In Vivo Measurement of Human Wrist Extensor Muscle Sarcomere Length Changes

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SUMMARY AND CONCLUSIONS

1. Human extensor carpi radialis brevis (ECRB) sarcomere length was measured intraoperatively in five subjects using laser diffraction.

2. In a separate cadaveric study, ECRB tendons were loaded to the muscle’s predicted maximum tetanic tension, and tendon strain was measured to estimate active sarcomere shortening at the expense of tendon lengthening.

3. As the wrist joint was passively flexed from full extension to full flexion, ECRB sarcomere length increased from 2.6 to 3.4 μm at a rate of 7.6 nm/deg joint angle rotation. Correcting for tendon elongation during muscle activation yielded an active sarcomere length range of 2.44 to 3.33 μm. Maximal predicted sarcomere shortening accompanying muscle activation was dependent on initial sarcomere length and was always <0.15 μm, suggesting a minimal effect of tendon compliance.

4. Thin filament lengths measured from electron micrographs of muscle biopsies obtained from the same region of the ECRB muscles were 1.30 ± 0.027 (SE) μm whereas thick filaments were 1.66 ± 0.027 μm long, suggesting an optimal sarcomere length of 2.80 μm and a maximum sarcomere length for active force generation of 4.26 μm.

5. These experiments demonstrate that human skeletal muscles can function on the descending limb of their sarcomere length-tension relationship under physiological conditions. Thus, muscle force changes during joint rotation are an important component of the motor control system.

INTRODUCTION

The sarcomere represents the fundamental unit of force generation in skeletal muscle. Elucidation of the relationship between sarcomere length and isometric tension represents one of the great accomplishments in muscle biophysics (Gordon et al. 1966). Less-well understood, however, are sarcomere length changes that occur during normal movement. Attempts to define such changes have involved sarcomere length measurements from fixed tissues (Rack and Westbury 1969; Rome et al. 1988; Rome and Sosnicki 1991; Weijs and van der Wielen-Drent 1982), theoretical modeling based on geometric relationships (Delp et al. 1990), and the descending limb (Herzog et al. 1991; Lieber and Brown 1992; Lieber and Boakes 1988; Mai and Lieber 1990).

There is, therefore, no general agreement regarding the physiological operating range of sarcomeres. Understanding this range is important for a complete understanding of the physiological basis of neuromotor control. Although muscle force can be altered by the number and frequency of activated motor units, force can also be altered by sarcomere length changes that are effective and predictable. Understanding these relationships in the human upper extremity may provide unique insights into the design of the musculoskeletal system because hand and wrist function are highly specialized to perform manipulative tasks. Thus, the purpose of this study was to measure the sarcomere length-wrist joint angle relationship in patients undergoing surgical release of the extensor carpi radialis brevis muscle (ECRB).

METHODS

Patient inclusion criteria

The five patients included in the study were undergoing surgical lengthening of the ECRB tendon for treatment of chronic lateral epicondylitis (tennis elbow). Patients ranged from 35 to 50 years of age and included three men and two women (Table 1). All procedures performed were approved by the Committee on the Use of Human Subjects at the University of Umeå and University of California, San Diego.

Intraoperative laser device

The device used was a modification of that originally described by Lieber and Baskin (1985) and Fleeter et al. (1985). A 5-mW helium-neon laser beam (Melles-Griot, model LHR-007, Irvine, CA) was aligned with a specially designed prism such that the beam projected normal to one prism face and was reflected 90°, exiting the other prism face (Fig. 1). The prism reflective surface was aluminum coated (Melles-Griot, model 001PRA/001) to direct all available laser power through the muscle.

The device was calibrated using diffraction gratings of 2.50 and 3.33 μm grating spacings placed at the location of the muscle fiber bundle (see below) directly on the prism. Diffraction order spacings from the ±1st order and the ±2nd order were measured to the nearest 0.1 mm using dial calipers that corresponded to a spatial resolution of ~0.02 μm. In practice, repeated measurement of diffraction order spacing resulted in a sarcomere length variability of 0.10 ± 0.21 (SE) μm (n = 12 measurements from two separate orders on three muscle biopsy samples by a single observer). Repeatability of sarcomere length measurements between observers
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TABLE 1. Characteristics of experimental subjects

