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Experimental determination of sarcomere force–length relationship in type-I human skeletal muscle fibers

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ABSTRACT

The objectives of this study were to measure the active and passive force–length (*F–L*) relationships in type-I human single muscle fibers and to compare the results to predictions from the sliding filament model (the "standard model"). We measured isometric forces in chemically skinned fibers at different sarcomere lengths (SLs) in separate maximal activations. The experimental tolerance interval for optimal SL was calculated to be (2.37, 2.95 μm), which included the prediction by the standard model (2.64, 2.81 μm). Average passive slack length was 2.22 ± 0.08 μm, and the passive *F–L* relationship was well described by an exponential function. Best fit lines were used to estimate the ascending and descending limbs from the active *F–L* data using the average SL obtained from a digital image of the fiber. The experimental descending limb was also estimated using the shortest SL to address the possible effects of sarcomere inhomogeneity (SI). The experimental slopes of the ascending and descending limbs, 0.42 F_o/μm and −0.52 F_o/μm (vs. −0.55 F_o/μm with the shortest SL) respectively, F_o being the maximal isometric force, were significantly less in magnitude than those from the standard model. These results suggested that the difference between experimental and standard models was not fully explained by SI and other factors could be important. The broader experimental *F–L* curve compared to the standard model implies that human muscle has functionally a wider operating length range where its force-generating capacity is not compromised.

1. Introduction

The isometric relationship between muscle length and force is a fundamental property of skeletal muscle. It is used as a basis for almost all models of muscle contractile properties and can be used to predict the kinematic range of optimal function, such as the joint angles for maximal strength. Therefore, precise measurement of the force–length (*F–L*) property is crucial for understanding *in vivo* muscle function.

Measurement of the *F–L* curve at the most elemental level of muscle, i.e., the sarcomeric *F–L* curve, is important because it is assumed to be generally applicable to all skeletal muscles. In intact frog single fibers, measurements of the sarcomeric *F–L* curve by Gordon et al. (1966) matched the prediction from the sliding filament model with thick (myosin) and thin (actin) filament length estimates from electron micrographs. Specifically, the model predicted the length range over which force was maximal (i.e., the "plateau region") and the slopes of the ascending and descending limbs. In this paper, we will refer to the *F–L* curve based on the sliding filament model as the "standard model".

To our knowledge, there has been no direct experimental verification of the sliding filament model in *human* muscle based upon the work in frog fibers with filament length estimates from electron microscopy. Therefore, the objectives of this study were to measure the active and passive *F–L* curves within a limited sarcomere length (SL) range encompassing the plateau region in type-I chemically skinned human single muscle fibers and to compare our results with the standard *F–L* model. Specifically, our aims were to: (1) calculate tolerance limits for optimal sarcomere length (SL_o); (2) estimate the lines of the ascending and descending limbs; (3) estimate slack length (SL_p); and (4) estimate the passive exponential *F–L* curve.

2. Methods

2.1. Single fiber preparation and experimental apparatus

Two thin strips of the *lateral gastrocnemius* muscle were harvested from a human subject with no previous musculoskeletal abnormalities during an ankle

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fracture repair surgery. The strips were maintained at a length with slight tension and were immediately washed with a series of relaxing and skinning solutions (see below for respective compositions) containing 1% (w/v) Triton X-100 at ~3 °C for complete removal of the connective tissue. These strips were further dissected into smaller bundles and stored in skinning solution with 1% Triton X-100 at −20 °C. Protease inhibitors (phenylmethylsulfonyl fluoride, 0.2 M; leupeptin, 0.04 mM; E-64, 0.01 mM) were included in all solutions to prevent protein degradation. These procedures were approved by the Washington State University Institutional Review Board.

Single fibers measuring 2.0–3.0 mm long were separated out and the ends of each fiber were attached to small aluminum clips by superglue (cyanoacrylate). The fiber was then suspended between a force transducer (AE801, Sensor One Technologies, Sausalito, CA) and a servo motor (model 308, Aurora Scientific, Aurora, Canada) in a chamber containing relaxing solution.

