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NON-MYOTENDINOUS FORCE TRANSMISSION IN RAT EXTENSOR DIGITORUM LONGUS MUSCLE

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Summary

The extensor digitorum longus muscle (EDL) of the rat hindleg consists of four heads. The heads are named after their insertions on the digits of toes II, III, IV and V. The EDL heads share a proximal tendon and aponeurosis, but have separate distal aponeuroses and tendons. By cutting the distal tendons of selected heads, direct myotendinous force transmission within these heads is prevented. Therefore, force exerted by the muscle would be expected to decrease according to the physiological cross-sectional area disconnected if myotendinous force transmission were the only mechanism of force transmission.

The results indicate that EDL force production remained at high levels after acute tenotomy: muscle length–force curves did not alter significantly following cutting of the tendons of heads II and III. Cutting the tendon of head IV as well leaves only head V in its original condition. After tenotomy of head IV, length–force characteristics were altered significantly, but optimum force was maintained at 84 % of that of the intact muscle. After separation of head IV from head V intramuscularly for some distance along their interface, the force dropped to much lower levels, with optimum force approaching 50 % of that of the intact muscle.

The length of active proximal fibres (located within head II) did not remain constant but increased with increasing muscle lengths after tenotomy as well as after partial separation of heads IV and V. The amount of length change decreased after intramuscular separation of the heads, indicating declining reactive forces.

It is concluded that force transmission occurred from tenotomized heads to their intact neighbours and *vice versa*. The magnitude of the force transmitted from head to head was dependent on the degree of integrity of the connective tissue at the interface between heads.

Key words: non-myotendinous force, length–force characteristics, tenotomy, extensor digitorum longus, skeletal muscle, rat.

Introduction

In skeletal muscle, the force exerted by muscle fibres is transmitted through the aponeurosis and through the tendon onto the bones. The myotendinous junction (MTJ) is recognised as a specialised part of skeletal muscle that is able to transmit force. It is localised at the interface between the aponeurosis and the ends of skeletal muscle fibres. It consists of an extensively folded sarcolemma, forming invaginations of the muscle cell filled with extracellular matrix (ECM) (Gelber *et al.* 1969; Schippel and Reissig, 1968; Trotter, 1993) and collagen fibres of the aponeurosis. It is commonly thought that MTJs represent the major, or only, site of force transmission in skeletal muscle.

However, in classical studies (Street and Ramsey, 1965; and particularly Street, 1983), strong evidence was presented that the force exerted by a single active muscle fibre is also transmitted by a pathway parallel to the MTJ pathway. From *in vitro* studies on skeletal muscle, a substantial number of molecular structures are known that connect intracellular elements to the extracellular space (reviewed recently by Patel and Lieber, 1997). In addition, several studies directed at 'series fibred' muscle have inferred that the force exerted by a fibre may be passed *via* intramuscular connective tissue to adjacent muscle fibres that also end in the middle of the muscle (e.g. Trotter, 1993; Trotter *et al.* 1995).

The relative importance of MTJ and non-MTJ force transmission is not known for whole skeletal muscle; as a consequence, it is difficult to address their functional relevance.

The principal aim of the present study is to determine whether lateral force transmission to connective tissue is of sufficient magnitude in whole muscle to play an important role in muscular functioning *in vivo*. Because of its morphology, the

extensor digitorum longus muscle (EDL) is especially suited for this study: it consists of four heads. The heads, named after their insertions on the digits of toes II, III, IV and V, share a proximal aponeurosis and tendon attached to the muscle's origin at the femur, but have separate distal aponeuroses and tendons. The length–force characteristics of the muscle–tendon complex after sequential tenotomy of heads II, III and IV will indicate the degree of non-myotendinous force transmission.

A second aim is to determine whether interference with muscle fibre interfaces affects force transmission in muscle. In orthopaedic clinical practice, such interference spontaneously follows aponeurotomy, an intervention aimed at lengthening spastic muscle (Baumann and Koch, 1989; Brunner *et al.* 1997). If the aponeurotomized muscle is stretched, connective tissue tears, and a gap opens and extends along the fibre direction into the muscle belly at the location of the cut.

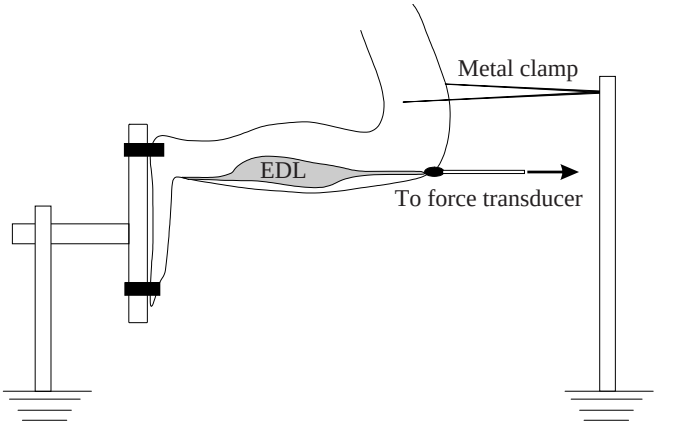

Fig. 1. Schematic representation of the experimental arrangement. The foot was strapped to a plastic plate, with the ankle at 90 °. The origin of the m. extensor digitorum longus (EDL) was attached to a force transducer. The femur was secured rigidly to a metal clamp.

