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Myofascial Force Transmission Causes Interaction between Adjacent Muscles and Connective Tissue: Effects of Blunt Dissection and Compartmental Fasciotomy on Length Force Characteristics of Rat Extensor Digitorum Longus Muscle

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Abstract

Muscles within the anterior tibial compartment (extensor digitorum longus: EDL, tibialis anterior: TA, and extensor hallucis longus muscles: EHL) and within the peroneal compartment were excited simultaneously and maximally. The ankle joint was fixed kept at 90°. For EDL length force characteristics were determined. This was performed first with the anterior tibial compartment intact (1), and subsequently after: (2) blunt dissection of the anterior and lateral interface of EDL and TA. (3) Full longitudinal lateral fasciotomy of the anterior tibial compartment. (4) Full removal of TA and EHL muscles.

Length-force characteristics were changed significantly by these interventions. Blunt dissection caused a force decrease of approximately 10% at all lengths, i.e., without changing EDL optimum or active slack lengths. This indicates that intermuscular connective tissue mediates significant interactions between adjacent muscles. Indications of its relatively stiff mechanical properties were found both in the physiological part of the present study, as well as the anatomical survey of connective tissue. Full lateral compartmental fasciotomy increased optimum length and decreased active slack length, leading to an increase of length range (by ≈47%), while decreasing optimal force. As a consequence an increase in force for the lower length range was found. Such changes of length force characteristics are compatible with an increased distribution of fiber mean sarcomere length. On the basis of these results, it is concluded that extramuscular connective tissue has a sufficiently stiff connection to intramuscular connective tissue to be able to play a role in force transmission. Therefore, in addition to intramuscular myofascial force transmission, extramuscular force transmission has to be considered within intact compartments of limbs. A survey of connective tissue structures within the compartment indicated sheet-like neuro-vascular tracts to be major components of extramuscular connective tissue with connections to intramuscular connective tissue stroma.

Removal of TA and EHL yielded yet another decrease of force (mean for optimal force ≈10%). No significant changes of optimum and active slack lengths could be shown in this case. It is concluded that myofascial force transmission should be taken into account when considering muscular function and its coordination, and in clinical decisions regarding fasciotomy and repetitive strain injury.

Keywords: Anterior tibial compartment, connective tissue, myofascial force transmission, length-force characteristics, fasciotomy.

Abbreviations

$\Delta 1_{oi}$	deviation from optimum length
ANOVA	analysis of variance
EDL	m. extensor digitorum longus
EHL	m. extensor hallucis longus
F_{ma}	active muscle force

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F_{mao}	optimal force
F_{mp}	passive muscle force
MI	membrane interossea
Per. long.	m. peroneus longus
SIA	septum intermusculare anterior
TA	m. tibialis anterior

Introduction

Anatomically, connective tissue of limbs is often considered (e.g., Le Gros Clark, 1975, p. 59) as 'an all pervading matrix, in which are embedded more highly organized structures such as muscles, nerves and vessels etc.'. Such a very general view of connective tissue leads to a rather global perception (e.g., Le Gros Clark, 1975, p. 59) that the connective tissue 'obviously provides support for these structures'. However in many cases the nature of the support is not further identified or discussed.

In order to obtain whole body movement, forces need to be transmitted from the muscles to the skeleton to cause acceleration of the limbs. A major location for force transmission is the well-studied myotendinous junction (e.g., Tidball, 1983, 1984, 1991, 1994; Tidball & Daniel, 1986; Tidball et al., 1986; Tidball & Quan, 1992). Such transmission involving transmission of force from one sarcomere to its serial neighbors and eventually onto the myotendinous junction. These junctions are located at the apical ends of muscle fibers, where sarcomeric forces are transmitted onto an aponeurosis or tendon by shearing.

In addition to myotendinous force transmission, a second pathway has been shown to exist, that uses the muscle's cytoskeletal lattice and trans-sarcolemmal and basal lamina connecting molecules to transmit force onto connective tissue (myofascial force transmission; Huijing et al., 1998; Huijing, 1999a, b). Such a mechanism of force transmission has been shown to be active in experiments on single isolated muscle fibers (Ramsey & Street, 1940), small fascicles (Street, 1983; Street & Ramsey, 1965). Its activity has also been inferred in non-spanning fibered muscle (Trotter, 1990, 1991, 1993; Trotter & Purslow, 1992; Trotter et al., 1995; Trotter et al., 1992). Non-spanning muscle fibers do not span the distance between proximal and distal muscle fiber attachment areas of a muscle on aponeuroses or bone, i.e., they end somewhere in a fascicle that is attached at both ends.

Recently, we reported evidence that myofascial force transmission plays an important role within isolated in situ muscles with spanning fibers (Huijing et al., 1998; Huijing, 1999a). In addition we hypothesized the possibility that myofascial force transmission may also play a role *extra*-muscularly (Huijing, 1998, 1999a, 2000). Some recent explorative experimental work (Huijing, 1999b) yielded at least some indications that this concept may be entertained and should be studied systematically.

Therefore, the purpose of present study was to make a first step in such systematic study. Two types of approaches were combined: (1) studying aspects of structure of intra- and extramuscular connective tissue and their interface within the anterior crural compartment of the rat and (2) relate that to acute functional consequences of interfering surgically with elements of extramuscular connective tissue structures. If extramuscular force transmission plays an important functional role, there should be significant changes in muscular length force characteristics depending on the interventions.

The specific interventions performed were: (1) blunt dissection of the lateral and anterior connective tissue interface between extensor digitorum longus and anterior tibial muscle and (2) fasciotomy of the lateral aspect of the anterior crural compartment. This second intervention resembles the surgery performed for the relief of compartment syndrome. Therefore a secondary purpose was to increase our understanding of acute effects of compartmental fasciotomy on muscular function.

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 were approved by a Committee on Animal Experimentation at the Vrije Universiteit.

Surgical procedure and preparation for experiment

Male Wistar rats (mean ± SD of body mass 309.7 ± 6.3 g) were anaesthetized by intraperitoneally injecting urethane solution (initial dose 1.2 mg/100 g body mass). Supplementary doses of the anesthetic agent (0.5 mg) were injected intraperitoneally if necessary to maintain deep anesthesia. The animals were placed on a heated water pad (37°C) during surgery and experimentation.

For the physiological part of the experiments (n = 5), the left anterior crural compartment was exposed by removing the skin, parts of the crural fascia and the biceps femoris muscle. In the rat, connective tissue associated with the biceps muscle covers the anterior compartment in order to reach a very long insertion along the tibia. Apart from such preparatory intervention, the anterior crural compartment and the lower leg in general were not interfered with to maintain physiological relations of intra- and extramuscular connective tissue.

