INTER- AND EXTRAMUSCULAR MYOFASCIAL FORCE TRANSMISSION FUNCTION AS A MAJOR DETERMINANT OF MUSCLE FORCE AND LENGTH RANGE OF FORCE EXERTION

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INTRODUCTION

Myofascial force transmission (Huijing, 1999) is transmission of the force generated by the sarcomeres, from the full perimeter surface of myofibers onto the intramuscular connective tissue. Force transmission between the extracellular matrices of adjacent muscles through direct connections is referred to as intermuscular myofascial force transmission. Force transmission between the extracellular matrix of a muscle and surrounding non-muscular structures is referred to as extramuscular myofascial force transmission. The goal of the present study is to test the following hypothesis: inter- and extramuscular myofascial force transmission has major effects on muscle force and length range of force exertion.

METHODS

Experiments

Male Wistar rats (n = 5, body mass = 304.4 ± 3.5 g) were anaesthetized by intraperitoneally injected urethane solution (initial dose: 1.2 mg 100 g⁻¹ body mass, extra doses if necessary: maximally 1.5 mg). To prevent hypothermia, the animals were placed on a heated water pad of approximately 37°C during surgery and experimentation. Ambient temperature (22 ± 0.5 °C) and air humidity (80 ± 2%) were kept constant by a computer controlled air-conditioning system (Holland Heating). Muscle and tendon tissue was further prevented from dehydration by regular irrigation with isotonic saline.

The left anterior crural compartment of the rat, which envelopes of the tibialis anterior (TA), extensor digitorum longus (EDL) and extensor hallucis longus (EHL) muscles, was exposed. Connective tissues at muscle bellies were left intact to maintain physiological relations of intra-, inter- and extramuscular connections (referred to as intact condition). TA and EHL lengths were kept at such a length that initially the total distal TA+EHL force equaled approximately 2 N. Isometric EDL force exerted at both proximal and distal tendons was measured after distal lengthening of EDL muscle in two conditions: (1) Intact condition (Fig. 1a) (2) after removing TA and EHL muscles, when EDL still had intact extramuscular connections to the compartment as well as to the tibia. The second condition is referred to as: EDL with extramuscular connections exclusively.

In both conditions, EDL was lengthened by moving exclusively the distal force transducer with 1 mm increments starting at muscle active slack length (i.e. the length at which active force approaches zero). After each tetanic contraction (all muscles were excited simultaneously) the muscles were allowed to recover for 2 minutes.

Figure 2: Schematic representation of the experimental setup and of the models.

(a) Experimental setup for EDL in intact condition. The Kevlar threads tied to the proximal and distal tendons of EDL, as well as to the tied distal tendons of TA+EHL complex were connected to force transducers (FT). (b) The model representing EDL in intact condition. One of the models that represents TA+EHL complex is kept at a fixed low length (i.e. 3.0 mm shorter than its initial length), and is referred to as restrained synergist. This muscle is connected intermuscularly to the modeled EDL muscle, which is lengthened distally, and referred to as lengthened muscle. Prior to distal lengthening, the proximal end of this muscle was moved 2.0 mm in the distal direction, as in the experiments. Both of the models were also connected extramuscularly: the nodes of the matrix mesh marked by a white “+” sign have connections to mechanical ground. The nodes marked also by a black square have stiffer extramuscular connections. Muscular geometry at the initial muscle length is represented by dotted lines. A proximal view of the models in the undeformed state is shown on the left hand side. (c) The model of EDL muscle with extramuscular connections exclusively. The nodes of the matrix mesh marked by a white “+” sign have extramuscular
connections to mechanical ground and the nodes marked also by a black square have stiffer connections. A proximal view in the undeformed state is shown on the left hand side. The sciatic nerve was stimulated supra-maximally using a pair of silver electrodes connected to a constant current source (3 mA, pulse width 100 µs). Two twitches were evoked and followed by a tetanic contraction after 300 ms (pulse train 400 ms, frequency 100 Hz). Simultaneously, images of the muscle in passive and active state were recorded using a digital camera (DVC, JAI CV–M10, shutter speed 1/50 s). The timing of stimulation, A/D conversion (12–bit A/D converter, sampling frequency 1000 Hz, resolution of force 0.01 N), and photography were controlled by a microcomputer.