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.

Surgical procedure and preparation for experiments

Eight male Wistar rats (body mass 303.6±19.7 g, mean ± S.D.) were anaesthetised with intraperitoneally injected sodium pentobarbital solution (initial dose 9.6 mg 100 g^{-1} body mass) and placed on a heated water pad (37 °C) during surgery and experimentation. Supplementary doses of pentobarbital solution were injected intraperitoneally throughout the experiment to maintain deep anaesthesia. The right EDL was exposed by removing the skin, the biceps femoris muscle and the tibialis anterior muscle. The EDL was dissected free of surrounding tissues, leaving the blood supply and innervation intact.

To avoid interfering with the physiological relationship between the length–force characteristics of the different heads of the EDL, force was measured at the 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. The knot was secured using a tissue glue (Histoacryl Blue, B. Braun AG, Melsungen, Germany). The Kevlar thread was connected to a force transducer (Hottinger Baldwin, maximal output error <0.1 %, compliance 0.0048 mm N^{-1}). For easy recognition of the distal tendons of the EDL during the experiment, differently coloured threads were used to mark the distal tendons of heads II, III and IV. Using an operating microscope (Carl Zeiss; magnification 6–40×), copper wires (diameter 0.1 mm) were inserted in the mid-sagittal plane of the muscle using a small needle. They were used post-experimentally as markers to provide points of reference for analysis of photographically obtained images. During surgery, four markers were placed at the most proximal and distal points of the muscle, at the most distal end of the proximal aponeurosis and at the proximal end of the distal aponeurosis. During the experiment (see below), two extra markers were placed at the most proximal point of the distal aponeurosis of head V and at the most proximal fibre end of head V.

The tibial nerve was dissected and cut as proximally as possible. The right foot of the rat was attached firmly to a plastic plate. After positioning the rat in the experimental apparatus (Fig. 1), the femur was secured by means of a metal clamp. The plate with the foot attached was manipulated such that the angle between the plate and the tibia was 90 °. 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). The EDL was covered with a layer of paraffin oil to prevent fluid loss.

Experimental procedure and data collection

The EDL was excited by stimulating the distal end of the severed tibial nerve supramaximally, using a pair of silver electrodes connected to a constant current source (4 mA, square pulse width 100 µs, pulse train 300 ms, 100 Hz). Isometric tetanic force was measured at various muscle lengths, starting below active slack length, defined as the smallest muscle length at which active muscle force approached zero.

Following each contraction, the muscle was allowed to recover near active slack length for 2 min. The muscle–tendon complex was then lengthened passively to a greater length than used for the previous contraction (1 mm increments). After stretching the muscle to the desired length, a twitch was evoked, to allow adaptation to the imposed length. 250 ms after this twitch, a photograph (Canon T90, 100 mm lens, extension tube 50 mm, 400 ASA slide film, shutter speed 1/60 s) was taken from the lateral side of the muscle. Passive force was measured simultaneously. The muscle was excited tetanically 550 ms later for 300 ms. During the tetanic plateau (i.e. 275 ms after evoking tetanic stimulation), total isometric muscle force was determined and a second photograph was taken. Force signals were acquired using a 12-bit A/D converter and

recorded on a microcomputer (sampling frequency 1000 Hz, resolution of force 0.01 N). A microcomputer controlled the timing of events related to stimulus generation as well as A/D conversion and photography.

Experimental protocol

During each experiment, several sets of length–force data were collected (see Fig. 2). (1) One set for the whole EDL (connected by four parallel tendons to its insertions) (N=6). (2) Three sets after sequential distal tenotomy of heads II, III and IV (tenotomy progressively lowers the number of parallel connections to insertions on the foot until only the tendon of head V remains as a single connection) (N=6). It should be noted that any distal tenotomy was performed as proximal as possible (i.e. close to the respective muscle bellies) to exclude any effects of mechanical interaction between the tendons. (3) In four animals, two additional sets were determined after progressive, but not total, intramuscular separation of heads IV and V by severing the connective tissue at their interface. The separation was performed in two steps. First, the tendon of head IV was separated from head V by lifting it until the most proximal point of the distal aponeurosis of head V could be seen. A marker was placed at this point (Fig. 2). The muscle fibres were traced visually to mark the most proximal end of head V on the aponeurosis. The second, more invasive, separation of heads IV and V was carried out by cutting intramuscularly with a scalpel along approximately 75–80 % of the length of the interface between the heads. No special techniques were used to ensure that muscle fibres were not damaged in this process, other than keeping the scalpel parallel to the direction of the muscle fibres. Deliberately cutting muscle fibres in pilot experiments caused the cut interface to be littered with clots of contractile proteins at the severed ends of muscle fibres. The absence of such features on visual inspection of the experimental muscles after myotomy makes it likely that muscle fibres were not extensively damaged.