In contrast, femoral compartments were opened in order to: (1) Attach a clamp to the femur for later fixation of the animal; (2) Reach the proximal tendon of m. extensor digitorum longus (EDL). In order to avoid interfering with the physiological relationship between the length-force characteristics of the different heads of the EDL, force was to be measured at this common proximal tendon. Therefore the proximal tendon was cut as proximally as possible and Kevlar thread (4% elongation at a break load of 800 N) was tied to it with a suture (Fig. 1, inset). The knot was secured using tissue glue (Histoacryl Blue, B. Braun AG, Melsungen.

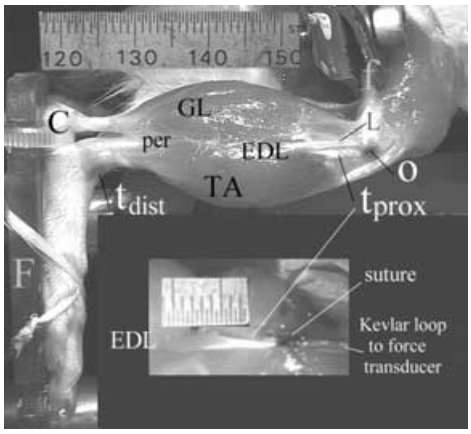

Fig. 1. Representation of the experimental position of the leg in a lateral view. It should be noted that in these figures a right leg is shown, but that for the experiments a left leg was used. The skin and m.biceps femoris were removed. The foot was secured to a foot plate (F) by a tie wrap around the calcaneus (C), anterior of the Achilles tendon) and a kevlar thread at the digits. The clamped femur is indicated (f). Foot plate and femur were manipulated so as to obtain an angle of approximately 90° at the knee and ankle joint. EDL indicates the muscle belly of m. extensor digitorum longus, t_{prox} its proximal tendon in un-dissected state, and O the marked origin of this tendon. t_{dist} indicates the location of the EDL distal tendon, remaining un-dissected within the anterior tibial compartment. Other muscles indicated are m. tibialis anterior (TA), muscles of the peronei group (PER) of which also tendons (unmarked) can be seen distally, and caput laterale of m. gastrocnemius (GL). L represents the collateral fibular ligament of the knee joint. In the inset the dissected proximal tendon of EDL and its connections for attachment to the force transducer are shown. EDL was lengthened between contractions by displacement of the proximal force transducer only. The remainder of the anterior tibial compartment was left initially as in the main figure. Later in the experiment full lateral compartmental fasciotomy was performed just anterior of the anterior intermuscular septum (see also Fig. 5).

BRD). The Kevlar thread was later to be connected to a force transducer (Hottinger Baldwin, maximal output error < 0.1%, compliance 0.0048 mm/N); (3) Dissect the n. Ischiadicus and cut it as proximally as possible and place a bipolar cuff electrode with stainless steel electrodes. All branches of the sciatic nerve, except the common peroneal nerve were denervated, so that by stimulating the sciatic nerve during the experiment, only the full motor segment of the common peroneal nerve were excited.

The left foot of the rat was attached firmly to a plastic plate using tie wraps around the calcaneus and Kevlar thread at the tip of the foot (Fig. 1). After positioning the rat in the experimental apparatus, the femur was secured by means of the metal clamp. The plate with the foot attached was manipulated such that the angle between the plate and the tibia was 90° (Fig. 1), after which the plate was firmly attached to the experimental apparatus. During the experiment, ambient temperature was kept constant at 22 ± 0.5°C and air humidity was kept at 69% ± 2% by a computer-controlled air conditioning system (Holland Heating) creating a down flow of air onto the experimental table. The crural compartments were rinsed regularly with saline to prevent fluid loss.

Experimental procedure and data collection

The anterior tibial compartment contains the following muscles: m. tibialis anterior (TA), m. extensor hallucis longus (EHL) and m. extensor digitorum longus (EDL). These muscles (all innervated via the deep peroneal nerve), as well as within the peroneal compartment (i.e., innervated via the superficial peroneal nerve) were excited simultaneously and maximally. This was done by stimulating the, distal end of the severed, sciatic nerve supra-maximally, using a pair of silver electrodes connected to a constant current source (2–5 mA, square pulse width 100 µs, pulse train 400 ms, 100 Hz).

It should be noted that ankle joint angle and the configuration of the foot on the footplate defined lengths of TA and EHL muscle tendon complexes, as well as of all muscles within the peroneal compartment. These variables were kept constant during the experiment.

Isometric tetanic EDL force was measured at various muscle lengths, obtained by moving the force transducer (1 mm increments) in between contractions, starting from below active slack length, defined as the lowest muscle length at which active muscle force approached zero.

Following each contraction the muscles were allowed to recover for 2 minutes, to minimize any effects of fatigue and potentiation. For EDL, recovery was allowed to occur at a length near active slack length.

After stretching the muscle to the desired length, two twitches were evoked (200 ms apart), to allow adaptation to the newly imposed length. Passive force was determined approximately 200 ms after the second twitch. Subsequently (i.e., 300 ms later), the muscle was excited tetanically, for 400 ms. During the tetanic plateau (i.e., 275 ms after evoking tetanic stimulation) total isometric muscle force was determined. Force signals were acquired using a 12-bit A/D converter and recorded on a microcomputer (sample frequency 1000 Hz, resolution of force 0.01 N). A special purpose microcomputer controlled timing of events related to stimulus generation as well as A/D-conversion.

Experimental protocol

During each experiment several sets of EDL length-force data were collected: (1) One set for the *in situ* 'intact' condition. (2) After blunt dissection of the interface between TA and EDL. For this blunt dissection a probe was introduced into the anterior crural compartment, from the proximal side along the proximal EDL tendon. Moving the probe around destroyed any local connective tissue connections on lateral and anterior sides between TA and EDL. (3) After fas-

ciotomy, in which the anterior crural compartment was opened by a lateral longitudinal section. (4) After full removal of TA and EHL, in which condition EDL was isolated in situ, in a comparable condition as in previous experiments (Huijing et al., 1998).

Post-experimental data collection and treatment of data

The individual length force data sets for passive muscle force and muscle length were fitted with an exponential curve (eq. 1), using a least squares criterion.

$$y = e^{a1+a2\cdot x}, \quad (1)$$

where y represents passive muscle force, x represents passive muscle-tendon complex length (Δl_{mtc}) and a_1 and a_2 are coefficients determined in the fitting process. Active muscle force (F_{ma}) was estimated by subtracting passive force calculated according to equation 1 for the appropriate active muscle tendon complex length from the total force exerted by the muscle at that length.