<table>
<thead>
<tr>
<th>Subject</th>
<th>Age, yr</th>
<th>Sex</th>
<th>Flexed angle, °</th>
<th>Neutral angle, °</th>
<th>Extended angle, °</th>
</tr>
</thead>
<tbody>
<tr>
<td>UF</td>
<td>50</td>
<td>M</td>
<td>-27</td>
<td>15</td>
<td>49</td>
</tr>
<tr>
<td>TL</td>
<td>36</td>
<td>M</td>
<td>-19</td>
<td>0</td>
<td>36</td>
</tr>
<tr>
<td>IS</td>
<td>35</td>
<td>F</td>
<td>-72</td>
<td>7</td>
<td>33</td>
</tr>
<tr>
<td>IA</td>
<td>43</td>
<td>M</td>
<td>-73</td>
<td>-8</td>
<td>18†</td>
</tr>
<tr>
<td>IM</td>
<td>41</td>
<td>F</td>
<td>-41</td>
<td>8</td>
<td>57</td>
</tr>
<tr>
<td>Average ± SD</td>
<td>40.5 ± 5.3</td>
<td></td>
<td>-46.4 ± 22.5</td>
<td>3.5 ± 8.5</td>
<td>51.0 ± 16.9</td>
</tr>
</tbody>
</table>

* —, value not obtained. †Patient with wrist arthrosis.

We made every effort to measure sarcomere length in the in vivo position of the fibers and not to elongate them artificially by elevation of the fiber bundle. Estimation of error due to fiber elevation was obtained in pilot experiments and never exceeded 0.2 μm. Only the distal 2 cm of muscle fibers were exposed, and thus sarcomere lengths reported represent these distal fiber regions. Currently we do not know the extent to which these are representative of the entire fiber length or of different fibers along the muscle length.

Experimental protocol

Immediately after administration of regional anesthesia, an electrogoniometer (Penny and Giles, model Z110, Blackwood, Gwent, UK) was placed on the palmar surface of the subject’s wrist and hand. The electrogoniometer was contained within a sterile plastic wrap and secured to the palmar skin using sterile tape. The wrist was allowed to assume its neutral position [a radiocarpal angle of 10 ± 2° (mean ± SD) of flexion] and this was defined as 0°. The small radiocarpal angle variability between subjects was considered negligible (see below).

The distal musculotendinous junction of the ECRB was exposed via a dorsoradial incision ∼10 cm proximal to the radiocarpal joint. The overlying fascia was removed exposing the underlying ECRB muscle fibers. A small fiber bundle was isolated at the insertion site using delicate blunt dissection, with care not to over-stretch muscle fibers.

The illuminating prism was inserted beneath the fiber bundle (Fig. 1) and approximated into the normal plane of the muscle.

\[ n \lambda = d \sin(\theta) \]

where \( \lambda \) is the laser wavelength (0.632 μm), \( d \) is sarcomere length, and \( n \) is diffraction order (2nd in all cases) and assuming that the 0th order bisected the orders on either side.

Sarcomere length was measured with the wrist placed in each of three positions: full flexion, neutral, and full extension. The actual angular value corresponding to each position was noted from the electrogoniometer’s digital display (Table 1). It was not technically possible to place the wrist in the same extreme angular position.
tensions for each subject due to physiological variations in patient range of motion and, in one case (subject LA), wrist arthrosis.

**Sarcomere length measurement from biopsies and micrographs**

After sarcomere length measurements, muscle biopsies (~15 mm long) were taken from the same region by isolating a small fiber bundle as in the diffraction experiment and securing it to a wooden stick using two silk sutures. Wrist angle at which the biopsy was obtained was noted. Extreme care was used to maintain the muscle biopsy in its in vivo configuration by suturing the biopsy to a stick before cutting the fibers. In two subjects (UF and LA), two biopsies were obtained with the wrist joint in different configurations yielding a total of 7 biopsies. Biopsies were obtained to corroborate sarcomere length values obtained by laser diffraction and were immediately passed to the operating room technician and immersed in 2.5% phosphate-buffered glutaraldehyde (0.1 M buffer adjusted to pH 7.4) for fixation.

One to two days after immersion in fixative, biopsies were rinsed in buffer and trimmed to remove obviously damaged muscle fibers, and sarcomere length was again measured using the identical laser diffraction device. In all cases, the presence of five diffraction orders suggested preservation of muscle structural integrity.

The mechanically undamaged portion of the biopsy was transversely cut into slices ~1 mm thick. From these slices, 8–10 tissue blocks were postfixed for 2 h in 1% osmium tetroxide, dehydrated in graded alcohols, and infiltrated with Spurr embedding resin (Polysciences, Warrington, PA). Blocks were oriented so that the muscle fibers could be sectioned either longitudinally or transversely. Survey sections of 1 mm were stained with toluidine blue, and a region was selected, trimmed, and sectioned for electron microscopy. Section thickness was kept as close to 60 nm as possible.

