effects of muscle relative position on length distributions of sarcomeres as well as of passive structures within the muscle. In the experimental conditions of the present study the muscle belly of EDL muscle is completely covered by TA muscle and its muscle fibers cannot be seen. To measure such length distributions within EDL muscle, the anterior crural compartment should be opened. Such fasciotomy will affect the results of the present study, because the pathways for inter- and extramuscular force transmission are affected or damaged. This problem may be solved by applying a mechanical model of the anterior crural compartment. A three-dimensional finite element model of skeletal muscle, which allows for intramuscular myofascial force transmission, was described recently (Yucesoy et al., 2002c). Effects of intermuscular myofascial force transmission on the distribution of lengths of sarcomeres, using a model of two linked muscles, have been reported (Yucesoy et al., 2001). Other noninvasive techniques to investigate skeletal muscle properties are ultrasonography (e.g.

Fukunaga et al., 1997; Maganaris et al., 1998a), and magnetic resonance imaging (MRI), such as magnetic resonance elastography (e.g. Dresner et al., 2001).

Intermuscular interactions on changing muscle length and muscle relative position
Based on previous experiments (Maas et al., 2001), it was expected and confirmed that distal lengthening TA+EHL decreased active force exerted at the distal tendons of EDL muscle. Force of adjacent muscles tends to be transmitted to the tendon of a muscle, which is at relatively high length. Lengthening one muscle changes the configuration (i.e. the length and angle) of its surrounding connective tissues in such a way that net force is transmitted via inter- and/or extramuscular myofascial pathways to the location at which the lengthening was imposed, as illustrated in Fig. 3C.

However, for some EDL muscle relative positions, distal EDL active force was unchanged on increasing TA+EHL length distally (Fig. 5B). These results indicate an interaction between length changes of the TA+EHL complex and relative position of EDL muscle. Note that both factors change the relative position of the different muscle bellies. Two explanations are suggested: (1) At those specific EDL muscle relative positions, the connective tissue that serves as a pathway for these intermuscular interactions is rather compliant. This is supported by the observation that at the same positions of EDL muscle (i.e. Δ EDL muscle position $\approx$ 3, 4 mm), changes of distal EDL force, as a function of EDL muscle relative position, were less pronounced than at other positions. (2) A decrease in distal EDL active force is cancelled by other factors, such as changes of the distribution of lengths of sarcomeres.

Relative position changes of muscles in vivo
In vivo, position changes of a muscle relative to adjacent synergists will be caused by (1) differences in moment arm between those muscles, and (2) the fact that some muscles span only one joint and adjacent muscles may span more than one joint. The position of a muscle relative to non-muscular (i.e. fixed) structures within a compartment (e.g. bone, fascia) will alter with any change of joint angle.

In rat, moment arms of the presently studied muscles at the ankle are expected to be different. For mice, it was found that the average moment arm at the ankle of TA muscle is 17% higher than of EDL muscle (Lieber, 1997). This indicates that length

changes of TA, as function of ankle angle, are 17% higher than of EDL muscle. Furthermore, the distal tendons of the muscles within the rat anterior crural compartment all cross the ankle joint. It should be noted that only the proximal tendon of rat EDL muscle crosses also the knee joint (knee extensor). Thus, movements of the knee joint involve length changes of EDL muscle without any changes of muscle-tendon complex length for TA and EHL muscles. Therefore, changes of relative position of (parts of) the muscles of the anterior crural compartment are expected to occur during *in vivo* rat movements.

Such changes of muscle relative position in rat anterior crural compartment have not been measured directly nor estimated using musculo-skeletal models. It has been reported that during treadmill stepping of rat the knee joint ranges between 54° and 125°, and the ankle joint ranges between 41° and 146° (Gruner et al., 1980). In addition, we have found that EDL muscle-tendon complex length increases by approximately 3 mm if the knee joint is moved from 45° to 135° and another 3 mm due to dorsal flexion of the ankle joint from 45° to 135° (unpublished observations). These observations indicate (1) that the length of EDL muscle can change proximally by 3 mm without any length change of TA and EHL muscles, and (2) that the position of whole EDL muscle relative to non-muscular fixed structures within the anterior crural compartment can change by 3 mm. During *in vivo* rat movements, the ankle and knee joints move simultaneously (Gruner and Altman, 1980; Gruner et al., 1980). In such conditions, a change of muscle relative position exclusively without any length changes of adjacent muscles, as imposed experimentally on EDL muscle in the present study, will not occur. However, the purpose of the present study was to distinguish effects of changing muscle relative position from effects of changing muscle length.

In the present study, the relative position of EDL muscle was changed by 5 mm for low length and by 7 mm for low length + 2 mm (Fig. 4). Note that a change of EDL muscle relative position of 3 mm still causes substantial changes of proximal and distal EDL forces. Furthermore, a 2 mm increase of EDL muscle-tendon complex length, caused a significant higher increase of distal EDL force compared to proximal EDL force, indicating a change of relative position of parts of EDL muscle (Fig. 4C). This suggests that also for *in vivo* length and position ranges, effects of muscle relative position may play an important role.

In addition, substantial changes of relative position of human muscles have been

measured *in vivo*, using cine phase-contrast magnetic resonance imaging (Asakawa et al., 2002). For a constant hip angle, length changes of bi-articular rectus femoris (RF) and mono-articular vastus intermedius (VI) muscles were compared during active knee extension. In healthy subjects, it was found that position changes of a square region of interest of the muscle belly were on average 31% greater in RF than in VI. The higher length changes of RF compared to VI are explained by the fact that RF has a higher moment arm than VI (Buford et al., 1997; Visser et al., 1990). These results show that *in vivo* the relative position of adjacent muscle bellies is changed by joint movements in humans.

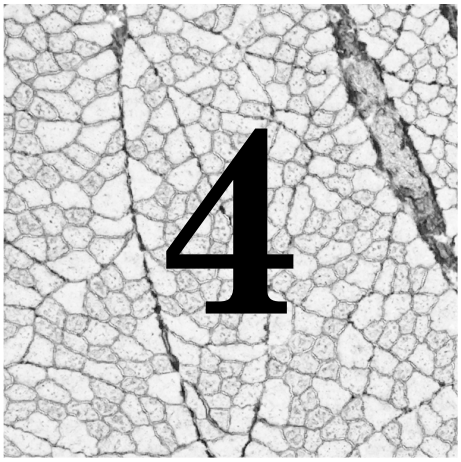
Implications and conclusions

The present results indicate that *in vivo* the assumption that isometric muscle force is constant, if muscle-tendon complex length is unchanged, is not tenable. Consequently, the assessment of length-force characteristics of mono- and bi-articular human muscles *in vivo* using this assumption (e.g. Herzog and ter Keurs, 1988b; Herzog and ter Keurs, 1988a; Huijing et al., 1986), may result in erroneous conclusions.

An important role of bi-articular muscles during multi-joint movements (e.g. running, jumping) is the capability to redistribute energy over the joints that are crossed, i.e. a transport of energy (e.g. Gregoire et al., 1984; Van Ingen Schenau et al., 1987). In rat for example, work performed by muscles in knee flexion, may be used for dorsal flexion of the ankle and/or toes via the poly-articular EDL muscle. Even if the length of the muscle would not change, the position of the muscle relative to surrounding tissues does change. The results of the present study suggest that in such a transport of energy not only the poly-articular muscle, as defined by location of origin and insertion, will play a role, but also adjacent muscles and other surrounding structures. Other implications of muscle relative position as a co-determinant of isometric muscle force have been described recently (Maas et al., 2003c).

In conclusion, the position of a muscle relative to adjacent muscles and relative to other connective tissue structures is a major co-determinant of isometric muscle force. Length changes of muscle involve also position changes of most parts of the muscle relative to surrounding structures. Therefore, potential effects of surrounding tissues should always be considered if *in vivo* muscle properties are studied.

The relative position of EDL muscle affects the length of sarcomeres within muscle fibers: experimental results and finite element modeling



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Journal of Biomechanical Engineering, **In press**

ABSTRACT

Background: Effects of extramuscular connective tissues on muscle force (experimentally measured) and lengths of sarcomeres (modeled) were investigated in rat. It was hypothesized that changes of muscle relative position affect the distribution of lengths of sarcomeres within muscle fibers.

Method of approach: The position of extensor digitorum longus muscle (EDL) relative to intact extramuscular connective tissues of the anterior crural compartment was manipulated without changing its muscle-tendon complex length.

Results: Significant effects of EDL muscle relative position on proximal and distal EDL forces were found, indicating changes of extramuscular myofascial force transmission. EDL isometric force exerted at its proximal and distal tendons differed significantly. Finite element modeling showed that the distribution of lengths of sarcomeres is altered by changes of muscle relative position.

Conclusions: It is concluded that forces exerted on a muscle via extramuscular myofascial pathways augment distributions of lengths of sarcomeres within that muscle.

INTRODUCTION

In vivo, skeletal muscles are surrounded by synergists and embedded within connective tissues of the compartment: connective tissue at the interface between muscle bellies (i.e. intermuscular connective tissue) as well as extramuscular connective tissues (connective tissue that supports the nerves and blood vessels and fascia forming the compartmental borders). Recent experiments on rat have shown that, in addition to the tendons, inter- and extramuscular connective tissues may also transmit a substantial fraction (up to 37%) of force from muscle fibers to bone (Huijing and Baan, 2001a; Maas et al., 2001).

In humans, it was found that position changes of a specified region of the muscle belly during knee extension were greater in rectus femoris muscle than in vastus intermedius muscle (Asakawa et al., 2002). These results show that the position of synergists relative to each other is changed. Effects of the relative position of extensor digitorum longus muscle (EDL), kept at a constant muscle-tendon complex length, on force transmission from that muscle with intact inter- and extramuscular connections have been studied recently (Huijing and Baan, 2003; Maas et al., 2002a). Proximally and distally measured EDL forces were affected significantly by EDL muscle relative position. It was concluded that muscle relative position is a co-determinant of isometric muscle force. The inter- and extramuscular connective tissues are connected to the intramuscular connective tissue (Huijing and Baan, 2001b; Maas et al., 2001), which is connected also to muscle fibers via trans-sarcolemmal linkages between cytoskeleton, extracellular matrix and endomysium (Berthier and Blaineau, 1997; Patel and Lieber, 1997). Therefore, it was hypothesized that changes of muscle relative position should affect the distribution of lengths of sarcomeres within muscle fibers. Presently, experimental confirmation of this hypothesis is not tenable for intact muscles.

Recently, a 3D finite element model of skeletal muscle (linked fiber-matrix mesh model: lfmm model) was developed (Yucesoy et al., 2002c). This model is used to calculate local fiber direction strain, which is a measure of sarcomere length. A finite element model study for two muscles connected by elastic elements, representing intermuscular connective tissue, indicated changes of the distribution of length of sarcomeres within both muscles due to length changes of one of the muscles only (Yucesoy et al., 2001). In another study, effects of extramuscular connective tissue on length-force characteristics of EDL muscle were investigated using the lfmm

model with extramuscular links (Yucesoy et al., 2002b). It was shown that extramuscular myofascial forces affect the distribution of length of sarcomeres.

In the above-described experimental studies (Huijing and Baan, 2003; Maas et al., 2002a), effects of relative position were investigated for EDL muscle with intact inter- and extramuscular myofascial pathways of the anterior crural compartment. As a consequence, the individual contribution of each pathway could not be distinguished. Therefore, the first aim of this study was to quantify the role of extramuscular connective tissue exclusively for force transmission from EDL muscle. For this purpose, EDL muscle was dissected free from surrounding synergists, but not from the extramuscular connective tissues that supports the nerves and blood vessels within the anterior crural compartment. Isometric forces exerted at proximal and distal tendons of EDL muscle were measured at several relative positions of EDL muscle, kept at constant muscle-tendon complex length. The second aim of the present study was to determine if changes of muscle relative position alters the distribution of lengths of sarcomeres within the muscle. For this purpose the lfmm model was used with extramuscular links exclusively. Local strains and stresses of intracellular components as well as of the extracellular matrix within EDL muscle were calculated.

MATERIALS AND METHODS

Experimental data

Surgical and experimental procedures were in strict agreement with the guidelines and regulations concerning animal welfare and experimentation set forth by Dutch law, and approved by the Committee on Ethics of Animal Experimentation at the Vrije Universiteit.

Surgical procedures

Male Wistar rats (n = 6, body mass = 309.5 ± 21.6 g) were anaesthetized using intraperitoneally injected urethane (1.2 ml/ 100 g body mass 12.5 % urethane solution, extra doses were given if necessary: maximally 1.5 ml). During surgery and data collection, the animals were placed on a heated water pad of approximately 37°C, to prevent hypothermia. Ambient temperature (22 ± 0.5 °C) and air humidity (80 ± 2%) were kept constant by a computer controlled air-conditioning system. Dehydration of muscle and tendon tissue was prevented by regular irrigation with

isotonic saline.