Data for active EDL force (F_{ma}) in relation to changes of active muscle-tendon complex length (Δl_{oi}) were fitted using a polynomial:

$$y = b_0 + b_1\cdot x + b_2\cdot x^2 + \ldots + b_n\cdot x^n, \quad (2)$$

where y represents F_{ma}, x represents length of the active muscle-tendon complex, n represents the order of the polynomial and b_0, $b_1 \ldots b_n$ are coefficients determined in the fitting process. The fitting started with a first order polynomial and the power was increased up to and including the sixth order. Polynomials that best described the experimental data were selected (see below). These polynomials were used for three purposes: (1) Averaging of data and calculation of standard errors, and (2) Determining EDL optimal force and optimum length. For each individual muscle optimal muscle force (F_{mao}) is defined as the maximum of the fitted polynomial for active muscle force and optimum length is defined as the corresponding active length, (3) Estimation of EDL active slack length (i.e., the lowest length at which active force approaches zero) by extrapolation of the curve to zero force.

Individual muscle-tendon complex length data were expressed as deviations from the corresponding optimum length for the 'intact' *in situ* muscle.

Anatomical survey of intra- and extramuscular connective tissue and their connections

During post-experimental surgery, for the experimental group, but also in a separate group of animals (n = 3) the lower limb connective tissue was assessed for connections between intra- and extramuscular connective tissue, as well as the structure of the intramuscular connective tissue.

One EDL muscle was fully isolated from the leg of an additional animal and frozen in isopentane (at −80°C). Frozen thin sections (thickness 15 µm) were cut approximately perpendicular to the muscle fibers, using a cryomicrotome. Starting at the distal end of the muscle belly, only that distal part of the muscle containing no proximal aponeurosis was sectioned (approximately one third of the muscle). Sections were thawed and fixed in Zenker solution for 30 min, and subsequently rinsed with water. After that the sections were stained for 30 min. with a solution of Sirius red (F3B, Brunswig Chemie, Amsterdam) in demineralized water, saturated with picric acid. The stained sections were dipped three times in absolute ethanol and subsequently put in xylene for some time. The sections were then embedded in Entallan.

Statistics

In the fitting procedure one-way analysis of variance (ANOVA) (Neter et al., 1990) was used to select the lowest order of the polynomials that still added a significant improvement of the description of changes of muscle tendon complex length and muscle force data for EDL.

Two-way ANOVA for repeated measurements (factors: muscle length and interventions) was performed to test for effects of interventions on muscle length force characteristics. If significant effects were found, post-hoc tests were performed using the Bonferroni procedure for multiple paired comparisons, to further locate significant differences. To test for differences in optimum and active slack lengths a one way ANOVA was performed. Any differences at $p \leq 0.05$ were considered significant.

Results

Effects of interventions on length force characteristics

Analysis of variance indicates significant changes of length force characteristics, as well as a significant interaction between the factor muscle-tendon complex length and intervention. Similar results were found for passive length force characteristics.

Blunt dissection of the anterior and lateral EDL-TA interface

After blunt dissection significant changes in length-force characteristics are found (Fig. 2). A general decrease in active muscle force (maximally approximately 10%) is seen at most lengths, but the general shape of the length force curve remains very similar. Optimum length (i.e., the length at which the active force is maximal), as well as active slack lengths (i.e., the lowest length at which active force approaches zero) were not significantly different. This is particularly clear from Figure 2b in which force is normalized for its value at optimum length: The initial curve and the curve after blunt dissection are almost superimposed. This means that, at a given length, the decrease in force is in some way proportional to the original force amplitude at that

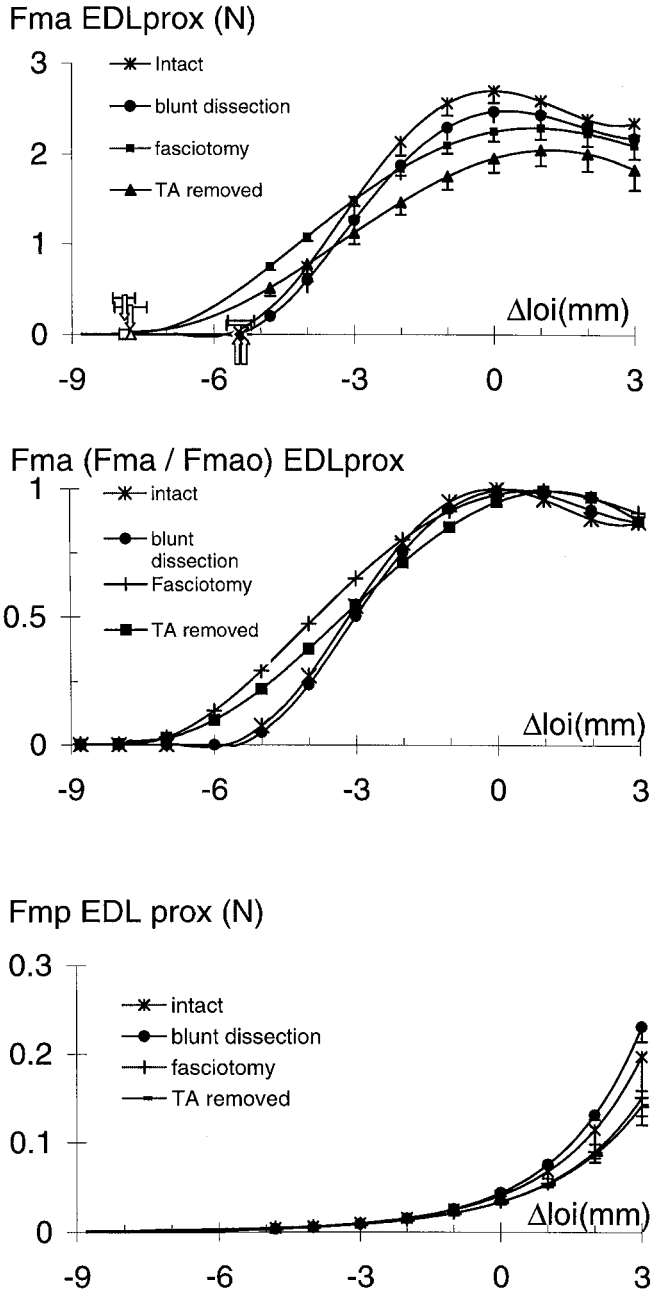

Fig. 2. Effects of interventions on length force characteristics of EDL muscle. (A) Absolute active force as a function of muscle tendon complex length; (B) Normalized active force as a function of muscle tendon complex length; (C) Passive force as a function of muscle tendon complex length. Muscle tendon complex length is expressed as deviation from optimum length ($\Delta 1_{oi}$) for the condition with 'intact' anterior tibial compartment. Mean values (n = 5) for force are indicated, as well as standard errors (vertical bars). Arrows indicate mean active slack lengths and nearby horizontal bars indicate standard errors of active slack lengths.

length. Any explanation of this result will have to be compatible with this result. It should be noted that neither a simple change in distribution of fiber mean sarcomere lengths nor simple elastic effects are compatible with such a property.