During each activation, SL was measured using two techniques: (1) a He–Ne laser beam was directed in the middle section of the fiber, perpendicular to its long axis. The resulting diffraction pattern was recorded at 50 Hz with a digital line scan camera (LD3500, PerkinElmer, Waltham, MA) and SL was computed from the images (Lieber et al., 1984); (2) a digital image of the central segment of the fiber (1.07 mm in length) was captured by a high-resolution camera (12 megapixels) (Fujifilm S7000) after the fiber attained the steady-state activation (defined as a change in the force less than 1% over a period of 1 s). The distribution of SLs along the section of the fiber was determined from the image (see *Data Analysis*). Fiber diameters at every 0.5 mm were also measured in relaxing solution to estimate the average cross-sectional area of each fiber, assuming the fiber was cylindrical (Debold et al., 2004; Metzger and Moss, 1987).

2.2. Experimental protocol

All experiments on each fiber were conducted at 20 °C with a maximum variation of ±0.5 °C (maintained by a water bath and monitored using a thermocouple). Fibers were bathed in three different solutions (relaxing, pre-activating, activating) with the following concentrations (in mM): 5.88 Di-sodium Adenosine tri-phosphate; 10 ethylene-glycol tetra-acetic acid; 40 N,N-bis (2-Hydroxyethyl)-2-aminoethanesulfonic acid 6.56 Mg^{2+}, 1 dithiothreitol; and 15 Di-sodium Creatine Phosphate (Fukuda et al., 2003). Ionic strength was adjusted by K-propionate to 180 mM, pH adjusted to 7.0, and 100 U/ml Creatine Phosphokinase added before the start of the experiments. pCa of each solution was adjusted by $CaCO_3$-EGTA for the relaxing (pCa = 9.0), pre-activating (pCa = 9.0; HDTA instead of EGTA), and activating solutions (pCa = 4.5). All the reagent concentrations were based upon the program by Fabiato (1988).

A fiber was first bathed in relaxing solution and then incubated in pre-activating solution for 3 min (to facilitate activation). The SL based upon laser diffraction was set to a desired value, e.g., 2.6 μm, by adjusting the fiber length. Time-based force and length recordings were initiated prior to replacing relaxing with activating solution and the recordings were continued for 40 s. The activating solution was then replaced with relaxing solution and the passive force was measured at the same SL recorded during activation. The fiber was relaxed for 5 min and the cycle was repeated at five other SLs.

For each fiber, forces were measured at six different SLs spanning from 2.0 to 4.0 μm in six separate activation/relaxation sequences (more than six activations could result in fiber degradation). This measurement range was determined by the lack of clear sarcomere patterns at shorter lengths and the disruption of orderly sarcomere patterns at the longer lengths. We randomized the order of the different SLs to avoid biasing from the sequence of lengths. Randomized SLs were chosen from a probability distribution where twice as many measurements were made from 2.5 to 3.0 μm over other lengths for the entire fiber population. This was done to improve the statistical analysis in the region where SL_o has been estimated previously (see Discussion). Upon experiment completion, the fiber was typed for myosin heavy chain (MHC) isoform by electrophoresis following the protocol of Bamman et al. (1999).

2.3. Data analysis

Large amounts of inhomogeneity in SLs can greatly influence the *F–L* relationship, causing a broadening of the curve (see Discussion). Our strategy to avoid this artifact was to: (1) only analyze fibers which had less than a certain amount of sarcomere inhomogeneity (SI) and (2) use methods from a previous study to determine the appropriate SL to plot with the recorded force.

The criteria for minimal SI were (justification of criteria is found in Discussion): (1) Creep, or a slow continuous increase in force, is a indication of inter-sarcomere dynamics (Julian et al., 1978; Julian and Morgan, 1979). We considered that a fiber did not exhibit creep if the force rise became less than 1% over a period of 1 s. (2) Due to compliance at the attached ends of the fiber, shortening of the central segment usually occurred (see Results). The acceptable amount of shortening upon activation of the diffraction-based SL was 10%. (3) SI was assessed directly by subdividing the digital image of the activated fiber into eight segments of equal length and determining the average SL of each segment by imposing a fast Fourier transform (FFT) (Slawnych et al., 1994). The acceptable amount of SI was a coefficient of variation (CV) of the SLs less than 5%. In addition, to ensure that a fiber activated maximally, we only analyzed fibers whose maximum isometric stress at optimal SL was greater than 100 kPa (Bottinelli et al., 1996; Galler and Hilber, 1994; Hilber and Galler, 1997; Julian and Morgan, 1979).