Data for total muscle force ($F_{mt}$) in relation to muscle tendon complex length ($l_{oi}$) were fitted by a polynomial function

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

where $y$ represents $F_{mt}$ and $x$ represents $l_{oi}$. $b_0$, $b_1$, $b_2$, $b_3$, $b_4$, $b_n$ are coefficients determined in the fitting process. Polynomials that best described the experimental data were selected by using one-way analysis of variance (ANOVA) (Neter et al., 1996), to be used for averaging of data and calculation of standard errors. One-way analysis of variance was also used in testing the length range of active force exertion. Two-way analysis of variance (ANOVA) was performed to test for length effects and for differences between the EDL force measured at the proximal and distal tendons. Bonferroni post-hoc tests were carried out to identify the significance of proximo-distal force difference at each $l_{oi}$ (Neter et al., 1996). Differences were considered significant at $p<0.05$.

**Finite Element Modeling**

Using a two-domain approach, a 3D-finite element muscle model (linked fiber-matrix mesh model: 1mm model) was developed (Yucesoy et al., 2002b). This model consists of two meshes, occupying the same space, that are linked elastically. These meshes represent the extracellular matrix domain (matrix mesh) and intracellular domain (fiber mesh). The two meshes are built using the self-programmed “myofiber” and “extracellular matrix” elements (Yucesoy et al., 2002b) that are introduced as user defined elements into the finite element program ANSYS 5.7.1. For the myofiber element, the total stress that acts only in the local fiber direction is equal to the sum of the active stress of the contractile elements and the stress due to intracellular passive tension. It is assumed that, at initial muscle length in the passive state, the sarcomeres arranged in-series within muscle fibers have identical lengths and material properties. The extracellular matrix element incorporates a strain energy density function that accounts for the non-linear and anisotropic material properties and the constancy of muscle volume.

Within the biological context, one muscle element is defined to represent a segment of a bundle of muscle fibers with identical material properties, its connective tissues and the links between them. This is realized as a linked system of extracellular matrix and myofiber elements. Both matrix and fiber meshes are rigidly connected to single layers of elements forming the muscles’ proximal and distal aponeurosis. To represent the aponeuroses, a standard element with a hyperelastic mechanical formulation (HYPER58) from the element library of ANSYS 5.7.1 was used. For the elastic links between the two meshes, which represent the transmembranous attachments of the cytoskeleton and extracellular matrix, a standard spring element (COMBIN39) was used. This element produces force in the direction of the deformation with linear stiffness characteristics.

In the present study, to study EDL in intact condition, two models for which the geometry is defined by the contour of a mid-longitudinal slice of the rat EDL muscle belly were intermuscularly connected: the corresponding nodes of the matrix meshes of the two models were linked elastically (Figure 1b). The extramuscular connections of both models to the surrounding structures were also included: using spring elements, a set of nodes of the matrix mesh of each model were linked to a set of fixed points, representing ‘mechanical ground’. Initially, the corresponding points of the mechanical ground and the nodes of the models were at identical locations (i.e., muscle length = 28.7 mm for both muscles, and before moving any of the tendon ends). The extramuscular connections of EDL muscle are located at approximately one-third of the fascicle length from the most proximal side of each fascicle (Huijing et al., 2001a). Embedded in this connective tissue, major nerves and blood vessels to the muscle and to the foot are found (neurovascular tract). Due to the collagen-reinforced structure, the proximal third of the extramuscular connective tissue sheet was shown to be much stiffer than the remainder of the sheet (Yucesoy et al., 2002a). This is taken into account in both models by making the three most proximal extramuscular links stiffer than the other links. For both inter- and extramuscular links, the spring element COMBIN39 was used with linear stiffness characteristics. A suitable stiffness ($k$) was determined for the intermuscular elements that provides a sufficiently good agreement between the experimental and modeled muscle forces. For the extramuscular links, the stiffness values determined previously (Yucesoy et al., 2002a) were used ($k = 0.286$ unit force/mm for stiffer part and, $k = 0.067$ unit force/mm for the remaining links).

