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Non-uniform Distribution of Strain during Stretch of Relaxed Skeletal Muscle Fibers from Rat Soleus Muscle

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Abstract

Tension and regional average sarcomere length (L_s) behavior were examined during repeated stretches of single, permeabilized, relaxed muscle fibers isolated from the soleus muscles of rats. We tested the hypothesis that during stretches of single permeabilized fibers, the global fiber strain is distributed non-uniformly along the length of a relaxed fiber in a repeatable pattern. Each fiber was subjected to eight constant-velocity stretch and release cycles with a strain of 32% and strain rate of $54\% \text{ s}^{-1}$. Stretch-release cycles were separated by a 4.5 minute interval. Throughout each stretch-release cycle, sarcomere lengths were measured using a laser diffraction technique in which 20 contiguous sectors along the entire length of a fiber segment were scanned within 2 ms. The results revealed that: (1) the imposed length change was not distributed uniformly along the fiber, (2) the first stretch-release cycle differed from subsequent cycles in passive tension and in the distribution of global fiber strain, and (3) a characteristic “signature” for the L_s response emerged after cycle 3. The findings support the conclusions that longitudinal heterogeneity exists in the passive stiffness of individual muscle fibers and that preconditioning of fibers with stretch-release cycles produces a stable pattern of sarcomere strains.

Keywords

sarcomere length non-uniformity; laser diffraction; preconditioning; relaxed fiber

Introduction

Results from studies of stretch-induced injury in activated whole muscles and single fibers suggest heterogeneity in the longitudinal distribution of the applied strain, giving rise to regional variations in sarcomere length and localized regions of damage (Brooks et al. 1995; Macpherson et al. 1997; Macpherson et al. 1996; Ogilvie et al. 1988; Talbot and Morgan 1996). Non-uniform distribution of strain during stretch of activated single muscle fibers has been confirmed using a high-speed laser-scanning technique (Panchangam et al. 2008). The net mechanical response of activated skeletal muscle to strain is the sum of the passive and active components. In past analyses of sarcomere dynamics during activation, heterogeneity

in the relative strength of sarcomeres is attributed only to the active component (Hill 1938; Julian and Morgan 1979; Martins et al. 1998; Meyer et al. 2010; Morgan et al. 1982; Telley et al. 2006b; Zajac 1989). Passive properties of muscle contribute up to 45% of the total force during plantar flexion in mice (Brooks and Faulkner 2001). Such large contributions suggest that regional heterogeneity in passive stiffness could be part of the cause of the heterogeneity observed in the response of an activated fiber to stretch. Despite the potential role of passive stiffness, the regional variation in the characteristics of relaxed skeletal muscle fibers has not been investigated. The purpose of this study was to determine the contribution of regional variations in the mechanical response observed during stretch of relaxed fibers to the response of activated fibers, with spatial resolution equivalent to that reported previously for activated fibers (Panchangam et al. 2008). This was done by monitoring the sarcomere lengths of 20 contiguous sectors measured using high-speed laser-scanning during stretches of relaxed fibers to test the hypothesis that, during stretch, global fiber strain is distributed non-uniformly along the length of a relaxed fiber in a repeatable pattern.

The velocity dependence, hysteresis, stress relaxation, and creep associated with viscoelastic materials have been demonstrated in whole muscle (Best et al. 1994), single fibers (Granzier and Wang 1993; Mutungi et al. 2003), myofibrils (Bartoo et al. 1997; Wang et al. 1993), and the titin protein primarily responsible for the elastic response of relaxed single fibers (Kellermayer et al. 2001; Minajeva et al. 2001). The viscoelastic properties of muscle tissue suggest that there is potential for variation and systematic drift in the mechanical response during testing, particularly in protocols involving repeated stretches. Preconditioning of a viscoelastic material is effective in minimizing the variability of the response as well as establishing a baseline state in the material for characterizing its behavior (Best et al. 1994; Ciarletta et al. 2006; Fung 1993; Giles et al. 2007; Grover et al. 2007). Moreover, during physical activity, relaxed muscle fibers routinely undergo large strain cycles *in vivo*. Therefore, stretch of an activated muscle occurs against the background of a passive component that is closer to a “preconditioned” rather than a naïve state. Among the studies describing effects of stretches on mammalian single fibers, there are no reports in which preconditioning cycles have been incorporated in the experimental protocols. Hence, a secondary objective of this study was to determine the effect of repetitive strain cycling on the distribution of strain along a relaxed fiber.

