Intraoperative Single-Site Sarcomere Length Measurement Accurately Reflects Whole-Muscle Sarcomere Length in the Rabbit

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Purpose: To compare single-site intraoperative sarcomere length values with sarcomere lengths measured from systematic sampling of the entire transferred muscle.

Methods: The tendon of the rabbit second toe extensor muscle was transposed to the ankle extensor retinaculum under levels of stretch over the sarcomere length range of about 2.5 μm to about 4.0 μm. Intraoperative sarcomere length was measured at a single site with a laser diffraction device. Whole-muscle sarcomere length measurement was then determined by sampling across the muscle in the proximal, middle, and distal regions. Linear regression analysis and intraclass correlation coefficients were used to validate single intraoperative sarcomere lengths relative to whole-muscle sarcomere lengths.

Results: Single intraoperative sarcomere lengths correlated strongly with average whole-muscle sarcomere length, although there was a systematic tendency to overestimate intraoperative sarcomere length. Intraoperative sarcomere length also matched well with all regions sampled, indicating that there was no tendency for intraoperative sarcomere length to better represent one region of the muscle compared with another.

Conclusions: These results show that intraoperative sarcomere lengths accurately represent the entire muscle. The relatively small sarcomere length variations validate the use of intraoperative sarcomere length measurement during tendon transfer in which the entire muscle is not available for measurement because of limited surgical exposure. (J Hand Surg 2007;32A:612–617. Copyright © 2007 by the American Society for Surgery of the Hand.)

Key words: Sarcomere length, validation, tendon transfer.

Tendon transfer surgery restores the loss of limb function that may occur, for example, after spinal cord injury, head injury, and peripheral nerve injury.[1] In this procedure, the tendon of a healthy, functional muscle is translocated to a new anatomic site to restore or augment lost function. During surgery, surgeons must define the route of transfer, donor muscle length, and method of tendon repair.[2] Although each of these factors is functionally important, there is evidence that setting donor muscle length affects the functional outcome of the transfer.[3] Although human skeletal muscle function is known to be very sensitive to length, not all muscles are equally sensitive to length. This is because the absolute length of fibers within a muscle varies substantially throughout the human upper extremity.[4–6] This complicates the decision regarding setting muscle length, because no single amount of stretch works for all types of human muscle, and decisions are made instead based on the posture of the joint being addressed by the surgery. The process of setting muscle length during surgery is known as *tensioning* in the vernacular of the hand surgeon.[1] This is based on the intraoperative sense that muscle tension is set to a certain level to achieve the anatomically appropriate surgical correction.

There are very few objective tools available to assist the surgeon in skeletal muscle tensioning.

Table 1. Architectural Properties of the Rabbit Toe Extensor

	Raw Fiber Length, mm	Pennation Angle, °	Sarcomere Length, μm	Serial Sarcomere Number	Normalized PCSA, mm²*
Proximal	10.93 ± 0.22	5 ± 1	2.73 ± 0.03	4003 ± 80	
Middle	10.70 ± 0.17	11 ± 1	2.80 ± 0.02	3823 ± 51	43.07 ± 1.94
Distal	10.51 ± 0.20	19 ± 1	2.75 ± 0.02	3825 ± 81	
Average	10.71 ± 0.17	12 ± 1	2.76 ± 0.02	3882 ± 59	

PCSA, physiological cross-sectional area.
*The PCSA can be determined only for the entire muscle as a whole.

Quantitative intraoperative mechanical studies pioneered by Freehafer and colleagues[7] were primarily intended to define the properties of commonly transferred muscles and showed tremendous variability among muscles. Subsequent quantitative biomechanic studies have shown that muscle tension levels selected by experienced hand surgeons are variable,[8] are highly influenced by the information provided to the surgeon,[9] do not accurately reflect the physiologic excursion of the muscle,[10] and affect functional outcome.[3]

Given the theoretical and practical ambiguities associated with intraoperative tension measurements, we have instead focused on muscle sarcomere length as the most objective parameter on which to rely for optimizing muscle function.[11] The sarcomere is the functional unit of force generation in skeletal muscle[12] and is an excellent predictor of active muscle force generated in species ranging from flies to humans.[13] Intraoperative sarcomere length measurements have shown the tendency by surgeons to overstretch muscles during routine tendon transfers.[8] The ultimate goal of intraoperative sarcomere length measurements is to be able to predict postoperative function with very high fidelity.[14,15] A valid concern, however, that can be raised regarding the use of intraoperative sarcomere length measurements to make surgical decisions is that sarcomere length is measured only at a single site in the muscle but is then considered representative of the entire muscle. The single-site measurement is often necessitated by the limited exposure of most muscles during reconstructive surgery.