The anterior crural compartment, which envelops tibialis anterior (TA), EDL and extensor hallucis longus (EHL) muscles, was exposed by removing the skin and most of the biceps femoris muscle from the left hind limb and opened by full lateral fasciotomy. Subsequently, TA and EHL muscles were almost fully excised. This involved cutting intermuscular connective tissue as well as nerves and blood vessels. Proximally, a small piece of TA muscle was not cut to prevent damage to the neurovascular tract of EDL muscle (Fig 1A). The remaining extramuscular connective tissue, e.g. a sheet all along EDL muscle that supports its nerves and blood vessels (Huijing and Baan, 2001b; Huijing et al., 2003), was left intact. To free EDL distal tendons from surrounding tissues, the transverse crural ligament and the crural cruciate ligament (retinaculae near the ankle joint) were severed. With the knee joint at 100° and the ankle joint at 90° (referred to as reference position) the four distal EDL tendons were tied together. Proximally, the femoral compartment was opened to detach the proximal tendon of EDL from the femur and to secure the femur (at a knee angle of 100°) with a metal clamp to the experimental apparatus (Fig. 1A). The tied distal tendons as well as the proximal tendon of EDL muscle were connected to force transducers (maximal output error < 0.1 %, compliance of 0.0048 mm/N) mounted on a single axis micropositioner (IEF Werner GMBH, Domino 50, total travel = 25 mm, advancement = 0.05 mm / division) with Kevlar thread (4% elongation at a break load of 800 N). The foot was attached to a plastic plate with tie wraps. To create a free passage for the Kevlar thread, connecting the distal EDL tendons to the force transducer, the footplate was positioned in maximal plantar flexion of the ankle, and approximately 40° pronation. Proximal and distal EDL tendons were aligned with the line of pull (Fig. 1A). Experimental data indicating that differences in force between the two force transducers greater than 0.7 % cannot be ascribed to this measurement system have been reported previously (Maas et al., 2001).

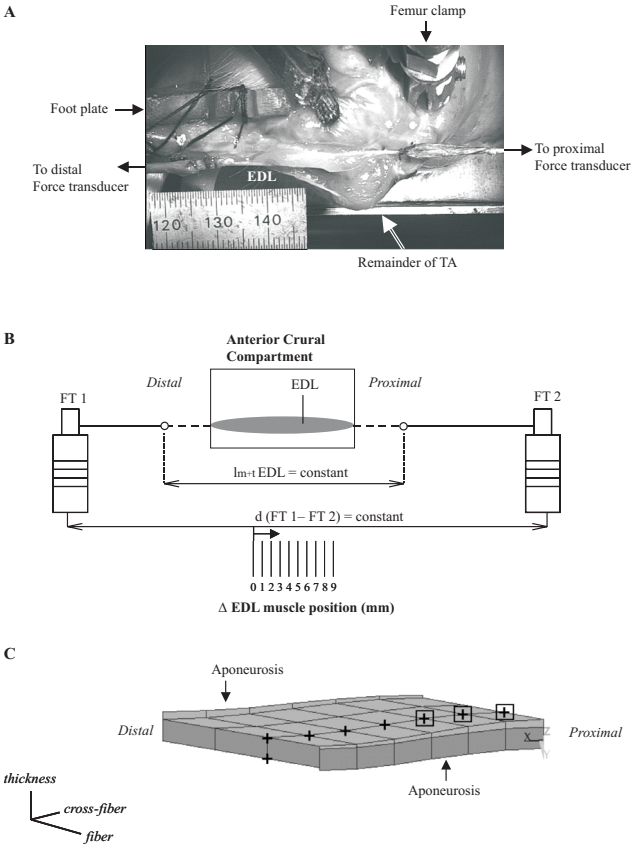


Fig. 1. The experimental set-up and the muscle model. (A) Lateral view of the anterior crural compartment in the experimental set-up after TA and EHL muscles were almost fully removed. The intervention involved fasciotomy of the anterior crural compartment and excising TA and EHL muscles. A small piece of TA muscle was left attached to the compartment to prevent damage to the neurovascular tract of EDL muscle. A connective tissue sheet all along EDL muscle supporting its nerves and blood vessels was left intact. Thus all intermuscular myofascial pathways and a part of the extramuscular myofascial pathways were excluded. The footplate and the femur clamp are indicated. The smallest division of the ruler shown represents 0.5 mm. (B) A schematic view of the experimental set-up, seen from above. FT 1 indicates the force transducer connected to the distal tendons of EDL muscle, FT 2 indicates the force transducer connected to the proximal tendon of EDL. The force transducers were mounted on a single axis micropositioner. Each relative position of EDL muscle (Δ EDL muscle position) was obtained by repositioning FT 1 as well as FT 2, in a variable order, one mm in proximal direction. Δ EDL muscle position = 0 refers to the most distal position of EDL muscle. The distance between FT 1 and FT 2, i.e. d(FT1-FT2) and, thus also the muscle-tendon complex length of EDL muscle (l_{m+t} EDL) remained constant. (C) Geometry of the muscle model. The geometry is defined by the contour of a longitudinal slice of the rat EDL muscle belly. The muscle model is composed of three muscle elements in series, representing a fascicle, and six muscle elements in parallel. A 3D local coordinate system is used for the analysis and presentation of the results. At the nodes indicated with a "+" sign the extramuscular connections are made and the ones marked also with a square are stiffer as they represent the connective tissues supporting the neurovascular tract of EDL muscle.

Within the femoral compartment, the sciatic nerve branches into the tibial nerve, the sureal branch and the common peroneal nerve. The common peroneal nerve enters the anterior crural compartment from the peroneal compartment through a fenestration within the anterior intermuscular septum (Huijing and Baan, 2001b). Branches of the intact common peroneal nerve innervate EDL muscle as well as the muscles in the peroneal compartment. The tibial nerve, the sureal branch as well as all other more proximal branches of the sciatic nerve were cut. The sciatic nerve, with only the common peroneal nerve branch left intact, was dissected, cut as proximally as possible and placed in a pair of silver electrodes. The nerve was prevented from dehydration, by covering it with tissue saturated with isotonic saline and a thin piece of latex. In all experimental conditions, the sciatic nerve was stimulated supramaximally using electrodes connected to a constant current source (3 mA, pulse width 100 μs).

EDL muscle relative position – force characteristics

Before acquiring data, EDL muscle was preconditioned by isometric contractions at several high lengths until proximally and distally measured isometric forces at low and high length were reproducible. Isometric force exerted at the proximal and distal EDL tendons was measured at various positions of EDL muscle relative to its surroundings. While EDL muscle was kept at constant muscle-tendon complex length, the position of EDL muscle was changed (Δ EDL muscle position), from a distal one to a proximal one, in steps of 1 mm. This was done by moving both the force transducer connected to the proximal EDL tendon and the force transducer connected to the distal EDL tendons, in a variable order, 1 mm in proximal direction (Fig. 1B). To make sure that the muscle will exert passive force at its tendons, this muscle relative position–force characteristics was assessed for EDL muscle kept at high length (i.e. 2 mm over proximally determined optimum length: l_o+2 mm).

Prior to each contraction, passive EDL muscle was brought to the desired relative position. Two twitches were evoked (200 ms apart) to let the muscle adjust to the new condition. After 400 ms, this was followed by a tetanic contraction (pulse train 400 ms, frequency 100 Hz). In between contractions, EDL muscle was allowed to recover near active slack length for 2 minutes. Isometric forces were measured just before (i.e. passive muscle force, Fmp) and during the tetanic contraction of the muscles (i.e. total muscle force, Fm). Simultaneously, images of EDL muscle in

passive and active state were recorded using a digital camera (DVC, JAI CV-M10, shutter speed 1/50 s). Stimulation of the nerve, A/D conversion (12-bit A/D converter, sampling frequency 1000 Hz, resolution of force 0.01 N), and photography were time-controlled by a computer.

Treatment of experimental data and statistics

For all data mean ± standard error of the mean (SE) were calculated. To test for the effects of EDL muscle relative position on proximal and distal EDL forces one-way ANOVA's for repeated measures (factor: EDL muscle relative position) were performed (Neter et al., 1996). P values < 0.05 were considered statistically significant.

Model data

Description of the "linked fiber matrix mesh model"

The lfmm model (Yucesoy et al., 2002c) consists of two meshes, occupying the same space, that are linked elastically. The two meshes represent the extracellular matrix domain (matrix mesh) and intracellular domain (fiber mesh). The fiber and matrix mesh are built using the self-programmed "myofiber" and "extracellular matrix" elements respectively, that are introduced as user defined elements into the finite element program ANSYS 5.7.1. The elements have eight nodes, linear interpolation functions and a large deformation analysis is employed. A 3D local coordinate system representing the fiber, cross-fiber (normal to the fiber direction), and thickness directions is used (Fig. 1C). For the myofiber element, the total stress that acts exclusively in the local fiber direction is the sum of the active stress of the contractile elements and the stress due to intracellular passive tension. It is assumed that, at initial muscle length and in the passive state, the sarcomeres arranged in series within muscle fibers have identical lengths and material properties. The extracellular matrix element incorporates a strain energy density function that accounts for the non-linear and anisotropic material properties and the constancy of muscle volume.

Within the biological context, one muscle element is defined to represent a segment of a bundle of muscle fibers with identical material properties, its connective tissues and the links between them. This is realized as a linked system of extracellular matrix and myofiber elements. Both matrix and fiber meshes are

connected rigidly to single layers of elements forming the muscle's proximal and distal aponeurosis. To represent the aponeuroses, a standard 3D, 8-node element HYPER58, from the element library of ANSYS 5.7.1 was used. This element is a hyperelastic element for which the strain energy density function is defined using the Mooney-Rivlin material law. For the elastic links between the two meshes, which represent the trans-sarcolemmal attachments of the cytoskeleton and extracellular matrix, another standard element, COMBIN39 was used. This is a 2-node spring element, which is set to be uniaxial and have linear stiffness characteristics.

The geometry of the model (Fig.1C) is defined by the contour of a longitudinal slice at the middle of the isolated rat EDL muscle belly. Three muscle elements in series and six in parallel fill this slice. Based on experimental measurements of rat EDL muscle (Van der Linden, 1998), the length of fibers located proximally in EDL muscle was lower than the length of distally located EDL muscle fibers (9.26 mm and 11.99 mm, respectively, in the passive condition of the model at optimum length). All aponeurosis elements have identical mechanical properties but using a variable thickness in the fiber-cross fiber plane, the increasing cross-sectional area of the aponeurosis toward the tendon (Zuurbier et al., 1994) is accounted for.

Model of EDL with extramuscular connections

In the present study, the lfmm model was extended to include extramuscular connections of EDL muscle. An extramuscular sheet of connective tissue connects EDL all along the muscle to the tibia, part of interosseal membrane and anterior intermuscular septum (for images see Huijing and Baan, 2001a; Huijing et al., 2003; Maas et al., 2001). This sheet defines the anatomical path of extramuscular myofascial force transmission. In our previous experimental study (Yucesoy et al., 2002b), the location of the extramuscular sheet was determined to be at one-third of the fascicle length from the most proximal side of each fascicle of EDL muscle. In that study it was also shown that the extramuscular connective tissues supporting the neurovascular tract to EDL muscle are much stiffer than the rest of the connective tissues. The extramuscular connections were modeled by linking a set of nodes of the matrix mesh (Fig. 1C) to a set of fixed points, representing the "mechanical ground". The corresponding fixed points of the mechanical ground and the nodes of the model were at identical locations initially (i.e., muscle length = 28.7 mm, and before moving any of the tendon ends). To represent the muscle's extramuscular

connections, the spring element COMBIN39 was used and set to be uniaxial. As connective tissue structures (e.g. tendons, aponeuroses, muscle fiber bundles, isolated epimysium) have non-linear force-deformation characteristics (Banus and Zetlin, 1938; Ettema and Huijing, 1989; Kawakami and Lieber, 2000; Scott and Loeb, 1995; Strumpf et al., 1993; Woo et al., 1980), the extramuscular linking elements were defined to have nonlinear stiffness. Force as a function of deformation was fitted by a third order polynomial. Suitable coefficients were selected in order to obtain a reasonably good agreement between the modeled and experimental forces exerted at the proximal and distal tendons of EDL muscle. The higher stiffness of the connective tissues supporting the neurovascular tract to the EDL muscle is taken into account by making the three most proximal extramuscular links stiffer than the remainder. This model is referred to as model of EDL with extramuscular connections.

The effects of relative position of EDL muscle with respect to its surrounding tissues were tested at high length (i.e. l_o+2 mm). Muscle length was kept constant throughout the analysis. However, the relative position of the muscle with respect to the mechanical ground was altered. The most distal relative position of the muscle belly was referred to as 0 mm. The effects of changes of relative position were assessed by moving both distal and proximal tendon ends of the muscle model in the proximal direction by 1 mm increments at a time, to a maximum of 9 mm.