In contrast to active force, results regarding passive force after blunt dissection (Fig. 2c) no significant changes could be located for this intervention.

It is concluded that an important interaction between active EDL and TA is active in the intact anterior tibial compartment through some direct intermuscular mechanical connections.

Extramuscular fasciotomy

Subsequent lateral extramuscular fasciotomy of the anterior crural compartment caused a further drop of optimal force (by approximately 10%). In contrast to effects of blunt dissection, this decrease in force at and near muscle optimum length is accompanied by a substantial increase (by ≈47%) of the length range between muscle optimum and active slack lengths: (1) Active slack length shifted significantly to lower lengths (by more than 2 mm, increasing the length range from the original optimum length by more than 30%). As a consequence the slope of the ascending limb of the length force curve becomes less steep, and an increase of force in the length range below $\Delta l = -4$ mm could be shown to be significant. (2) In addition, a significant increase of optimum length was found ($\Delta lo \approx 0.9$ mm, causing a further increase in length range between optimum and active slack lengths of approximately 17%). Such changes in length force characteristics are compatible with a less uniform muscle regarding active sarcomere lengths. The effects of such changes per se on optimum length are likely to have been even greater, since they must have been partially compensated by elastic effects: At lower forces, elastic effects will shift optimum length to lower lengths.

Significant changes could also be located in the passive length force curves (Fig. 2c). Passive force decreased significantly particularly at higher lengths. No significant changes in passive slack length were observed.

Removal of tibialis anterior muscle

A significant decrease in active force was found at most lengths after removal of TA. Considering the effect of removing TA, after fasciotomy the mean data suggests possible changes in length force characteristics (Figs. 2a and b). However, statistical analysis does not support that suggestion: optimum and active slack lengths could not be shown to be significantly different. Therefore it must be concluded that these results resemble, qualitatively, the effects of blunt dissection: i.e., generally decreased forces without major changes the shape and length range of the length force curve For individual muscles, the amplitude of force decrease was rather variable (ranging from ~ −8% to ~ −33%). The expla-

nation for such individual variability is not immediately apparent.

Anatomical survey of major connections of connective tissue of the anterior crural compartment to EDL

Within the anterior crural compartment, three muscles are present in very close approximation: m. tibialis anterior (TA), EDL and extensor hallucis longus (EHL). The compartment (see schematic representation in Fig. 6) is delimited by the following structures: (1) the anterio-lateral aspect of the tibia. (2) The anterior part interosseal membrane connecting the tibia and fibula, which, in rat, is oriented rather anterior – posteriorly. (3) The anterior intermuscular septum (SIA), separating the anterior crural and peronei compartments and (4) the frontal part of the epimysium of TA, which may be connected with the epimysium of m. biceps femoris and the anterior part of the crural fascia. It should be noted that in the present experiments the extramuscular aponeurosis of the biceps muscle with parts of the fascia cruris had been removed prior to physiological measurements.

Intramuscular connective tissue of EDL

We considered the arrangement of the intramuscular connective tissue in cross-sections of a distal third of an isolated EDL muscle (Fig. 3). In a more proximal cross-section of the muscle (located just distal of the proximal aponeurosis within this distal third) the orientation of perimysial network to the medial side is quite apparent (Fig. 3a). At that side, the epimysium does not seem as thick as on the lateral, anterior and dorsal sides. In an EDL cross-section obtained more distally (Fig. 3b), such an orientation is not apparent. This is probably related to the fact that in the very distal region no major nerves and or blood vessels enter the muscle, which allow for thinner perimysia surrounding the fascicles and a more longitudinal orientation rather than a specifically medially oriented one.

Extramuscular connective tissue

Two major types of extramuscular connections can be distinguished:

• *Direct intermuscular connections: TA to EDL connections*
The direct connections by connective tissue between TA and EDL are not readily illustrated, because they are short and can be very easily broken for example by blunt dissection. It should be noted, however, that being 'loose' and or relatively weak under the stresses of blunt dissection does not necessarily mean that force transmission, in which shear forces are expected to play a major role, can not occur. After opening the compartment by lateral fasciotomy, and deflecting the lateral edge of TA in medial direction, EDL was seen to rotate with TA. This can only occur if mechanical connections exist between the epimysia of EDL and TA. Figure 4 shows some

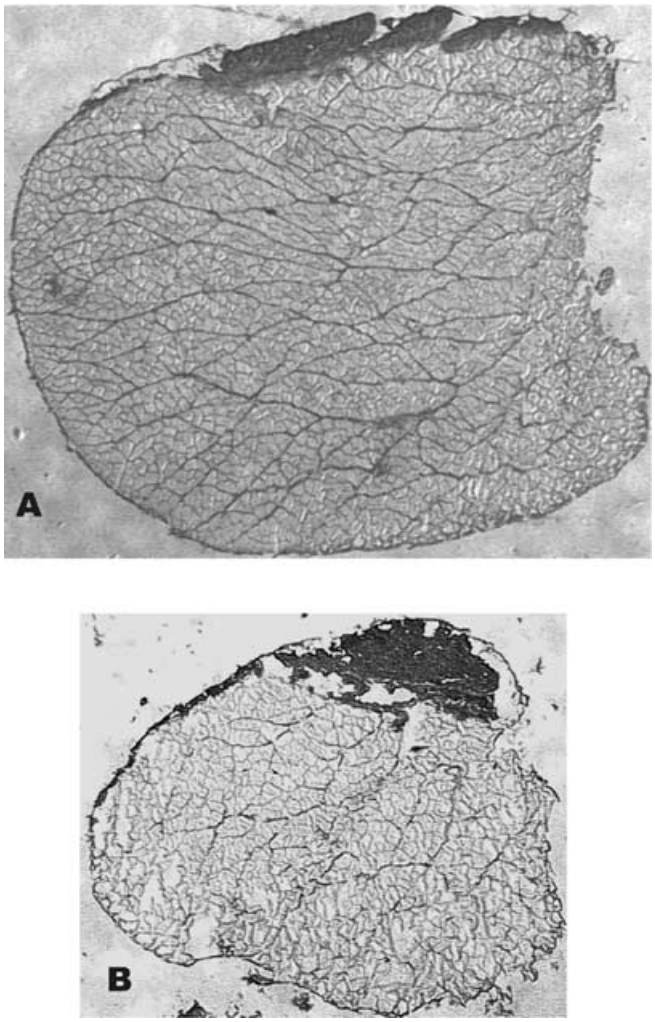

Fig. 3. Cross-sections of the distal third of an isolated rat EDL muscle showing the organization of intramuscular connective tissue. Frozen thin sections (thickness 15 µm) were cut approximately perpendicular to the muscle fibers, fixed and stained with a solution of Sirius red and embedded in Entallan. (A) A more proximal section within the distal third of the muscle. The horizontal midline of this figure represents approximately 3.2 mm life size. The medial side is on the right, and on top four distal aponeuroses and or tendons are showing as separate entities. Note the specific orientation of the perimysia to the medial side of the muscle, presumably due to the entrance of parts of the neuro-vascular tracts. At the medial side of the muscle all the way to the proximal end of the muscle belly, the intramuscular connective tissue is connected to its extramuscular counterpart of the neuro-vascular tract. (B) A more distal section within the distal third of the muscle. Magnification is identical to Fig. 3A. The decreased diameter is due to the tapering of the muscle in distal direction. The medial side is on the right and on top a cluster of distal tendons is showing. No specifically medially oriented structure of the perimysial stroma is observed, presumably because no neuro-vascular tracts enter this part of the muscle.

