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Progressive surgical dissection for tendon transposition affects length–force characteristics of rat flexor carpi ulnaris muscle

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Abstract

Extramuscular connective tissue and muscular fascia have been suggested to form a myo-fascial pathway for transmission of forces over a joint that is additional to the generally accepted myo-tendinous pathway. The consequences of myo-fascial force transmission for the outcome of conventional muscle tendon transfer surgery has not been studied as yet. To test the hypothesis that surgical dissection of a muscle will affect its length–force characteristics, a study was undertaken in adult male Wistar rats. During progressive dissection of the flexor carpi ulnaris muscle, isometric length–force characteristics were measured using maximal electrical stimulation of the ulnar nerve. After fasciotomy, muscle active force decreased by approximately 20%. Further dissection resulted in additional decline of muscle active force by another 40% at maximal dissection. The muscle length at which the muscle produced maximum active force increased by approximately 0.7 mm (i.e. 14% of the measured length range) after dissection. It is concluded that, in rats, the fascia surrounding the flexor carpi ulnaris muscle is a major determinant of muscle length–force characteristics.

Keywords: Muscle; Connective tissue; Rats; Biomechanics; Tendon transfer; Force transmission

Introduction

Understanding of biomechanical aspects of muscle transposition aimed at restoration of function of the human upper extremity has increased dramatically over the last three decades. Research has predominantly been focused on the specific structure and functional mechanics within the muscle [12,13]. In addition, intramuscular connective tissue has been shown to play an important role in transmission of force from the contractile proteins within muscle fibers to the extracellular matrix [5,15,17,19–24]. This means that forces produced within sarcomeres are transmitted not only via sarcomeres in series toward the myo-tendinous junction, but also laterally toward the fascia. The latter direction of transmission is termed myo-fascial force transmission [5,10]. Also, attention has been paid to possible functions of the extramuscular connective tissues that interconnect adjacent muscles as well as muscles and bone. Recently, Riewald and Delp presented indications that the connective tissue surrounding a muscle may also be involved in transmission of muscle forces [18]. Huijing hypothesized that the connections of the intramuscular fascia to extramuscular structures and to adjacent muscles may form a pathway for transmission of forces that fully bypasses the myo-tendinous pathway [5]. The possible consequences of myo-fascial force transmission for the effects of conventional transposition surgery, in which these connective tissues are largely interfered with or destroyed, have yet to be studied. The acute effects of the interventions on muscular properties will determine (1) the optimization of variables for transposition and (2) the starting conditions of post-surgical recovery and adaptation.

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Materials and methods

Experiments were performed on seven adult male Wistar rats with a mean body mass of 302 g (SD 4.0 g). All experiments were performed in accordance with Dutch law and the guidelines of the Vrije Universiteit Ethics Committee on Animal Research. The experimental setup and methods were similar to those described previously [10]. The animals were anesthetized by intraperitoneal injection of urethane (Sigma Chemical Co., St Louis MO, USA) (0.15 g/100 g body weight) and placed on a warm pad during surgery and experimentation. Secondary injections of urethane (0.05 g/100 g body weight) were supplied as needed.

Preparatory surgery

The left forelimb was shaved and the skin was resected from the axilla down to the carpus. The FCU was identified at its insertion on the accessory carpal bone. In order to access the distal end of the FCU, the antebrachial compartment was opened distally over approximately 2 mm. The FCU insertion was released by removing the accessory bone from the carpus, leaving the insertion of the tendon on it intact. A suture loop was placed just proximal of the accessory bone and knotted to a very stiff, kevlar thread (4% elongation at a break load of 800 N) which was to be connected to a force transducer with a maximal output error <0.1% and a compliance 0.0048 mm/N [25]. On the medial side of the humerus, the distal brachial plexus was dissected out of the sulcus in between the biceps and tensor fascia antebrachii muscles. The ulnar nerve was identified and cut from the plexus. Part of the tensor fascia antebrachii muscle was resected.

General experimental conditions

To fix the humerus to the experimental setup, a fixation clamp was positioned on its deltoid tuberosity between the biceps muscle and the lateral head of the triceps muscle. The left forelimb of the rat was fixed in neutral position using suture wire. The ulnar nerve was placed on a pair of silver electrodes connected to a constant current source. The nerve was prevented from dehydration by application of an isotonic saline-wetted tissue and measurements were carried out at room temperature (22–24 °C) and an air humidity of at least 92%. The muscle and tendon were also prevented from dehydration by regularly applying isotonic saline.