Treatment of model data

Local fiber direction strain is used as a measure of change of sarcomere length: positive strain indicates lengthening and negative strain indicates shortening of sarcomeres relative to optimum length of isolated muscle. Zero strain in the model represents the undeformed state of sarcomeres (i.e., sarcomere length ≅ 2.5 μm) in the passive condition at optimum muscle length. Differences of strain between elements arranged in series indicate a distribution of lengths of sarcomeres in series within muscle fibers. Mean fiber direction strain of a fascicle is calculated as a measure of fiber mean sarcomere length. Differences of mean strains between fascicles indicate a distribution of fiber mean sarcomere length.

RESULTS

A. Effects of EDL muscle relative position on isometric forces: experimental results

Figure 2A shows effects of the position of EDL muscle relative to extramuscular tissues of the anterior crural compartment on proximally and distally measured EDL forces. Because EDL was kept at constant length (i.e. l_o + 2 mm), force is plotted as a function of EDL muscle relative position rather than muscle-tendon complex length.

Passive muscle. Proximally measured passive EDL force increased from 0.06 ± 0.02 N to 0.13 ± 0.01 N (mean ± SE) on changing the relative position of EDL muscle from a distal to a more proximal location. Simultaneously, distally measured EDL passive force decreased from 0.43 ± 0.03 N to 0.06 ± 0.01 N (mean ± SE). These results indicate length changes of passive components within EDL muscle as a function of its position relative to other structures within the anterior crural compartment. As the surrounding synergists of EDL (i.e. TA and EHL) had been excised, the only pathways that can mediate these length-independent effects are connections between the intact intra- and extramuscular connective tissue of EDL muscle. These results indicate that those connections are sufficiently stiff to transmit passive force.

Active muscle. ANOVA indicated significant effects of EDL muscle relative position for proximal and distal EDL total muscle forces. At the most distal EDL muscle relative positions studied (i.e. Δ EDL muscle position = 0, 1 and 2 mm), force exerted at the distal tendons was significantly higher than force exerted at the proximal tendon of EDL muscle. For the present experiment, any proximo-distal force difference indicates extramuscular myofascial force transmission. Changing the relative position of EDL muscle from a distal to more proximal locations, decreased distal EDL force and increased proximal EDL force, significantly. For the more proximal EDL muscle relative positions (Δ EDL muscle position > 3 mm), proximal and distal EDL force did attain similar values, which indicates that no net extramuscular myofascial force transmission did occur.

These results show that the position of EDL muscle relative to extramuscular connective tissue structures of an opened anterior crural compartment is a co-determinant of isometric force exerted at the proximal as well as the distal tendons of EDL muscle. This proves that the intact extramuscular connective tissue is a pathway for extramuscular myofascial force transmission. Changing the relative position of EDL muscle alters the configuration (i.e. the length and angle) of this connective

tissue and, consequently, its effect on force transmission from EDL muscle.

It is hypothesized that a proximo-distal EDL total force difference may lead to differential lengths of sarcomeres as well as extracellular structures (e.g. extracellular matrix, endomysium) located proximally and distally within muscle fibers (i.e. distribution of length of sarcomeres arranged in series within muscle fibers). A higher proximal EDL total force than distal EDL total force may be explained by the fact that proximal sarcomeres within muscle fibers attain higher lengths than distal sarcomeres within muscle fibers. As long as the sarcomeres are operating on the ascending limb of the length-force curve, this will be the result of predominantly active forces. Over optimum length, passive force of intracellular passive elements (e.g. cytoskeleton, titin) as well as of the extracellular matrix will also play a role. As the proximo-distal EDL total force difference changed as a function of EDL muscle relative position, changes of the distribution of lengths of sarcomeres are expected.

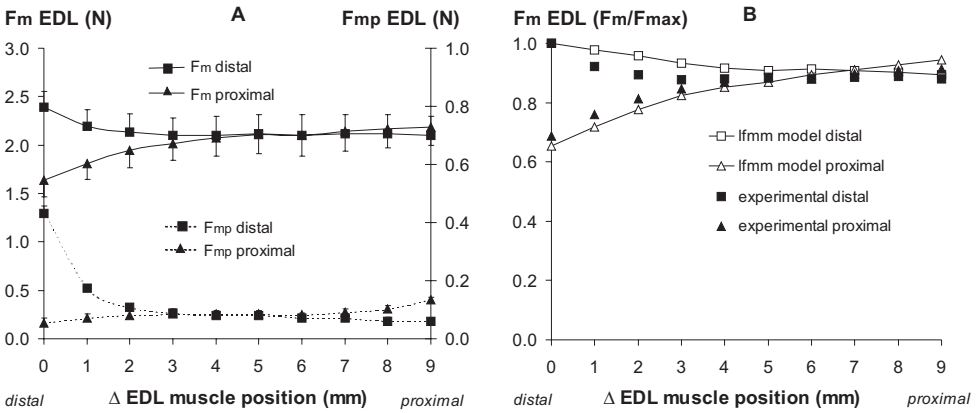


Fig. 2. Experimentally measured as well as modeled forces of EDL muscle, kept at constant high length (i.e. l_0+2 mm), with varying EDL muscle relative positions. (A) Experimentally measured proximal and distal total and passive forces of EDL muscle plotted as a function of EDL muscle relative position. (B) Experimental data and lfmm model data for total proximal and distal EDL muscle force normalized for maximal force (F_{max}) plotted as a function of EDL muscle relative position. EDL muscle relative position is expressed as the deviation from the most distal position. The F_m, F_{mp}, and F_{max} represent total, passive and maximal force, respectively. Note that in A different y-axes with different scaling factors are shown for total (left axis) and passive forces (right axis). Values are shown as mean ± SE (n = 6).

B. Effects of EDL muscle relative position on lengths of sarcomeres: finite element model results

Effects of EDL muscle relative position on the length of sarcomeres as well as their surrounding connective tissue were investigated, using the lfmm EDL model equipped with extramuscular connections exclusively. Local strain within the fiber mesh is used as an estimate of sarcomere length: positive strain indicates sarcomeres over optimum length and negative strain indicates sarcomeres below optimum length. Due to the high stiffness of the intramuscular links between fiber and matrix mesh, strains within the matrix mesh did not differ substantially from those within the fiber mesh and are, therefore, not shown.

Normalized total muscle forces calculated using the lfmm model are compared to the experimentally measured EDL muscle relative position-normalized total force characteristics (Fig. 2B). The general pattern of normalized experimental and modeled curves is reasonably similar. For more distal EDL muscle relative positions only, normalized modeled proximal forces are overestimated and distal forces are underestimated. Consequently, for those positions the modeled differences between proximal and distal muscle force are higher than the experimentally measured force differences.

Strains in fiber direction at different locations within the fiber mesh are shown in Fig. 3, for selected EDL muscle relative positions. Considerable differences of strain between elements arranged in series are found. These distributions in series within elements comprising a fascicle are altered by changes of EDL muscle relative position. At the most distal EDL muscle relative position (Δ EDL muscle position = 0 mm), negative strains are found throughout the muscle near the proximal aponeurosis (fascicle section 4, minimally –0.26) and positive strains are found throughout the muscle near the distal aponeurosis (fascicle section 1 and 2, maximally 0.41). In contrast, at the most proximal EDL muscle relative position (i.e. Δ EDL muscle position = 9 mm), strains located near the distal aponeurosis (fascicle section 1, minimally –0.02) are lower than strains near the proximal aponeurosis (fascicle section 4, maximally 0.43), for almost all fascicles.

Differences of mean strains between fascicles are found also (Fig. 4). For each EDL muscle relative position, mean fiber direction strain is lowest for the middle fascicle (fascicle number 4) and highest for the outermost fascicles (fascicle number 1 and 7). Changing the relative position of EDL muscle from distal to more proximal

locations decreased the mean strain of the more distal fascicles (i.e. fascicle number 1 to 5) but increased the mean strain of the most proximal fascicles (i.e. fascicle number 6 and 7). It is concluded that the distribution of mean strain of fascicles arranged in parallel is a function of EDL muscle relative position. The difference between maximum and minimum mean strain increased (from 6 % to 10 %) on changing EDL muscle from the most distal to the most proximal relative position.

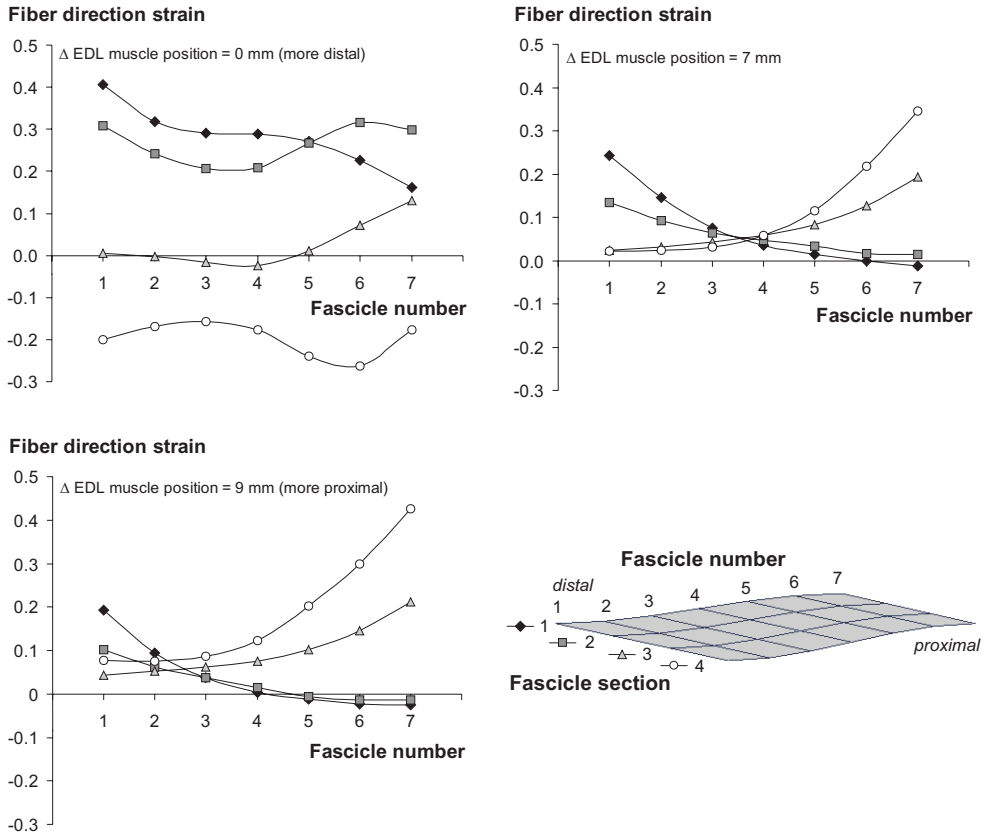


Fig. 3. Fiber direction strains at different locations within the fiber mesh. Comparison of strains between elements arranged in series of EDL model kept at constant high length (i.e. l_o+2 mm), for selected EDL muscle relative positions (i.e. Δ EDL muscle position = 0, 7 and 9 mm). For four fascicle sections, the fiber direction strains are plotted as a function of fascicle number. Each fascicle is indicated by a number from 1 to 7. Differences of strain between sections arranged in series within a fascicle provide a measure of the distribution of lengths of sarcomeres arranged in series within muscle fibers. Strain is defined as the ratio of the change in length to the original length. Zero strain in the model is assumed to represent the undeformed state of sarcomeres (i.e., sarcomere length ≅ 2.5 μm) in the passive condition, at initial muscle length (28.7 mm). Positive strain shows lengthening and negative strain shows shortening of the sarcomeres with respect to this undeformed state.