Pilot experiments also showed that a complete separation of heads IV and V was not achievable without damaging the nerve and blood supply of head V.

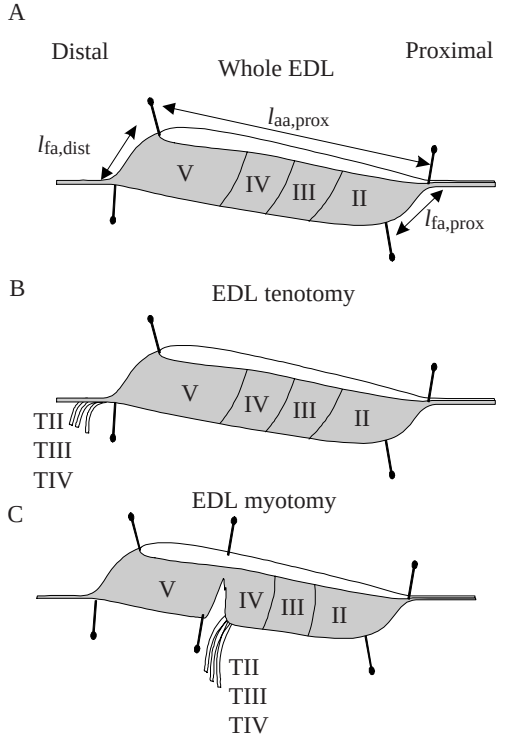

Fig. 2. Schematic representation of the rat m. extensor digitorum longus (EDL) and experimental protocol. (A) Whole EDL with markers inserted at strategic locations for estimating distal fibre length ($l_{fa,dist}$), proximal fibre length ($l_{fa,prox}$) and proximal aponeurosis length ($l_{aa,prox}$). Note that fibre angles drawn are highly exaggerated for clarity. (B) The EDL after the second stage of the experimental protocol: distal tenotomy of heads II, III and IV. Note that the muscle was only disturbed at its distal end. Tenotomies were performed sequentially with intermittent determination of muscle length–force characteristics. (C) The effects of the third stage of the experimental protocol: partial myotomy at the interface between heads IV and V. Only head V is still connected to its insertion by its distal tendon. Myotomies were performed sequentially in two stages with intermittent determination of muscle length–force characteristics. TII–TIV, tendons for heads II–IV.

Number of sarcomeres in series within fibres

In a separate group of EDL muscles (N=7), the number of sarcomeres in series within muscle fibres was determined according to methods described previously (Huijing, 1985). For the most proximal and distal bundle of heads II–V, four fibres were isolated from each bundle after treatment with dilute nitric acid. Mean fibre sarcomere lengths were determined by sampling sarcomere lengths for 80 μm lengths of the fibres every 800 μm along their length. The number of sarcomeres was calculated by dividing fibre length by mean fibre sarcomere length.

Post-experimental data collection and treatment of data

Post-experimentally, complete separation of the heads was performed and muscle mass was determined for each head. Photographic images of the EDL were projected at approximately 10 times life-size on a translucent screen. On the screen, appropriate distances between the markers were measured using a digitiser system (Science Accessories) with an accuracy of 0.05 mm. These measurements gave estimates for active muscle length (l_{ma}), distal and proximal fibre length ($l_{fa,dist}$ and $l_{fa,prox}$, respectively) and proximal aponeurosis length ($l_{aa,prox}$) (Fig. 2).

The individual relationships between passive muscle force and muscle length were fitted with an exponential curve (equation 1), using a least-squares criterion:

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

where y represents passive muscle force, x represents passive muscle length 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 length from the total force exerted by the muscle at that length.

Data for several variables (e.g. F_{ma}, $l_{aa,prox}$, $l_{fa,dist}$ and $l_{fa,prox}$) with respect to active muscle length (l_{ma}) were fitted using a polynomial:

$$y = b_0 + b_1x + b_2x^2 + ... + b_nx^n, \quad (2)$$

where y represents the particular variable, x represents active muscle length, n represents the order of the polynomial and b_0, b_1... 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 two purposes: (1) averaging of data and calculation of standard deviations, and (2) determining optimum force and optimum muscle length. For each individual muscle, optimum muscle force (F_{mao}) is defined as the maximum of the fitted polynomial for active muscle force, and optimum muscle length is defined as the corresponding active muscle length.

