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Extramuscular myofascial force transmission for *in situ* rat medial gastrocnemius and plantaris muscles in progressive stages of dissection

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Summary

The aim of this study was to establish the extent of extramuscular myofascial force transmission for dissected rat medial gastrocnemius (GM) and plantaris (PL) muscles. Initially, this was done with GM still connected to extramuscular connective tissue (general fascia, neuro–vascular tract and compartmental fascia). Neighbouring muscles were also connected to these tissues. In a later stage, it was dissected progressively until finally a fully dissected *in situ* GM was obtained, for which the neuro–vascular tract (i.e. the nerves, bloodvessels and the surrounding connective tissue) was the only extramuscular tissue left intact. Force of GM was measured not only at its distal tendon in progressive stages of dissection, but also at its dissected proximal tendon. In the stage where GM was still connected to extramuscular tissues, the experiments showed that up to 40.5±5.9% (mean ± S.E.M.) of the force exerted by the neighbouring PL muscle was transmitted onto the calcaneal bone, even when the PL tendon was not connected to this bone. After distal PL-tenotomy, a difference between proximally and distally measured forces of GM constituted evidence for myofascial force transmission. In the fully dissected *in situ* GM muscle, no relevant myofascial force transmission occurred in the reference position (the position of the GM origin corresponding to a knee angle of 120°). However, some myofascial force transmission occurred when the relative position of the origin of the fully dissected GM muscle was changed with respect to the neuro–vascular tract.

Key words: dissection, epitendinous tissues, extramuscular myofascial force transmission, *in situ* muscle, neuro–vascular tract, tenotomy.

Introduction

Muscles are often considered to be functionally independent of each other and of surrounding tissues and it is assumed that all muscle force is transmitted *via* its tendon to the bone. Many characteristics of skeletal muscle, such as force–length and force–velocity relations, have been studied using fully dissected *in situ* animal muscle (e.g. De Ruiter et al., 1995; Meijer et al., 1998; Rack and Westbury, 1969; Rijkelijkhuizen et al., 2003). In such conditions, force at the proximal tendon should be equal to force at the distal tendon of that muscle. Therefore, force has been measured exclusively at one tendon.

In addition to myotendinous force transmission (i.e. force is transmitted *via* the myotendinous junctions to the tendon (e.g. Tidball, 1984; Tidball, 1991), there are other paths by which force can be transmitted from a muscle. Force can be transmitted from muscle fibres onto the continuous endomysial fascia of the muscle (Street and Ramsey, 1965; Street, 1983). This type of force transmission is called myofascial force transmission. Myofascial force transmission can occur *via* several pathways. (1) If force is transmitted within a muscle, from the endomysial–perimysial network onto adjacent fibres (e.g. Purslow and Trotter, 1994) or onto the aponeurosis (Huijing et al., 1998; Huijing, 1999a), it is referred to as intramuscular force transmission. (2) If force is transmitted *via* the connective tissue at the interface between the muscle bellies of adjacent muscles (Maas et al., 2001), it is called intermuscular force transmission. (3) If force is transmitted *via* other, extramuscular, connective tissues (Huijing, 1999b) (e.g. compartmental fascia, general fascia or connective tissue reinforcing nerves and blood vessels), we refer to it as extramuscular force transmission. Differences between proximally and distally measured forces have been observed in rat extensor digitorum longus (EDL) muscle when measured within an intact connective tissue environment (Huijing and Baan, 2001), indicating that force is transmitted not only *via* the myotendinous junction, but also *via* myofascial pathways.

In vivo, skeletal muscles are surrounded by neighbouring

muscles and connective tissue. *In situ* experiments are usually performed on dissected muscles freed from their surrounding tissues with the exception of the neuro–vascular tract (i.e. the nerves, bloodvessels and the surrounding connective tissue). If force is measured at only one tendon of the muscle, usually the distal one, the assumption is made implicitly that extramuscular myofascial force transmission does not occur. However, the intact neuro–vascular tract is at least a potential path for transmission of passive or active force from the muscle (Yucesoy et al., 2003).

The aim of the present study was to test the hypothesis that extramuscular myofascial force transmission occurs for rat medial gastrocnemius (GM) muscle and its neighbouring plantaris (PL) muscle, and that such transmission will decrease in progressive stages of dissection of the GM muscle–tendon complex. In addition, the hypothesis will be tested that force of PL may be exerted on the calcaneal bone *via* extramuscular connective tissues around GM and PL tendons (epitendinous tissues) and muscle bellies. Active and passive force exerted by GM will be measured not only at its distal tendon, but also simultaneously at its proximal tendon. Any difference between proximally and distally measured forces constitutes evidence for extramuscular myofascial force transmission. To test if the extent of myofascial force transmission *via* the neuro–vascular tract varied with the position of the muscle belly relative to this tract, effects of moving the GM origin were studied as well.

Materials and methods

Surgical and experimental procedures were approved by the Committee on Animal Experimentation of the Vrije Universiteit Amsterdam and complied with Dutch law.

Muscle dissection and experimental set-up

Male Wistar rats, *Rattus norvegicus albinus* Berkenhaut 1769 (*N*=7; body mass 250–289 g), were anaesthetised with 1.5 g kg^{-1} body mass urethane (administered intraperitoneally). Supplemental injections of 0.63 g kg^{-1} body mass were given if necessary. During surgery as well as during the experiment, the animal was placed prone on a heated pad of 32°C to prevent hypothermia.

The lateral (GL) and medial (GM) parts of the gastrocnemius muscle were exposed by removing the skin and most of the biceps femoris muscle from the limb. Semitendinosus and gracilis posticus muscles were removed as well. The soleus, deep flexors, peroneal muscles and muscles in the anterior crural compartment were left intact, but were denervated. Only extramuscular connections of GM were left intact. GM and GL of the rat are parts of one muscle. GL and GM were separated carefully without separating them from any other surrounding tissues. This was done by cutting the GL loose from the GM in such a way that GM was not damaged. Thus, the intermuscular connection between the GM and GL was severed. The reason that only the GM was studied was to be able to investigate the extent of extramuscular force transmission for *in situ* rat GM muscle in various stages of dissection. This is of interest because the fully dissected rat GM *in situ* muscle model is a widely used model. The plantaris (PL) muscle is facing the aponeurosis of GM muscle, which prevents intermuscular connections (i.e. direct connections between two intramuscular connective tissue stromata). Therefore, the connection between GM and PL is extramuscular and was left intact. The rat PL muscle is a muscle of a shape similar to the rat GM muscle, but of smaller size. Around the GM and PL muscle bellies, extramuscular connective tissue (i.e. remnants of the general fascia and epimysium) was left intact.

The tendons of the GL muscle and the soleus muscle were cut from the calcaneal bone without damaging extramuscular tissues around the tendons. This intervention was performed from a proximal direction through the space between the separated GM and GL muscles. These extramuscular tissues around the tendons involve remnants of general fascia, epimysium, neuro–vascular tract and compartmental fascia; these tissues will be further referred to as epitendinous tissues. In contrast to the proximal tendons, the distal tendons of GM and GL are not completely separate. Therefore, the tendon of GL was cut from the tendon of GM. After this intervention, GL and soleus muscles were not connected to the calcaneal bone in any way. The calcaneal bone, with the neighbouring tendons of GM and PL muscles still attached, was cut without removing any of the epitendinous tissues. The calcaneal bone was connected to a force transducer with a small metal rod. A representation of the experimental set-up is shown in Fig. 1.