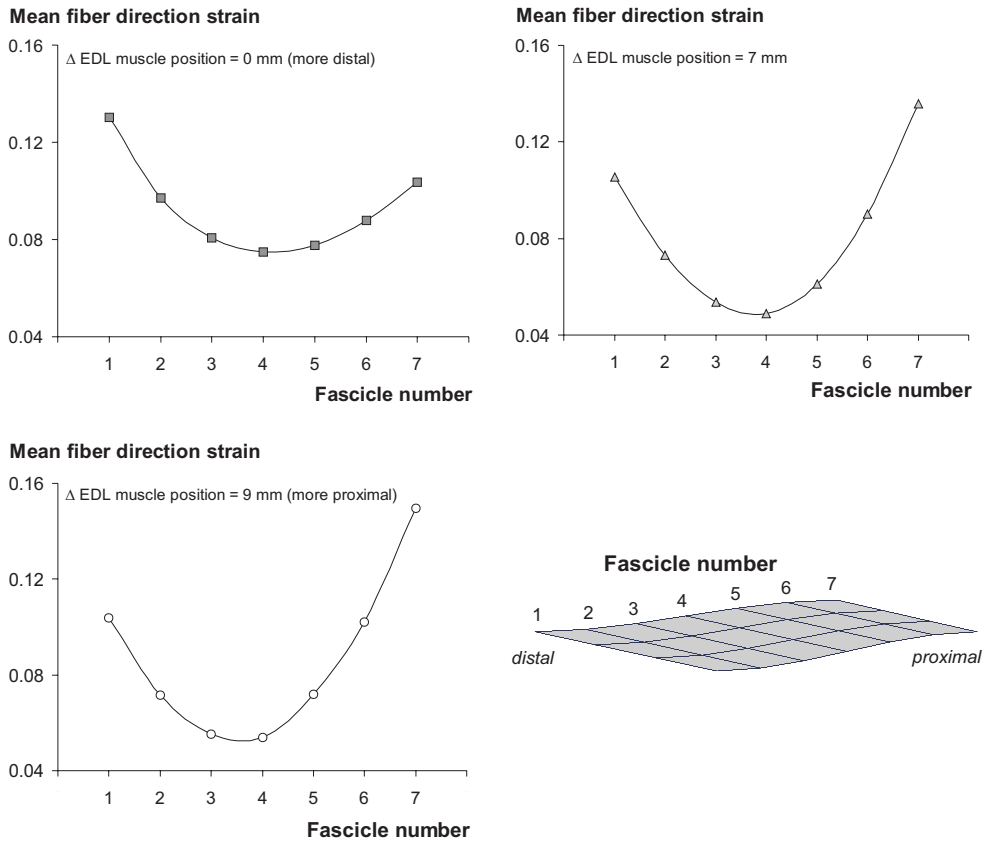


Fig. 4. Fiber direction mean strains of different fascicles within the fiber mesh. Comparison of mean strains between fascicles of EDL model kept at high length (i.e. l_o+2 mm), for selected EDL muscle relative positions (i.e. Δ EDL muscle position = 0, 7 and 9 mm). Mean fiber direction strains are plotted as a function of fascicle number. Each fascicle is indicated by a number from 1 to 7. Mean fiber direction strain was calculated at nodes of the myofiber elements (in the fiber mesh) in series representing a fascicle. Mean fiber direction strain indicates the distribution of fiber mean sarcomere length in parallel among muscle fibers. Strain is defined as the ratio of the change in length to the original length. Zero strain in the model is assumed to represent the undeformed state of sarcomeres (i.e., sarcomere length ≅ 2.5 μm) in the passive condition, at initial muscle length (28.7 mm). Positive strain shows lengthening and negative strain shows shortening of the sarcomeres with respect to this undeformed state.

In accordance with fiber direction strains, considerable differences of total fiber direction stress in the fiber mesh between elements arranged in series comprising a fascicle are found (Fig. 5). For the most distal EDL muscle relative position, stress near the distal aponeurosis was higher (maximally 1.00) than near the proximal aponeurosis (minimally 0.51) for all fascicles. These distributions of stress are

affected also by changes of EDL muscle relative position. For the most proximal EDL muscle relative position, stress distributions are found only in fascicles proximally within the muscle (maximal stress = 1.00, minimal stress = 0.59).

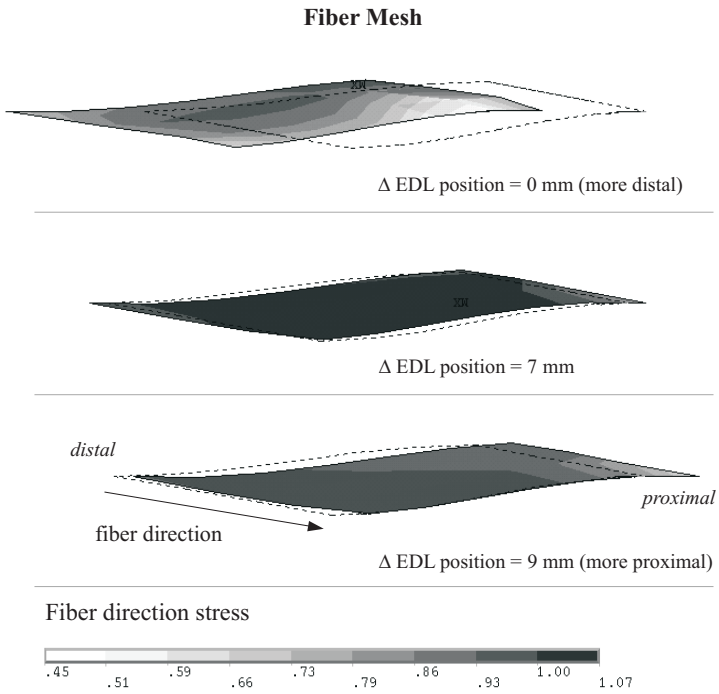


Fig. 5. Fiber direction stress within the fiber mesh of the EDL model kept at high length (i.e. l_o+2 mm), for selected EDL muscle relative positions (i.e. Δ EDL muscle position = 0, 7 and 9 mm). The dotted line contour indicates muscle geometry of isolated EDL model at the initial length. The fiber direction (arrow) as well as proximal and distal ends of the model is shown.

As local strain within the fiber mesh is a measure of sarcomere length, it is concluded that the position of EDL muscle relative to extramuscular connective tissues within the anterior crural compartment alters the distribution of lengths of sarcomeres arranged in series within muscle fibers as well as the distribution of fiber mean sarcomere length among muscle fibers. Due to a distribution of lengths of sarcomeres arranged in series within muscle fibers, forces exerted by a muscle fiber at the proximal aponeurosis or at the distal aponeurosis by the same muscle fiber are not equal, as local stress within the fiber mesh is a measure of force generated by sarcomeres. As strains within the matrix mesh are similar to strains within the fiber

mesh, passive forces exerted at the proximal aponeurosis by connective tissue around a muscle fiber are also not equal to passive forces exerted at the distal aponeurosis by connective tissue around the same muscle fiber.

DISCUSSION

Force is transmitted from muscle to bone via several pathways: (a) via the tendons (i.e. myotendinous force transmission), (b) via intermuscular connective tissue to adjacent muscles (i.e. intermuscular myofascial force transmission), (c) via structures other than muscles (i.e. extramuscular myofascial force transmission) (Huijing, 1999b; Huijing and Baan, 2001a; Maas et al., 2001).

It has been reported that the fraction of force that is transmitted via these pathways is affected by the position of a muscle relative to adjacent muscles as well as relative to other surrounding tissues (Huijing and Baan, 2003; Maas et al., 2002a; Maas et al., 2003c). Force exerted at proximal and distal tendons of EDL muscle as well as the proximo-distal force difference changed if the position of EDL muscle, kept at constant muscle-tendon complex length, relative to surrounding tissues within the anterior crural compartment was altered. The difference between force exerted at the proximal and distal tendons of a muscle is a measure of the net force that is exerted on the muscle via structures other than the tendons. As this force must be born by structures within the muscle, it was hypothesized that the relative position of EDL muscle is a determinant of the distribution of length of sarcomeres within EDL muscle.

In the present study, EDL muscle was measured after TA and EHL muscles were almost fully excised, leaving only the extramuscular tissues, such as supporting the nerves and blood vessels of EDL muscle, intact. It was found that the position of dissected EDL muscle relative to surrounding non-muscular tissues affected force transmission from EDL muscle. As the surrounding synergists of EDL had been removed, the only pathways that can mediate these length-independent effects are connections between the intact intra- and extramuscular connective tissues of EDL muscle.

The hypothesis regarding effects of muscle relative position on lengths of sarcomeres within EDL muscle was tested using the finite element lfmm model of EDL muscle equipped with extramuscular connections. The distribution of lengths of sarcomeres arranged in series within muscle fibers as well as the distribution of fiber

mean sarcomere length among muscle fibers was changed as a function of muscle relative position. It is concluded that forces exerted on a muscle via extramuscular myofascial pathways augment distributions of lengths of sarcomeres within that muscle.

Distributions of lengths of sarcomeres within muscle

We found that the distribution of lengths of sarcomeres arranged in series within EDL muscle fibers as well as the distribution of mean sarcomere length of muscle fibers changed as a function of muscle relative position (Fig. 4). Previous experiments (for a review see Huijing, 1995) have shown that changes of such distributions affect the muscle length range of active force exertion (i.e. the length range between the minimum and maximum length at which the muscle can still exert force). An increase of the distribution of lengths of sarcomeres arranged in series within an isolated muscle fiber has been reported to bring about a higher length range between optimum length and maximal length of active force exertion (Granzier and Pollack, 1990; Pollack et al., 1993). An increased distribution of fiber mean sarcomere length within a fully dissected whole muscle has been associated with higher length ranges between active slack length and optimum length as well as between optimum length and maximum length of force exertion, at the expense of optimal force (e.g. Ettema and Huijing, 1994; Willems and Huijing, 1994).

Recent results indicated effects of inter- and extramuscular connective tissues on the distribution of fiber mean sarcomere length (Huijing and Baan, 2001b; Huijing et al., 2003). Interfering with the connective tissues surrounding EDL muscle (i.e. blunt dissection of the EDL-TA interface and extramuscular fasciotomy of the anterior crural compartment) changed EDL length-proximal force curves in a way compatible with an increased distribution of fiber mean sarcomere length. In another study (Yucesoy et al., 2002b), length-distal force characteristics of EDL muscle were measured in two conditions: (a) after full lateral fasciotomy of the anterior crural compartment (i.e. intact intermuscular connective tissues and extramuscular connective tissue supporting the nerves and blood vessels of EDL muscle) and (b) after removal of TA and EHL muscles (i.e. intact extramuscular connective tissue of the neurovascular tract of EDL muscle exclusively). It was found that the length range between active slack length and optimum length was higher for the first condition (a) compared to the second one (b). Finite element modeling of both

conditions indicated that the increased length range of active force exertion could be explained by a higher distribution of fiber mean sarcomere length among muscle fibers. These findings are in agreement with the results of the present study that extramuscular connections affect the distribution of fiber mean sarcomere length.

Experimental studies are needed to confirm the finite element model results of the present study. Fiber mean sarcomere lengths have been calculated for a limited number of locations in dissected rat muscle by dividing fiber length by the number of sarcomeres in series of fibers at those locations (e.g. Willems and Huijing, 1994). In humans, fiber length can also be measured non-invasively, using ultrasonography (e.g. Fukunaga et al., 1997). Distributions of strain within the muscle have also been confirmed experimentally for dissected rat muscle *in situ* (Savelberg et al., 2001; Van Bavel et al., 1996), using a number of fluorescent spheres on the muscle. For different human muscles *in vivo* this has been done as well (Maas et al., 2003c; Pappas et al., 2002), using magnetic resonance imaging (MRI). For investigations regarding effects of muscle relative position on the distribution of lengths of sarcomeres within a muscle, MRI scanning of muscle within its *in vivo* context of connective tissue is indicated. If possible, this should be combined with measurements of tendon forces.

The proximo-distal force difference is associated with distributions of lengths of sarcomeres

A difference between isometric force exerted at the proximal and at the distal tendons of a muscle, as found in the present study, indicates that an additional force must act on the muscle. As a result of force equilibrium, this net force is equal to the proximo-distal force difference. Inter- and extramuscular connective tissues appear to have sufficiently stiff connections to intramuscular connective tissue (Huijing and Baan, 2001b; Huijing et al., 2003; Maas et al., 2001). Muscle fibers are linked to this connective tissue network via trans-sarcolemmal linkages between cytoskeleton, extracellular matrix and endomysium (for review see Berthier and Blaineau, 1997; Patel and Lieber, 1997). It has been indicated that that these myofascial connections are capable of bearing force (Goldberg et al., 1997; Huijing, 1999b; Huijing et al., 1998; Jaspers et al., 2002; Jaspers et al., 1999; Monti et al., 2001; Monti et al., 1999; Street, 1983; Street and Ramsey, 1965). Therefore, this additional force is expected to affect distributions of length of active (i.e. sarcomeres) and/or passive components

(i.e. epimysium, perimysium and endomysium, trans-sarcolemmal linkages, non-contractile proteins within the sarcomeric cytoskeleton, such as titin) within muscle.