All force data were normalised for the optimum force of the intact muscle, and all muscle length data were expressed as deviations from the corresponding optimum muscle length.

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 added a significant improvement to the description of muscle length, muscle force and fibre and aponeurosis length.

To test for the effects of tenotomy and separating muscle heads on muscle force, two-way ANOVAs (factors: muscle length and muscle configuration) for repeated measurements were performed. If significant effects were found, *post-hoc* tests were performed using the Bonferroni procedure for multiple paired comparisons to identify differences between the muscle force produced for each muscle configuration.

A regression analysis was performed for data from individual muscles relating the length change of proximal fibres to the muscle length for the experiments involving separation of heads IV and V along two-thirds of the length of their interface. The significance of the positive slope was tested using a *t*-test.

Differences were considered significant at $P<0.05$.

Results

Table 1 shows the mass of the individual heads expressed as a percentage of total EDL mass. Note that head V is the biggest of the EDL heads, representing approximately 45 % of total EDL mass. As the number of sarcomeres in series within fibres of different heads (Table 1) did not differ substantially, values of normalised mass may be considered as estimates of normalised physiological cross-sectional area and thus of maximal relative contribution to total EDL muscle force.

Acute effects of distal tenotomy

Tenotomy did not cause any apparent changes in the integrity of the experimental muscles, with the exception of one case in which a rupture of the interhead III–IV interface in the direction of the fibres occurred spontaneously during contraction after tenotomy of head III.

Because of this material failure, this particular muscle was excluded from the present study.

Table 1. *Properties of the rat hindlimb m. extensor digitorum longus (EDL) heads*

EDL segment	Mean relative mass (% total EDL)	Sublocation	Number of sarcomeres in series
II	23	Proximal	5096±190.98
		Distal	4910±164.70
III	17	Proximal	4643±252.36
		Distal	5071±254.03
IV	15	Proximal	4760±186.82
		Distal	5045±186.92
V	45	Proximal	5348±164.92
		Distal	5047±196.76
Whole EDL	100		4990

Values for number of sarcomeres in series in the proximal and distal location of the heads are mean ± S.D. (N=28).

Length–force characteristics

Fig. 3 shows mean length–force curves (normalised to relative length) before and after sequential tenotomy of heads II, III and IV (four uppermost curves). Corresponding values of optimum force normalised to whole-EDL optimum force and the deviation of optimum muscle lengths from whole-muscle optimum length

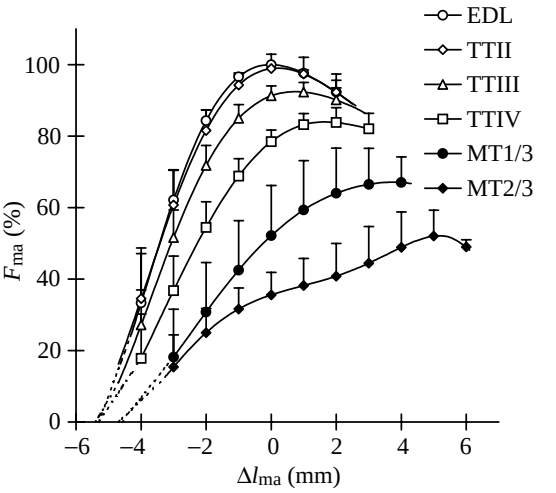

Fig. 3. Length–force characteristics of the rat m. extensor digitorum longus (EDL). From top to bottom the following curves are shown: whole EDL (EDL), after distal tenotomy of heads II, III and IV (TTII, TTIII and TTIV, respectively) and after subsequent partial separation (myotomy) of heads IV and V for approximately one-third and two-thirds of the fibre length (MT1/3 and MT2/3, respectively). Values are means + S.D., N=6 for tenotomy experiments and N=4 for myotomy experiments. Active muscle length (l_{ma}) is shown as the deviation from optimum length, and force (F_{ma}) is normalised to the optimum force of the whole EDL.