Five micrographs were printed from each biopsy at approximately ×30,000 magnification. An image analysis system (IBAS, Zeiss, New York, NY) was used to measure A-band length (corresponding to the myosin filament length), actin filament length, and sarcomere length. Micrograph magnifications were calculated using the M line repeat distance of 220 Å within the M-band and sarcomere length. Micrographs were imaged using the identical laser diffraction device. In all cases, the presence of five diffraction orders suggested preservation of muscle structural integrity.

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**ECRB tendon biomechanical testing**

Muscle-tendon units from five fresh-frozen cadaveric specimens were removed and muscle architectural properties measured as previously described (Lieber et al. 1990). ECRB fiber length-to-muscle length ratio was ~0.4 with a cross-sectional area of 2.4 cm², in agreement with our previous study. ECRB maximum tetanic tension (P0) was then predicted for each specimen by multiplying the calculated physiological cross-sectional area by a specific tension of 2.5 kg/cm², which yielded an average P0 of 6 kg (Table 2). This procedure has been shown to predict muscle tetanic tension accurately (Powell et al. 1984).

ECRB tendons were dissected free of the muscle and transverse dye lines were placed at a 10-mm spacing along the tendon length for measurement of strain as previously described (Lieber et al. 1991). The marked ECRB tendon was then placed in a bath of normal saline at a temperature of 37°C and slowly loaded (over a 30-s interval) to P0 during which time surface strain was recorded. This relatively slow strain rate (0.1%/s) was chosen to permit maximum tendon strain and permit calculation of maximum sarcomere shortening. Because tendons are somewhat viscoelastic (Herrick et al. 1978), demonstrating increased stiffness with increasing strain rate, it is likely that sarcomere shortening during physiological contraction rates would be slightly less. Load strain relationships were calculated for each tendon and averaged across tendons to yield a single load-strain equation in the form

\[ \text{Load } (\% P_0) = a + 10^b - c \]

where a represents tendon strain and b and c are regression constants. This relationship was then used with muscle architectural properties to predict sarcomere shortening at the expense of tendon lengthening during muscle activation by using the computer model previously described (Lieber et al. 1992a).

**Statistical analysis**

Comparison between wrist angles and sarcomere lengths in the different wrist configurations was made using one-way analysis of variance with repeated measures and multiple paired comparisons performed using Fischer’s least significant difference test (Statview 4.0, Abacus Concepts, Berkeley, CA). Differences between sarcomere lengths measured in vivo were compared with those measured from biopsies and electron micrographs by calculating the root mean square (RMS) difference between the data sets and using a one-sample t test to determine whether the RMS difference was significantly different from zero. Significance level was set to α = 0.05. Based on the experimental coefficient of variation of 21%, statistical power (1 − β) was calculated as 81% (Sokal and Rohlf 1981).

**RESULTS**

**Diffraction pattern characteristics**

In all cases, multiple diffraction orders were observed on either side of the 0th order with approximately equal intensities. Typically, three diffraction orders were seen, but in several cases, up to five diffraction orders were observed implying excellent preservation of the normal sarcomere lattice. In two specific cases, intensity fluctuation of even and odd orders was seen as would be expected from thick grating effects (Magnusson and Gaylord 1977). It was easy to see that wrist flexion caused diffraction orders to come closer together, and wrist extension caused diffraction orders to spread apart as expected.

**Sarcomere length change with joint rotation**

Despite of the difficulty in achieving uniform joint angles for the flexed, neutral, and extended positions, there was a significant difference (P < 0.001) between joint angles in

**TABLE 2. ECRB muscle and tendon properties**

<table>
<thead>
<tr>
<th>Muscle Mass, g</th>
<th>Muscle Length, mm</th>
<th>Fiber Length, mm</th>
<th>Estimated Muscle P0, kg</th>
<th>Muscle Cross-Sectional Area, mm²</th>
<th>Tendon Length, mm</th>
<th>Tendon Strain at P0, %</th>
</tr>
</thead>
<tbody>
<tr>
<td>11.8 ± 1.09</td>
<td>176 ± 4.4</td>
<td>70.8 ± 1.7</td>
<td>6.0 ± 0.51</td>
<td>240 ± 21</td>
<td>204.0 ± 4.4</td>
<td>2.0 ± 0.2</td>
</tr>
</tbody>
</table>