To be able to measure force exerted by the GM muscle at its proximal tendon, a piece of the femur with the origin of the muscle attached was cut. This piece of bone was connected to a second force transducer using a small metal rod. The femur was clamped in such a way that the knee could be fixed at an angle of approximately 120°, with the lower leg horizontally. Muscle temperature was controlled by a water-saturated airflow of approximately 33°C around the hind limb. The blood supply remained intact. Within the upper leg, the sciatic nerve was cut as proximally as possible, placed on a bipolar stimulating electrode and used for stimulation. All distal branches of this nerve were cut except for the branches to GM and PL, which were clearly distinguishable from the other branches. By cutting these other distal nerve branches carefully through the space between the separated GM and GL, without pulling on tissues that enclose the GM and PL nerve branches, damaging these nerve branches was prevented. The supramaximal stimulation current was 1 mA with a pulse width of 0.05 ms for maximal stimulation of all fibres of GM and PL. The stimulation frequency used was 200 Hz, which was sufficient to obtain maximal isometric force at the experimental temperature. After the experiments, the rats were killed by cervical dislocation.

The distal force transducer (custom made, compliance 8 μm N^{-1}) used was part of an isovelocity measuring system (for more details about this system see (De Haan et al., 1989). The transducer was mounted on a servomotor. Motor movements and stimulation were computer controlled. The

proximal force transducer (serial number 17053, compliance 16.2 μm N^{-1}; BLH Electronics Inc., Canton MA, USA) remained in a constant position. The proximal and the distal force transducer were positioned in the line of pull of the GM muscle. Force and length data were digitised (1000 Hz) and stored on disk for later analysis.

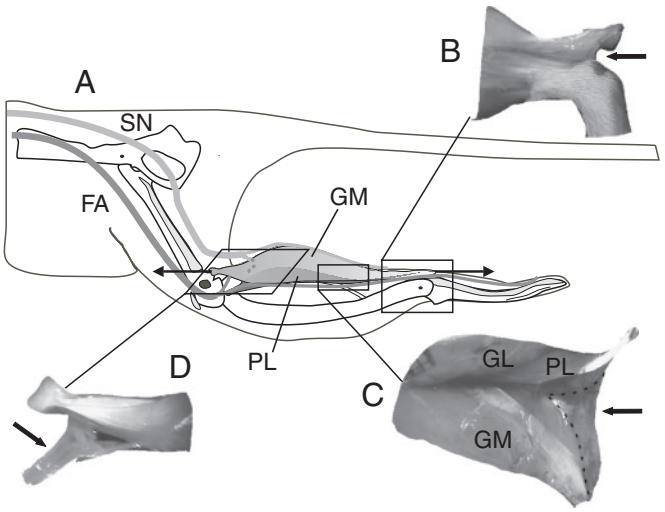

Fig. 1. Representation of the experimental set-up. (A) Medial view of the GM and PL in the experimental set-up after biceps femoris muscle, semitendinosus and gracilis posticus muscles were removed. The GM and GL were separated by cutting fibres in such a way that the GM was not damaged. The GM is made transparant in the image to show the position of the PL. The soleus, deep flexors, peroneal muscles and muscles in the anterior crural compartment were left intact, but for clarity are not shown. Only extramuscular tissues around the GM were left intact (see B–D). Image A also shows the sciatic nerve (SN) and the femoral artery (FA) approaching GM laterally and medially respectively and entering GM proximally as a neuro–vascular tract (i.e. nerve, bloodvessel and surrounding connective tissue). Proximally, the dissected origin of the GM was attached to a force transducer using a metal rod (represented by an arrow). In the initial condition, the distal tendons of the GM and PL, the epitendinous tissues and a piece of the calcaneal bone were connected to a force transducer with a metal rod (represented by an arrow). The femur was clamped in such a way that the knee could be fixed at an angle of approximately 120°, with the lower leg horizontally. (B) Extramuscular tissues around the distal GM and PL tendons, i.e. remnants of the general fascia, epimysium, neuro–vascular tract and compartmental fascia (referred to as epitendinous tissues and indicated by an arrow). Medial view. Note that these tissues are exposed only for clarity by lifting the piece of the calcaneal bone. (C) Extramuscular tissues around the GM and PL muscle bellies, i.e. remnants of the general fascia and epimysium. Dorsomedial view. This image does not provide a representation of the position of the muscles in the experiment. For clarity, the GM and PL are pulled apart to expose the extramuscular tissues (indicated by an arrow and broken lines). (D) Medial view of the dissected origin of GM. The origin of GM was dissected with a piece of the femur. The image also shows a part of the neuro–vascular tract embedding the femoral artery approaching and entering GM proximally (indicated by an arrow). The sciatic nerve approaches the GM from the lateral side and is therefore not visible.