One of the modeled muscles was distally lengthened; this procedure models the EDL muscle in the experiment (referred to as the lengthened muscle). In the anterior crural compartment, EDL muscle is connected to the tibia only by extramuscular tissues such as the anterior intermuscular septum. As the tibia is located medially, the extramuscularly connected face of the lengthened muscle is referred to as the “medial face”. Accordingly its intermuscularly connected face is referred to as the “lateral face”.

The other model muscle, which was kept at a fixed length (3 mm shorter than the initial length) represents the TA+EHL muscle complex (referred to as the restrained synergist). Mimicking the experimental conditions, before distal lengthening, the proximal end of the lengthened muscle was displaced 2 mm distally, and was then fixed at that position. Throughout the analysis, both muscles were modeled as activated maximally.

Another EDL model with extramuscular connections exclusively was used in order to compare its results to the
results of the lengthened muscle with both inter- and extramuscular connections. The extramuscular connections of this model were also represented by spring elements that link a set of nodes of the matrix mesh at medial and lateral faces (as described above) to mechanical ground (Fig1.c). Its proximal end was displaced 2 mm distally before distal lengthening and the activation was maximal.

Local strain, as a measure of change of length, reflects the lengthening or shortening of sarcomeres. Note that zero strain in the model represents the undeformed state of sarcomeres (i.e., sarcomere length \(\cong 2.5 \mu m\)) in the passive condition. Fiber direction strain within the fiber mesh of the lftmm model was used to assess the non-uniformity of sarcomere lengths arranged in-series within muscle fibers (referred to as serial distribution). Mean fiber direction strain (mean of nodal strain values) was used to assess heterogeneity of mean sarcomere lengths of different fibers within the muscle (referred to as parallel distribution).

RESULTS

Both for EDL in intact condition and EDL with extramuscular connections exclusively, significant differences were found between isometric EDL forces measured at the distal and proximal tendons. In both conditions, distal isometric EDL forces were significantly higher than proximal forces after distal lengthening (Figure 2a). The proximo-distal force difference increases as a function of the muscle length. For example in condition 2 the difference maximally equals 22 % of the proximal force. Such force differences indicate that substantial extramuscular myofascial force transmission occurs.

To identify the specific effects of intermuscular myofascial force transmission, length-force data of EDL were compared for the two conditions (Figure 2a). The proximo-distal force difference is enhanced for EDL in intact condition (maximally equals 46 % of its proximal force). This proves that force is transmitted both inter- and extramuscularly. Distal optimum force as well as optimum length of EDL in intact condition was higher than that of EDL with extramuscular connections exclusively. The length range of active force exertion between muscle slack and optimum lengths is significantly higher for EDL in intact condition (mean ± SD: 12.7 ± 0.67 mm,) than that of EDL with extramuscular connections exclusively (10.2 ± 0.74 mm).

The modeled forces agree reasonably well with the experimental data for EDL muscle in intact condition (Figure 2b), provided that suitable stiffness values are selected for inter- and extramuscular linking elements. However, in contrast to modeled forces, at high lengths (i.e., \(\Delta l \text{EDL} > 11\) mm) the experimental forces reach a plateau region. This is because, over optimum length, passive intramuscular connective tissues are modeled stiffer than that of the experimental muscle.

The strain distributions shown in Figure 3 are studied to address two points: (1) the intact inter- and extramuscular connections of EDL cause substantial serial distribution of sarcomere lengths within muscle fibers (2) parallel distribution within muscle is more pronounced for EDL in intact condition compared to EDL with extramuscular connections exclusively.

Serial distribution of length of sarcomeres within muscle fibers
For model of EDL in intact condition, the strain distributions of the medial face and the lateral face of the fiber mesh were different.

Medial face.
Due to the stiffer proximal extramuscular links, the strain distributions are highly non-uniform in the proximal part of the medial face (Figures 3a and b, upper panels). The sarcomeres located at the proximal ends of the muscle fibers are shorter than the ones located more distally. The serial distribution is more pronounced at higher lengths. For example, within the most proximal fascicle at high length, the most proximally located sarcomeres remained slightly shortened (by 0.7 %) whereas the more distal sarcomeres are lengthened up to 22 % (Figure 3b, upper panel).

![Figure 2](image-url): The isometric muscle length-force curves of EDL muscle.