Values are means ± SE for n = 5 samples. ECRB, extensor carpi radialis brevis; P0, maximum tetanic tension.
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FIG. 2. A: sarcomere length vs. wrist joint angle relationship determined for the 5 experimental subjects. Negative angles represent wrist flexion relative to neutral whereas positive angles represent wrist extension. One-way analysis of variance revealed a significant difference between wrist joint angles and sarcomere lengths in the three positions. ○, flexed angles; ■, neutral angles; △, extended angles. Note that one point is missing from subject 1A at a neutral joint angle (Table 1). B: comparison of the sarcomere length vs. wrist joint angle relationship in one subject for which both the human extensor carpi radialis (ECRB) and extensor carpi radialis longus (ECRL) were exposed. Slope of sarcomere length-joint angle curve for ECRB was 7.4 nm/deg whereas that for the ECRL was only 5.2 nm/deg. C: comparison between sarcomere lengths measured in vivo (●), those measured from muscle biopsies (+), and those measured from electron micrographs prepared from muscle biopsies (○).

ECRB tendon biomechanical properties

Load-strain relationships within each of five samples were highly reproducible and consistent between specimens. All correlation coefficients obtained from curve-fit of the raw data exceeded 0.7, suggesting that the exponential fit was appropriate. The averaged load strain relationship (Fig. 3A) was described by the equation: Load (% P₀) = 2.415 × 10⁻⁰°× 2.415. Using this relationship along with muscle architectural properties and tendon dimensions (Table 2), the computer model predicted slight sarcomere shortening at the expense of tendon lengthening (Fig. 3B). Maximum sarcomere shortening was 0.15 μm at intermediate sarcomere lengths ranging from ~1.8 to 2.7 μm with less shortening at longer and shorter sarcomere lengths. At the measured sarcomere length extremes, passive sarcomere length of 2.6 μm shortened to 2.41 μm and passive sarcomere length of 3.4 μm shortened to 3.33 μm (Fig. 3B).

Electron microscopic sarcomere and filament lengths

Sarcomere lengths measured directly from micrographs were found to be both shorter and longer than those measured by laser diffraction from biopsies (Fig. 2C). The aver-
Given the measured actin filament length of 1.30 µm and myosin filament length of 1.66 µm, these data suggest that the muscle operates primarily on the plateau and descending limb of its sarcomere length-tension curve (Fig. 5). Assuming that human muscles generate force as do frog skeletal muscles (for which the sarcomere length-tension relationship has been elucidated; Gordon et al. 1966), optimal sarcomere length would occur between 2.60 and 2.80 µm, which agrees well with the optimal sarcomere length of 2.64 to 2.81 µm predicted by Walker and Schrodt (1973) on the basis of filament length measurements.

These data suggest that the ECRB muscle would develop near maximal isometric force at full wrist extension, force would remain relatively constant as the sarcomeres lengthened “over” the plateau region, and then force would decrease to −50% maximum at full wrist flexion. This result contrasts with the generally accepted notion that skeletal muscles generate maximum forces with the joint in a neutral position. We conclude, therefore, that muscle force change due to sarcomere length changes during joint rotation is “built-in” as part of the control in the musculoskeletal system and not simply a consequence of muscle microanatomy. Of course, the actual muscle force generated at a given angle depends not only on sarcomere length, but also on the number and firing frequency of motor units. Thus, the change in sarcomere length might be viewed as setting the “upper limit” for force production at a given joint angle.

**Muscle sarcomere lengths in vivo**

Previous studies relating sarcomere length to in vivo movement have produced a variety of results. In part this may be due to the variety of methods used, including fixation and manual sarcomere counting (Rack and Westbury 1969; Rome et al. 1988; Weijs and van der Wielen-Drent 1982), theoretical modeling based on geometric considerations (Delp et al. 1990; Herzog et al. 1991; Hoy et al. 1990), and direct laser diffraction (Lieber and Boakes 1988; Lieber et al. 1992b; Mai and Lieber 1990). Studies of mammalian muscle have suggested that the muscles operate both on the ascending and descending limb of their length-tension curve. In the cat soleus muscle, this corresponded to a predicted sarcomere length range of 2.1–3.2 µm [cf. Fig. 2 of Rack and Westbury (1969)]. In the human rectus femoris, sarcomere lengths were not calculated, but it was suggested that fibers operated at lengths corresponding to the descending limb (Herzog et al. 1991).