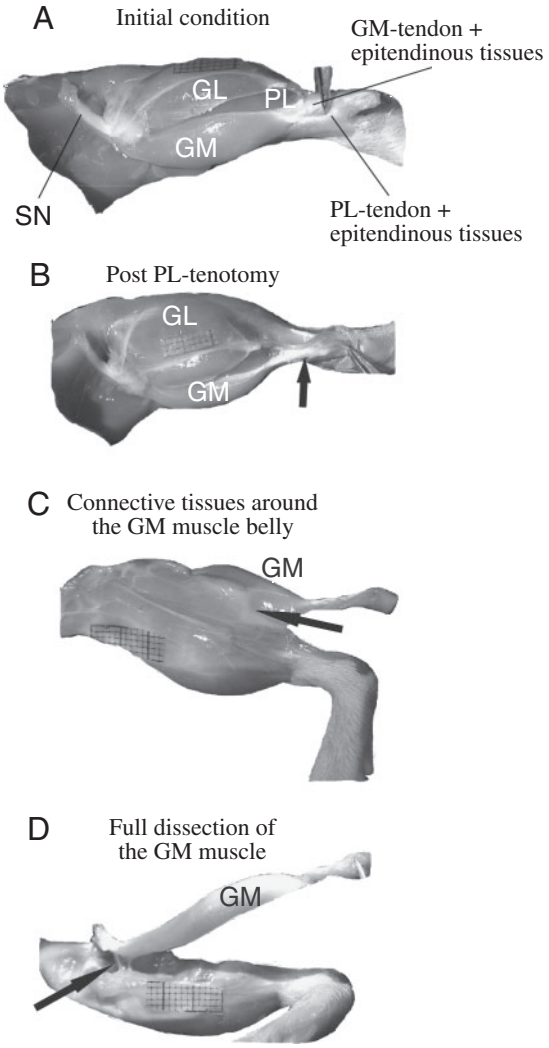

Fig. 2. Overview of the experimental conditions in progressive stages of dissection. Note that images do not show the position of the rat hind limb in the experimental set-up. (A) *Initial condition.* Dorsal view of the lower hind limb of the rat after the GM and GL have been separated. Below the GM and GL, the PL is visible. The distal tendons of the GM and PL were the only tendons connected to the calcaneal bone. The tip of a pair of tweezers is shown in the image, pointing at the GM and PL tendons. The cut calcaneal bone was connected to a force transducer (not shown). The dissected proximal origin of GM is not visible in this image. SN indicates the sciatic nerve. (B) Post PL-tenotomy. Dorsolateral view. A very small area of the connective tissue (barely visible but indicated by an arrow) was dissected to perform the PL-tenotomy. (C) Connective tissues around the GM muscle belly. Medial view. After dissection of the epitendinous tissues, the GM muscle belly is still connected to extramuscular tissue (i.e. remnants of the general fascia and epimysium). These tissues, holding the muscle belly, are indicated by an arrow. (D) Full dissection of the GM muscle. Ventral view. The GM muscle belly has been dissected free from its extramuscular tissues except for the neuro–vascular tract (i.e. nerves, blood vessels and the connective tissue). The arrow indicates the femoral artery entering the GM proximally and medially; the nerve is entering GM proximally and laterally and is therefore not visible in this image. In this view, the dissected GM origin, with a piece of the femur, is visible.

Prior to each experiment, the two force transducers were calibrated to make sure that there were no differences in forces measured. The two force transducers were connected to each other using a metal spring. The output was recorded with the identical measurements system as used in the animal experiment. The slope of the regression line (r^2=0.999) of the simultaneously measured forces deviated 0.41% from the expected 45°. Therefore, any differences between proximally and distally measured forces exceeding 0.41% cannot be ascribed to the measurement system used.

Determining force–length characteristics

Force–length characteristics were determined at various muscle–tendon complex lengths with tetani of 200 ms with a stimulation frequency of 200 Hz. Optimum length (L_0) of the GM was defined as the muscle length at which proximally measured active force was maximal. Measurements started from 13 mm below L_0 (i.e. near active slack length). Passive force at this length and position, if any, was indicated as zero by the measurement system. The actual passive force at the proximal force transducer was zero at this length and position, but the actual passive force at the distal force transducer may have been slightly higher because of the epitendinous tissues left intact around the distal tendon, exerting a passive force. All forces are expressed relative to this zero value. Before each contraction, GM was brought to the desired length passively by distal lengthening using the (computer controlled) distal measurement system. The proximal force transducer remained at a fixed position. Contractions were performed for a range from 13 mm below to 3 mm over L_0 with 1.0 mm increments). The passive force measured after the contraction was subtracted from measured total force to obtain active force. After each contraction, the muscles were allowed to recover at L_0–13 mm for 2 min.

Experimental conditions

1. Initial condition

Proximal (GM) and distal (GM+PL) forces were measured after performing the dissection as described above. Thus, the GM and GL were separated after removing the biceps femoris muscle. The soleus, deep flexors, peroneal muscles and muscles in the anterior crural compartment were left intact but were denervated. The distal tendons of the GM and PL muscles and the epitendinous tissues were still attached *via* the calcaneal bone to the distal force transducer. Only the proximal GM tendon was connected to the proximal force transducer. An overview of this stage of dissection is presented in Fig. 2A.

2. Post PL-tenotomy

After determining the forces of the proximal GM and the distal GM+PL-complex at different muscle–tendon complex lengths, a distal tenotomy of PL muscle was performed. The distal tendon was cut from the calcaneal bone without removing the animal or moving the hind limb within the experimental set-up. A very small area of the connective tissue around the calcaneal bone had to be dissected to detach the PL tendon from the calcaneal bone. The PL tendon retracted after it had been cut. The majority of the epitendinous tissues was left intact (Fig. 2B). Subsequently, the proximal (GM) and distal (GM) forces exerted by GM were measured.

3. In the reference position

(A) *With connective tissues present exclusively around the GM muscle belly.* Following the previous measurements, all epitendinous tissues surrounding the distal tendon of GM was dissected without moving the hind limb within the experimental set-up (Fig. 2C). The proximal (GM) and distal (GM) force–length characteristics were determined without any connective tissue around the distal tendon. Connective tissue (i.e. remnants of the general fascia and epimysium) around the GM and PL muscle bellies was still present (Fig. 2C). The proximal reference position of GM was defined as the position in which the origin of the muscle is situated as the knee angle is set at 120°.

(B) *After full dissection of GM muscle.* Subsequently, the GM muscle belly was dissected free from extramuscular tissues without removing the hind limb from the experimental set-up. The extent of this dissection is limited, because GM innervation and blood supply need to be kept intact (Fig. 2D). This means that connective tissues associated with the neuro–vascular tract remained connected to the GM muscle at its proximal region. This situation is referred to as the *in situ* situation.

4. The effects of moving GM origin

Finally, the origin of the fully dissected GM muscle was moved towards a 3 mm more-proximal or a 3 mm more-distal position prior to measuring force–length characteristics for distal lengthening. Moving the origin of the fully dissected muscle changes the relative position of the muscle belly with respect to the neuro–vascular tract. The direction in which the proximal origin was moved first was imposed randomly for different muscles. After moving the distal force transducer over an identical distance and direction, identical initial muscle lengths are obtained but with different positions of the muscle relative to the neuro–vascular tract.

Statistics

Values are presented as means ± S.E.M. Two-way analyses of variance (ANOVA) for repeated measures (factors: proximal/distal location of force exertion and muscle–tendon complex length) were performed to test for differences between proximally and distally measured forces and length effects. One-way ANOVA for repeated measures (factor: muscle–tendon complex length) was used to test for effects of length on proximo–distal force differences. Differences were considered significant if P<0.05.

Results

1. Initial condition

With the distal tendons of the GM and PL muscles and their epitendinous tissues attached *via* the calcaneal bone to the