Table 2. *Experimental conditions and summarised results for optimum force and length of the rat hindlimb m. extensor digitorum longus (EDL)*

Whole EDL	*N*	F_{mao}	Δl_{mao}
Heads II, III, IV, V connected to tendon	6	2.2±0.65	0
Post-tentomies			
Heads III, IV, V connected to tendon	6	99.0±4.2	0.3±0.4
Heads IV, V connected to tendon	6	92.4±2.6	1.0±0.5
Head V connected to tendon	6	84.0±3.8	1.6±0.6
Post-'myotomies'			
Head V connected to tendon, one-third partial separation of heads IV and V	4	67.0±7.6	3.4±1.0
V connected to tendon, two-thirds partial separation of heads IV and V	4	52.1±6.3	5.3±0.7

F_{mao} , optimum muscle force; Δl_{mao}, deviation of optimum length from whole EDL optimum length.
Values are means ± S.D.
F_{mao} values for whole EDL are in N, all other F_{mao} values are expressed as percentage of this value.

are shown in Table 2. Analysis of variance revealed that the length–force curves were not significantly different from that of whole EDL after tenotomy of heads II and III, despite the fact that, for up to 40 % of the muscle mass, direct myotendinous force transmission was removed. Subsequent tenotomy of head IV resulted in significant differences in the length–force characteristics. *Post-hoc* tests identified significant differences in force at muscle lengths from Δl_{ma}=−3 mm up to l_{mao}, but the decrease was always considerably less than 45 %. For example, optimum force decreased to 84 % of whole-muscle optimum force as optimum length increased by 1.6 mm (Table 2). This shift in optimum length is compatible with changes in active fibre length (see below).

Fibre and aponeurosis lengths in relation to muscle length

The relationship between the length of the most distal fibre (located in head V) and active muscle length was not altered significantly by acute tenotomy of heads II, III and IV (Fig. 4A), nor was the relationship between proximal aponeurosis length and muscle length (Fig. 4C). In contrast, significant changes were found in the active length of the most proximal fibres (located in head II) (Fig. 4B). Despite the fact that these fibres were no longer connected to the muscle's insertion *via* their myotendinous junction, they were still elongated at higher muscle lengths, albeit progressively less so after sequential tenotomy of heads II, III and IV than in the intact EDL. It is concluded that, despite tenotomy, a reactive force of sufficient magnitude is exerted to maintain proximal active fibres at relatively high lengths, allowing them to contribute to total muscle force. As the source of the reactive force is not the myotendinous junction after tenotomy, it is hypothesised that the intramuscular connective tissue is the carrier of such forces.

Acute effects of partial myotomy of the interface between heads IV and V

Length–force characteristics

Fig. 3 shows the length–force curves obtained before and after two stages of physical intramuscular separation of the interface between heads IV and V (lower three curves). It should be noted that, for this part of the experiment, no further tendinous tissue was cut, leaving the myotendinous force transmission capability of head V unchanged. Also note that there is a gradual progression in the changes in length–force characteristics after sequential tenotomies and partial myotomies.

The initial phase of 'myotomy' was obtained by lifting tendon IV from head V until the most proximal point of the distal aponeurosis of heads V could be seen. Despite the fact that this movement was performed against minor resistance, the intervention acutely caused a partial separation (by approximately 30 %) of head IV and V at the fibre interface. This lesion was accompanied by significant changes in the length–force characteristics.

Both optimum and active slack length moved to higher muscle lengths by several millimetres, and force decreased significantly over the length range from −2 to +1 mm relative to whole EDL optimum length. Note that force decreased by a similar amount as it did following tenotomy of head IV (by 17 %). It is concluded that lifting of tendon IV from head V interfered with the interface between these heads, which caused substantial interference with force transmission.

The second phase involved a more thorough, but still partial, separation of heads IV and V, by cutting the intramuscular connective tissue at the interface between the heads along approximately 75–80 % of its length. Again, substantial and significant changes in the length–force characteristics were found. Optimum length shifted several millimetres more to higher lengths, and force decreased significantly for the muscle length range −3 to +3 mm. Optimum force decreased to 52.1 % of whole-muscle optimum force. It should be noted that, even though this value is close to the level of 45 % predicted on the basis of reduced muscle mass following tenotomy, it is still more than 7 % higher. This indicates that even a partially intact interface can still transmit substantial levels of force between the muscle heads. It is concluded that interfering with the