The present results showed that the difference between total force exerted at the proximal and distal tendons of a muscle is accompanied by differences of lengths of sarcomeres within muscle fibers (Fig. 3). If distal total force is higher than proximal total force (Δ EDL muscle position = 0 mm, Fig. 2), sarcomeres located distally within muscle fibers attained higher lengths than sarcomeres proximally within muscle fibers (Fig. 3). However, due to the fact that the length of sarcomeres near the distal aponeurosis was well beyond optimum length (maximal strain = 0.41), force is exerted at the distal tendon by active sarcomeres, but also by intracellular passive elements (e.g. cytoskeleton, titin) as well as by the extracellular matrix. As the lengths of sarcomeres near the proximal aponeurosis were below optimum length (minimal strain = –0.26), force exerted at the proximal aponeurosis is the result predominantly of active force. Moreover, for the most distal EDL muscle relative position, model results show that only 11% of the proximo-distal force difference originates within the fiber mesh and 89% originates from the matrix mesh. Thus, for this high EDL length, the extracellular matrix is the most important source of the proximo-distal force difference. It is likely that the relative contribution of intracellular and extracellular structures is length dependent. In conclusion, the present results indicate that the higher distal force compared to proximal force was born both intracellularly (sarcomeres and intracellular passive elements) and extracellularly (extracellular matrix).

Repositioning EDL muscle in proximal direction decreased the proximo-distal force difference (Fig. 2). Force exerted at the proximal aponeurosis increased as well as the length of sarcomeres proximally within muscle fibers. All sarcomeres located proximally within muscle fibers attained lengths beyond their optimum length (Fig. 3). As a consequence, intracellular passive elements as well as the extracellular matrix will exert force at the proximal aponeurosis. Thus the increase of proximal force is the result of an increase of active as well as passive force. Moreover, model results for the most proximal EDL muscle relative position (Δ EDL muscle position = 9 mm) show that 43% of the increase of force exerted at the proximal aponeurosis originated intracellularly (sarcomeres and intracellular passive elements) and 57% within the extracellular matrix.

Simultaneously, the length of sarcomeres located distally within muscle fibers

decreased towards optimum length. Consequently, fiber mesh normalized force exerted at the distal aponeurosis increased (from 0.71 to 0.88) but matrix mesh normalized force decreased (from 0.29 to 0.01). These length changes of intracellular components and extracellular matrix explain the finding that the decrease of distal EDL normalized force (i.e. from 1 to 0.89) was lower than the increase of proximal EDL normalized force (from 0.65 to 0.95).

It is concluded that changes of the proximo-distal force difference as a function of EDL muscle relative position are accompanied by changes of the length of sarcomeres as well as changes of the length of extracellular matrix elements.

CONCLUSIONS AND IMPLICATIONS

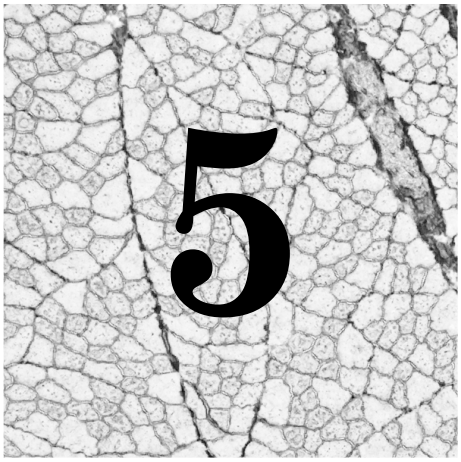
The position of a muscle relative to surrounding connective tissues alters the distribution of lengths of sarcomeres within muscle. In the present study, these position effects are mediated by the intact extramuscular connective tissues. In previous experiments (Huijing and Baan, 2003; Maas et al., 2002a), position effects were investigated within the anterior crural compartment, leaving the connective tissue at the muscle bellies of TA, EHL and EDL muscles intact. In addition to extramuscular myofascial force transmission, intermuscular myofascial force transmission was allowed. Based on the results of the present experiment, it is concluded that also for those studies, the distribution of lengths of sarcomeres was affected by muscle relative position.

To investigate the range of sarcomere lengths that is used when human muscles operated *in vivo*, laser diffraction has been used to measure sarcomere length directly (Lieber et al., 1997; Lieber et al., 1994). The main limitations are (a) that sarcomere length is measured of a bundle of fibers, which is dissected free from surrounding connective tissues and (b) that sarcomere length is measured of a small region of the fibers only. The present results demonstrated that the connective tissues surrounding muscle fibers and whole muscle affect the length of sarcomeres within muscle. Furthermore, it was shown that due to myofascial force transmission the length of sarcomeres in series within muscle fibers varies considerably. Therefore, non-invasive techniques should be applied to measure accurately the *in vivo* range of sarcomere lengths.

In vivo, joint movements involve position changes of synergists relative to each other due to (a) differences in moment arm between muscles and (b) the fact that

some muscles span only one joint (i.e. mono-articular muscles) and other muscles span more than one joint (i.e. bi- or poly-articular muscles). The position of (parts of) any muscle relative to non-muscular fixed structures within a compartment (e.g. bone, fascia) will also be altered with each change of joint angle. This indicates that also *in vivo*, effects of muscle relative position on force transmission as well as on the distribution of the length of sarcomeres within muscle may play an important role functionally. Accordingly, experiments that investigate or model muscle properties *in vivo* should consider the role of muscle relative position.

Myofascial force transmission between a single head of multi-tendoned muscle and adjacent tissues: length effects of head III of rat EDL muscle



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Journal of Applied Physiology, **In press**

ABSTRACT

Force transmission from muscle fibers via the connective tissue network (i.e. myofascial force transmission) is an important determinant of muscle function. This study investigates the role of myofascial pathways for force transmission from multi-tendoned EDL muscle within an intact anterior crural compartment. Effects of length changes exclusively of head III of rat extensor digitorum longus muscle (EDL III) on myofascial force transmission were assessed. EDL III was lengthened at the distal tendon. For different lengths of EDL III, isometric forces were measured at the distal tendon of EDL III, as well as at the proximal tendon of whole EDL and at the distal tendons of tibialis anterior and extensor hallucis longus muscles (TA+EHL).

Lengthening of EDL III caused high changes in force exerted at the distal tendon of EDL III (from 0 to 1.03 ± 0.07 N). In contrast, only minor changes were found in force exerted at the proximal EDL tendon (from 2.37 ± 0.09 N to 2.53 ± 0.10 N). Increasing the length of EDL III decreased TA+EHL force significantly (by 7%, i.e. from 5.62 ± 0.27 N to 5.22 ± 0.32 N). These results show that force is transmitted between EDL III and adjacent tissues via myofascial pathways.

Optimal force exerted at the distal tendon of EDL III (1.03 ± 0.07 N) was more than twice the force expected on the basis of the physiological cross-sectional area of EDL III muscle fibers (0.42 N). Therefore, a substantial fraction of this force must originate from sources other than of EDL III.

It is concluded that myofascial pathways play an important role for force transmission from multi-tendoned muscles.

INTRODUCTION

Several human tasks, such as typing on a keyboard and playing a musical instrument, require movements of fingers relative to each other. In general, multi-tendoned muscles of the forearm contribute to finger movements. In both humans (Kilbreath and Gandevia, 1994) and monkeys (Schieber, 1991) it has been shown that it is hardly possible to move a single digit without movements of adjacent digits. Furthermore, voluntary exertion of force by one finger is in most cases accompanied by force exertion by the non-target fingers (Kilbreath et al., 2002; Li et al., 2000; Zatsiorsky et al., 2000).

It has been hypothesized that mechanical interactions at the level of the muscle belly could be one of the factors limiting the independent control of the position and force of the fingers (Maas et al., 2001). Previous studies on fully dissected rat extensor digitorum longus muscle (EDL), a multi-tendoned muscle with distal insertions on digit II to V within the foot (Balice-Gordon and Thompson, 1988), yielded clear evidence of transmission of force between muscle heads via connective tissues at their interfaces (Huijing et al., 1998; Jaspers et al., 2002). Within a muscle there are several pathways via which force generated by a sarcomere is transmitted to the tendon (for reviews see Huijing, 1999b, 1999a; Monti et al., 1999). (a) Via sarcomeres arranged in series within a muscle fiber and the myotendinous junction (i.e. myotendinous force transmission). (b) Via complexes of structural proteins connecting parallel sarcomeres onto the subsarcolemmal cytoskeleton and endomysium (Berthier and Blaineau, 1997; Patel and Lieber, 1997). From the endomysium force is transmitted to neighboring fibers or directly onto the aponeurosis (i.e. intramuscular myofascial force transmission).

In vivo, skeletal muscles are surrounded by synergists and embedded within the connective tissues of a compartment, which comprises (a) intermuscular connective tissue (i.e. connective tissue at the interface between muscle bellies) as well as (b) extramuscular connective tissues (e.g. connective tissue that supports the nerves and blood vessels and fascia representing the compartmental borders). Recent experiments on rat EDL muscle (Huijing and Baan, 2001a; Maas et al., 2001) have shown that inter- and extramuscular connective tissues may be the pathways for transmission of a substantial fraction (up to 37%) of force to or from the muscle. Such transmission is referred to as inter- and extramuscular myofascial force transmission. In those experiments, whole EDL muscle was studied and the four

heads of EDL muscle were manipulated as one unit. However, movements of digits relative to each other require the individual heads of a multi-tendoned muscle to be active at different lengths and thus to move with respect to each other.

In the present study, effects of changing the length of only one head of rat EDL muscle (head III, referred to as EDL III) on myofascial force transmission are studied. For different lengths of EDL III, isometric forces were measured at the distal tendon of EDL III as well as at the proximal tendon of whole EDL and at the distal tendons of tibialis anterior (TA) and extensor hallucis longus muscles (EHL).

We tested the following hypotheses: (a) force is transmitted via myofascial pathways to the distal tendon of EDL III; (b) changes in force exerted at the proximal tendon of whole EDL muscle as EDL III is lengthened are not equal to changes of force exerted at the distal tendon of EDL III; (c) force exerted by synergistic muscles (i.e. TA and EHL) is affected by length changes of EDL III.

METHODS

Surgical and experimental procedures were in strict agreement with the guidelines and regulations concerning animal welfare and experimentation set forth by Dutch law, and approved by the Committee on Ethics of Animal Experimentation at the Vrije Universiteit.

Surgical procedures

Male Wistar rats (n = 6, body mass = 305.2 ± 6.8 g) were anaesthetized using intraperitoneally injected urethane (1.2 ml/ 100 g body mass 12.5 % urethane solution, w/v, extra doses were given if necessary: maximally 1.5 ml). To prevent hypothermia during surgery and data collection, the animals were placed on a heated water pad of approximately 37°C. Ambient temperature (22 ± 0.5 °C) and air humidity (80 ± 2%) were kept constant by a computer controlled air-conditioning system (Holland Heating, Waalwijk, The Netherlands). Dehydration of muscle and tendon tissue was prevented by regular irrigation with isotonic saline.

Removing the skin and most of the biceps femoris muscle from the left hind limb exposed the anterior crural compartment, which envelopes the tibialis anterior (TA), extensor digitorum longus (EDL) and extensor hallucis longus (EHL) muscles. Connective tissues of the compartment at the muscle bellies of TA, EHL and EDL as well as the retinaculae at the ankle (i.e. transverse crural ligament and the crural

cruciate ligament) were left intact.

In the foot, the distal tendon of head III of EDL muscle (EDL III) was dissected free from surrounding tissues. Limited crural fasciotomy was performed only distally to reach the distal tendons of TA and EHL muscles. The distal tendons of TA and EHL muscles are, for a substantial part of their length quite close to one another. As it is difficult to measure force exerted at each tendon individually without friction between them, the distal tendons of TA and EHL were tied together using polyester thread, with the ankle joint at 90°. This complex of muscles will be referred to as the TA+EHL complex.