Materials & Methods

Permeabilized fiber preparation, fiber mounting, regional average sarcomere length (L_s) measurements, solutions and activation, and laser sweeping protocols have been described in detail by Panchangam et al. (Panchangam et al. 2008). Briefly, permeabilized single fibers from soleus muscles of adult male rats ($n=3$; age 6–10 months) were harvested and stored at 20°C. The experimental procedures were approved by the University of Michigan Committee on the Use and Care of Animals and in accordance with the *Guide for the Care and Use of Laboratory Animals* [DHHS Publication No. 85–23 (NIH), Revised 1985, Office of Science and Health Reports, Bethesda, MD 20892]. Fibers from rat soleus muscles were chosen for these experiments because of prior experience with the preparation and their long-term viability during mechanics experiments (Brooks and Faulkner 1996; Macpherson et al. 1997; Macpherson et al. 1996; Ogilvie et al. 1988; Panchangam et al. 2008). On the day of an experiment, a bundle of fibers was removed from the storage solution and placed in relaxing solution for ~30 min at 4°C. Permeabilized single fiber segments (‘fibers’) were then pulled gently from the bundle using fine forceps and transferred to a fluid-exchange chamber mounted on the laser scanning apparatus. One end was then attached to a force transducer and the other to a servomotor via monofilament nylon ties. Care was taken to avoid excessive stretch of a fiber during isolation and attachment procedures. Once the fiber

was mounted, the remainder of the protocol was performed at 15°C. The approximate resting L_s of the fiber was determined by projection of the laser diffraction pattern produced by the middle section of the fiber onto a calibrated target screen. The fiber length was then adjusted to obtain the desired L_s by translating the servomotor using a micrometer drive. Fiber length (L_f) was defined as the distance between the innermost nylon ties when the approximate resting L_s was 2.5 m. Fiber cross-sectional area was calculated at 5 discrete locations along the fiber by measuring the width and depth of the fiber and assuming an elliptical cross-section. Fiber forces were divided by the average of the 5 cross-sectional area measurements to obtain specific force (kPa). The diffraction patterns produced by each fiber were inspected visually during the course of an experiment and a fiber was rejected if the diffraction pattern was faint or diffuse. Additional rejection criteria were gross markers of damage, such as tears, or other disruptions, although no fiber was rejected based on these criteria.

The hypothesis that the stretch of a relaxed skeletal muscle fiber is distributed non-uniformly along the fiber in a repeatable pattern was tested using rapid measurements of the sarcomere lengths of 20 contiguous sectors along the fiber before and at the peak of each of the stretches. The L_s measurements were made from diffraction patterns that resulted from trans-illumination of a small volume of the fiber with a laser beam operating at a wavelength of 650 nm having e^{-2} beam width of $\sim 180 \mu\text{m}$. Using an acousto-optic beam deflector (AOBD), the laser beam was passed sequentially through 20 evenly-spaced sectors along the fiber, stopping briefly (100 μs) at each sector. Diffraction patterns for each sector were captured with a charge-coupled device linear array sensor and digitized with a frame grabber. The time taken to capture the diffraction patterns from a full cycle of 20 sectors (a 'sweep') was 2 ms. When fiber length was changed, the sweeping was adjusted to track sectors as described by Panchangam et al. (Panchangam et al. 2008). For each experimental protocol, servomotor and AOBD command arrays were pre-calculated and updated to the respective instruments at a rate of 10 k updates s^{-1} . Diffraction patterns captured at the rate of 10 k patterns s^{-1} were stored by the computer for post-processing. Fiber length and force measurements were also sampled simultaneously at 10 k s^{-1} and stored by the computer. A custom LabVIEW™ (National Instruments Corp.) program controlled the instruments and acquired and saved the data.