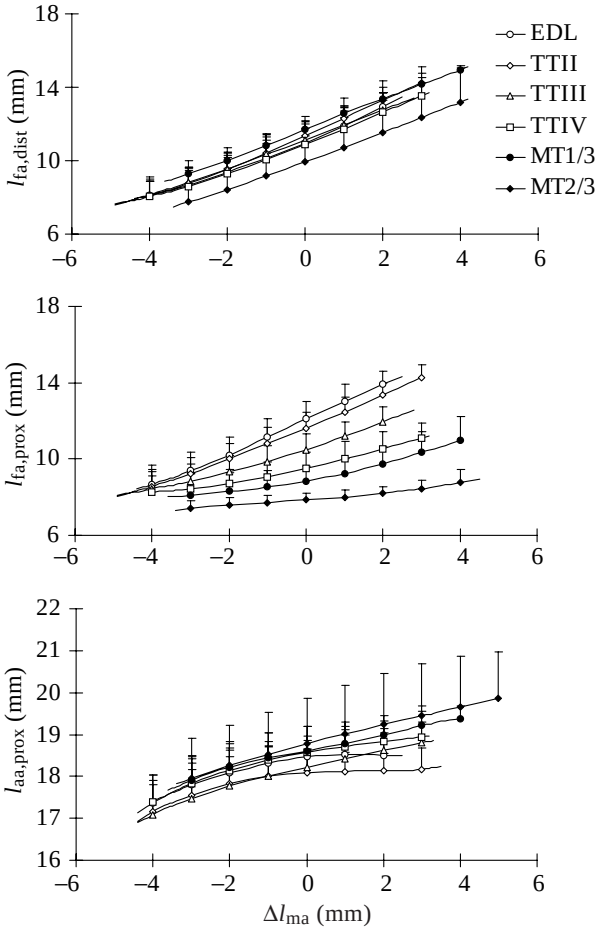

Fig. 4. The relationships between fibre and aponeurosis lengths and muscle length. (A) Distal fibre length ($l_{fa,dist}$), (B) proximal fibre length ($l_{fa,prox}$) and (C) the length of the proximal aponeurosis shared by all EDL heads ($l_{aa,prox}$). Curves connecting mean values are shown for whole EDL (EDL), after sequential distal tenotomies of heads II, III and IV (TTII, TTIII and TTIV, respectively) and after subsequent separation of heads IV and V (myotomy) along approximately one-third and two-thirds of the fibre length (MT1/3 and MT2/3, respectively). Values are means + S.D., N=6 for tenotomy experiments and N=4 for myotomy experiments. Active muscle length (l_{ma}) is shown as the deviation from optimum length.

interface between heads IV and V resulted in significant interference with force transmission from the other heads to head V. The magnitude of this effect seems to be proportional to the length along which the heads are separated.

Fibre and aponeurosis lengths in relation to muscle length

Partial separation of heads IV and V also caused changes in muscle geometry in head V (Fig. 4). The distal fibre length and proximal fibre length curves were altered significantly, as was the curve for the length of the proximal aponeurosis. Increasing the degree of separation of heads IV and V caused the curve for the distal fibre length to shift to higher muscle lengths. This also occurred for the curve for the proximal fibre length. However, it should be noted that, at high active muscle length,

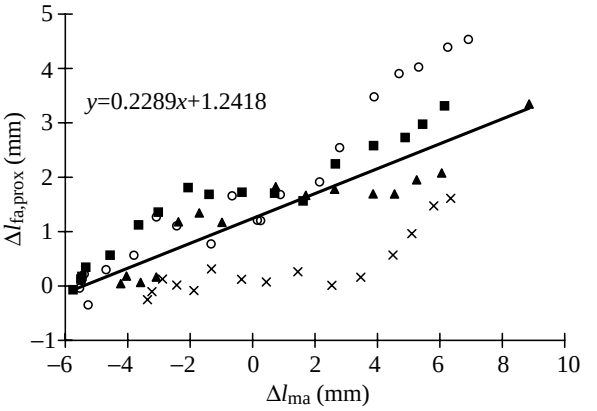

Fig. 5. Proximal fibre length change after myotomy ($\Delta l_{fa,prox}$) as a function of muscle length. Heads IV and V were separated along approximately two-thirds the fibre length. Data for four individual rat hindlimb extensor digitorum longus muscles (EDL) are shown (different symbols) as a function of active muscle length (l_{ma}). Active muscle length is shown as the deviation from the optimum length. The least-squares regression line and its equation are shown (r^2=0.6). The positive slope of the regression line indicates proximal fibre length change (P<0.01), and thus transmission of force, even in this experimental condition.

significant lengthening of proximal fibres did still occur, even if the heads were separated for more than two-thirds of the length of their fibres. For this degree of separation, regression analysis of the change in the proximal fibre length after myotomy *versus* the deviation of active muscle length from optimum length yielded a significant positive slope (Fig. 5). This indicates that some reaction forces are still exerted onto muscle fibres at a distance quite proximal to the location of the lesion.

It is concluded that interference with the interface between EDL heads IV and V does reduce the degree of force transmission, but does not abolish it completely.

Discussion

We distinguish two components of force transmission in skeletal muscle: myotendinous and non-myotendinous transmission. The latter component may be referred to as myofascial transmission since the force is thought to be transferred through the continuous endomysial fascia of the muscle. In the present study, we could abolish myotendinous force transmission in large parts of the EDL because of its specialised morphology.

