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Journal of Biomechanics 34 (2001) 927–940

JOURNAL OF BIOMECHANICS

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

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Accepted 18 March 2001

Abstract

Force transmission in rat anterior crural compartment, containing 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.

Keywords: Myofascial; Force transmission; Connective tissue; Rat; Dorsal flexors

1. Introduction

Individual muscles or muscle groups are frequently investigated in vivo. Properties of muscle groups have been determined by 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

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0021-9290/01/$ - see front matter
PII: S0021-9290(01)00055-0

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 and Ramsey, 1965; Street, 1983) 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. Trotter and Purslow, 1992; Trotter, 1993; Purslow and Trotter, 1994) or, more likely, from the network directly onto the aponeurosis (intramuscular myofascial force transmission) (Huijing et al., 1998; Huijing, 1999a). Recent observations (Huijing, 1999b, 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, 1999b).

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 extensor hallucis longus (EHL) 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.

2. 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 Animal Experimentation at the Vrije Universiteit.

2.1. Surgical procedures

Male Wistar rats ($n = 15$) were anaesthetized using intraperitoneally injected urethane (initial dose: $0.15\,g\,100\,g^{-1}$ 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 ($80 \pm 2\%$) 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 consists of the 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 $0.0048\,mm\,N^{-1}$) 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 force transducer (FT 3, Fig. 1C) was equal to the weight used. Note, however, that both EDL