Previous arguments regarding the generalizability of single-site sarcomere length measurements come primarily from cadaveric studies of upper-extremity muscles. It was shown in the functionally complex pronator teres muscle[16] and in the simpler flexor carpi ulnaris muscle[17] that sarcomere length did not vary significantly from proximal to distal and medial to lateral (coefficients of variation of ~10%) despite fiber length variations of more than 30% measured in those directions.[18] In addition, the relationship between single-site sarcomere length and active force production was measured in the relatively large canine flexor muscle mass[19] and shown to be well correlated. The purpose of this study was to compare the single-site intraoperative sarcomere length values obtained with sarcomere length measured from the same muscle after systematic sampling across the entire muscle.

Materials and Methods

Experimental Model

The hindlimbs of male New Zealand White rabbits (mean body mass ± SD = 2.57 ± 0.30 kg; n = 10 subjects, 20 hindlimbs) were used for this study. All experimental procedures were approved by the University of California and Department of Veterans Affairs, San Diego Institutional Animal Care and Use Committees. The extensor digitorum of the second toe (EDII) muscle was used to create the tendon transfer based primarily on its small thickness that allowed application of the laser diffraction method through the full muscle thickness without the need for any tissue dissection. Whole-muscle diffraction alleviated the concern that distortion of the muscle, which may occur with dissection, would corrupt intraoperative measurements. The EDII muscle runs along the medial aspect of the tibia and inserts onto the second toe using the medial malleolus as a pulley. Its main action is as a toe extensor/abductor. Pilot experiments showed that this muscle has an average fiber length of 10.7 mm, a 2.76-μm sarcomere length with the ankle in neutral (90° flexion), and a maximum anatomic sarcomere length range from 2.6 μm to 3.1 μm (Table 1; M. Takahashi, S. R. Ward, R. L. Lieber, unpublished data). Animals were anesthetized with isoflurane (IsoFlo; Abbott Laboratories, North Chicago, IL), and the ankle and knee joints were secured at 90° of flexion in a custom-made jig. The full EDII muscle length was exposed and muscle

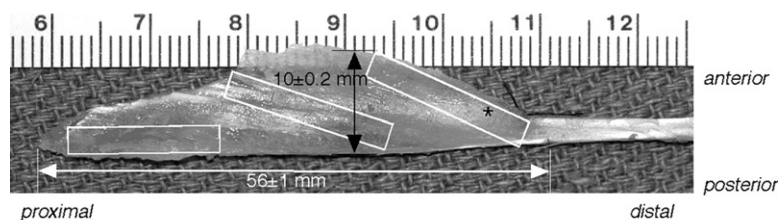

Figure 1. A EDII muscle isolated from the right tibia showing the 3 specific regions for architectural L_s measurement (white rectangles). This muscle has variable pennation angles by region (Table 1). The muscle border is normally intimately associated with the periosteum. Average dimensions are given for the muscles used in this study, and the site used for laser diffraction is shown with an asterisk. This area is very thin and can be transilluminated with a low-power laser as is evidenced by the translucency in which the calibration scale can be seen through the muscle.

length (L_m) was measured with digital calipers (accuracy, ±0.05 mm) as the distance between the origin of the most proximal muscle fibers and the insertion of the most distal fibers (Fig. 1). The EDII tendon was then released at the level of first metatarsal, passed deep to the ankle extensor retinaculum, looped back, and sutured to itself with a 5-0 suture material (Ethibond; Ethicon, Sommerville, NJ).

Single-Site Sarcomere Length Measurement

Intraoperative sarcomere length (L_s) was measured after transposition (addressed later in text) in the most distal fiber bundle of the EDII with a laser diffraction device as previously described (asterisk, Fig. 1).[20] Great care was taken not to stretch the muscle in any way, and because no tissue dissection was required, no tissue distortions were introduced by this procedure. The diffraction pattern was analyzed and converted to L_s by using the standard equation $n\lambda = d\sin\theta$, where λ is the laser wavelength (0.632 μm), d is L_s, θ is the diffraction angle, and n is the diffraction order.[21] Diffraction order position was measured with dial calipers, and calipers measurement error (0.05 mm) corresponded to a sarcomere length resolution of less than 0.01 μm. Muscle length at the time of transposition was intentionally varied from 0 to 4 mm longer than the original muscle length by varying tension during the transfer and forcing sarcomere length to vary over the range of about 2.5μm to about 4.0 μm.