Myotendinous force transmission

Direct evidence that force is transmitted onto the tendon at the myotendinous junction (MTJ) is difficult to obtain. Nevertheless, it is generally accepted that such transmission is an important factor in muscle function. Because of the morphology of the MTJ, it seems likely that force is transmitted by shear (e.g. Tidball, 1991). The invaginations of the sarcolemma increase the contact surface area and have a

configuration ideal for shear transmission of force. The basal membrane of the muscle fibre is thought to play an important role in this process.

The average breaking stress of the MTJ was reported to be only 20 % greater than average maximum isometric tension (Tidball and Chan, 1989). This small safety margin between maximum junction load and breaking strength suggests that structures parallel to muscle fibres may also be important in load-bearing, especially in eccentric contractions where the muscle is loaded heavily by external forces (Tidball, 1991).

Myofascial force transmission

A paradox in the present experiments is that very little force is needed to interfere with the integrity of the intramuscular connective tissue at the interface of heads IV and V (simply lifting tendon IV from head V in the first stage of our 'myotomy' experiments had an effect), and yet it is proposed that a large force is transmitted onto and through that same tissue. This may be explained by two considerations: the nature of the force exerted onto the connective tissue and the distribution of this force. First, in intact muscle, the force is exerted by shearing, while during the process of manual separation of the heads a tensile force is exerted at much higher angles. The tensile length–force properties of the connective tissue network are likely to be quite different from its length–force characteristics under shear (e.g. Purslow and Trotter, 1994). It is apparently much easier to bring the network to its failing point using tensile forces than using shear forces. When studying the properties of so-called 'loose' connective tissue, this phenomenon should be taken into account.

Second, after progressive tenotomy, it is clear that the EDL force is distributed onto the whole endomysial network, whereas during the actual 'myotomy' the force exerted is concentrated on a small fraction of the whole network.

Myofascial force transmission was first inferred from the results of single-fibre experiments (Street and Ramsey, 1965) and was shown very convincingly by Street (1983) from experiments on small bundles of fibres. In such lateral force transmission from muscle fibres, force must be transferred across the sarcolemma onto the lateral surface of the connective tissue tunnels in which muscle fibres are located. Possible routes are considered below.

Lateral trans-sarcolemmal connections

In striated muscle, costameres couple myofibrils to the sarcolemma by providing lateral actin–membrane associations, and integrins (a super-family of extracellular cell surface glycoproteins) participate in linking the cytoskeleton to the extracellular matrix through the cell surface (Hemler, 1990; Hynes, 1992; Sastry and Horwitz, 1993). Both these molecular structures are intimately associated with the sarcolemma (Pardo *et al.* 1983; Craig and Pardo, 1983; Burridge *et al.* 1988; McDonald *et al.* 1995). Examples of costameric constituents are vinculin (Pardo *et al.* 1983), talin (Tidball *et al.* 1986), spectrin (Repasky *et al.* 1982; Craig and Pardo, 1983) and dystrophin, which has been implicated as playing an important role in Duchenne and Becker muscular dystrophies (e.g. Ozawa *et al.* 1995; Worton, 1995). Dystrophin has been implicated in the transmission of force to the sarcolemma (Ibraghimov-Beskrovnaya *et al.* 1992). For further details on extra-sarcomeric protein connections, see a recent review by Patel and Lieber (1997). In cultured cardiac muscle, evidence has been found to indicate that the costameres are the sites of contractile force transmission to the substratum (Danowski *et al.* 1992). Vertebrate skeletal muscle expresses a large variety of different integrins (Terracio *et al.* 1989).

Other evidence for myofascial force transmission in whole muscle

Balice-Gordon and Thompson (1988) examined the innervation of intact rat EDL, and distinguished two extramuscular branches. They thought that this compartmentalisation of the EDL might allow for some degree of control of individual foot digits, but when stimulating one branch of the nerve, they found that force could be measured at the digits connected to parts of the muscle innervated by the second and unstimulated branch. Without further interpretation, they concluded that the different heads of the EDL must have considerable 'mechanical coupling'.

On the basis of morphological work and biomechanical modelling, Trotter (1990), Trotter and Purslow (1992) and Purslow and Trotter (1994) inferred that lateral force transmission across the sarcolemma is necessary in 'series fibred' muscles. In these muscles, fibres do not extend from one bony or aponeurotic fibre attachment location to the next, but have tapered ends in the middle of the muscle belly. Therefore, these fibres cannot transmit force directly onto an aponeurosis. These authors indicated that, in such muscle, force could be transmitted between adjacent muscle fibres *via* their common endomysium.

Muscle fibres that are not exposed to tendinous reaction forces *via* their aponeurosis will shorten (Fig. 4C), probably because of the shear strain of the loaded lateral basal membrane and endomysial structures. In effect, a continuous distribution of fibre lengths is expected in which fibre length decreases with distance from the interface of the muscle heads, without distal tenotomy, to the most proximal fibre. Any distribution of EDL fibre mean sarcomere length, due to differences in actual fibre lengths (Huijing *et al.* 1994), will be increased by tenotomy. Because of this increase in the distribution of the mean sarcomere length of the fibres, the muscle length range for active force generation will increase at the expense of optimum force (Huijing, 1995, 1996, 1998*b*), which will occur at higher muscle lengths (Fig. 3; Table 2).