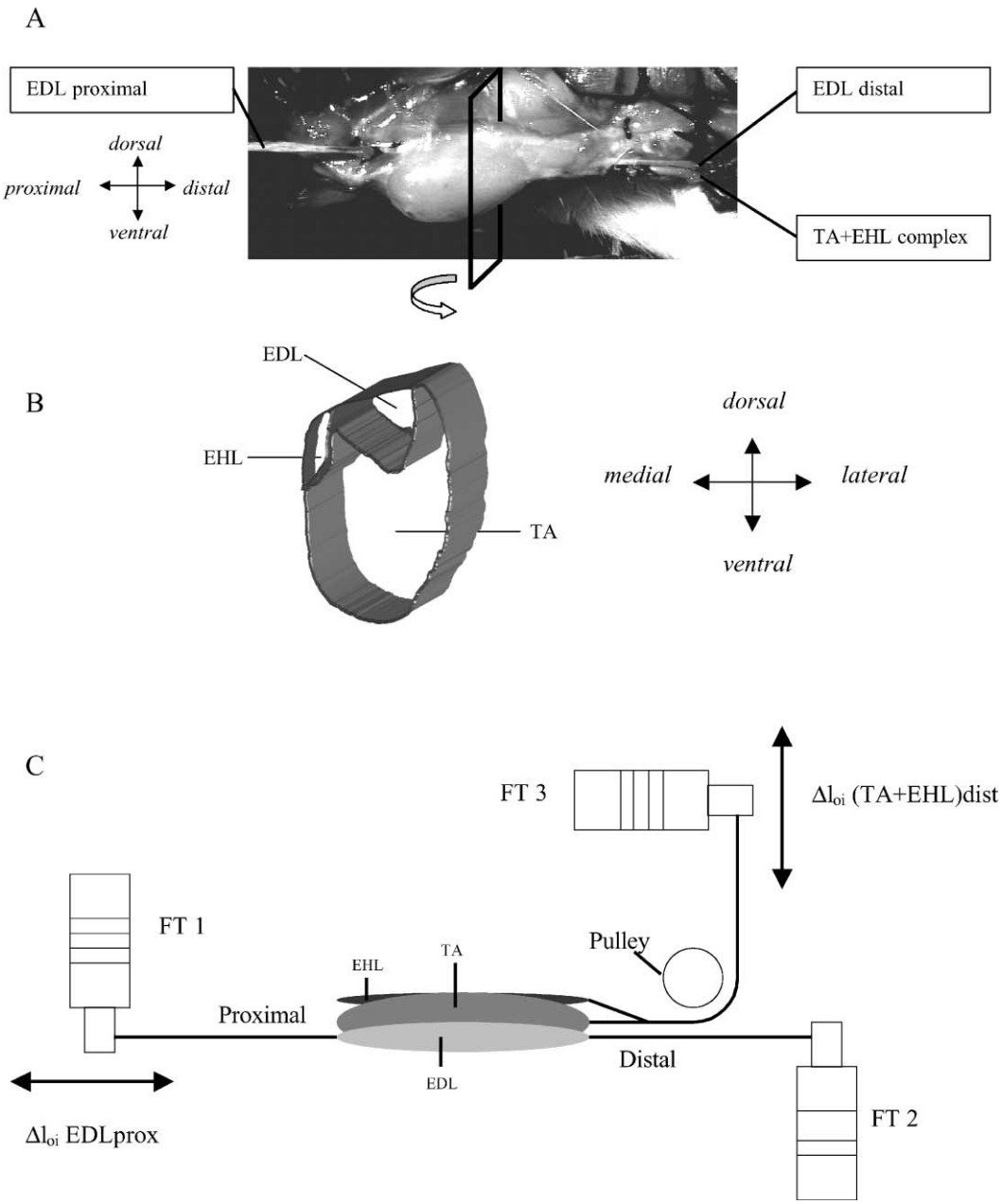

Fig. 1. The anterior crural compartment as in the experimental setup. (A) Lateral view of the compartment in the experimental setup. The Kevlar thread connecting the different tendons to force transducers and the anatomical orientation (cross of arrows) are indicated. (B) A schematic outline of the muscles 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 setup, 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.

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.

2.2. 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 min. The force transducer connected to the distal EDL tendons was kept at a constant position in all experimental protocols.

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).

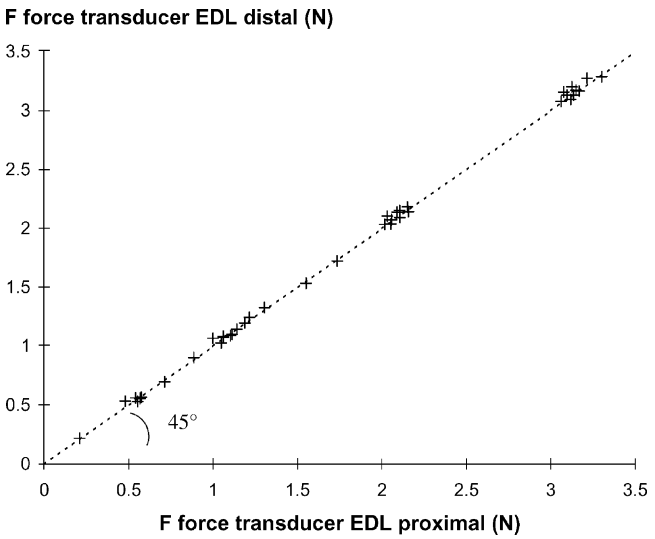

Fig. 2. An example of a comparison of isometric characteristics of the force measurement system. In the animal experiment, the proximal and distal tendons of EDL muscle were connected to the system (force transducers, amplifiers, A/D converter). The two force transducers were connected to each other 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.

2.3. Length–force characteristics of EDL muscle

EDL isometric force was measured at various muscle–tendon complex lengths ($n = 8$, body mass $= 304.8 \pm 23.1$ 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.

2.4. 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 previously assessed optimal active force of proximal measured EDL. Isometric length–force characteristics of the TA + EHL complex were measured ($n = 7$, body mass $= 307.3 \pm 23.7$ g). The TA + EHL complex was lengthened distally with increments of 1 mm from active slack length to approximately 3 mm over optimum length.

2.5. EDL force at different forces of TA + EHL

For a separate group of animals ($n = 7$, body mass $= 319.4 \pm 11.6$ 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.