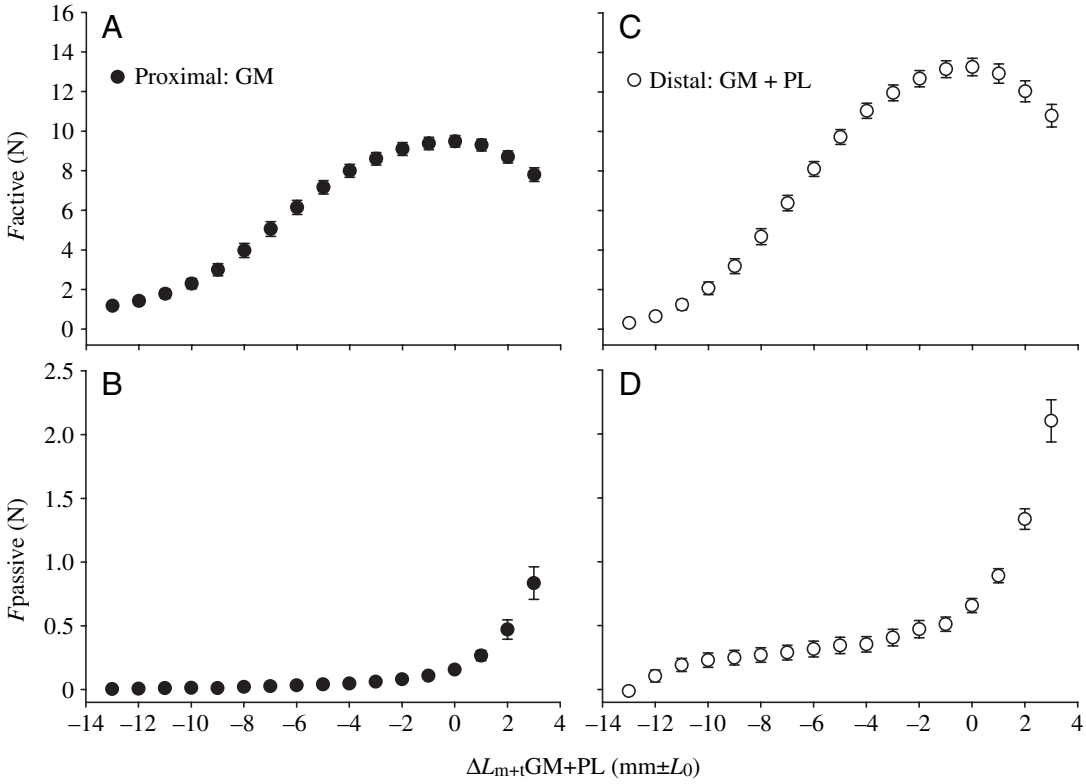

Fig. 3. Forces exerted at the proximal and distal transducers by rat medial gastrocnemius (GM) and plantaris (PL) muscles connected to extramuscular connective tissue at the level of the tendons and the muscle bellies (initial condition), within a dissected compartment. (A,B) Proximal active and passive force exerted at the GM tendon. (C,D) Distal active and passive force exerted at the calcaneal bone. Proximal force (closed symbols) represents force exerted predominantly by the GM muscle. Distal force (open symbols) represents predominantly the summed force exerted by the GM and PL muscles. Note that, at short lengths, the summed distal active force was lower than the proximal active force. Muscle–tendon complex length (L_{m+t}) is expressed as deviation from optimum length (L_0). Force is shown as mean ± S.E.M.

distal force transducer, and only the proximal GM tendon connected to the proximal force transducer, differences should exist between forces at the two locations of force exertion, which were confirmed in the experiment (Fig. 3). For example at L_0, proximally measured active force, ascribable to the GM, amounted to 9.5±0.3 N and distally measured force predominantly ascribable to the GM+PL was 13.3±0.4 N. Such differences were confirmed for other lengths ($-8<\Delta L<+3$). Surprisingly and in contrast to this: at lengths shorter than L_0–9 mm, distally measured active force (related to GM and PL activity) was significantly lower than proximally measured active force (related to GM activity exclusively). In accordance with this finding, a significant interaction of the effects of the factors muscle–tendon complex length and location of exertion of active force was found (ANOVA, $P<0.01$). It is concluded that effects of distally changing the length of the GM–PL complex differed for proximally and distally exerted forces.

Significant effects (i.e. main effects of location of force exertion and muscle–tendon complex length as well as an interaction) were also found for passive forces ($P<0.01$). Passive forces measured at the distal force transducer were consistently higher than the proximally measured forces (Fig. 3B,D).

Extramuscular myofascial force transmission from PL

2. Effects of PL-tenotomy

After distal tenotomy of the PL muscle, the GM distal tendon was the only tendon attached to the distal force transducer. Despite that fact, proximally and distally measured active forces were not equal at the shortest and longest lengths studied (Fig. 4A). For example at L_0, proximally exerted active force amounted to 9.2±0.3 N and distally exerted active force was 10.4±0.3 N. Note, that at long muscle–tendon complex lengths, distal forces dominated proximal forces. Whereas at the shortest lengths, lower distal forces than proximal forces were measured. A significant ANOVA main effect of location of force exertion could not be shown ($P=0.08$). By contrast, significant effects of length ($P<0.01$) and a significant interaction between effects of the factors location of force exertion and muscle length ($P<0.01$) were shown. Expressing the difference between proximal and distal active forces as a function of muscle length (Fig. 5A) allows assessment of the contribution of force of the PL muscle and the significance of this contribution. This proximo–distal force difference was calculated by subtracting the proximal force from the distal force. ANOVA showed a significant length effect on the force difference. At longer GM lengths ($\Delta L>-5$ mm) such PL contribution for active force rises with increasing GM length

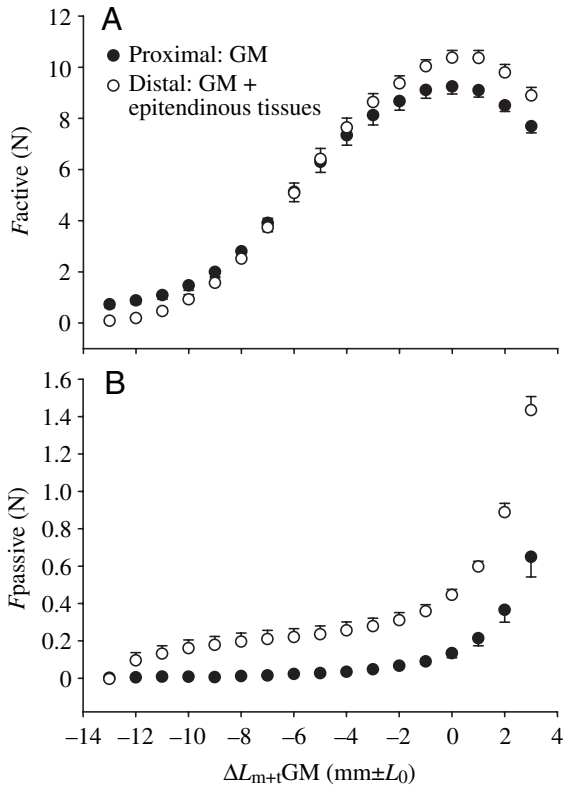

Fig. 4. Forces exerted at the proximal and distal transducers by rat GM and PL muscles connected to extramuscular connective tissue, within a dissected compartment after distal PL-tenotomy. Note that after distal PL-tenotomy, epitendinous tissues connected to the calcaneal bone were left intact. (A) A comparison of proximal active force exerted at the GM tendon (closed symbols) and distal active force exerted at the calcaneal bone (open symbols). (B) A comparison of proximal passive force exerted at the GM tendon (closed symbols) and distal active force exerted at the calcaneal bone (open symbols). Note that, despite PL-tenotomy, distal active force was higher at long muscle lengths and lower at short muscle lengths than proximal active force. Distal passive force was higher than proximal passive force. Muscle–tendon complex length (L_{m+t}) is expressed as deviation from optimum length (L_0). Force is shown as mean ± S.E.M.