Whole-Muscle Sarcomere Length Measurement

Immediately after transfer, animals were killed with pentobarbital (Euthasol; Virbac AH, Fort Worth, TX). Hindlimbs were skinned and amputated at the level of femur, and the transferred intact muscle–tendon–bone unit was fixed in 10% buffered formalin for 48 hours. This ensured that sarcomere length was measured in the fixed tissue while in the same geometric configuration as during intraoperative measurement. After rinsing in isotonic phosphate-buffered saline, the complete EDII muscle was detached from the tibia and digested in 15% H_2SO_4 for 20 minutes to facilitate muscle fiber bundle microdissection. Each EDII muscle was divided into 3 regions (proximal, middle, distal) to yield representative architectural L_s values across the entire muscle (white rectangles, Fig. 1). Small fiber bundles (5–15 fibers) with tendinous ends intact were teased from each region under a dissecting microscope. Fiber bundle length was measured under the dissecting microscope to the nearest 10 μm from 2 different fiber bundles in each of 3 regions, providing 6 measurements of bundle length across the entire muscle. The L_s of each bundle was measured by laser diffraction in 3 regions of each bundle. Thus, whole-muscle sarcomere length was calculated as the average of 18 L_s measurements (3 regions/muscle × 2 bundles/region × 3 L_s measurements/bundle) obtained from the entire muscle.

Data Analysis

Linear regression was used to quantify the correlation between L_s values obtained by single-site intraoperative laser diffraction and L_s values obtained from microdissected bundles taken across the entire muscle. This analysis was performed for L_s averaged across the entire muscle and for each of the 3 muscle regions to detect possible regional effects. In addition, because we were interested in the specific numeric agreement between these 2 measures in addition to their correlation, the intraclass correlation coefficients (ICCs) were calculated for the same comparisons.[22] The ICC is similar in use to the kappa statistic but is used for continuous data.[23] Data are reported in the text and figures as mean ± standard error of the mean (SEM) unless otherwise noted.

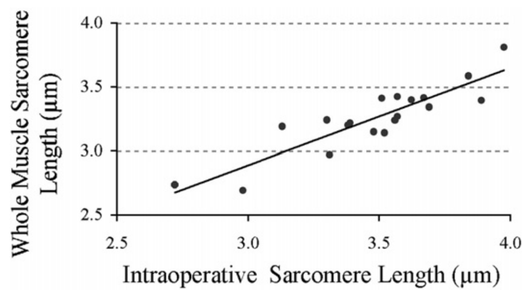

Figure 2. Relationship between single-site L_s measured during surgery and L_s measured by averaging L_s values from the 6 fiber bundles sampled across the entire muscle in areas shown by rectangles in Fig. 1. Data are highly correlated ($p < .001$, $r^2 = 0.794$), with an ICC of 0.805.

Results

Intraoperative L_s values obtained from a single site in the distal EDII region agreed well with the average L_s calculated from fibers sampled across the entire muscle (Fig. 2). This agreement was reflected by regression analysis, which showed a strong correlation between the single-site intraoperative L_s values and L_s averaged across the entire muscle ($p < .001$, $r^2 = 0.794$). Although the single-site measurement matched the whole-muscle technique well, there was a systematic tendency to overestimate intraoperative L_s (on average, 6% ± 1%). More importantly, the absolute numeric agreement between L_s obtained from a single site and L_s calculated from fibers sampled across the entire muscle was excellent (ICC = 0.805). This systematic comparison thus shows the validity of generalizing from a single site to the whole muscle for this experimental model.

No dramatic variability in L_s was observed across the entire muscle. This was important to quantify, because a possible weakness of the whole-muscle analysis presented earlier is that large variations between regions could average to equal the single-site L_s measurement, thus masking a whole-muscle effect. To test for this effect, comparisons were made between single-site L_s measurements and each region. First, very small L_s variation was observed among the 3 muscle regions, as indicated by a coefficient of variation of only 3% ± 0.17% across the whole muscle. The average L_s values measured in the proximal, middle, and distal regions were nearly the same at 3.30 ± 0.06 μm, 3.26 ± 0.06 μm, and 3.19 ± 0.07 μm, respectively. When intraoperative L_s was regressed against each region individually, all regions had nearly the same degree of correlation ($p < .001$ for all regions; r^2 range, 0.713–0.883) (Fig. 3). Thus, the single-site intraoperative measurement was equally representative of all 3 regions.

Discussion

The purpose of this study was to compare L_s values obtained from a single site, as is currently performed intraoperatively, with a panel of L_s measurements obtained across the entire muscle based on the microdissection of small fiber bundles. The results clearly show that intraoperative L_s values are representative of the entire muscle. This agreement even extends to the different regions, all of which correlated significantly with single-site L_s values to approximately the same extent. It can be concluded, therefore, that the relatively small sarcomere length variations across this muscle (coefficient of variation = 3%) are randomly distributed and easily estimated by L_s measured during surgery.