2.6. 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_{mt}) at equal muscle–tendon complex length. The relationship of F_{ma} with l_{oi} was fitted by the polynomial

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

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 Section 2.8). 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}).

2.7. 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 s in absolute ethanol and then put in xylene for 5–15 min, and finally embedded in Entallan (Merck, Für Mikroskopie).

2.8. 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 $p < 0.05$.

3. Results

3.1. 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 $\approx -18\%$ 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 ($\Delta l_{oi} \approx -4$ to $+2$ 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).

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.

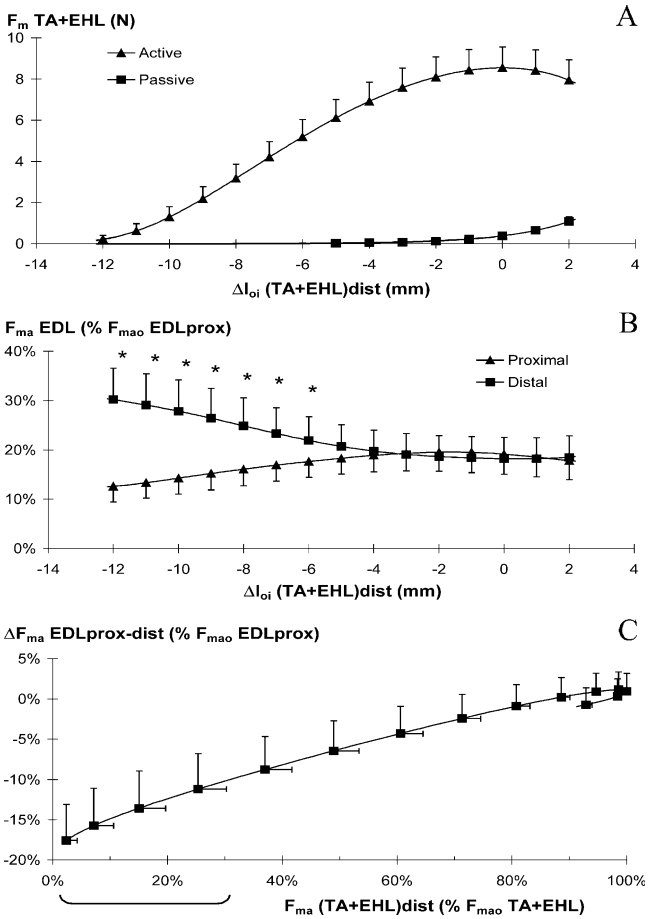

Fig. 3. Length–distal force characteristics of the TA + EHL complex and simultaneously measured EDL forces at constant muscle–tendon complex length. (A) Absolute values of active and passive TA + EHL forces as a function of TA + EHL muscle–tendon complex length (Δl_{oi}). (B) Proximal and distal active 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 force 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 ± 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).