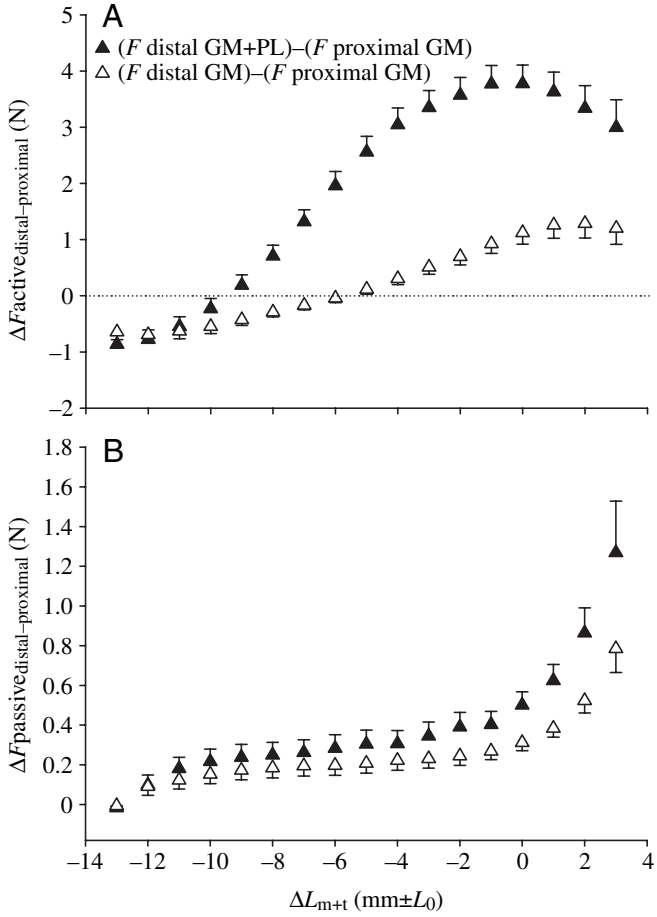

Fig. 5. Effect of distal plantaris-tenotomy on proximo–distal force differences. (A) Proximo–distal active force differences. (B) Proximo–distal passive force differences. Closed symbols represent the data before tenotomy (i.e. both medial gastrocnemius (GM) and plantaris (PL) distal tendons connected to calcaneal bone). Open symbols represent post-tenotomy data. Note that a substantial fraction of PL force was still transmitted to the distal force transducer. Muscle tendon complex length (L_{m+t}) is expressed as deviation from optimum length (L_0). Force is shown as mean ± S.E.M.

from 0 N to a substantial level of approximately 1 N. Such levels of force difference correspond to fractions of approximately 0–40% of the active force difference present with the GM and PL both exerting force also by myotendinous pathways (shown for comparison in Fig. 5). It is concluded that despite the fact that PL distal tendon is no longer connected to the calcaneal bone, active force exerted by the PL muscle is still transmitted onto this bone *via* alternative paths.

Significant differences were observed between passive forces exerted proximally and distally (P=0.001, Fig. 4B): distal forces being consistently higher. Also, significant effects of length (P<0.01) and a significant interaction between effects of the factors location of passive force exertion and muscle length (P<0.01) were observed.

For GM passive force the proximo–distal force difference ranged from 0 to 0.8 N (Fig. 5B). Except at the shortest lengths, this was no less than 60% of the passive force difference present with both GM and PL exerting force also by myotendinous pathways (shown for comparison in Fig. 5). It is concluded that effects of distally changing GM length differ for proximally and distally exerted active and passive forces.

Extramuscular myofascial force transmission from GM

3. In the reference position

(A) *With connective tissues present exclusively around the GM muscle belly.* All epitendinous tissues surrounding the distal tendon of GM were dissected to isolate the distal tendon fully. After this intervention, the active force measured distally did not differ significantly from the force measured proximally (ANOVA: no main effect of location of force exertion P=0.38). For example at L_0 (Fig. 6A), proximal and distal active forces were 9.1±0.3 N and 9.2±0.3 N, respectively). However, significant effects of muscle length (P<0.01) and a

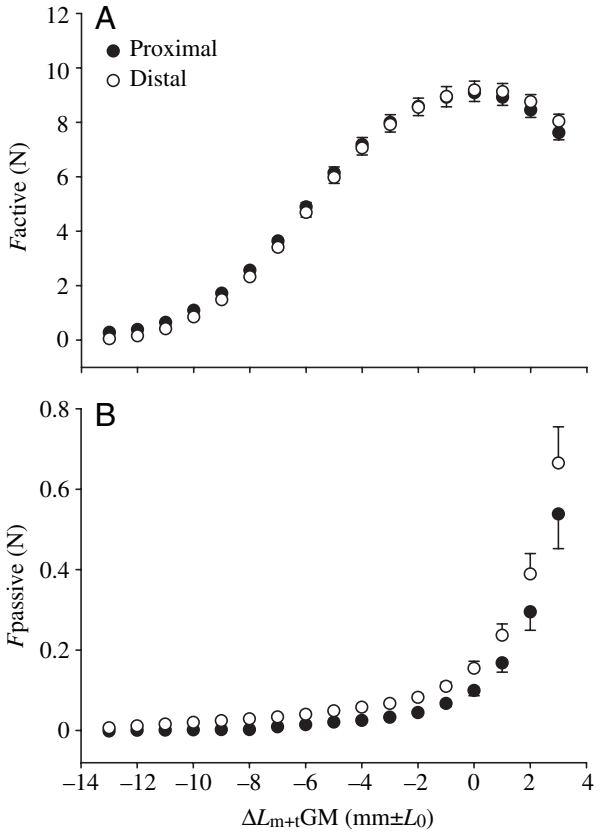

Fig. 6. Force–length characteristics of partially dissected rat medial gastrocnemius (GM) muscle connected only at the level of the muscle belly by extramuscular connective tissue (i.e. remnants of the general fascia and epimysium). (A) Active forces exerted at the proximal tendon (closed symbols) and at the distal tendon (calcaneal bone) (open symbols). (B) Passive forces exerted at the proximal tendon (closed symbols) and at the distal tendon (calcaneal bone) (open symbols). Muscle–tendon complex length (L_{m+t}) is expressed as deviation from optimum GM muscle–tendon complex length (L_0). Force is shown as mean ± S.E.M.

significant interaction of location of active force exertion and muscle length (P<0.01) were observed for active force. This significant interaction is an indication for some myofascial force transmission from GM. In accordance, ANOVA also showed a significant length effect on the GM proximo–distal active force difference (P<0.01). However, for the present experimental conditions, this effect is considered too small in absolute and normalised terms (Fig. 6A) to attribute a functional relevance. It is concluded that in the reference position, the extramuscular connections of the GM belly (i.e. remnants of the general fascia and epimysium) are hardly capable of sustaining GM proximo–distal active force differences under these experimental conditions. This means that most of the proximo–distal active force difference present after PL-tenotomy, but before dissection of the GM tendon (Figs 4A, 5A), must be ascribed predominantly to the epitendinous tissues. These tissues are forming the path for extramuscular transmission of active force from the PL onto the tendon and calcaneal bone.