(a) Comparison of experimental normalized length-total force characteristics of EDL muscle in intact condition to that of EDL muscle with extramuscular connections exclusively. Length is expressed as a function of deviation (\(\Delta l \text{EDL}\)) from active slack length. Both sets of data are normalized for distal optimum force of EDL in
intact condition. (b) Comparison of experimental and modeled forces of EDL muscle in intact condition (Fm) normalized for optimal force (Fmo).

**Lateral face.**

More pronounced strain distributions were found throughout the lateral face (Figures 3a and b, lower panels). The sarcomeres at the proximal ends of the muscle fibers are generally shorter than the ones located distally. At low length, the most distal parts of the muscle fibers show almost no length changes (i.e., strain values close to zero), although the proximal parts are shortened by up to 31% (Figure 3a lower panel). Even at high muscle lengths, the proximal ends of fibers remained shortened (by 14%) while the most distally located sarcomeres are lengthened by up to 51% (Figure 3b lower panel). It is concluded that a major serial distribution of lengths of sarcomeres within muscle fibers does occur in EDL muscle in intact condition.

**Figure 3:** Distributions of fiber direction strain within modeled EDL muscle in intact condition at selected muscle lengths.

The Strain distributions within the fiber mesh are shown for (a) $\Delta l_{EDL} = 7.5$ mm and (b) $\Delta l_{EDL} = 11.5$ mm. The distributions at the medial face of this model are shown in the upper panels whereas the lower panels show the distributions in the lateral face. Length is expressed as a function of deviation ($\Delta l_{EDL}$) from active slack length. The dotted line contour indicates muscle geometry at the initial length and position. Prior to distal lengthening, the proximal end of the muscle model was moved 2.0 mm in the distal direction, as in the experiment of the present study. The fiber direction as well as the proximal and distal ends of the muscle is shown in the lower panel of part (b).

**Parallel distribution of length of sarcomeres among different muscle fibers**

For model of EDL in intact condition, the differences in local strain between the lateral and medial faces are an additional indication for a sizable parallel distribution of length of sarcomeres. In Figure 4 the mean fiber direction strain distributions for different fascicle groups are shown for the medial and lateral faces of model of EDL in intact condition, as well as for EDL with extramuscular connections exclusively. The collections of nodes along the longitudinal sides of myofiber elements represent fascicle groups (numbered from 1 to 7, Figure 4a). At all lengths studied, the mean strain values for the medial and lateral faces of EDL in intact condition are clearly different for all fascicles (see Figure 4b and c for a comparison between the mean strain values at a low and a high muscle length respectively). This is consistent with our hypothesis of increased heterogeneity of mean fiber sarcomere lengths for this muscle, particularly at high lengths. It explains the shift in optimum length to higher lengths (Figure 2a).

**Figure 4:** Distributions of mean fiber strains in fiber direction for model of EDL in intact condition and for EDL with extramuscular connections exclusively.

(a) Mean fiber direction strain was calculated at nodes of the myofiber elements (in the fiber mesh) in-series representing a muscle fascicle. Each fascicle is indicated by a number from 1 to 7. Mean fiber direction strains are plotted as a
function of fascicle number for the selected EDL lengths: (b) \( \Delta l \text{EDL} = 7.5 \text{mm} \), and (c) \( \Delta l \text{EDL} = 11.5 \text{mm} \). EDL length is expressed as a function of deviation (\( \Delta l \text{EDL} \)) from active slack length.

**DISCUSSION**

**Effects on muscle length force characteristics**

A major experimental result of the present study was the significant proximo-distal EDL force measured, after distal lengthening. Such force difference was reported recently for rat EDL muscle with intact inter- and extramuscular connections (Huijing et al., 2001a; Maas et al., 2001) after proximal lengthening. The proximal EDL force was shown to be higher than the distal force. Note that, in the present study, after distal lengthening, the sign of the proximo-distal force difference was reversed favoring the distal force. Our model results show that the force difference is caused by the proximally directed forces acting on the muscle via its inter- and extramuscular connections. This confirms the role of myofascial force transmission.