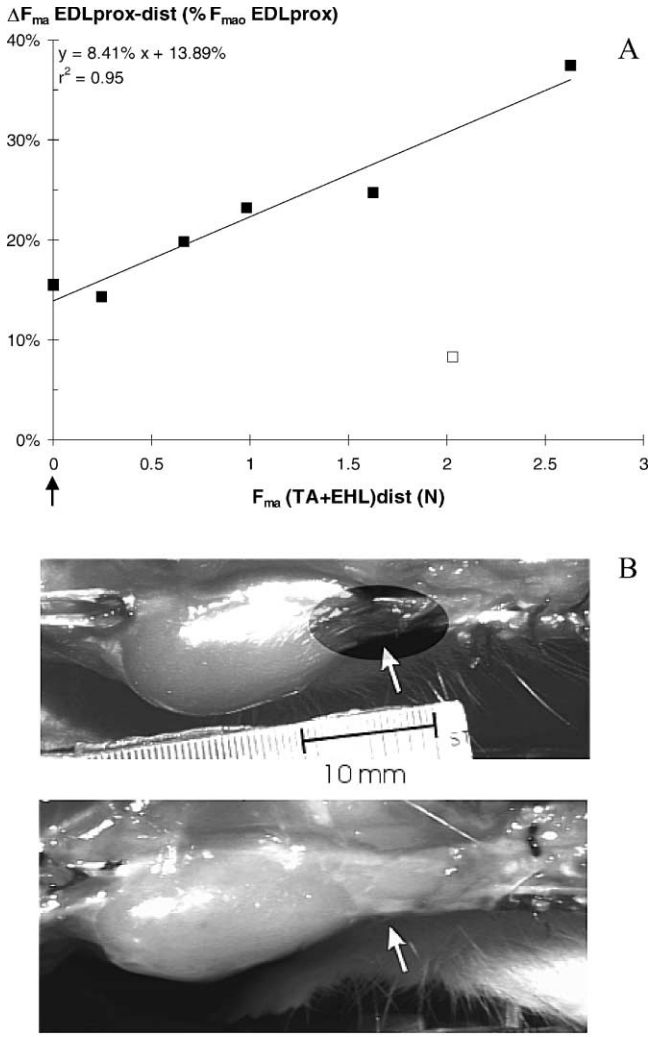

Fig. 4. (A) The relationship between active force of the TA + EHL complex and the proximo-distal EDL active force difference. EDL forces were measured at proximally determined optimum length and normalized for proximal optimal active EDL force ($F_{mao} = 2.60 \pm 0.24$ N, mean ± SD). Individual data 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.

3.2. 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

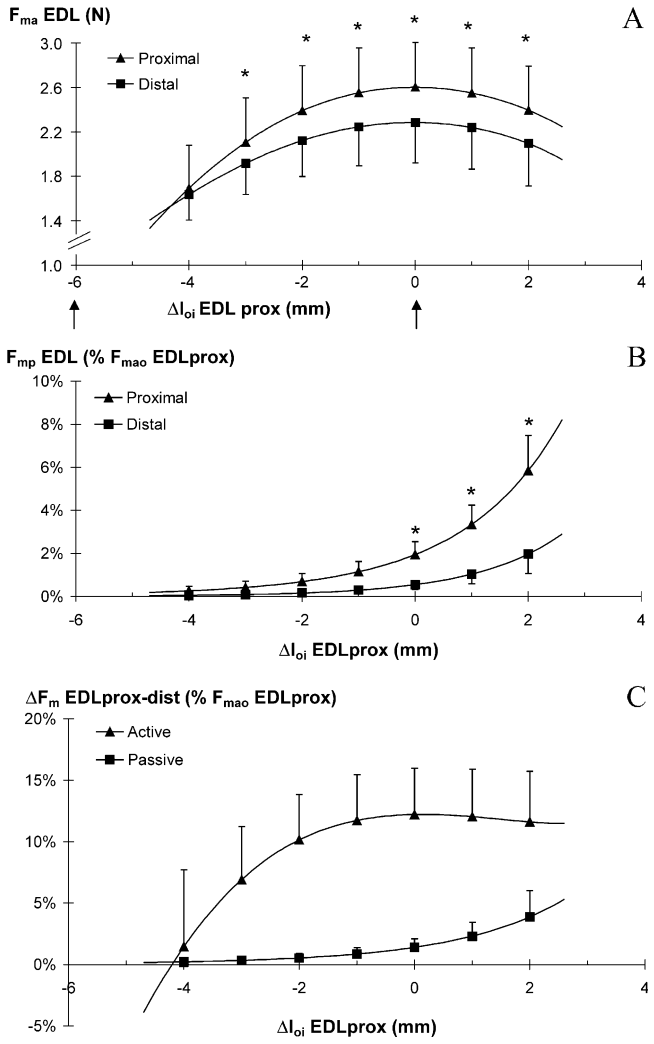

Fig. 5. Simultaneously measured length–force characteristics of EDL as measured at proximal and distal tendons. (A) Absolute values of active forces (F_{ma}) at proximal and distal tendons of EDL muscle–tendon complex as a function of its length (Δl_{oi}). (B) Passive forces (F_{mp}) at proximal and distal tendons of EDL muscle–tendon complex as a function 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 ± 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 ($F_{mao} = 2.60 \pm 0.40$ 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.