Experimental Protocol

The mean initial resting sarcomere length was set to 2.7 μm to match the protocol of Panchangam et al. (Panchangam et al. 2008). Each fiber was stretched by 32% L_f at a strain rate of 54% $L_f \text{ s}^{-1}$ and released at the same speed. A strain of 32% L_f was chosen because higher strains sometimes caused the passive length-tension curve to become sigmoidal, suggesting either the onset of plastic deformation (Wang et al. 1991), or significant stress relaxation in the fiber. Immediately upon the return to initial length, the true zero for the force transducer was obtained by rapidly shortening the fiber (step shortening) by a length of 22% L_f followed by a hold for 40 ms. Each fiber was then returned rapidly to its initial length (step stretch). The sequence of stretch-release followed by a find-transducer-zero maneuver was repeated 8 times with a 4.5 min interval between successive sequences as shown in Fig. 1A. A set of typical force responses of a fiber to this protocol is shown in Fig. 1B and superimposed force responses to the protocol are shown in Fig. 3. Following the eighth preconditioning cycle, a single isometric activation was performed at a mean L_s of 2.5 μm by first equilibrating the fiber for 3 min in a pre-activating solution and then rapidly introducing a high- $[\text{Ca}^{2+}]$ activating solution (pCa 4.5) to obtain the maximum isometric active force (F_o) for the fiber. In a separate group of permeabilized fibers from rat soleus muscles, F_o was assessed both before and after five passive conditioning cycles. The F_o measured after passive conditioning in those fibers was $99.2 \pm 4.1\%$ ($n=16$) of the F_o

measured immediately after mounting the fiber, indicating that passive cycling using the velocity and amplitude parameters described here do not adversely affect the active force-generating capability of the fibers.

Statistical Analysis

To determine the effect of repeated stretches on the passive response, fibers were grouped based on the number of the pre-conditioning cycle. A linear regression analysis was performed to analyze the passive force as a function of the cycle number. A repeated measures ANOVA was performed to determine differences between cycles. The group means were assessed by a univariate one-way analysis of variance (ANOVA). If the F-statistic indicated significance, a more detailed analysis was performed using Tukey's HSD *post hoc* tests for multiple comparisons. The level of significance was set *a priori* at $p < 0.05$.

Results

The lengths of the fibers ($n = 9$) were $L_f = 1.52 \pm 0.08$ mm, CSA was 6840 ± 1770 m², and the specific force was 157 ± 33 kPa. For each fiber, the L_s for all sectors was computed from diffraction patterns captured just prior to the stretch and at the peak of the stretch. The fiber-mean pre-stretch L_s ranged from 2.49 to 2.69 μ m (mean = 2.62 ± 0.06 m) and increased between 0.69 and 0.80 m (mean = 0.76 ± 0.03 m) at the peak of the stretch (Fig. 2). At the pre-stretch length, the passive stress was 11.1 ± 3.5 kPa for cycle 1 and 3.9 ± 1.2 kPa for cycle 8. At the peak of the stretch, the passive stress was 180 ± 38 kPa for cycle 1 and 137 ± 32 kPa for cycle 8. No fibers in this study exhibited signs of yield or plastic deformation at the peak of the stretch (Wang et al. 1991).

Superimposed force responses of a representative fiber subjected to the stretch-release protocol are shown in Fig. 3. For each cycle, the passive force normalized by the maximum isometric force for the fiber is plotted as a function of the mean fiber L_s . A single cycle of stretch and release manifests a hysteresis pattern consistent with previous reports (Kellermayer et al. 1997; Minajeva et al. 2001). Successive stretch-release cycles showed a decline in the nominal stress at the peak of stretch concomitant with a decline in the amount of hysteresis during each stretch cycle. Although both stretch and release portions of the curve showed a systematic decline with cycling, the change in the release portion of the curves was minimal. Consequently, the effect of cycling on the energy absorbed by the fiber during each cycle is due primarily to the decreases in the nominal stress for the fiber during the stretch portion of the cycle.