A pilot experiment suggests that the effect of tenotomy is not influenced substantially by the order in which tenotomy is performed. When tendons III and IV were cut simultaneously, length–force values were quantitatively similar to those after tenotomy IV following tenotomy III (optimum force decreased to approximately 84 % and optimum length shifted to higher muscle lengths in both cases).

If the integrity of the intramuscular connective tissue is damaged, the muscle's capability for myofascial force transmission decreases accordingly. In one experiment, not included in the present results and partially in contrast with them, the interface between EDL heads III and IV tore spontaneously during a contraction for the determination of length–force characteristics after tenotomy of head III. Such ruptures are an inevitable result after experimental aponeurotomy followed by stretch (Brunner *et al.* 1997), but not after tenotomy (present study). In agreement with the major conclusions of the present study, such a rupture caused different changes in the length–force characteristics from the changes caused by tenotomy alone because of interference with lateral force transfer (optimum force decreased to approximately 70 % of the initial whole-EDL value in contrast with a decrease to 92 % after tenotomy of head III without rupture). However, after subsequent partial 'myotomy' at the head IV–V interface, optimum force was similar to that reported above in the Results section. We believe that the spontaneous interface rupture is an indication that safety margins for lateral force transmission may be relatively small, as in myotendinous force transmission (Tidball and Chan, 1989).

Intra- and extramuscular routes of force transmission

In tenotomized EDL

Since tenotomy was performed as close as possible to the distal end of the whole EDL muscle belly, it is conceivable that connections between, or shearing of, the remaining tendon ends could contribute to force transfer between the heads. However, a pilot experiment indicated that length–force characteristics were quantitatively affected in a similar way if tenotomy were performed as close as possible to the distal end of each EDL head, leaving no possibility of tendon interactions. Shearing between distal intramuscular aponeuroses is not possible since these structures do not overlap. Therefore, in the present experiments, any force exerted locally on the endomysial fascia of tenotomized heads must be transferred laterally to the tendon(s) of heads with intact tendons. Similar to the events proposed for 'series fibred' muscles (Trotter, 1990, 1993; Trotter *et al.* 1995), it is conceivable that this force could be transmitted back onto the active fibres of the untenotomized heads and exerted through the myotendinous junctions onto the tendon(s). In our experiment, this would mean that, after tenotomy IV, almost twice the force has to be carried by the fibres of head V. Since there is no indication of altered distal fibre lengths following tenotomy (Fig. 4A), it seems more likely that force is exerted onto the distal aponeurosis through a parallel path bypassing the muscle fibres. This indicates transmission of force onto the aponeurosis of head V through the integral connective tissue apparatus of the muscle (the endomysial–perimysial–epimysial complex) or aspects thereof.

Our present results show that the myofascial pathway can transmit all the force exerted by the EDL heads within the muscle.

In intact EDL

Is myofascial force transmission also a quantitatively important mechanism in intact EDL? The conclusive way to determine this would be to consider the mechanical stiffness of the two parallel components: the sarcomere-to-sarcomere-to-myotendinous junction pathway as well as the myofascial pathway. Most force will be carried by the stiffer component. However, little is known about the relative stiffness of these components. Nevertheless, the observations of Balice-Gordon and Thompson (1988), described above, would indicate a substantial role for non-myotendinous force transmission in intact EDL. As there is no evidence that rat EDL connective tissue is specialised in this way, myofascial force transmission is likely to be a general feature of muscle.

If force is transmitted to the fascial apparatus of a muscle and not necessarily from fibre to fibre, and if this process is similar in so-called 'series fibred' muscles, then the fibres of such muscles are actually arranged in parallel rather than in series. This would mean that the functional relevance of such architecture would have to be reconsidered.

Possible extramuscular paths of force transmission

If a substantial proportion of *in vivo* muscle force is transmitted laterally to the fascial apparatus of muscle, is this force transfer limited to intramuscular connective tissue or can force be transferred out of the muscle onto the bones *via* the general connective tissue apparatus of the limbs? This question cannot be answered on the basis of the present results, since the muscle in these experiments was dissected from its surrounding connective tissue structures. However, Riewald and Delp (1996), using intramuscular stimulation, found that the rectus femoris muscle is still capable of acting as a knee extensor after distal tendon transfer to flexor sites in human patients. If the functional relevance of this phenomenon is confirmed, the concept of muscle as a functional unit of force generation and function will have to be revised (Huijing, 1998*a*).

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