When there is a distribution of SLs along the length of the fiber, deciding which SL value to use for plotting the *F–L* curve is problematic. In our initial analysis, we used the average of the calculated SLs of the eight subdivided segments. In order to correct for SI at SLs greater than the estimated plateau region, the shortest SL of the eight segments was used to plot the *F–L* curve (see Discussion for method rationale).

We assumed that the force measured in activating solution was the combination of both active and passive forces (i.e., the total force). The active force was calculated as the difference between the total and passive forces. The active and passive *F–L* curves for each fiber were normalized by the maximum active force from all the activations of that fiber. SL_o was estimated as the length where the peak force occurred in each active *F–L* curve and the 95% ($\alpha = 0.05$) tolerance interval computed from the pooled SL_os. Tolerance intervals statistically represent the range of possible SL_o, as opposed to an estimate of the mean value (i.e., a confidence interval).

The standard *F–L* model was calculated using the sliding filament model of Gordon et al. (1966) and the electron microscopy estimates of Walker and Schrodt (1974) (I segment length = 2.64 μm, thick filament length = 1.6 μm, bare zone = 0.17 μm, Z-line thickness = 1000 Å). Four points on the standard *F–L* curve were determined following the calculations of Gordon et al. The force at SL = 1.7 μm (when Z-line meets the thick filament) was calculated as length of thick–thin filament overlap without thin–thin filament overlap (interference) divided by length of the thick filament where myosin is present (1.47 μm). All tests in this study were performed above this SL (1.7 μm) because of experimental limitations, and hence we did not have to consider frictional forces which occur when the thin filaments overlap extensively (Trombitas and Tigyi-Sebes, 1985). The shortest SL of the plateau region was where thin filaments meet. The longest SL of the plateau was where the thin filaments are no longer in the bare zone. Lastly, the force at SL = 4.24 μm (no filament overlap) was assumed to be zero. Forces at SLs between the four points were determined by linear interpolation. To compare the experimental ascending and descending limbs to the standard model, the 95% confidence intervals (CI) of the best fit lines were calculated from the pooled data for SLs shorter and longer than the plateau region of the standard model (2.64 and 2.81 μm)

To estimate the beginning of the passive *F–L* curve, we fitted the passive *F–L* data of each fiber to an exponential function (Zajac, 1989):

$$F(\mathrm{SL}) = Ae^{b(\mathrm{SL}-\mathrm{SL_p})} \quad (1)$$

where *F* is the passive force, SL is the sarcomere length, SL_p is the slack length at which the passive force begins to be nonzero, and *A* and *b* are the fitted constants.

3. Results

We obtained data which satisfied the criteria for quality control from 10 type-I fibers out of 31 fibers tested. The mean diameter of the fibers was 0.103 ± 0.006 mm, and mean maximal isometric stress was 133 ± 26 kPa (at an average SL = 2.7 μm). More measurements were made within the range of 2.4–3.0 μm (Fig. 1). Upon fiber activation, force rise was marked by an initial rapid phase followed by a plateau in all trials (Fig. 2) and sarcomeres in the central region shortened from their passive lengths (Fig. 3). In general, shortening upon activation was greater for shorter than longer lengths (average amount of shortening was 6.4% for SLs < 2.8 μm and 1.5% for SLs > 2.8 μm).

The scatter plot of the pooled *F–L* data showed qualitatively ascending limb, plateau, and descending limb regions (Fig. 4). Estimates of SL_o ranged from 2.54 to 2.78 μm (Fig. 5), with a mean and standard deviation of 2.66 and 0.085 μm, respectively. The 95% tolerance interval for SL_o was (2.37, 2.95) μm. A scatter plot of the passive data resembled qualitatively an exponential function (Fig. 6). Passive *F–L* relationships from individual fibers were fitted well by exponential functions (average r^2 of 0.894 ± 0.085), with a mean and standard deviation of 2.22 and 0.08 μm, respectively, for SL_p.

The 95% CIs for the lines of the ascending and descending limbs based upon the experimental data were compared to the standard model (Fig. 7). When the average SL was used for the *F–L* plot, the slope for the ascending limb was 0.42 F_o/μm (CI = (0.31, 0.52) F_o/μm), and the slope for the descending limb was −0.52 F_o/