For the passive force, both main effects of location of force exertion and muscle length as well as the interaction between both factors were significant (all P<0.01). Thus, a proximo–distal difference of passive GM force is sustained with the distal GM passive force being consistently higher than proximal GM passive force (Fig. 6B). Despite its small absolute size, the difference remains a sizable fraction of force exerted. For example at L_0, the proximo–distal force difference is approximately 56% of the proximal passive force, whereas at shorter muscle lengths, this percentage is even higher. It is concluded that, in contrast to active force transmission, passive GM extramuscular myofascial force transmission persists at a considerable degree.

(B) In situ *GM force–length characteristics after full dissection of the GM muscle.* The GM muscle was subsequently dissected free from its extramuscular tissues except for its neuro–vascular tract. In such conditions, with the GM origin at the reference position, proximally measured active forces were not different from distally measured forces (no ANOVA main effect for location of force exertion, P=0.14). For example at L_0 (Fig. 7A), both proximally and distally measured forces equalled 8.9±0.3 N. A significant effect of muscle length was observed (P<0.01), but no significant interaction effect between location of force exertion and muscle length was found (P=0.14). It is concluded that no evidence was found indicating extramuscular transmission of active GM force.

Passive forces were still significantly different for the different locations of force exertion (P<0.01; Fig. 7B). A significant effect of muscle length (P<0.01) and a significant interaction effect (P=0.03) were also detected. Therefore, it is concluded that in experimental conditions with fully dissected GM with its origin kept at reference position, myofascial force transmission of active force does not play a role whereas extramuscular myofascial force transmission of passive force still does.

4. Effects of moving GM origin

The origin of the fully dissected GM muscle was moved by 3 mm to a more-proximal or more-distal position, before measuring force–length characteristics for distal lengthening. The distal force transducer was moved over similar distances to obtain identical initial lengths as above. This changed the relative position of the muscle belly with respect to extramuscular tissues, i.e. the neuro–vascular tract.

(A) *More proximal position.* Subsequent to moving the GM origin towards a more proximal position, proximal GM active force exerted was significantly higher than distal GM active force. A main effect of location of force exertion (P=0.02), a significant effect of muscle length (P<0.01) and a significant interaction between effects of location of force exertion and muscle length (P=0.01) were found. For example at L_0 (Fig. 8A), proximal and distal active forces were 8.3±0.5 N and 8.0±0.4 N respectively.

For the passive forces, a main effect of location of force exertion was found (P<0.01), distal GM passive force being

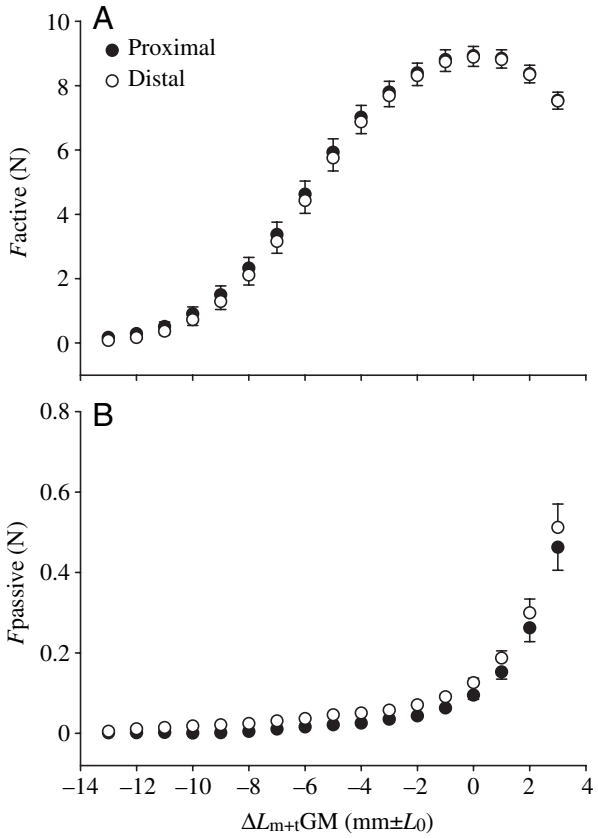

Fig. 7. Force–length characteristics of fully dissected rat medial gastrocnemius (GM) muscle with only the neuro–vascular tract as extramuscular connective tissue. GM had been dissected free from extramuscular tissues except for its innervation, blood supply and associated connective tissues. (A) Active forces exerted at the proximal tendon (closed symbols) and at the distal tendon (calcaneal bone) (open symbols). (B) Passive forces exerted at the proximal tendon (closed symbols) and at the distal tendon (calcaneal bone) (open symbols). Muscle–tendon complex length (L_{m+t}) is expressed as deviation from optimum GM muscle–tendon complex length (L_0). Force is shown as mean ± S.E.M.

higher than proximal GM passive force. Also significant main effects of muscle length (P<0.01) were found, but no significant interaction between these factors could be shown (P=0.13; Fig. 8B).

(B) *More distal position.* After moving the GM origin towards a more distal position, the distally measured active forces (e.g. 7.7±0.5 N at L_0) were higher than the proximally measured forces (e.g. 7.2±0.6 N at L_0; Fig. 8C). Despite the fact that qualitatively similar results were found for all muscle lengths studied, we could not show a significant ANOVA main effect for location of force exertion (P=0.095). A significant effect of muscle length was detected (P<0.01). The effect of muscle length was not significantly different for the different locations of force exertion (P=0.08). It is concluded that a proximo–distal difference of active force of fully dissected GM reappears as the relative position of GM with respect to the neuro–vascular tract is altered.

Distal GM passive forces were significantly higher than proximal passive forces at most muscle lengths (Fig. 8D). A significant main effect of location of force exertion (P<0.01), a significant effect of muscle length (P<0.01) and a significant interaction between effects of location of force exertion and muscle length (P=0.01) were observed. Thus, in both conditions distal GM passive forces were higher than proximal GM passive forces.

Discussion

Two pathways for force transmission are distinguished. (1) Myotendinous force transmission: force is transmitted from the muscle fibres to the muscle tendon *via* the myotendinous junction. (2) Myofascial force transmission: force is transmitted *via* the continuous endomysial fascia of the muscle. In the present study, myofascial force transmission from rat GM and PL muscles was studied. With intact extramuscular connective tissue around GM and PL, active force of the PL muscle was transmitted onto the calcaneal bone, even when the PL tendon was not connected to this bone. In the dissected *in situ* GM muscle, after full dissection except for its neuro–vascular tract, no appreciable myofascial transmission of active force occurred in the reference position. However, evidence was found for some myofascial force transmission when the relative position of the origin of the GM muscle with respect to the neuro–vascular tract was changed.

Extramuscular connective tissue

Extramuscular connective tissue is continuous with intramuscular connective tissue. Muscle fibres are linked to this intramuscular connective tissue network *via* connections between cytoskeleton, extracellular matrix and endomysium (e.g. Berthier and Blaineau, 1997; Patel and Lieber, 1997). Many studies indicated that these tissues are capable of transmitting force (Goldberg et al., 1997; Huijing, 1999a; Monti et al., 2001; Street and Ramsey, 1965; Street, 1983). Therefore, changes in the position of the extramuscular connective tissue relative to the muscle may also result in changes within intramuscular connective tissue and muscle fibres (i.e. myofascial force transmission).