The normalized force (F/F_0) as a function of cycle number is shown in Fig. 4. The normalized forces at both the onset of stretch and peak of stretch show a decline with increasing cycle number. An Analysis of Variance (ANOVA) showed that there was a significant difference among groups. Tukey's Honestly Significant Difference post hoc tests for multiple comparisons (Tukey's HSD) indicated that at the peak of stretch, Cycles 3 through 8 all have normalized stress values that are significantly different ($p < 0.05$) from cycle 1. The ANOVA for cycles 2 through 8 indicated that there was no significant difference in peak stress. The trend for the decline in stress with repeated cycling is consistently less than 2% per cycle after four cycles. The average rate of decline decreases with repeated cycling reaching a value of 1.2% between cycles 7 and 8.

Heterogeneity in the length of sarcomeres was observed along the fiber prior to the onset of stretch and at the peak of stretch. Sarcomere length profiles are shown for a representative fiber in Fig. 5. Note that, for both the onset of stretch and peak of stretch, the sarcomere lengths of the individual sectors are nearly coincident for cycles 4 and 8, whereas the

sarcomere lengths for individual sectors in cycle 1 differ from the other cycles. Fig. 5 also demonstrates that the stretch is not distributed evenly across the sectors within a fiber. The distribution of the global fiber strain among sectors from all fibers was determined by normalizing the change in sarcomere length for a sector within a fiber by the mean change in sarcomere length for that fiber. The data from all fibers are plotted in Fig. 6A ($n = 180$ sectors) for Cycle 1 and Cycle 8. The range in the normalized change in L_s for Cycle 1 and Cycle 8, respectively, were -17% to $+18\%$ (SD 5%) and -14% to $+13\%$ (SD 4%). Therefore, preconditioning reduced both the range and the spread in the distribution of the global strain across sectors. The data from all sectors ($n=180$) for all fibers are plotted in Fig. 6B showing the normalized change in sarcomere length as a function of the initial sarcomere length. Consistent with Fig. 6A, the graph indicates that the range in the normalized change in sarcomere length for cycle 1 is 83% to 118% and the range in cycle 8 is 86% to 113%. Fig. 6B also indicates that the change in sector sarcomere length is independent of the initial sarcomere length.

Additional insight into the behavior of sectors across all fibers was achieved by comparing the relative pre-stretch L_s and relative post-stretch L_s for all sectors ($n=180$). The relative pre-stretch L_s for a given sector was calculated as the ratio of its pre-stretch L_s to the mean of all the pre-stretch L_s values for the sectors within a fiber. The relative post-stretch L_s for a sector was calculated in a similar manner using the mean of all the post-stretch L_s values for the sectors within a fiber. Fig. 7 shows a comparison of sector level sarcomere lengths between pairs of cycles at the onset of stretch and at the peak of stretch. Based on the statistical analyses (Fig. 4), comparisons of cycles are made relative to Cycle 4. The broken lines in the graphs correspond to the line of identity. If the sectors maintained the same sarcomere lengths in each of the cycles being compared, then all data points would fall along this line. Fig. 7A shows the relative pre-stretch L_s for cycle 1 compared with those for cycle 4. The graph indicates that the relative pre-stretch sarcomere lengths did not fall on the line of identity and that there was a weak correlation between cycle 1 and cycle 4. In contrast, Fig. 7B indicates that there is a strong correlation between the relative pre-stretch values for cycle 4 and cycle 8. A similar relationship was observed for the relative sarcomere lengths at the peak of stretch (Fig. 7C and Fig. 7D). A weak correlation was observed in the relative peak sarcomere lengths between cycle 1 and cycle 4. In contrast, a strong correlation was observed in the relative peak sarcomere lengths between cycle 4 and cycle 8.

To further quantify the change in sarcomere lengths for a sector in response to passive cycling, the root mean square deviation (RMSD) was calculated. For each fiber, the RMSD was determined between two cycles using the profile from the lower of the two cycle numbers as a reference. Fig. 8 shows a graph of the mean and standard deviation of the RMSD across all fibers for each successive pair of cycles. The data show that the change in sarcomere length profiles between cycles at the onset of stretch for cycles 2 through 8 are significantly different from the change in sarcomere length profile between cycle 1 and cycle 2. At the peak of stretch, the change in sarcomere length profiles between cycles for cycles 4 through 8 are significantly different from the change in sarcomere length profiles between cycle 1 and cycle 2. The mean RMSD at the peak of stretch from cycle 4 to cycle 8 is $0.005 \pm 0.002 \mu\text{m}$ representing less than 0.2% of the average sarcomere length for each fiber.