examples of such connections after some dissection and during only minimal loading. The connections become apparent as reflections of light at the interface between the lateral side of the EDL and the medial side of TA along the intermuscular interface. This rotation of EDL occurred even though no force is exerted on it *directly* by the experimenter. This is a clear indication of the relative stiffness of the connections between EDL and TA, despite the relative flimsy appearance of the connective tissue. Again it should be emphasized that this appearance is not necessarily related to the mechanical strength and stiffness if loaded under shear.

• *Other extramuscular connections* Major connective tissue components are formed by structures that encapsulate major blood vessels and nerves. We will refer to such structures as neurovascular tracts. The major blood vessels and nerves enter the anterior crural compartment, from the peroneal compartment, through a proximally located fenestration of the anterior intermuscular septum (Figs. 5a and b). In Figure 6a, which is a dorsal view of the anterior crural compartment, three major directions of collagen fibers can be distinguished and are indicated by arrows: (1) collagen constituting the proximal EDL aponeurosis (seen below the septum and through the fenestration); (2) collagen of the intermuscular septum: (a) in the longitudinal direction of the septum and (b) in circular direction to delineate the fenestration. Note that the proximal aponeurosis of EDL and the septum are in close approximation. However, they are not directly connected to each other, as they can be moved independently.

After passing through the fenestrated intermuscular septum, the nerves and blood vessels are embedded in sheets of connective tissue, that reach EDL and the other muscles of the compartment. These sheets are continuous with the intramuscular connective tissue of these muscles reaching from rather proximal locations, to as far distal as the level of distal aponeuroses. In EDL the intra- extramuscular connection is made on the medial side of the muscle belly (Fig. 5c). In this figure, the sheet is made visible, for one of the muscles of the length force experiments, after removal of TA by pulling it downward and rotating it laterally.

A schematic representation of these sheets containing the neuro-vascular tracts within the anterior crural compartment is shown in Figure 6. Note the tri-folar appearance: one common sheet of connective tissue appears from the fenestration of the intermuscular septum and splits into one sheet of connective tissue connecting to the EHL and EDL and one sheet connecting to TA.

Considering the effects of blunt dissection as well as anterior crural compartmental fasciotomy, on length force characteristics of EDL, it is concluded that extramuscular connective tissue structures, described above, are playing an important role in myofascial force transmission. In addition to affecting intramuscular force transmission, they may be involved in transmitting force from muscle onto adjacent muscle or onto non-muscle connective tissue structures.

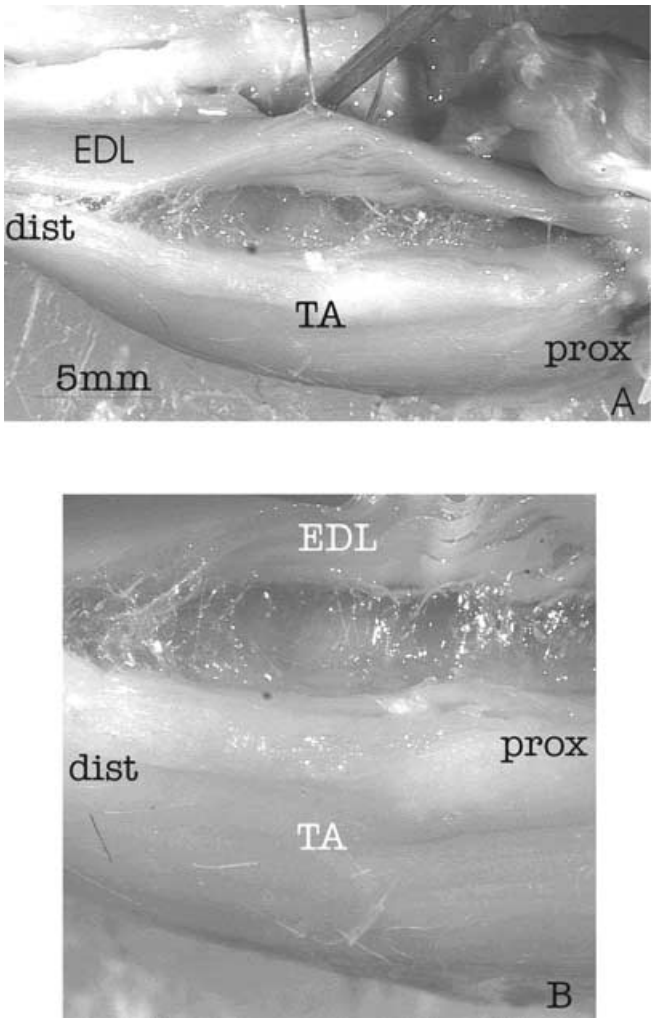

Fig. 4. Examples of direct intermuscular connective tissue connections between TA and EDL muscles in rat. The images (lateral views of a right lower limb) were obtained after compartmental fasciotomy. EDL is very slightly deflected in dorsal direction to expose a part of the anterior interface of EDL with the dorsal aspect of TA. (A) Panel A shows most of the EDL muscle length. (B) Panel B shows an enlargement of the distal part of panel A. 'prox' and 'dist' indicate proximal and distal directions respectively.