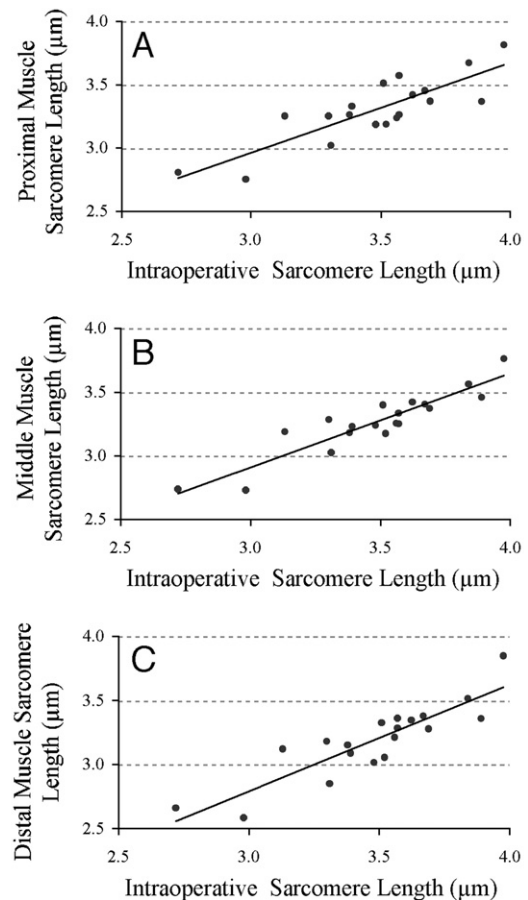

Figure 3. Relationship between L_s measured during surgery and L_s measured in a specific muscle region shown by rectangles in Fig. 1. (A) Proximal muscle region. (B) Middle muscle region. (C) Distal muscle region. There was no tendency for one region to be more highly correlated to the intraoperative measurement, showing that intraoperative L_s was equally representative across the entire muscle.

Although intraoperative L_s values were measured from the most distal region, the intraoperative measurement most highly correlated with L_s measured in the proximal region (Fig. 3A). This argues against a situation in which fibers closest to the measurement site are more closely represented by the intraoperative measurement. This point was recently used to argue against acceptance of intraoperative laser diffraction values measured in the flexor carpi ulnaris muscles of children with cerebral palsy.[24]

Although intraoperative L_s values were linearly related to L_s measured from microdissected fibers, intraoperative L_s values were also systematically longer (ie, the slope of regression relationship in Figure 1 is significantly less than 1). We believe that the most likely explanation for this difference is the muscle fiber shrinkage that is known to occur during formalin fixation.[25] Unfortunately, laser diffraction is the only method currently available to measure L_s in fresh tissue, although other promising methods are currently under development.[26]

The results of the current study are limited to the extent that this animal model accurately represents the use of laser diffraction as applied in human surgery. The most obvious limitation is that the EDII is a relatively small muscle compared with human upper-extremity muscles. This relatively small size, however, is still huge compared with the single site sampled by laser diffraction. The area of muscle sampled during surgery is based on the area transilluminated by the laser beam itself. For this 5 mW laser diode, the effective beam diameter profile is a circle with a diameter given by its $1/e^2$ distribution that is approximately 0.55 mm^2.[27] The muscle profile projection shape is approximately triangular (Fig. 1) with an average length of 55.9 $\pm$ 0.9 mm and an average height of 9.8 $\pm$ 0.2 mm, yielding a projection area of 335.2 $\pm$ 8.9 mm^2. Thus, in terms of area, only about 0.2% of the muscle was sampled. In addition, the thickness of the muscle is not constant, and we sampled from one of the thinnest regions (asterisk, Fig. 1); therefore, including thickness as a factor yields an even a smaller fraction of muscle sampled. Based on shape and thickness variations, we estimate that less than 0.1% of the muscle by volume was actually sampled. Thus, the key question is whether sampling 0.1% of the muscle (as performed here) is qualitatively different from sampling 0.005% of a human muscle (estimated based on human muscle volumes previously measured).[5,6]

In addition to limitations of our model based on its small volume, it is also relatively simple from an architectural point of view (Table 1). This is in contrast to some human muscles that, based on magnetic resonance imaging results, have been shown to be more complex with multiple subbellies and high fascicle length variability.[28,29] At this point, it is impossible to know the extent to which these complexities apply to all human muscles. At least in upper-extremity muscles, however, there is not yet current evidence of this level of fiber length heterogeneity. The few studies[16,17,30] that have been performed at the muscle subregion level have shown no such L_s heterogeneity despite relatively high fiber length heterogeneity. This study indicates that single-site laser diffraction sarcomere length measurements are representative of the sarcomere length found throughout the entire muscle.

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