The force transducer was set in a starting position on a vertical metal shaft in such a fashion that it could run freely in vertical direction. On exertion of muscle force the transducer was pulled down until it passed the intersection of the muscle line of pull with the gliding rod. Special care was taken that the position of the FCU muscle always resembled as much as possible the in vivo situation (Fig. 1) [25].

Experimental surgery

All experimental surgery was performed with a biocular operation microscope with the animal mounted in the experimental setup.

Four stages of dissection were studied:

(1) *Intact situation*: the compartment as well as intracompartmental fascial surroundings of the FCU were intact, with the exception of the compartment's most distal 2 mm, where the accessory bone of the carpus had been dissected.

(2) After *fasciotomy*: the distal part of the antebrachial compartmental fascia was incised longitudinally, dorsally over the muscle belly over two-thirds of the muscle length and without damaging the underlying muscle fibers.

(3) After *clinical dissection*: the flexor carpi ulnaris muscle was meticulously dissected from its environment until it was mobilized sufficiently to perform a smooth transposition to the extensor carpi radialis tendon at the dorsal aspect of the forelimb. This meant that the muscle was dissected to a point that was half way up the muscle belly. Note that the actual transposition was not performed.

(4) After *maximal dissection*: the flexor carpi ulnaris muscle was dissected further in the proximal direction toward its origin on the humerus. The antebrachial fascia was incised completely, up to the medial epicondyle of the humerus. Care was taken to leave the superficial vasculature on the radial–volar side of the muscle intact.

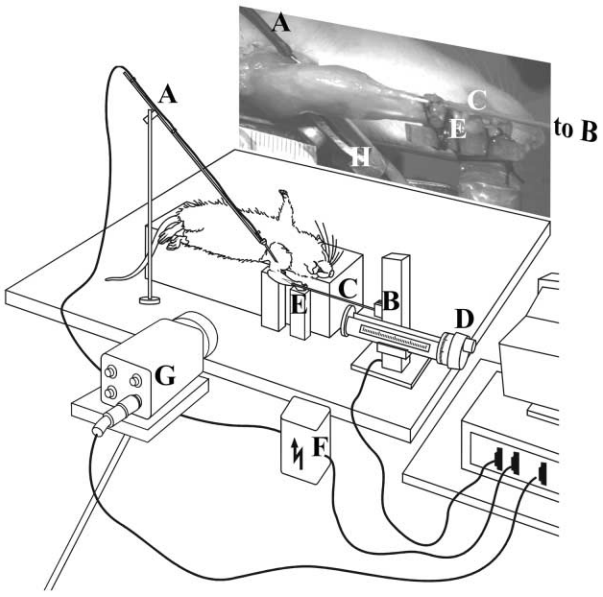

Fig. 1. Schematical representation of the experimental setup. A glass rod (A) holds a set of silver electrodes at a certain location, the severed end of the ulnar nerve is placed on the electrodes. The electrodes are connected to a constant current stimulator (F). The force transducer (B) is mounted on a vernier mechanism (D). This vernier is used for controlled lengthening of the muscle in between contractions. The distal tendon of the FCU is connected to the force transducer with a stiff kevlar wire (C). The left forefoot is fixed in neutral position (E). A clamp (F) fixes the humeral bone. A camera placed with its imaging surface parallel to the FCU muscle (G). An example of one actual experimental image, zoomed in on the FCU muscle within its almost intact compartment, is shown as backdrop. The timing of stimulation and imaging are controlled by a computer, which also records A–D converted force data.

Measurement of length–force characteristics

The passive muscle was stretched to the desired target length, using a screw mechanism with a vernier (read to the nearest 1/10 of a mm). The FCU was activated maximally by supra-maximal electrical stimulation of the ulnar nerve, with square wave pulses from a constant current source (duration 1000 ms, amplitude 3 mA, frequency 50 Hz), using an electrical nerve stimulator (Stimulator 50–4977, Harvard Apparatus, South Natick MA, USA). Two hundred milliseconds prior to the tetanic stimulation, a single electrical pulse was applied which elicited a single twitch, with the purpose to tighten the kevlar thread and to allow some adjustment of the muscle to the target length. The interval between two subsequent tetanic contractions was 2 min, during which the muscle was allowed to recover at its resting length.

The stimulation procedure was repeated at a consecutive series of muscle lengths starting at low length, with increments of 1 mm until the active force decreased rather than increased, followed by two more length increments of 0.5 mm to allow a better estimation of muscle optimum length. For every individual FCU, at least seven data-points were obtained in this manner.