In our present study, only extramuscular connective tissue around the GM and PL was left intact initially and was subsequently removed in phases. The neuro–vascular tract of the GM, consisting of nerves, blood vessels and their surrounding connective tissues, is one of the extramuscular connections and it enters into the GM proximally. Between the PL and GM muscles, no intermuscular connections (i.e. direct link between two intramuscular stromata) are present due to the fact that the PL muscle is ventral to the distal aponeurosis of GM muscle. Ventrally, the epimysium of the GM covers the aponeurosis but is not attached to it, other than *via* fascicle insertions at the dorsal aspect of this aponeurosis. Therefore, any connective tissue between the aponeurosis of GM and the PL muscle must constitute extramuscular tissue. At the level of the PL and GM tendons, various extramuscular connective tissues (remnants of general fascia, epimysium, neuro–vascular

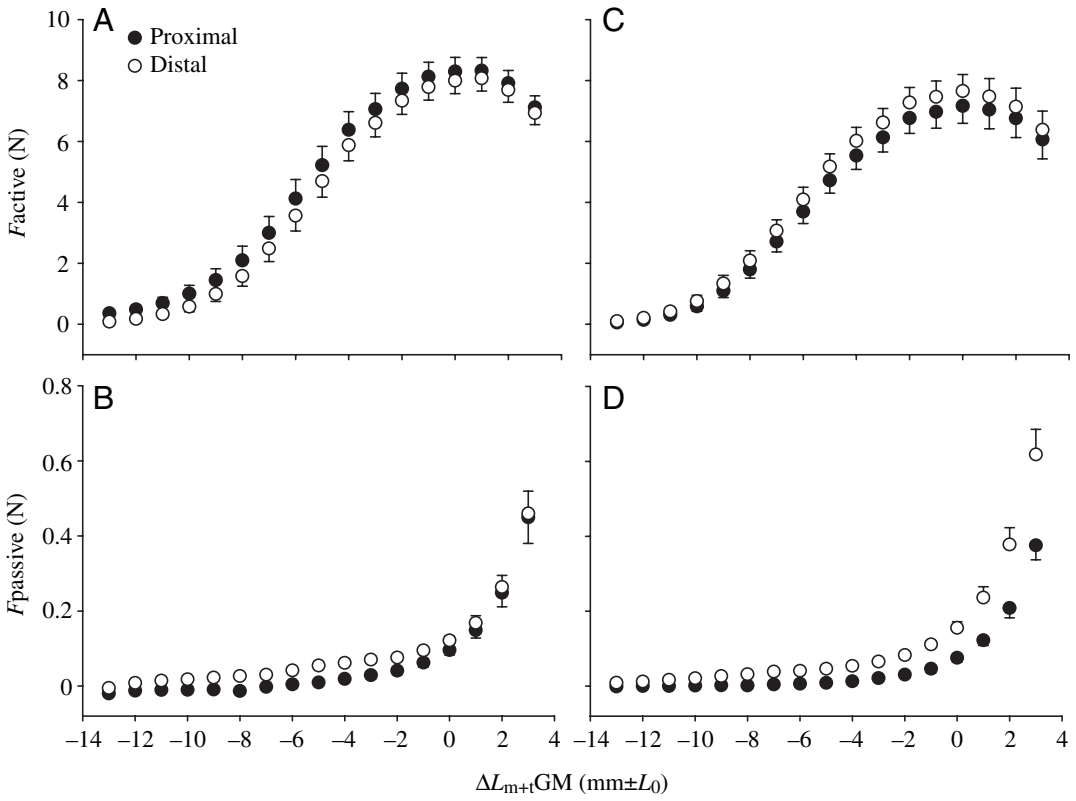

Fig. 8. Effects of muscle relative position of fully dissected *in situ* rat medial gastrocnemius (GM) muscle on force. Muscle relative position was manipulated by moving the origin 3 mm proximally or distally from the reference position (i.e. the position corresponding to a knee angle of 120°). (A) Active force–length characteristics after moving the origin in the proximal direction. (B) Passive force–length characteristics after moving the origin in the proximal direction. (C) Active force–length characteristics after moving the origin in the distal direction. (D) Passive force–length characteristics after moving the origin in the distal direction. Proximal and distal forces (mean ± S.E.M.) are represented by closed and open symbols, respectively. Muscle–tendon complex length (L_{m+t}) is expressed as deviation from optimum length (L_0).

tract and compartmental fascia) form connections between the epitenons of those tendons. These epitendinous tissues also attach to the calcaneal bone.

Extramuscular myofascial force transmission via *epitendinous tissues*

Because PL exerts force proximally at its origin on the lateral epicondylus of the femur, this force was not measured in the present experiment. Therefore, a difference in force exerted on the distal and proximal force transducers (by summed effects of GM and PL at the calcaneal bone and by the proximal GM tendon, respectively) was expected and observed with intact extramuscular connective tissues. However, the fact that a proximo–distal force difference was still observed after distal tenotomy of PL without removing the epitendinous tissues indicates extramuscular myofascial force transmission. At longer GM lengths, the force at the distal transducer was higher than GM proximal force. Thus, a fraction of the active force generated by PL was transmitted onto the calcaneal bone *via* other tissues than its tendon. This

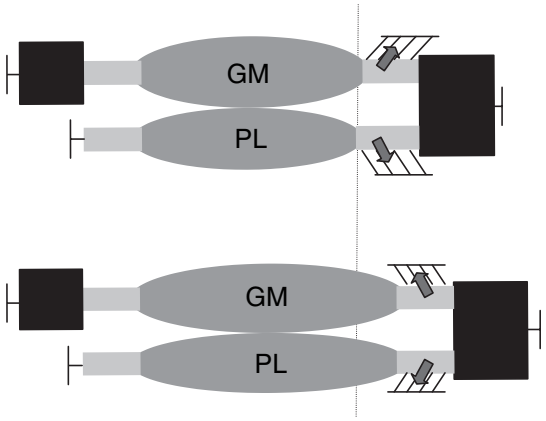

Fig. 9. Representation of the orientation of the epitendinous tissues relative to GM- and PL-tendons, being dependent on muscle length. Proximally, GM is attached to a force transducer, whereas distally, GM and PL are both attached to one force transducer. Force transducers are indicated by black squares. Epitendinous tissues are represented by solid lines attached to the tendons. At short muscle lengths (upper panel), the epitendinous tissues are oriented in such a way that the force borne by these connections (indicated by arrows) is directed distally, but not to the force transducer. In contrast, at long muscle lengths (obtained by distal lengthening), the epitendinous tissues are oriented in an opposite direction, bearing force to a proximal direction (lower panel).

extra force exerted at the distal force transducer was up to 40% of the extra force generated with the PL tendon still attached to the calcaneal bone.

After dissection of the epitendinous tissues, the force difference disappeared (Fig. 6A), indicating that those tissues must have formed the path for myofascial force transmission. It is concluded that the epimysium of GM and PL are connected to the epitendinous tissues. Force exerted distally by PL is transmitted *via* these epitendinous tissues directly onto the calcaneal bone, because the tendon does not seem to be connected to its epitenon, and therefore also no connections exist between the tendon and the epitendinous connective tissues. This view is compatible with observations that serious problems do occur within the tendon if such connections are formed pathologically by collagen fibre reinforcements of nerves and blood vessels entering the tendons perpendicularly (Alfredson, 2004). Thus, a novel result of the present study is that extramuscular myofascial force transmission also occurs *via* epitendinous tissues.