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 (e.g. Woo et al., 1980; Rack and Westbury, 1984; Huijing and Ettema, 1988-89; Ettema and Huijing, 1989; Scott and Loeb, 1995). 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.

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.

3.3. 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 $\approx -4\%$ to $\approx +12\%$ 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 $\approx 5\%$ 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.

3.4. Intermuscular and extramuscular connections

Images of intra-, inter- and extramuscular connective tissue within the anterior crural compartment are shown (Figs. 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.

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.

In summary, the difference between proximally and distally measured EDL force 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

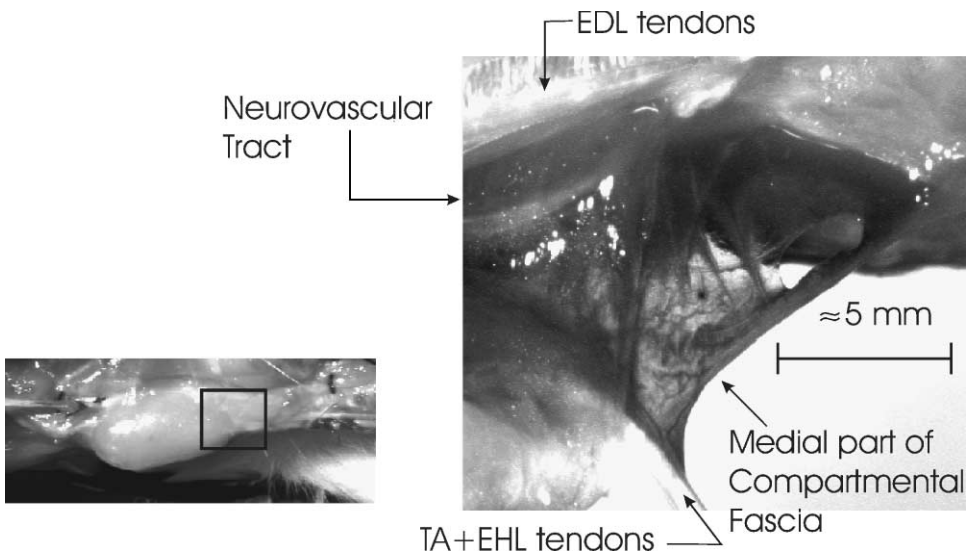

Fig. 6. Extramuscular connective tissue of the anterior crural compartment that supports the neuro-vascular tract and compartmental fascia (latero-distal–ventral view of the lower rat leg). This fascia is connective tissue that encloses the muscles within the compartment. The overview image (left) is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