After each series of measurements, one more control stimulation was performed at a muscle length that had yielded maximum active force. If the control measurement showed a similar result it is was assumed that measurements were not influenced unduly by muscle fatigue, disturbances in nerve conduction, or by damage to muscle fibers or the intramuscular connective tissue by lengthening the muscle to high lengths. If these factors would have played a role, the control-measurement would have showed a decreased force output. Two images were taken from the lateral side, using a digital camera (CV-M10 CCD Camera, JAI, Glostrup, Denmark) perpendicular to the rat's forelimb: one was taken just prior the single twitch, and one during the tetanic contraction.

Force signals and image synchronisation signals were A–D converted at a sampling frequency of 1000 Hz and a resolution of force of 0.0071 N and stored on a hard disk.

Treatment of data

Mathematical functions were fitted to the experimental data for further treatment and averaging [10]. In short, passive length–force data were least square fitted using an exponential function and active muscle force was calculated by subtracting the measured passive force from total force during muscle activity. Active length–force data were fitted with a polynomial function. The degree of the polynomial function that most adequately described a particular set of length–force data was selected using an analysis of variance. The maximum of the selected polynomial was defined as optimal FCU force, and its corresponding length as optimum length (l_{opt}).

Initial experimental length and muscle optimum length were determined from the digital images that were taken. The initial experimental length as well as FCU optimum length differed individually per animal. Therefore, muscle length was expressed as deviation from l_{opt}, determined for the intact situation (stage 1) in each rat, and allow for comparison of individual FCU muscles.

Two way ANOVA for repeated measurements were performed to test for differences between the four experimental conditions. The Bonferroni procedure was used in post hoc testing to locate significant differences [1]. Active and passive force data at normalized lengths were averaged for the seven rats. A standard SPSS 8.0 (SPSS Inc., Chicago, USA) package was used for all statistical calculations.

Results

General effects of experimental surgical interventions

Analysis of variance indicates that length–active force characteristics were affected significantly by the progressive stages of dissection ($p < 0.001$). This is most obvious at low muscle length where force decreased most (Fig. 2). Length–passive force characteristics were not affected significantly ($p = 0.150$). Besides a decreased force output, the length–force profile was shifted to a higher length (Fig. 2 inset), and the steepness of the curvature changed after dissection, which is shown after normalization of muscle force and muscle length for their maximums in every separate stage of dissection (Fig. 3). Compartmental fasciotomy is the main intervention that causes these changes. The control measurement, which was obtained during the extra stimulation after each series of measurement, showed not to be different from the first measurement (Fig. 4). All effects found may therefore be attributed to the experimental conditions.

(1) Intact situation

Individual characteristics of the rats are shown in Table 1. The mean optimal force of the FCU measured within the intact compartment (stage 1) was 2.53 N ± 0.45 (SD). The mean optimum length of the muscle, including the distal tendon in stage 1 of dissection was 3.09 cm ± 0.24 (SD).

(2) Fasciotomy

Compartmental fasciotomy affects FCU length–active force characteristics significantly (post hoc test

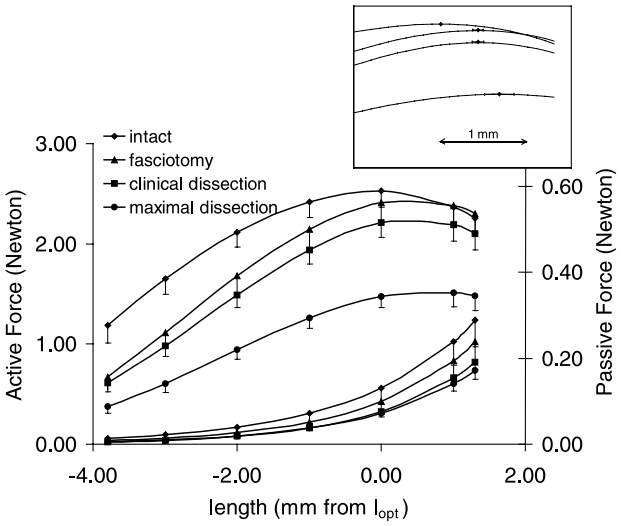

Fig. 2. Effects of stages of dissection of rat FCU on absolute length–force characteristics. Active (top four curves) and passive (bottom four curves) length–force characteristics of rat flexor carpi ulnaris muscle during four progressive stages of dissection. Muscle length is expressed as deviation from optimum length during intact situation. Means and standard error (vertical bars) are shown ($n = 7$). Inset: close-up near optimum length. The mean optimum length in every stage of dissection is shown by the dots in the graph, joined the standard error of the mean (horizontal bars). Note the significant shift in optimum length to a higher length after dissection.