In the present study, keeping the intermuscular connections of EDL muscle intact along with its extramuscular connections allowed muscle optimum length to be significantly higher. In recent studies, systematic manipulation of the extra and inter-muscular connective tissue structures of EDL muscle (i.e., isolation of EDL gradually) significantly altered muscle length range of force exertion for proximal lengthening (Huijing et al., 2001b). These results indicate that the determinants of muscle length range of active force exertion, are not specific properties of muscle related only to filament overlap in sarcomeres, but are a function of the conditions in which the muscle is operating. Moreover, our present results suggest that insufficient stiffness of the intermuscular connective tissues may be a cause for an overly low muscle length range and therefore, for a limited range of joint motion.

A higher length range due to heterogeneous mean fiber sarcomere lengths was argued to cause decreased optimum force (Huijing, 1996) for isolated muscle in situ. However, in the present study the distal optimum force of EDL in intact condition was higher than that of EDL with extramuscular connections exclusively. This is explained by intermuscular myofascial force transmission from TA+EHL muscle complex to EDL muscle. The study on the principles of intermuscular myofascial force transmission exclusively (Yucesoy et al., 2001) showed that the distal force of an intermuscularly connected muscle was significantly higher than that of an isolated muscle after identical distal lengthening. However, the mean fiber stress found in the isolated muscle was well above that of the intermuscularly connected muscle at high lengths. It is concluded that force generated within sarcomeres of one muscle may be exerted at a tendon of an adjacent synergist.

**Distribution of sarcomere lengths**

The continuity of intramuscular connective tissues to inter- and extramuscular connective tissues is expected to lead to distributions of sarcomere length arranged “in-series” within muscle fibers. The design of lmm model allows testing this hypothesis (Yucesoy et al., 2002b). The present modeling results indicate substantial serial distributions of length of sarcomeres within muscle fibers.

Serial sarcomere length distribution (Wohlfart et al., 1977; Morgan et al., 2000) and heterogeneity of mean sarcomere length of different fibers (Willems et al., 1994; Huijing, 1998) was shown to extend the muscle length range. Our present results confirm the earlier findings and show that myofascial force transmission is a major contributor to such heterogeneity.

**Limitations and implications of this study**

Our results indicate that a significant determinant of the myofascial effects is the relative position of a muscle with respect to its neighboring muscles and nonmuscular connective tissue structures. However, in vivo, not only the muscle studied, but also the surrounding muscles may change length with joint motion. Differences in moment arm will contribute to relative movement. For bi- or polynarticular muscle, relative movement of muscles will be much larger. If myofascial force transmission would be found for antagonists, effects larger than described here may be found. Therefore, studies aiming at identification of the relative positions of in vivo muscles with respect to their surroundings, for given tasks are indicated.

The experimental conditions of the present study included maximal activation whereas this is hardly the case in vivo. The mechanical interaction between adjacent muscles and other structures strongly depends on the stiffness of the muscular tissues. Maximally activated muscle is stiffer than sub-maximally activated muscle. Therefore, level of activation is expected to significantly affect myofascial force transmission. This needs to be studied in the future.

Using linearly stiff spring elements to represent the inter- and extramuscular connections must be considered as a significant simplification. Such connective tissues feature non-linear length-force characteristics. The modeled effects of myofascial force transmission are expected to be more pronounced at lower lengths (because of a higher stiffness than reality), and less pronounced at higher lengths (because of a lower stiffness than reality). In future modeling, possible nonlinear behavior of the linking elements should be accounted for.

A study on unintended force exertion showed that production of isometric force with one, two, or three fingers caused the other fingers of the hand also to exert a certain force (Li et al., 2000). Our present results could be extrapolated to explain those findings. Moreover a possible role of myofascial effects on such pathologies as musicians arm and repetitive strain injury are indicated. Deformations due to length changes of only one muscle, or even only a part of a muscle while all the surrounding muscles are restrained (as in the precision task of clicking the mouse button) may contribute to such conditions.

**CONCLUSIONS**

Inter- and extramuscular myofascial force transmission affects substantially isometric muscle force and length range of force exertion, which confirms our hypothesis.
Moreover, myofascial interaction of a muscle with its surrounding structures and muscles causes substantial distributions of lengths of sarcomeres both for sarcomeres arranged in-series within myofibers and among myofibers arranged in-parallel. Such effects are expected to have major implications for joint movement potential as well as neuromuscular coordination.

REFERENCES