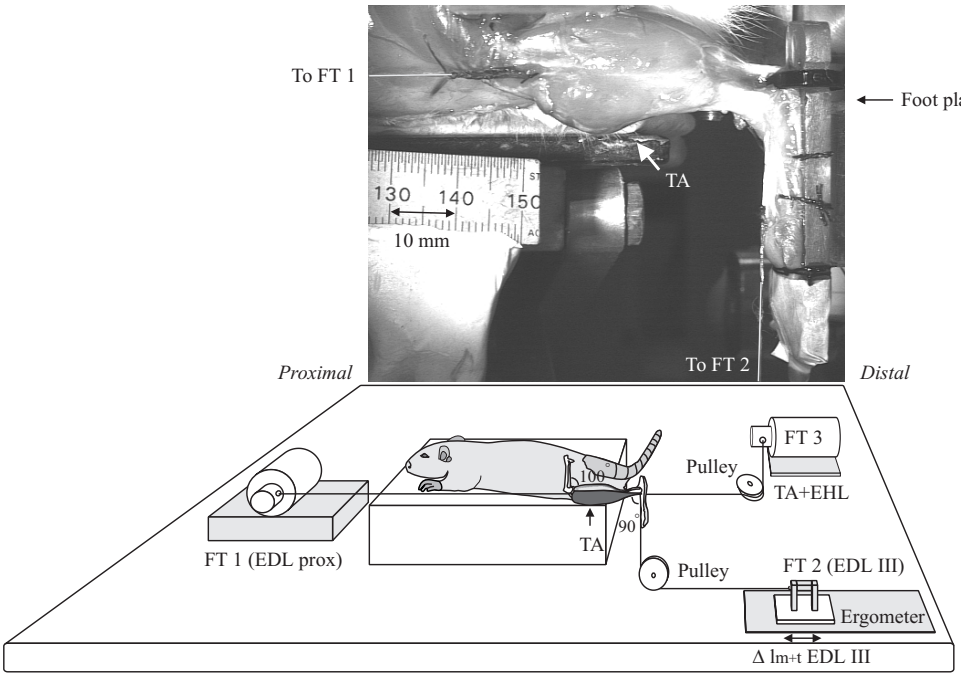

Fig. 1. Schematic representation of the experimental set-up. FT1 indicates the force transducer connected to the proximal tendon of EDL muscle, FT2 indicates the force transducer mounted on a multipurpose muscle ergometer connected to the distal tendon of EDL III, FT3 indicates the force transducer connected to the tied distal tendons of TA and EHL muscles. Kevlar thread was used to connect the muscles to force transducers. A low friction pulley guided the Kevlar thread from TA+EHL to FT3 that, for reasons of space, was placed perpendicular to the other force transducers. The proximal tendon of EDL muscle was connected directly to the force transducer, which was positioned in the line of pull. The distal tendon of EDL III was connected to the force transducer via a low friction pulley. Various muscle-tendon complex lengths of EDL III were obtained by repositioning FT2, as indicated by the double arrow. A lateral view of the experimental set-up, zoomed in on the lower leg of the rat left hind limb, is shown as backdrop. Bar = 10 mm. As EDL and EHL muscles are enclosed by TA muscle, only the latter muscle is visible.

The distal tendon of EDL III and the distal tendons of the TA+EHL complex were cut and Kevlar threads (diameter = 0.5 mm, tensile modulus = 58 GPa, 3.7% extension to breaking; Goodfellow, Cambridge, England) were tied to them. The foot was attached to a plastic plate with tie wraps. The femoral compartment was opened in order to (a) cut a small piece of the lateral epicondyle of the femur (i.e. the origin of EDL muscle) for attaching it to Kevlar thread, (b) to secure a metal clamp to the femur for later fixation at a knee angle of 100° in the experimental apparatus, and (c) to dissect the sciatic nerve.

Within the femoral compartment, the sciatic nerve branches into the tibial nerve, the sureal branch and the common peroneal nerve. The common peroneal nerve enters the anterior crural compartment from the peroneal compartment through a fenestration within the anterior intermuscular septum (Huijing and Baan, 2001b). Branches of the intact common peroneal nerve innervate EDL, TA and EHL muscles as well as the muscles in the peroneal compartment. The tibial nerve, the sureal branch as well as all other more proximal branches of the sciatic nerve were cut. The sciatic nerve, with only the common peroneal nerve branch left intact, was dissected and cut as proximally as possible.

Mounting the animal in the experimental apparatus

The rat was placed on a platform. The metal clamp was used to secure the femur at a knee angle of 100°. The plastic footplate was positioned in such a way that the ankle angle was 90° (Fig. 1). Using the Kevlar threads, the proximal tendon of EDL muscle as well as the distal tendons of the TA+EHL complex were connected to force transducers (maximal output error < 0.1 %, compliance of 0.0048 mm/N; Hottinger Baldwin, Darmstadt, Germany) mounted on single axis micropositioners. The distal tendon of EDL III was also connected to a force transducer (compliance 0.014 mm/N) (Woittiez et al., 1987). The other distal tendons of EDL muscle were left attached to their insertions on the digits (i.e. II, IV and V) within the foot. For TA+EHL force measurements, the Kevlar thread was connected to the force transducer via a low friction pulley that, because of limited space, was placed perpendicular to the other force transducers. For EDL III, the retinaculae at the ankle causes the line of pull to be in parallel with the foot. To connect the Kevlar thread to the force transducer on the ergometer, an additional pulley was needed. The proximal tendon of EDL muscle was connected directly to the force transducer, which was

positioned in the line of pull.

The sciatic nerve, with only the common peroneal nerve branch left intact, was placed in a pair of silver electrodes. The nerve was prevented from dehydration, by covering it with paper tissue saturated with isotonic saline covered by a thin piece of latex. In all experimental conditions, the sciatic nerve was stimulated supramaximally using electrodes connected to a constant current source (3 mA, pulse width 100 μs).

Isometric length-distal force characteristics of EDL III

The length of the TA+EHL complex (corresponding to an ankle joint at 90°) was kept constant. This was also the case for the position of the proximal tendon of EDL muscle (corresponding to a knee joint at 100°). In addition, the distal tendons of EDL head II, IV, and V were left attached to their insertions. Therefore, their lengths were also kept constant.

Length-distal force characteristics of EDL III were assessed. Isometric force was measured at various lengths of EDL III. EDL III was lengthened at its distal tendon (Fig. 1) with 1 mm increments starting at active slack length (i.e. the lowest length at which active force approaches zero) until approximately 2 mm over optimum length. Subsequently, measurements of length-force characteristics of EDL III were repeated two times to assess the reproducibility.

Prior to excitation of the muscles, EDL III was brought to the target length. Two twitches were evoked, followed by a tetanic contraction of the muscles after 500 ms (pulse train 400 ms, frequency 100 Hz). Muscle force was measured just prior to the tetanic contraction (i.e. passive force) and during the tetanic plateau (i.e. 275 ms after evoking tetanic stimulation, total force). Simultaneously, images of the anterior crural compartment with muscles in the passive and active state were recorded using a digital camera (DVC, JAI CV-M10, shutter speed 1/50 s). Timing of stimulation of the nerve, A/D conversion (12-bit A/D converter, sampling frequency 1000 Hz, resolution of force 0.01 N), and photography were controlled by a special-purpose microcomputer. After each contraction EDL III was allowed to recover near active slack length for 2 minutes.

Treatment of isometric length-force data

The individual data for length of the muscle-tendon complex (l_{m+t}) and passive muscle force (F_{mp}) were fitted with an exponential curve (equation 1), using a least-squares criterion:

$$y = e^{a_1 + a_2 x} \quad (1)$$

where y represents F_{mp}, x represents l_{m+t}, and a_1 and a_2 are coefficients determined in the fitting process. For each muscle-tendon complex length studied, active muscle force (F_{ma}) was assessed by subtracting fitted F_{mp} from total muscle force (F_m). These length–active force data were fitted by a polynomial (equation 2):

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

where y and x represent F_{ma} and l_{m+t} respectively, and b_0 through b_n are coefficients determined in the fitting process. The order of the polynomial most adequately describing the relationship was selected (see statistics). Fitted curves were used to calculate mean data and standard errors of the mean (SE) as well as to determine optimal force and optimum muscle-tendon complex length. Muscle-tendon complex length was expressed as the deviation from active slack length (Δl_{m+t}).

Although forces exerted at the distal tendons of EDL III and the TA+EHL complex are in opposite direction to forces exerted at the proximal tendon of EDL, all forces will be presented as positive values.

Morphology of EDL muscle

After the experimental measurements, the masses of the individual EDL heads were measured and expressed as a percentage of total muscle mass. As the number of sarcomeres in series within fibers of different heads has been shown to be rather similar (Huijing et al., 1998), values of normalized mass may be considered as adequate estimates of normalized physiological cross-sectional area and, thus, of relative contribution of individual heads to total EDL active muscle force. Mean and standard error of the mean (SE) were calculated.

Two additional animals (body mass = 304 and 324 g, respectively) were used to study the morphological characteristics of EDL muscle. EDL muscle was excised

from the left hind limb. Intramuscular fasciotomy was performed to separate the heads of EDL muscle. Images were made of the heads and their corresponding distal aponeuroses and tendons. Prior to cross-sectioning, a layer of graphite powder was applied at the interface between the different muscle heads. The distal tendons were tied together and the muscle, kept at a constant length, was fixed in a buffered formaldehyde solution (0.4% formaldehyde, v/v). The muscle was dehydrated in a series of buffered ethanol dilutes (70% – 95% ethanol, v/v) and embedded (70-2218-500 Historesin Embedding Kit, Jung, Heidelberg, Germany). Subsequently, serial transverse sections (10 mm) of the midbelly region were cut using a microtome (type K, HK3 knife, Reichert-Jung, Heidelberg, Germany). The sections were stained for 2 min in a 0.02% (w/v) Toluidine Blue (Merck Art. 1273, Amsterdam, The Netherlands) solution and finally sealed with a cover slip using Entellan (Merck Cat.No. 1.07961, Amsterdam, The Netherlands).

Statistics

For fitting the length-active force data, a stepwise polynomial regression procedure was used. In this procedure, the curve fit is determined by increasing the order of the polynomial as long as this yields a significant improvement to the description of the length-active force data, as determined by one-way analysis of variance (ANOVA).

To test for effects of EDL III length on force exerted at the proximal tendon of whole EDL muscle, at the distal tendon of EDL III force as well as at the distal tendons of TA+EHL, one-way ANOVA's for repeated measures were performed (factor: l_{m+t} EDL III). Two-way ANOVA for repeated measures (factors: l_{m+t} EDL III and repetition of length-force assessment) was performed to test for differences between subsequent measured length-force curves. If significant effects were found, Bonferroni post-hoc tests were performed to locate significant differences. P values < 0.05 were considered significant.

RESULTS

Morphological characteristics of EDL muscle

Fig. 2A shows the four EDL heads, which are separated partially after dissection. Muscle fibers of these heads insert distally to different aponeuroses and tendons and proximally to a common aponeurosis and tendon.

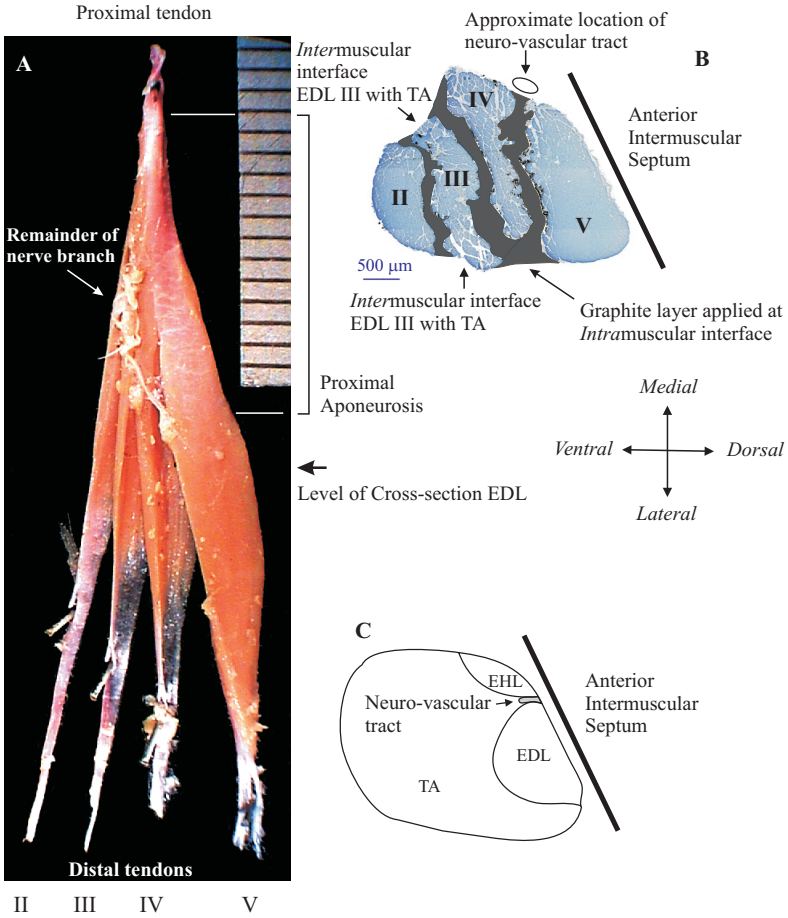

Fig. 2. Morphology of multi-tendoned EDL muscle of the rat. (A) Muscle fibers of four muscle heads insert distally onto different aponeuroses and tendons and proximally onto a common aponeurosis and tendon. The heads are named after their insertions on digit II to V of the toes. The distal aponeuroses are a continuation of the distal tendons and located at the opposite site of the muscle bellies. A remainder of the nerve branch innervating EDL muscle is indicated (arrow). Within an intact EDL muscle, these muscle heads are connected to each other by intramuscular connective tissue. Therefore, intramuscular fasciotomy was performed to make the different heads distinguishable in the figure. The approximate level of cross-section (B) is indicated. The division of the ruler shown represents 1 mm. (B) Distal view of a cross-section of EDL muscle after the muscle heads were separated using a layer of graphite powder. The section (thickness = 10 µm) was stained with a Toluidine Blue solution. It should be noted that within an intact EDL muscle, the layer of connective tissue between muscle heads is rather thin. The thickness of the graphite layer between muscle heads as shown in the cross-section is caused by the applied method. It shows that head III shares intramuscular connective tissue with head II, IV and V. EDL III interfaces intermuscularly with TA. Furthermore, EDL muscle is connected to non-muscular structures of the anterior crural compartment via extramuscular connective tissues (e.g. connective tissue that supports the nerves and blood vessels of the compartment). The approximate location of the neuro-vascular tract and the anterior intermuscular septum is indicated. Bar = 500 µm. (C) A schematic outline of the muscles within the anterior crural compartment as well as the location of the neurovascular tract and the anterior intermuscular septum. Anatomical orientation for B and C is indicated (cross of arrows).