Discussion

Connective tissues of the limb: a revised vision

In any science, measuring usually means interfering with the object of measurements. However, particularly the study of intra- and extramuscular connective tissue is severely affected by this. The unavoidable fact is that parts of the connective tissue compartments and/or their content have to be destroyed in order to gain experimental access, even for simple visualization or measurement of characteristics. In

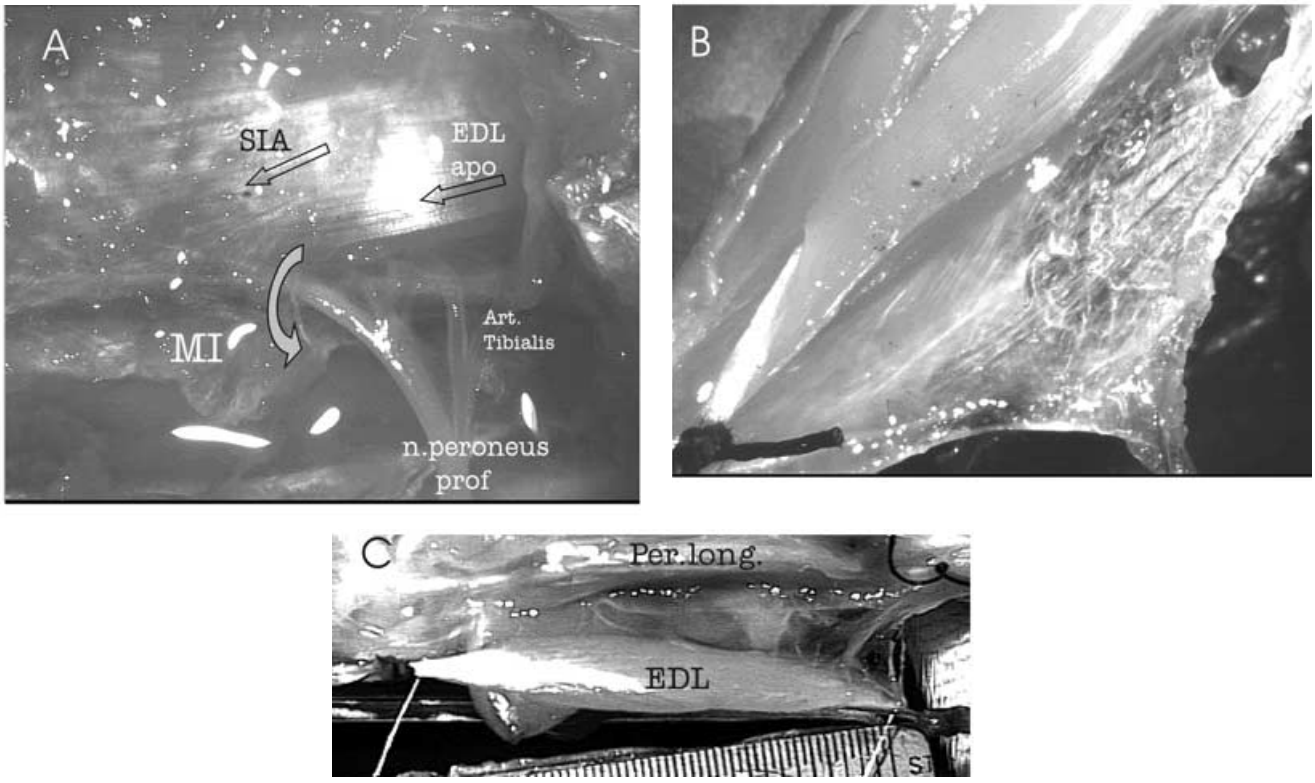

Fig. 5. Major elements of extramuscular connective tissue of the anterior tibial compartment of the rat, that is connected with intramuscular connective tissue. (A) Dorsal view of the unopened anterior crural compartment of a left leg, with the fibula removed. Note the dissected neuro-vascular tract entering the anterior tibial compartment through a fenestration of the anterior intermuscular septum (SIA) en route to the muscles within the compartment. Three arrows indicate major directions that can be distinguished for collagen fibers. These directions correspond to (1) the longitudinal direction of the proximal EDL aponeurosis (EDL apo), (2) SIA and (3) delimitation of the fenestration within SIA (curved arrow). It should be noted that the proximal aponeurosis and SIA can be moved independently and are not connected to each other. MI indicates the remainder of the severed interosseal membrane, that is deflected from a plane approximately perpendicular to the plane of the page. Note the tibial artery (art. tibialis) and the deep peroneal nerve (n. peroneus prof.). (B) Latero-distal view of the opened anterior crural compartment of a left leg, with the fibula removed. Note the fenestration of the anterior intermuscular septum (SIA, showing its ventral side) through which the neuro-vascular tract enters the compartment. The tension exerted compresses this tract that passes under the EDL to its medial side. Therefore the tract is difficult to see in this image. The latero-dorsal faces of TA and EDL are labeled (EDL and TA respectively). The severed interosseal membrane and the anterior intermuscular septum (SIA) are deflected together, exposing the proximal aponeurosis of EDL (prox apo). One of the four distal aponeuroses of EDL is shown (EDL dist apo), together with a ligature tied to the distal EDL tendons in the lower left hand corner. (C) One of the experimental EDL muscles after the physiological experiment, deflected and rotated ventrally and laterally, to expose the proximal aponeurosis sheet (prox apo) and more important the sheet-like neuro-vascular tract that connects to EDL intramuscular connective tissue on the medial side of the muscle. The ruler at the bottom indicates half mm divisions. Within the intact peroneal compartment, the peroneus longus muscle is indicated (Per. long.).

experiments and surgery alike, too often effects of such intervention are not considered fully, because they are not known, and are (often implicitly) assumed to be negligible. This has lead, for example, to a rather general underweighing of the importance of the role of connective tissue in muscular function. A major contributor to such a process may have been the conviction that if connective tissue can be easily broken, for example by blunt dissection, it can not play a major role in force transmission. For intramuscular connective tissue, previous work from our laboratory (Huijing et al., 1998), has shown this conviction to be false: Connective tissue that could easily be broken was shown to be able to transmit all force of muscle compartments. Those results and their interpretation also indicate that shear forces and shear stiffness need to be considered in detail. It has also led us to doubt the view that intermuscular connective tissue may be too weak or too compliant to play some role in force transmission.

Combination of views from extracellular matrix biology (e.g., Comper, 1996) and material sciences dealing with synthetic composites (e.g., Powell, 1993; Termonia, 1987) have created vision of limb tissues and/or muscle as composite materials (Trotter & Baca, 1987; Danowski et al., 1992; Trotter et al., 1992; Wang et al., 1993; Purslow & Trotter,