Before dissection of epitendinous tissues and even with PL still attached to the calcaneal bone, at shorter lengths the distal force was lower than GM proximal force. This surprising result indicates that not all force of both muscles is exerted at the calcaneal bone, but is partly transmitted in a distal direction *via* the extramuscular tissues of the posterior compartment and the epitendinous tissues to the underlying tissues of the lower hind limb (Fig. 1B). The results after PL-tenotomy and after removing the epitendinous tissues indicate that more force was transmitted *via* the epitendinous paths than *via* other extramuscular tissues, since the effect became much smaller after removal of the epitendinous tissues. The fact that the proximo–distal force difference is negative only at short GM muscle lengths (Fig. 5A) is explained by the varying position of the epitendinous tissues relative to GM with changes in GM muscle length. A changed configuration of such connective tissue leads to changes in the degree and direction of myofascial force transmission (Huijing and Baan, 2003). At short GM lengths, the force borne by these connections between the epitendinous tissues and underlying tissues of the lower hind limb is oriented in a distal direction but away from the calcaneal bone (Fig. 9). At long GM muscle lengths, the direction of the connection is reversed. Therefore, the force borne by these connections is oriented towards a proximal direction (Fig. 9).

Myofascial transmission of passive force

Passive forces are usually assumed to be either very small or absent when the muscle is below its optimum length. This is true for fully dissected *in situ* muscle, but for a muscle surrounded by other muscles and connective tissue, this may be different. In the present study, passive forces of approximately 0.3 N were measured on the distal side of the muscle at short muscle lengths. The passive forces are probably the result of the surrounding (extramuscular) tissues, which may be at a long length while the muscle is at a very short length. It could also be a contribution from the PL muscle. At the proximal side, passive forces were absent because the GM muscle was dissected free from extramuscular tissues at that side. When the distal side of the GM muscle was also dissected free from extramuscular tissues, there was still a small difference between proximally and distally measured passive forces. The distally measured forces were slightly higher than the proximally measured forces. Some passive force could be transmitted *via* the proximal part of the neuro–vascular tract to GM.

The neuro–vascular tract as a pathway for extramuscular force transmission in dissected in situ *GM muscle*

In the final stage of preparation in the present study, the GM was dissected from almost all extramuscular tissues, leaving only the neuro–vascular tract intact. In this stage of the experiment, this tract contains the only remaining extramuscular connective tissue. The neuro–vascular tract is composed of the tibial nerve approaching and entering GM from the lateral side and the anterior tibial vein and artery approaching and entering GM from the medial side with a connective tissue sheets enveloping all. With the knee flexed at 120°, this proximal aspect of neuro–vascular tract near the GM is positioned almost perpendicular to the line of pull of the muscle. An important purpose of the collagen fibres within this tract is to protect the nerves and blood vessels from high strains. To perform this function, this connective tissue has to be fairly stiff and it will provide a potential path for myofascial force transmission. In the reference position of GM, no appreciable myofascial force transmission was apparent in the present study. This indicates that the neuro–vascular tract is in such a condition that it is either rather compliant or perpendicular to the muscle and therefore not able to transmit force.

Changing the relative position of a muscle or its origin will result in a different length and configuration of the connective tissue connecting the muscle with the proximal aspect of the neuro–vascular tract. As the origin of GM was repositioned to a more-proximal or more-distal position, a proximo–distal force difference was present, indicating extramuscular myofascial force transmission *via* the neuro–vascular tract. The sign of the proximo–distal difference of active force was dependent on the direction of repositioning of the muscle. This is explained by that fact that repositioning the muscle towards a more proximal position causes the proximal aspect of the neuro–vascular tract to approach the muscle from a more-distal direction. Then, some force is transmitted in a distal direction between the neuro–vascular tract and the GM. This extra force constitutes an additional load on sarcomeres located proximally within the GM muscle fibres. As a result, proximal active force is higher than distal active force. By contrast, in both conditions (moving the GM from the reference position towards either a more-proximal or a more-distal relative position) the GM distal passive forces were higher than the GM proximal passive forces. Therefore, it is concluded that the contraction of the GM on activation is responsible for the reversal of the direction of force transmission.

Extramuscular force transmission in vivo

One may question if force is transmitted *via* extramuscular pathways *in vivo*. In the literature, some indications are found that this in indeed the case. Gregor et al. (1988) measured forces at the Achilles tendon during gait of a cat and found that the measured forces were higher than maximal force of a muscle in a fully dissected *in situ* set-up, despite the fact that during gait, the muscles are expected to contract submaximally. The unexplained high force might be explained by extramuscular force transmission (Huijing, 1999b). Furthermore, Riewald and Delp (1997) and Asakawa (2002) found that the rectus femoris muscle was still able to generate an extensor moment after a distal tendon transfer to the flexor side of the knee. Although it is not clear if this is the result of extramuscular myofascial force transmission, it seems plausible that some type of myofascial force transmission is involved. It may be argued that scar formation after surgical intervention could alter myofascial force transmission. However, at the long term, scar formation may also lead to formation of new or regular tissues (for a review see e.g. Liu et al., 1995), presumably leading to new myofascial paths.

In situ experiments

In physiological experiments, many characteristics of muscle have been studied using fully dissected *in situ* preparations of animal muscle, with only the neuro–vascular tract left intact. Dissection is performed on inter- and extra-muscular connective tissues to gain access to the muscle. Denny-Brown (1929) is one author who mentioned explicitly that dissection was performed to exclude the interaction of the surroundings of a muscle with that muscle, but generally, the effects of connective tissue are not taken into account. For the fully dissected GM in the present study, it is concluded that no myofascial force transmission occurred *via* the neuro–vascular tract in the reference position. However, after moving the GM origin, some myofascial force transmission reappeared. From these results, it is concluded that regarding *in situ* experiments, one has to be aware of the position of the muscle with respect to the extramuscular tissues. If an appropriate position is chosen, myofascial force transmission will not be effective and therefore not influence the results. However, when applying conclusions of such experiments to *in vivo* conditions, it should be realised that myofascial force transmission may play a role in altering muscular characteristics (Huijing, 2003).

In conclusion, extramuscular myofascial force transmission within the posterior compartment of the rat lower leg plays a role even after substantial dissection has been performed on the compartment. With intact extramuscular connective tissues around the whole GM and PL muscle–tendon complex, active force of the PL is transmitted onto the calcaneal bone, even after the PL tendon has been cut from this bone. After dissection of all of the epitendinous and the majority of the epimuscular connective tissues, myofascial transmission of passive force remains important, but myofascial transmission of active force is either absent or becomes so small that its effects may be neglected for most purposes. However, this is only true if the GM origin is kept in a position corresponding to a knee angle of approximately 120°. After moving GM origin from this position, some myofascial transmission of active force occurred.

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