The cross-section of EDL muscle (Fig. 2B) shows that, within EDL muscle, head III shares intramuscular connective tissue predominantly with head II and head IV. However, laterally within EDL muscle, the injected graphite layer indicates that head III is also connected to head V. It is shown also that EDL III shares intermuscular connective tissue with TA muscle. Note that the surface of the intermuscular TA-EDL III interface is considerably smaller than the intramuscular interface of EDL III. In addition, EDL muscle is connected to non-muscular structures of the anterior crural compartment (e.g. bone, fascia, anterior intermuscular septum) via extramuscular connective tissues (e.g. connective tissue that supports the neuro-vascular tract of the compartment).

The images of EDL muscle show that the cross-sectional area of head II, III, and IV is smaller than of head V. Physiological cross-sectional area of head III of EDL muscle, normalized for the physiological cross-sectional area of whole EDL, was estimated to be only 16 ± 2.2 %, whereas head II, IV and V represent 20 ± 1.4 %, 19 ± 1.8 %, and 45 ± 0.6 %, respectively.

Acute effects of changes of EDL III length

EDL proximal force (Fig. 3A). ANOVA revealed significant effects of EDL III length on active and passive force exerted at the proximal tendon of whole EDL. For low lengths of EDL III, a small passive force ($F_{mp,\,min}$ = 0.02 ± 0.00 N, right axis in Fig. 3A) was exerted at the proximal tendon. Proximal passive EDL force increased exponentially as a function of EDL III length ($F_{mp,\,max}$ = 0.10 ± 0.03 N). Proximal active EDL force increased also as a function of EDL III length: from 2.37 ± 0.09 N (at $\Delta\, l_{m+t}$ = 0 mm) to a maximum of 2.53 ± 0.10 N (at $\Delta\, l_{m+t}$ = 5.9 mm). A further increase of EDL III length caused a decrease in proximal active EDL force (to 2.36 ± 0.15 N). These results show that substantial changes of length of EDL III ($\Delta\, l_{m+t}$ = 9 mm) cause only minor effects on isometric force exerted at the proximal tendon of EDL muscle.

Distal EDL III force (Fig. 3B). ANOVA revealed significant effects of EDL III length on passive and active forces exerted at the distal tendon of EDL III. Passive force was zero at low EDL III lengths and increased exponentially after lengthening EDL III ($F_{mp,\,max}$ = 0.17 ± 0.04 N). EDL III optimum length deviated 8.3 mm from active slack length ($\Delta\, l_{m+t}$ EDL III = 0 mm) and optimal force was 1.03 ± 0.07 N.

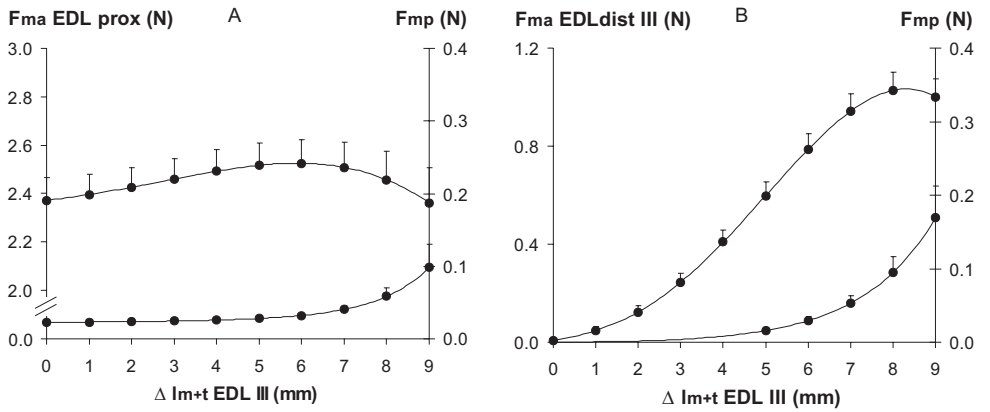

Fig. 3. Length-distal force characteristics of EDL III and the simultaneously measured proximal EDL force. (A) Active (F_{ma}) and passive (F_{mp}) forces exerted at the proximal EDL tendon plotted as a function of muscle-tendon complex length (Δl_{m+t}) of EDL III. (B) Length-distal force characteristics of EDL III. Note that different y-axes with different scaling factors are shown for active (left axis) and passive force (right axis). Values are shown as mean ± SE (n = 6).

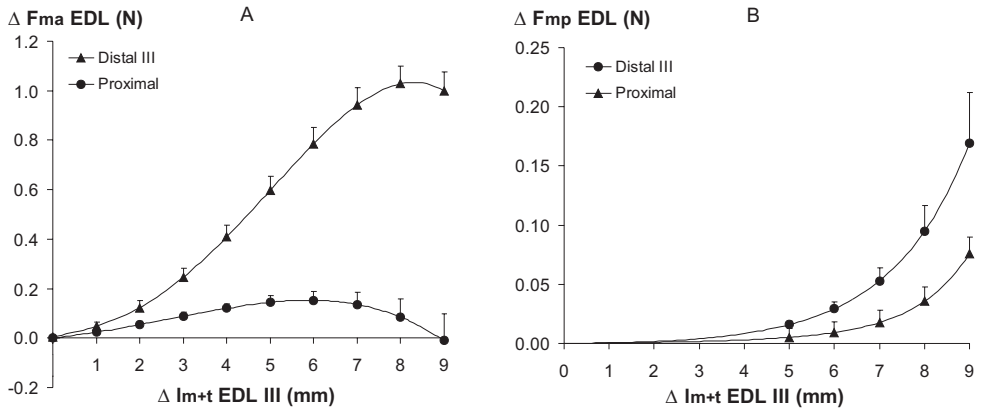

Fig. 4. A comparison of changes in proximal EDL force and EDL III distal force. (A) Changes of active (F_{ma}) force and (B) changes of passive (F_{mp}) force, both plotted as a function of muscle-tendon complex length (Δl_{m+t}) of EDL III. Forces are expressed as the deviation from the initial value (i.e. measured at active slack length of EDL III, i.e. Δl_{m+t} EDL III = 0 mm): for proximal EDL F_{mp} = 0.02 ± 0.00, F_{ma} = 2.37 ± 0.09 N, for EDL III F_{mp} and F_{ma} are zero. Values are shown as mean ± SE (n = 6).

A comparison between changes of proximal EDL force and of distal EDL III force, both expressed as a function of EDL III length, reveals three remarkable features (Fig. 4). (a) Between Δl_{m+t} EDL III = 0 mm and 5.9 mm (i.e. the length of EDL III that corresponds to maximal force of proximal EDL), proximally measured active EDL force increased by only 0.15 ± 0.03 N (Fig. 4A), while for the same EDL

III length range, force exerted at the distal tendon of EDL III increased by 0.77 ± 0.06 N (i.e. 5 times higher than the increase of proximal EDL force). (b) The increase of EDL III passive force (ΔF_{mp} = 0.17 ± 0.04 N) was also significantly higher than the increase of proximally measured EDL passive force (ΔF_{mp} = 0.08 ± 0.03 N) (Fig. 4B). (c) Maximal proximal EDL active force was found at lower length of EDL III (Δ l_{m+t} = 5.9 mm) than optimal force of EDL III (Δ l_{m+t} = 8.3 mm). As a consequence, proximal EDL force is decreasing while distal EDL III force is still increasing on lengthening EDL III (from Δ l_{m+t} = 5.9 mm to 8.3 mm).

Thus, length changes of EDL III cause different effects on proximal EDL and distal EDL III forces. If force transmission between EDL III and adjacent tissues was absent, changes of force exerted at the proximal tendon of EDL would equal the changes of force exerted at the distal tendon of EDL III. Therefore, these results show that force is transmitted via intra-, inter- and/or extramuscular myofascial pathways from or to EDL III.

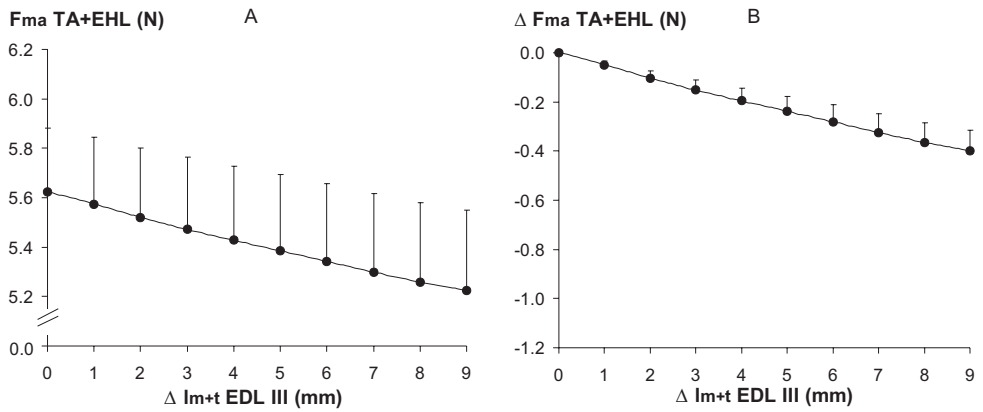

Fig. 5. Changes of distally measured active force of the TA+EHL complex, kept at constant muscle-tendon complex length, after length changes of EDL III. (A) Absolute values of active forces (F_{ma}) and (B) forces expressed as the deviation (ΔF_{ma}) from the initial value (i.e. measured at active slack length of EDL III, Δ l_{m+t} = 0 mm): F_{ma} = 5.62 ± 0.27 N. In both graphs force is plotted as a function of EDL III muscle-tendon complex length (Δ l_{m+t}). Values are shown as mean ± SE (n = 6). The high standard errors in A are caused by differences in the initial level of TA+EHL force. Note that the standard errors of the change of TA+EHL force (ΔF_{ma}), as shown in B, are decreased substantially.

TA+EHL complex force (Fig. 5). ANOVA revealed significant effects of EDL III length on active force exerted at the distal tendons of TA+EHL. Since the TA+EHL complex was kept at a constant moderate length, passive force exerted by the

TA+EHL complex remained negligible. Increasing the length of EDL III decreased TA+EHL force significantly (i.e. from 5.62 ± 0.27 N to 5.22 ± 0.32 N) (Fig. 5A). These results indicate mechanical interactions between EDL III and the TA+EHL complex.