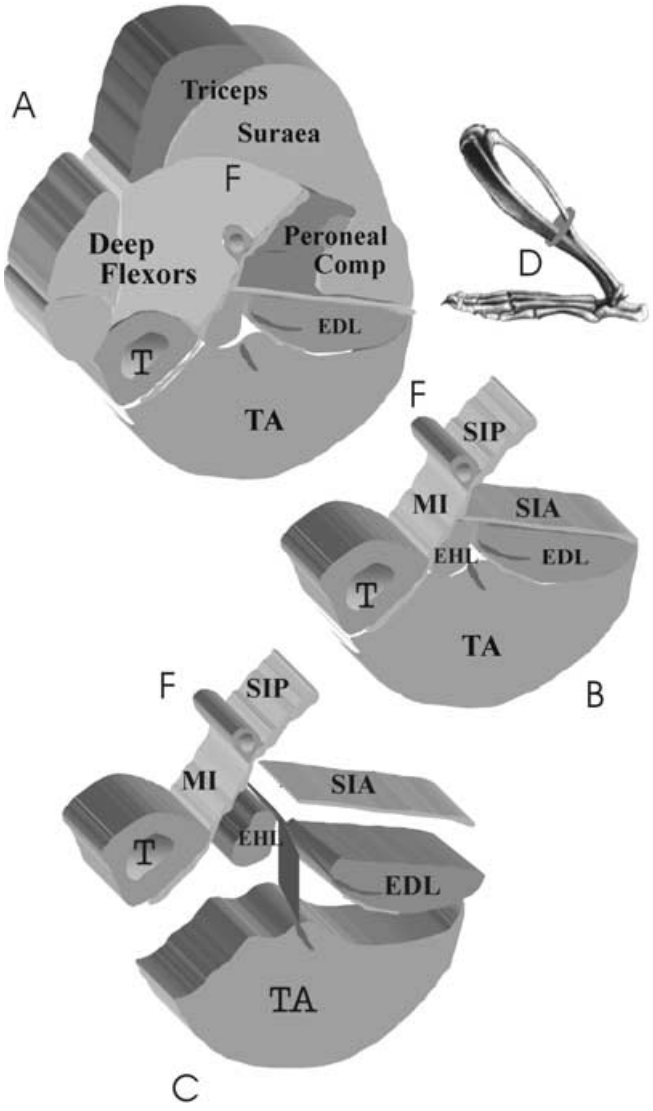

Fig. 6. Schematic representation of major neuro-vascular tracts within the rat anterior tibial compartment of the left leg. (A) The full lower leg after removal of biceps femoris muscle. Showing the peroneal compartment (Peroneal comp), deep flexors and triceps surae muscles. T indicates the tibia and F the fibula. (B) The selection of relevant elements in relation to the anterior tibial compartment, with EDL, TA and EHL muscles. MI the interosseal membrane, and SIA and SIP the anterior and posterior intermuscular septum respectively. (C) Exploded view of the anterior tibial compartment showing the sheet-like neuro-vascular tracts. (D) Indicates the approximate level of the cross-section shown, relative to tibia and fibula.

1994; Huijing, 1999a). In combination with detailed anatomical work (e.g., Trotter et al., 1983; Trotter et al., 1985a; Trotter et al., 1985b; Wal, 1988) and experimental work on non-myotendinous force transmission (Street, 1983; Huijing et al., 1998; Huijing, 1999a, b; Jaspers et al., 1999), a new window on feasible functions of connective tissue has been created and drawn attention to a connective tissue role in force transmission.

As a consequence of the above, limb connective tissue should be considered more as a unit, regardless if it is located intra- or extramuscularly. Thus the connections shown in Figure 4 are not to be considered separate sheets, but integral, albeit most likely reinforced, parts of a total network, the interactions of which determine its function. A main purpose of such an integral connective tissue structure is expected to be that of creating friction or resistance to movement, unless its layers are specifically lubricated at certain locations (e.g., tendineal vaginae). In locations where more connective tissue is concentrated, one should also be alert for possible concentrations of stress. This may mean that reinforcement of neurovascular tracts is not only present for protection of rather delicate structures, but also to transmit force.

Effects of lower limb connective tissue on muscular function

Our present results allow for an improved description of the mechanical effects of lower limb connective tissue on muscular function. There are two ways to approach to such mechanical effects, which in a final analysis should lead to identical conclusions, but may yield different views and insights:

• *A direct mechanics approach, in which we focus on transmission of force from muscle fibers to the outside world, (e.g., such approach has been employed in the introduction of the present article)*

Non-spanning muscle fibers do not span the distance between proximal and distal muscle fiber attachment areas of a muscle on aponeuroses or bone. Such muscle fibers end, in a variety of morphologies (e.g., Hijikata & Ishikawa, 1997; Hijikata et al., 1993; Huijing, 1999b), somewhere in the middle of the muscle belly. For non-spanning fibers, it has been argued (Trotter, 1990, 1991, 1993; Trotter & Purslow, 1992; Trotter et al., 1992; Hijikata et al., 1993; Trotter et al., 1995; Hijikata & Ishikawa, 1997), that shearing of intact endomysial -basal lamina -sarcolemma complexes of adjacent muscle fibers is determinant for intramuscular transmission of force from muscle fiber onto adjacent muscle fiber. In such a case, after transmission of force from a source fiber to target fiber, force could be transmitted further by serial transmission via sarcomeres onto the myotendinous junction of the target fiber.

In contrast, within the concept of intramuscular myofascial force transmission, shearing of the endomysial -basal lamina -sarcolemma complex has been indicated as very important for force transmission, not from muscle fiber to muscle fiber, but from the muscle fibers onto the connective tissue stroma of the muscle (e.g., Huijing, 1999a). This means that force may be transmitted onto the tendon without passing the myotendinous junctions. In such a case, force generated within a muscle fiber has to be distributed along *at least* two paths: (a) tensile transmission of force from sar-

comere to sarcomere followed by shear transmission onto the aponeurosis or tendon at the myotendinous junction and (b) shear transmission of force to the endomysial network. The stiffness of each of these paths determining the fraction of force transmitted by it: Stiffer paths will transmit more force. For pure intramuscular transmission the rule governing the separation of fractions of force is that the sum of in series and myofascially-transmitted force is constant.

It should be noted that intramuscular myofascial force transmission is likely to be active in all muscles, consisting of either spanning, or non-spanning muscle fibers or a combination of both. A description as provided above would work well for intramuscular force transmission within an isolated muscle.

However, in vivo additional connections of the intramuscular connective tissue need to be taken into account. If the tensile or shear stiffness of intra- to extramuscular connections is high enough, force may be transmitted through such connections: (a) from the muscle directly to bone (extramuscular force transmission) or (b) inter- muscularly (i.e., transmission from the intramuscular connective tissue of one muscle to the other). The latter means that, in principle, force generated within one muscle may be exerted at the tendon of another muscle.

• *An inverse mechanics approach, in which the mechanical effects are expressed in terms of 'support' for active muscle fibers*

In this approach the connective tissue performs a scaffold function by either preventing the muscle fibers from shortening on activation or very much limiting that shortening to small length changes. As shearing of the interface between the endomysium and muscle fiber's cytoskeleton (i.e., basal lamina, sarcolemma, and subsarcolemmal supramolecular system) is the determining mechanism, the degree of shortening of active muscle fibers will be determined by the shear stiffness of those structures. Finite element modeling of isolated skeletal muscle, implicitly, takes into account such mechanical interaction (e.g., Linden, 1998) by definition of a shear stiffness of the muscle elements of the model. Such modeling shows that a limited distribution of lengths of in series sarcomeres is possible in isolated muscle.