In contrast to mammalian muscle, studies of swimming fish have suggested that active physiological sarcomere lengths lie almost exclusively on the plateau of their sarcomere length-tension relationships, resulting in maximum efficiency and muscle power output (Lieber et al. 1992b; Rome et al. 1988; Rome and Sosnicki 1991). This concept was supported experimentally by Rome and Sosnicki (1991), who compared in vitro contractile properties to predicted in vivo velocities and claimed that sarcomere velocity corresponded to 0.3 Vmax, the peak power and efficiency point of these muscles. In contrast with fish locomotion studies, in situ optical diffraction studies of frog semitendinosus muscle demonstrated that sarcomere lengths well onto the descending limb of the length-tension curve.

occur in normal joint configurations (Lieber and Boakes 1988; Lieber and Brown 1993; Mai and Lieber 1990). It thus appears that various muscle-joint systems operate on different portions of the isometric length-tension curve. This could be a result of the different types of movements initiated by these muscles: oscillatory for the fish, propulsive for the frog, and manipulative for the human ECRB. It is possible that operating on the descending limb allows the ECRB to “automatically” decrease the muscle’s maximum force in the configuration where it is less often used (i.e. flexion). However, the data may also suggest that the most important design constraint of the musculoskeletal system is not simply to maintain constant sarcomere length. As more data are acquired, the underlying design constraints for determination of sarcomere length range will undoubtedly be better understood. It is interesting to note, however, that the slope of the ECRB sarcomere length-joint angle relationship (7.6 nm/deg) is completely within the range of similar values measured in a variety of frog muscle-joint combinations (Lieber and Brown 1993).

ECRB/ECRL comparison

Although we acknowledge the paucity of data available for ECRB/ECRL comparison, it is interesting to note that, in the one subject for whom ECRB and ECRL sarcomere lengths were measured, there was a significant difference between the slopes of the sarcomere length-joint angle relationships (Fig. 2B). The slope for the ECRB relationship was ~40% greater than that of the ECRL, which is about the same proportion as the ratio between fiber lengths: ECRL fibers (76 mm) are ~50% longer than the ECRB fibers (48 mm; Lieber et al. 1990). Because the ratio of sarcomere length change is nearly the same as the fiber length ratio, these data suggest that the moment arm of the two muscles at the wrist joint are approximately equivalent. Experimental data from Brand (1992) and Buchannan et al. (1993), however, suggest that the ECRL moment arm is ~50% larger in flexion-extension than the ECRB. It is not clear why our sarcomere length-joint angle data do not reflect the combination of anatomical muscle differences (ECRL fibers 50% longer than ECRB fibers) with moment arm differences (ECRL moment arm 50% greater than ECRB moment arm). Using the anatomical and moment arm data from the literature, we would predict that the ECRL sarcomere length-joint angle relationship would actually have a smaller slope than that of the ECRB.

Nevertheless, based on our measured sarcomere length-joint angle data, we would predict that the ECRL would generate greater active force over a greater range of motion than would the ECRB. This type of fiber length disparity between synergists is not unprecedented. For example, the rabbit tibialis anterior (TA) and extensor digitorum longus (EDL) have approximately the same moment arm at the
ankle joint but have muscle fibers that are significantly different in length (Lieber and Blevins 1989). We thus speculate that the musculoskeletal system may be designed such that "high gear" and "low gear" muscles are juxtaposed in order to permit generation of a significant joint moment at a variety of angular velocities (Gans and deVree 1987). Such a musculoskeletal interaction could control joint stiffness and improve control. This type of muscle-joint interaction might be a parameter defining the properties of a muscle-joint combination in the same way that muscle architecture defines a muscle's contractile properties (Walmsley and Proske 1981). Additional studies of this sort in a variety of muscle-joint systems may a more clear understanding of the rationale for the design of muscle-joint combinations.

The authors thank the many individuals whose support made this project possible: L. Bergfors (OR nurse), B. Bush (machine shop foreman), U. Ranggård (electronics technician), M. Jacobson, and S. Shoemaker (UCSD), F. Mjärmdal (head nurse), B. Chamberlain (medical illustrator), U. Hedlund (electron microscope technician), and M. Schmitz (micrograph analysis).

This work was supported by the Veterans Administration and National Institute of Arthritis and Musculoskeletal and Skin Diseases. Grant AR-35192.

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Received 10 August 1993; accepted in final form 5 November 1993.

REFERENCES


Lieber, R. L., Brown, C. G., and Trestik, C. L. Model of muscle-tendon

FIG. 5. Hypothetical length-tension curve obtained using measured filament lengths and assuming the sliding filament mechanism proposed by Gordon et al. (1966). Shaded area represents sarcomere length change during wrist flexion (causing sarcomere length increase) and wrist extension (causing sarcomere length decrease). Top: schematic of filament lengths measured in the current study. Numbers over graph represent calculated inflection points based on filament lengths measured and a Z-disk width of 1,000 Å.