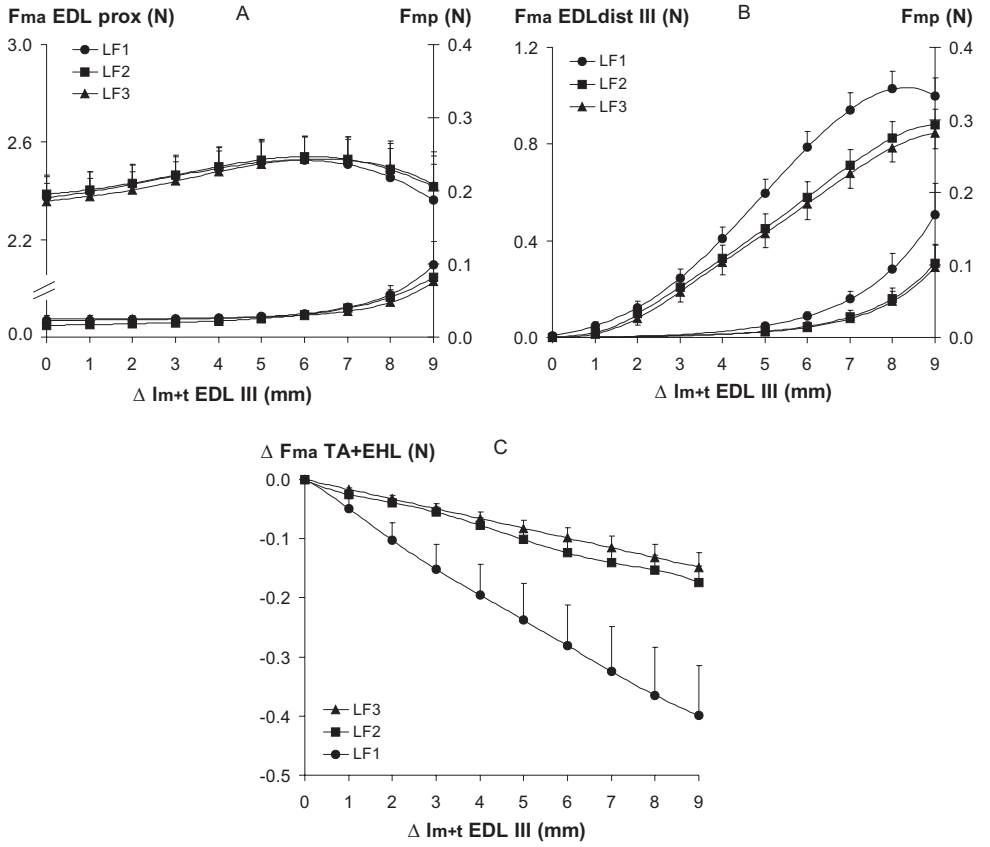

Fig. 6. Effects of repeated length-force measurements of EDL III on proximal EDL force, distal EDL III force and distal TA+EHL force. (A) Active and passive EDL proximal forces plotted as a function of EDL III muscle-tendon complex length (Δ l_{m+t}) of repeated measurements (LF1, LF2 and LF3). (B) Active and passive forces exerted at the distal tendon of EDL III plotted as a function of EDL III muscle-tendon complex length. (C) Distally measured active forces of the TA+EHL complex plotted as a function of EDL III muscle-tendon complex length. Note that TA+EHL force is expressed as the deviation from the initial value (i.e. F_{ma} LF1 = 5.62 ± 0.26 N, F_{ma} LF2 = 5.29 ± 0.32 N, F_{ma} LF3 = 5.21 ± 0.34 N, measured at Δ l_{m+t} EDL III = 0 mm). F_{ma} and F_{mp} represent active and passive force, respectively. Note that in A and B different y-axes with different scaling factors are shown for active force (left axis) and passive force (right axis). Values are shown as mean ± SE (n = 6).

Repeated isometric length-force measurements of EDL III

EDL proximal force (Fig. 6A). For active and passive forces, ANOVA indicated no significant differences between repeated measurements on proximal EDL force curves. Therefore, it is concluded that proximal EDL force expressed as a function of EDL III length is reproducible.

Distal EDL III force (Fig. 6B). In contrast to proximally measured EDL force, length-force characteristics of EDL III were altered significantly in subsequent length-force measurements. Passive force of EDL III measured at high lengths (Δ l_{m+t} EDL III = 7 to 9 mm) was decreased significantly subsequent to measurements for the first length-force curve. Particularly at higher lengths (Δ l_{m+t} EDL III = 5 to 9 mm), active forces differed significantly. In contrast, for the lower lengths (Δ l_{m+t} EDL III = 0 to 4 mm), no significant differences in active force were shown. Furthermore, EDL III optimum length was shifted to a higher length (at least by 0.7 mm), whereas active slack length was not changed. A subsequently measured length-force curve (i.e. LF3) was not significantly different from the previous one (i.e. LF2).

These results indicate that previous isometric contractions, particularly at high lengths of EDL III, modify the length-distal force characteristics of EDL III. As muscle fibers of head III insert also to the proximal aponeurosis of EDL muscle, it is remarkable that these effects were not measured at the proximal tendon of EDL muscle. This is another indication of effects of myofascial force transmission between EDL III and adjacent tissues.

TA+EHL complex force (Fig. 6C). ANOVA revealed significant effects of EDL III length-force measurements on active force of the TA+EHL complex. The slope of the EDL III length – TA+EHL force curve decreased subsequent to measurements for the first length-force curve of EDL III. As a consequence, the decrement of TA+EHL force after lengthening EDL III by 9 mm was less pronounced, but significant, during the measurements for the second and third length-force curves of EDL III (i.e. 0.17 ± 0.03 N and 0.15 ± 0.03 N, respectively) if compared to the force decrement during the first measurement (i.e. 0.40 ± 0.08 N).

These results indicate that the degree of intermuscular interaction between EDL III and TA+EHL was decreased. As such interaction is attributed to myofascial force transmission, this is explained by a decreased stiffness of the structures representing the myofascial pathways between the muscle belly of EDL III and the muscle bellies of TA and EHL muscles.

DISCUSSION

The most important results of the present study are that (a) changes in isometric force exerted at the proximal tendon of whole EDL muscle as function of EDL III length are not equal to changes in force exerted at the distal tendon of EDL III, (b) isometric force exerted at the distal tendons of TA+EHL decreased significantly after lengthening of EDL III, and (c) isometric activity, particularly at high lengths of EDL III, changed EDL III length-force characteristics as well as TA+EHL force, whereas no such changes of proximal EDL force were found.

These results are explained in terms of myofascial force transmission between EDL III and adjacent tissues. Excitation of a muscle leads to shortening of sarcomeres until active force exerted by cross-bridges between the actin and myosin filaments together with passive force exerted by intra-sarcomeric passive elements (e.g. titin) is equal to the sum of opposing forces (i.e. the reaction force). The reaction force may originate from (a) sarcomeres in series, which are linked to each other by the Z-lines, or from (b) the endomysium, which is linked to the subsarcolemmal cytoskeleton and the sarcomeres via complexes of structural proteins (i.e. the myofascial pathway). Proteins that may be involved have been reviewed recently (Berthier and Blaineau, 1997; Patel and Lieber, 1997). Within a muscle, force exerted at a particular location of the endomysium can in principle be transmitted onto the tendon (a) via neighboring fibers and their myotendinous junction or (b) via the endomysial-perimysial network.

If a muscle is isolated from surrounding tissues, muscle force can only be transmitted via the muscular origin and insertion onto bone. *In vivo*, however, muscles are embedded within connective tissues of a compartment and surrounded by synergists. It has been shown that muscle force exerted at the endomysial-perimysial network is also transmitted via intermuscular connective tissue onto the intramuscular connective tissue network of adjacent muscles (Huijing, 1999a; Maas et al., 2001, 2003a). In addition, force is transmitted between muscle and extramuscular connective tissues (Huijing and Baan, 2001a; Huijing et al., 2003; Maas et al., 2003d). Effects of inter- and extramuscular myofascial force transmission on muscle properties have been reviewed recently (Maas et al., 2003c).

In the present study, a surprisingly high optimal force of EDL III was found (i.e. 1.03 ± 0.07 N). In a previous study, optimal force for whole EDL muscle of rats with similar body mass was 2.60 ± 0.14 N (Maas et al., 2001). If a comparable optimal

EDL force is assumed for the present study, at optimum length of EDL III 40% of active force of whole EDL muscle is exerted at the distal tendon of EDL III. This is in sharp contrast with the value for relative physiological cross-sectional area of EDL III, which was only 16 ± 2.2 % of whole EDL muscle. Therefore, a substantial fraction of active force exerted at the distal tendon of EDL III originated from sources other than from muscle fibers of head III. As the endomysial-perimysial stroma of EDL III is a part of the continuous connective tissue network of the anterior crural compartment (Huijing and Baan, 2001b; Huijing et al., 2003; Maas et al., 2001), such sources may be (a) fibers within surrounding muscles (i.e. TA and EHL) and/or (b) fibers within the other heads of EDL. Therefore, it is concluded that force is transmitted to the distal tendon of a single muscle head of multi-tendoned muscle via inter- and extramuscular myofascial pathways as well as via intramuscular myofascial pathways.

Moreover, we showed that an increase of length of a single head of multi-tendoned EDL muscle simultaneously decreased force exerted at the distal tendons of synergistic muscles (i.e. TA and EHL) (Fig. 5). This means that partitioning of force over the different pathways of force transmission is altered by length changes of EDL III. As EDL III is lengthened, force previously exerted at the distal tendons of TA+EHL is exerted at the distal tendon of EDL III. As the other EDL heads are also connected to EDL III, it is expected that, in the present experiment, forces exerted at distal tendons of the other heads of EDL (not measured) were affected also.

In vivo, movement of a single finger requires a change of length of the corresponding head of multi-tendoned muscle. If the antagonist forces remain equal, changes of force exerted at the distal tendons of muscle heads other than the target one will result in joint movements of the corresponding fingers. This indicates that myofascial force transmission is one of the mechanisms, which explains that during single finger tasks changes of force or movements are measured also in the non-test fingers. The limited independent control of the position and force of the fingers has been attributed to central neural factors as well as to mechanical factors at the muscle-tendon level (e.g. Kilbreath and Gandevia, 1994; Li et al., 1998; Zatsiorsky et al., 2000). Until now, mechanical factors that have been postulated are: (1) transmission of force from fibers, inserting on the same tendon, via the intramuscular connective tissue network to adjacent heads (i.e. intramuscular myofascial force transmission), (2) force transmission via tendinous connections between the distal

tendons of multi-tendoned extensors and flexors of the fingers. The present results indicate that (3) intermuscular and (4) extramuscular myofascial force transmission should also be considered as an important factor. It has been reported that surgically removing those intertendinous connections improves finger independence (Kaplan, 1959; Leijnse, 1995, 1997; Lombardi et al., 1988; McGregor and Glover, 1988; Moore et al., 1987; Von Schroeder and Botte, 1993). However, for most cases full independent digit movement was not obtained, even if the central neural factors were excluded (Kaplan, 1959; Moore et al., 1987; Von Schroeder and Botte, 1993). This indicates that both intertendinous and myofascial force transmission contribute to involuntary movements of the fingers.

It should be noted that in the present experiment tendon forces were measured while all four EDL muscle bellies as well as TA and EHL muscles were excited simultaneously and maximally. For most movements *in vivo*, such activation pattern is not found. It may be argued that the stiffness of intra-, inter- and/or extramuscular connective tissues in the present study was higher than during *in vivo* patterns of muscle activation. Nevertheless, effects of myofascial force transmission were also found for passive muscles (Fig. 4B). Furthermore, during normal rat behavior, motor units of EDL muscle are occasionally activated at approximately 100 Hz (i.e. the stimulation frequency imposed on EDL muscle in the present study) (Hennig and Lomo, 1985, 1987). Also co-activation of synergists during normal limb movements (e.g. Nakazawa et al., 1993) and co-activation of non-target muscle heads of multi-tendoned muscle during voluntary flexion of one finger (Kilbreath and Gandevia, 1994) have been reported in humans. Therefore, it is expected that, also *in vivo*, myofascial force transmission is an important feature for multi-tendoned muscles.

Repeated isometric length-force measurements

We found that isometric contractions, particularly at high lengths of EDL III, shifted optimum length of EDL III to higher lengths (Fig. 6B) and, consequently, increased the length range between active slack length and optimum length. There are three possible explanations for such an increase of length range: (a) an increase of the compliance of elastic components in series with the sarcomeres (Huijing, 1992; Kawakami and Lieber, 2000; Lieber et al., 1992; Zajac and Gordon, 1989). The present finding of decreased passive forces of EDL III (Fig. 6B), subsequent to previous isometric contractions at high lengths, is in agreement with an increase of

series elastic compliance for EDL III.

(b) An increase of the distribution of lengths of sarcomeres arranged in series within muscle fibers (Granzier and Pollack, 1990; Huxley and Peachey, 1961; Pollack et al., 1993) or (c) an increase of the distribution of fiber mean sarcomere length (i.e. higher differences of mean sarcomere length between fibers within a muscle) (Ettema and Huijing, 1994; Willems and Huijing, 1994). It has been shown that interfering with the connective tissues surrounding EDL muscle (i.e. blunt dissection of the EDL-TA interface and full lateral fasciotomy of the anterior crural compartment) changes the length-force curves of EDL muscle in a fashion compatible with an increased distribution of fiber mean sarcomere length (Huijing and Baan, 2001b; Huijing et al., 2003). These results suggest that inter- and extramuscular connective tissues affect the distribution of fiber mean sarcomere length.

It was found that the slope of the EDL III length – TA+EHL force curve decreased after the measurements for the first length-force curve of EDL III (Fig. 6C), indicating a decrease of the degree of intermuscular interaction between EDL III and TA+EHL. This is explained by a decreased stiffness of the connective tissues between these muscle bellies. Such a decreased stiffness may have changed the distribution of fiber mean sarcomere length and, consequently, the length-force characteristics of EDL III.

Conclusions

The present study shows that a substantial amount of force can be transmitted between a single head of a multi-tendoned muscle and surrounding muscle heads, adjacent synergists and/or extramuscular structures. Such myofascial force transmission should be considered as a mechanism that contributes to the inability of humans to move a single finger without movements of adjacent fingers.

ACKNOWLEDGEMENTS

The authors wish to acknowledge Dr. Bente R. Jensen for raising questions from the field of occupational health, regarding force transmission from different muscles and muscle heads of multi-tendoned muscle during movements of fingers relative to each other. This stimulated us to design the protocol of the present study.