On the basis of that argument and experimental evidence in support of myofascial force transmission, the hypothesis of popping sarcomeres (Morgan, 1990), i.e., the overstretching of single sarcomeres of a myofibril to lengths higher than those involving minimal overlap between filaments, can be classified as unlikely. Such events are likely to be prevented by the mechanical interaction of intramuscular connective tissue and muscle fibers. It also shows that modeling of intersarcomere dynamics within a muscle fiber (e.g., Edman et al., 1993) should not be performed without taking into account the shear forces exerted by the endomysium or entire stroma of intra-muscular connective tissue.

Effects of interference with intra-muscular connective tissue

Experimental interference with the integrity of intramuscular connective tissue (not performed directly in the present experiment), has been obtained previously in two ways. (1) By cutting purposefully along the direction of the muscle fibers in an experiment (e.g., Huijing et al., 1998) or (2) by allowing the muscle to tear in this same direction due to intramuscular aponeurotomy (Brunner, 1998; Jaspers et al., 1999). The latter intervention is a surgical technique applied to spastic muscles, to counteract the functional effects of their overly short length (e.g., Thom & Asperger, 1982; Baumann & Koch, 1989). Any such destruction of intra-muscular connective tissue will allow substantial shortening of a segment of the population of fibers in a muscle. Such interventions will therefore result in a very much limited muscular force, but yield increases in muscle length range of active force (Huijing et al., 1998; Jaspers et al., 1999). This means that as a consequence of such intervention the distribution mean sarcomere length of different fibers of the muscle (i.e., fiber mean sarcomere length see also Huijing, 1995, 1998) is increased. It should be noted that such effects qualitatively resemble our present results for compartmental fasciotomy.

Interaction of intramuscular connective tissues in neighboring muscle

As active force increases with increasing EDL lengths from its active slack length to optimum length, the stiffness of the intramuscular connective tissue is expected to increase as well, because of the (non linear) stress strain characteristics of this connective tissue. This could lead to increased transmission of force to the force transducer via the proximal EDL aponeurosis. If connections between intermuscular connective tissue and EDL intramuscular connective tissue are stiff enough, force may be transmitted by shearing from other muscle (i.e., TA) to the EDL. If this is the case in the initial experimental condition, a variable part of the TA force may have been transmitted onto the EDL tendon, depending on the stiffness of the EDL intramuscular connective tissue. It should be noted that our present results provide evidence of intermuscular connections, but no direct proof of such intermuscular force transmission. However, our results regarding the effects of blunt dissection could possibly be an indication of such an event. The approximately proportional decrease of force at different lengths measured at the EDL tendon may be related to decreasing intramuscular connective tissue stiffness as a function of EDL force (and length). However, alternative explanations involving complex interactions of interference with the distribution of fiber means sarcomere length and elastic effects can not be ruled out. Even alterations in distribution of lengths of sarcomere in series within fibers, remains a feasible explanation, since such decreasing effects on absolute muscle force were reported to

occur without major changes of characteristics of the normalized length force curve (e.g., Meijer et al., 1997).

Interaction of extra- and intramuscular connective tissue

The extra-muscular connective tissue is continuous with the intra-muscular connective tissue. Therefore, if the interface between the two is sufficiently stiff, also the supporting function of the intramuscular connective tissue described above will be influenced by the state of the extra-muscular connective tissue and vice versa. For example, removing the epimysium of the human gracilis muscle allows its passive muscle fibers to respond extensively to forces in the cross fiber direction (100% increase in size, Holle et al., 1995). Our present results regarding effects of compartmental fasciotomy (Fig. 2) are indicative of increased distributions of fiber mean sarcomere lengths, and thus are fully compatible with the concept of extramuscular myofascial force transmission. An intact compartment apparently does stiffen the intra-muscular connective tissue, which does impose an increased, but not absolute, uniformity on fiber length and fiber mean sarcomere length. The decrease in force (of maximally activated muscle) of approximately 10% with fasciotomy, reported presently, is in agreement with results of Garfin et al. (1981), for muscles of the dog hindlimb. However those authors did not study length force characteristics systematically. They did also report muscle pressure to be decreased 50% after fasciotomy (i.e., 84 ± 17 to 41 ± 8 mm Hg).

It is also conceivable that interactions between muscle fibers and connective tissue plays a role in the etiology of repetitive strain injury (RSI, e.g., 'mouse arm'). In such conditions, rather localized, but low, activity of fibers with a muscle may cause sizable and repeated deformations of local intramuscular and possibly extramuscular connective tissue particularly in the case of unusual positions of involved joints.

Human compartment syndrome and surgical 'release' of the compartment by fasciotomy

Compartment syndromes have many etiologies and may arise in any area of the body that has little or no capacity for tissue expansion (Moore & Friedman, 1989). Compartment syndromes of the lower limb occur as an over-use type of injury in athletes, resulting in a chronic affliction. The compartment syndrome is interpreted as an increase in intracompartmental pressure above the capillary pressure, preventing capillary flow (e.g., Allen & Barnes, 1986; Abramowitz & Schepsis, 1994). If symptoms of chronic compartment syndrome have persisted for longer than 6 months, definitive treatment is indicated. Such treatment usually consists of immediate removal of the constricting force (Moore & Friedman, 1989), most often accomplished by means of a subcutaneous fasciotomy (e.g., Allen, 1990; Almdahl & Samdal, 1989). Even though fasciotomy is clinically and experimentally very successful regarding decompression, Garfin et al. (1981) raised questions concerning the merits of performing a fasciotomy for athletes with a compartment syndrome, on the basis of their experimental work on dogs. In addition intact compartmental fascia may be of importance for venous return and venous pump function of muscle (Rosfors et al., 1988), even though reports of contrary conclusions can also be found (Ris et al., 1993). Our present results show that altered myofascial force transmission results in substantial changes in force transmission and length force characteristics. The effects of such changes on muscular performance in the athlete should be considered in the decisions about therapy.

In conclusion, we found indications of involvement of extramuscular connective tissue in transmission of force. The interactions between extra- and intermuscular connective tissue does affect length force characteristics significantly and are thus shown to be of functional importance. It is concluded that properties of an in situ but isolated muscle are considerably different from in vivo properties.

Length-force characteristics were changed significantly by surgical interventions.

Blunt dissection caused a general force decrease at all lengths, i.e., without changing EDL optimum or active slack lengths. This indicates that intermuscular connective tissue mediates significant interactions between adjacent muscles.

Full lateral compartmental fasciotomy increased optimum length and decreased active slack length, leading to an increase of length range, while decreasing optimal force.

On the basis of these results, it is concluded that extramuscular connective tissue has sufficiently stiff connections to intramuscular connective tissue, to be able to play a role in force transmission. Therefore, in addition to intramuscular myofascial force transmission, extramuscular force transmission has to be considered within intact compartments of limbs. Such myofascial force transmission should be taken into account when considering muscular function and its coordination, and in clinical decisions for example regarding fasciotomy and repetitive strain injury.

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