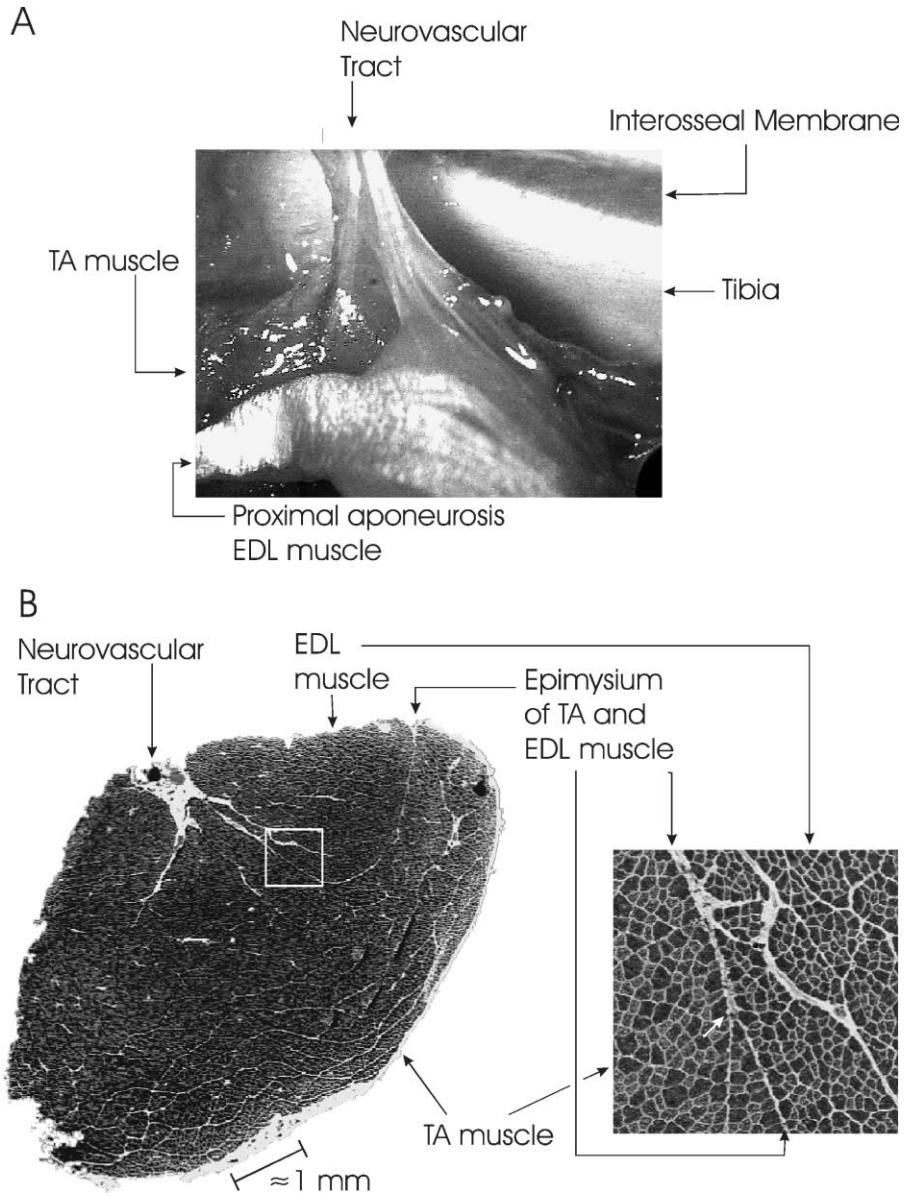

Fig. 7. Images of extramuscular connective tissue and of a cross section of the anterior crural compartment of the rat. (A) Proximal view of the neuro-vascular tract of EDL, TA and EHL muscles. TA, EHL and EDL muscles are isolated from their surrounding tissue, only the blood supply and innervation was 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.

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.

4. Discussion

Anatomical investigations revealed intermuscular mechanical connections between human gluteus max-

imus 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, 1999a). 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, 1999b, 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.

4.1. 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 (Rack and Westbury, 1969; Bobbert et al., 1990; Gareis et al., 1992; Huijing et al., 1998; Jaspers et al., 1999). For validation, Hill-type models to simulate the behavior of single skeletal muscles have been compared with such experimental results (e.g. Jewell and Wilkie, 1958; Van Ingen Schenau et al., 1988; Zajac, 1989; Van Soest et al., 1995). 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; Willems and Stauber, 1999; Gorselink et al., 2000).

Hill-type muscle models are used also to study human limb movements such as jumping (e.g. Hatze, 1981; Bobbert et al., 1986; 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.