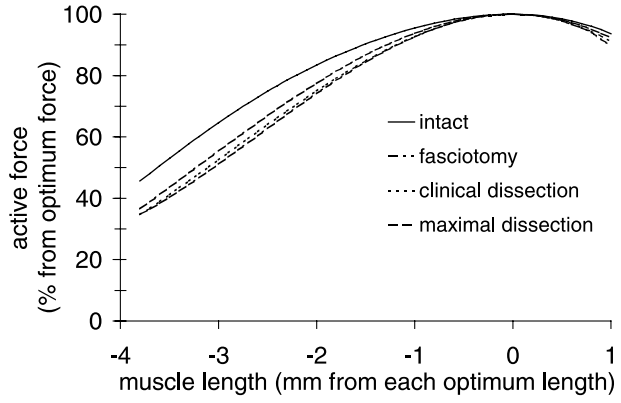

Fig. 3. Normalized active length–force curves of rat FCU in different stages of dissection. Force was normalized for its optimum value at each individual stage of dissection and expressed as a percentage of that optimum force. Optimum length was determined separately for every stage of dissection. Length is expressed as deviation from optimum length for each condition, so that different optimum lengths are superimposed in this figure to allow a good comparison of the shape of the curves.

$p < 0.05$). Active force decreased approximately by 40% at low lengths compared to the intact situation. If compared at the original optimum length, a small decrease in active force is seen. However, muscle optimum length shifts very substantially to higher lengths (approximately 0.5 mm i.e. 10% of the measured length

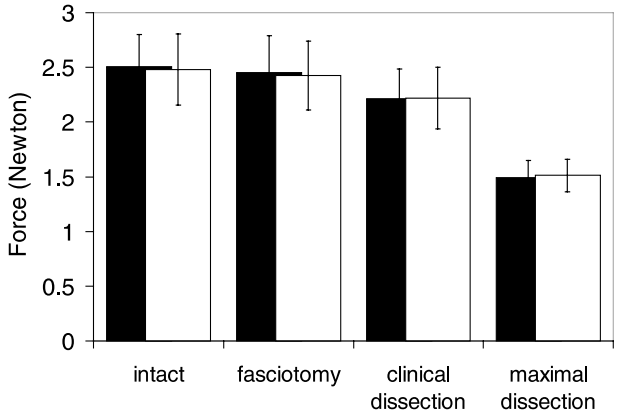

Fig. 4. Comparison of experimental and control measurements of force. Means of control measurements (white bars) and experimental measurements (black bars) of force during four stages of dissection are shown. These measurements were performed at experimental optimum length of every individual stage of dissection in every individual rat. The vertical bars indicate the standard error.

Table 1
Characteristics of the individual rats

Rat no.	Weight (g)	l_{opt} (cm)	F_{opt} (N)
1	308	2.79	2.57
2	300	3.35	2.97
3	307	3.03	2.01
4	305	3.19	3.23
5	312	3.12	2.02
6	290	3.27	2.41
7	300	2.99	2.48

range) after compartmental fasciotomy. A comparison of optimal active force before and after fasciotomy (each with different optimum lengths) indicates that this force level is not affected significantly ($p = 0.59$; paired student t-test).

(3) Clinical dissection

The curves of stage 2 and stage 3 did not significantly differ from each other, as post hoc testing showed that clinical dissection had no significant additional effect ($p = 0.239$) on active length–force characteristics. As a consequence clinical dissection does not result in a further shift of l_{opt}. This indicates that the stiffness of compartmental connective tissue is already decreased to such low levels due to compartmental fasciotomy that any further changes of force transmission by clinical dissection is prevented. The optimum force, compared at different optimum lengths after fasciotomy and after clinical dissection decreased on average with 0.19 N ± 0.18 SD (i.e. 8% of the maximum force after fasciotomy) after clinical dissection, which was statistically significant ($p = 0.04$; paired student t-test).

(4) Maximal dissection

Maximal dissection produced a substantial decrease also in optimum force by approximately 40%. The decrease in force is significant ($p < 0.01$). This is accompanied by a further major shift of l_{opt} to higher lengths (by approximately another 0.2 mm). Note also that at low lengths a further decrease of active force by approximately 60% was found. Also, the optimum active force (at different optimum lengths) decreased significantly after maximal dissection compared to clinical dissection ($p < 0.01$).

The conclusive result in this study is that progressive dissection of the surrounding connective tissue causes force output to decrease over the whole length range.

Discussion

To this point, isometric muscular length–force characteristics have been studied using isolated preparations of: (1) single isolated muscle fiber [3], (2) isolated fascicles [27], or (3) isolated whole muscle in situ [26]. Therefore, the possible role of the surrounding connective tissue in force transmission has largely been neglected. The present study shows that the forces that are measured in studies on isolated muscle (i.e. stage 4) may be a considerable underestimation of the forces that are produced by that same muscle in vivo. Furthermore, there was a shift of the length–active force profile to a higher length after dissection, which partly explains the dramatic decrease in force output specifically at low muscle length, since such a shift has the greatest effect at the steepest part of the profile. Because after dissection the curvature changes to an even steeper profile, the decline of force at low muscle length is enhanced. In addition to that, a steeper curvature after dissection implicates a decreased active length range of the FCU after dissection.

An explanation for all changes in force exerted during ongoing dissection should be sought in altered force transmission from the muscle fibers. In addition to myotendinous force transmission to the aponeurosis and tendon, force may be transmitted from muscle fibers onto the endo-, peri-, and epi-mysial network within muscle [5,7,10]. This system of intramuscular connective tissue is often referred to as loose connective tissue. Previously this system was thought to be too compliant [16] or too weak to be able to transmit force, but it has been shown recently that this loose connective tissue is capable of transmitting all muscle force in conditions of experimental tenotomy in rat EDL [5,6]. This type of force transmission onto the intramuscular network has been referred to as myo-fascial force transmission and is felt to take place by shearing of the connections between the sarcolemma and the endomysium [17,19–24]. As this intramuscular system is continuous with extramuscular

connective tissues as well as intramuscular connective tissue of adjacent muscles, force may also be transmitted from muscle without passing the tendon provided such connections are stiff enough [5]. If this happens, there should be a difference between force exerted between proximal and distal attachments of the muscle. Recent evidence [8,14] indicates that a substantial fraction of the force (up to 30–40%) may be transmitted from a muscle without passing either origin or insertion of the muscle. Therefore, force exerted at a specific tendon of a muscle is determined by two major factors: (1) force exerted by its own muscle fibers and (2) force transmitted from or onto the muscle by surrounding muscles. As ongoing dissection progressively removes the extra- and intermuscular connections depending on the actual conditions of the muscle with respect to its surroundings, force exerted at the tendon may either increase or decrease. Our present results for rat FCU indicate that the experimental conditions were such that progressive dissection removed transmission of force from adjacent sources onto the distal tendon of FCU. A similar drop in force measured at the proximal tendon with progressive dissection was found in rat EDL [9].

Extra- and intermuscular myo-fascial transmission may also explain why Riewald and Delp [18] found the moment of the rectus femoris muscle to produce extension of the knee even after the tendon was transferred to the semitendinosus muscle or iliotibial band to increase flexion forces. Even though the myo-tendinous pathway was rerouted, the rectus femoris forces may still have been transmitted laterally onto adjacent extensor muscles by way of proximal fascial connections that were left intact [5].

Clinical considerations

Results from the present study on the rat FCU may not be generalized to human muscle without further study. The rat FCU has different architecture and a much longer tendon than its human counterpart. Moreover, the function of the FCU in rats differs from that of man because a rat walks on four legs.

From a clinical point of view, it would have been interesting to have included a fifth stage i.e. the function of the muscle in a transposed position. However, to take the muscle out of its normal alignment to a new route would have changed the sarcomere distribution as a result of this new alignment, and not only as a result of the connections with their fascial surroundings. Since the purpose of this study was to investigate the latter, we therefore chose not to actually perform the transposition.

Still, our finding that length–force characteristics are altered with progressive surgical dissection of a muscle from its surroundings is likely to be indicative of a general physiological principle, which should be acknowledged in tendon transfer surgery. Standard surgical procedures [4] that include mobilizing a muscle through dissection of the surrounding connective tissue in order to increase the muscle–tendon's excursion [2,11] may also have dramatic effects on the muscle force production capacity. To date, the surgeon's subjective estimation of passive tension of the muscle is the only guideline to qualify muscle function. Since dissection of muscle does not seem to result in significant changes of passive tension while active tension does, qualification on this base seems not accurate.

Furthermore, it should be noted that the inevitable dissection of the connective tissue during transposition surgery is the starting condition at which the muscle and its surroundings will recover. The need for ongoing research is evident since, undoubtedly, new connective tissue will be created and the connections with the muscle will be restored in some way. The new route of the muscle gives opportunity to engage in new interaction with adjacent muscles and their connective tissue, and length–force relation is likely to be modified during rehabilitation as the muscle adapts to its new function.

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