4.2. 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, distal 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. 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 $\Delta l_{oi} = -4$ 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

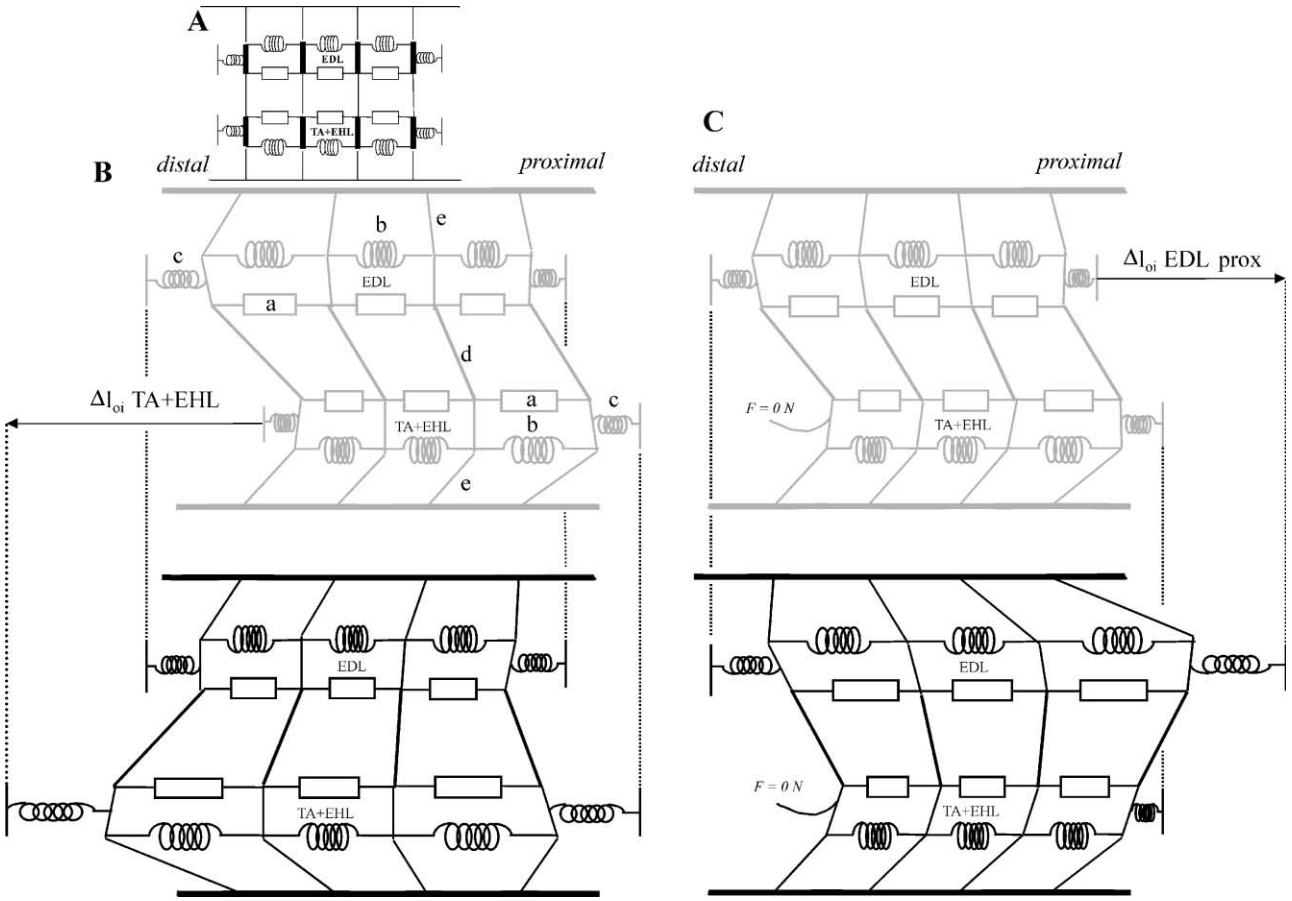

Fig. 8. Representation of a physical model illustrating effects of extra- and intermuscular connections of EDL muscle and the TA + EHL complex. (A) General structure of the model. The muscles are represented as to consist of (a) contractile elements (represented as rectangles), (b) intramuscular connective 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 the proximal direction; (2) rotations of the linking components between springs and (3) a length increase of extramuscular connections of the TA + EHL complex.

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.

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