Discussion

The sarcomere length distribution for a single permeabilized fiber during activation and stretch was explored previously by performing high speed scans using a laser diffraction system (Panchangam et al. 2008). That study reported isometric stresses of $135 \pm 25 \text{ kPa}$.

Fibers were stretched by a magnitude of $0.27 L_f$ at a rate of $0.54 L_f s^{-1}$ and the total stress at the peak of stretch was approximately double the peak isometric stress. The current study indicates that during stretches to sarcomere lengths of $1.27 L_f$ the passive tension reaches 80% F_o during cycle 1 and approximately 45 to 50% F_o in subsequent cycles. Therefore the passive tension may account for 25% to 40% of the total tension measured for the single fiber during an activated stretch corroborating reports from previous studies (Brooks and Faulkner 2001; Pinniger et al. 2006). Consequently, longitudinal variations in the passive stiffness of a fiber have the potential to contribute significantly to longitudinal variations in the total stiffness of a fiber during an active stretch.

In addition to the role of passive tension during a stretch, this study raises the question of repeatability in quantifying the behavior of skeletal muscle preparations. The measurement of passive tension at the single fiber level (Fig 3) and the distribution of strain among sectors (Fig 5-8) are both shown to be dependent on the number of stretch-release cycles for fewer than 4 cycles. After cycle 4, there is no significant difference in either the passive tension response or the distribution of strain among sectors. The implications of these findings for sarcomere dynamics and stretch-induced injury are discussed below.

A limitation of the laser diffraction technique is the inability to measure the lengths of individual sarcomeres. In this study, the beam diameter was approximately $180 \mu m$. The L_s measurements reported thus represent the diffraction contributions of approximately 70 series-connected sarcomeres from several thousand myofibrils. That is, L_s provides a convenient indication of global sector strain rather than individual sarcomere strain. A recent report on the direct visualization of individual sarcomeres *in vivo* (Llewellyn et al. 2008) indicates that sarcomere length variations are greater than suggested by regional average measurements such as those reported here.

Sarcomere length heterogeneity

Heterogeneity in the sarcomere length response to a strain imposed on a single fiber has been implicated in the patterns of injury observed in skeletal muscle fibers (Morgan 1990; Panchangam et al. 2008; Patel et al. 2004; Telley et al. 2006a). The sarcomere length profile of a fiber during the force plateau of an isometric activation predicts the sarcomere strain profile for the fiber during the course of a subsequent stretch, with the highest strains observed in sarcomeres at lengths corresponding to the descending limb of the length-tension relationship (Panchangam et al. 2008). The proposed basis for this finding is that increased sarcomere length results in reduced filament overlap, thereby reducing the number of attached crossbridges available to resist the strain (Morgan 1990). However, this argument does not consider the possibility that the sarcomeres along a fiber might have different passive elastic responses and manifest different passive stiffness characteristics. Figs. 5, 6 and 7 show that, although each sector is exposed to the same tension during the course of a stretch, the changes in the sarcomere length of sectors are not homogeneous, indicating that the passive length-tension response varies along the fiber. Moreover, the results of these experiments indicate that for stretches of a relaxed fiber, the initial sarcomere length does not predict the capability of a sarcomere to resist a change in length (Fig. 6). Morgan (Morgan 1990) hypothesized that failure at the level of sarcomeres occurs because “weak” sarcomeres are stretched to extreme lengths during lengthening contractions. If sarcomere strength is defined as resistance to a change in length during a stretch, then both the active and passive stiffness of a sarcomere contribute to sarcomere strength. For the active component, the initial sarcomere length determines the number of cross-bridges available to contribute to the stiffness, which is consistent with the length-based definition of sarcomere strength. Our findings that the passive stiffness can make a significant contribution to total sarcomere stiffness (Fig. 4) and that initial length is not a

strong predictor of passive stiffness (Fig. 6), indicate that variations in passive stiffness are an important factor contributing to sarcomere dynamics during an injury-inducing, lengthening contraction.

Relative Stiffness of Sarcomeres

The results indicate that stretch of a relaxed fiber is distributed non-uniformly along the length of the fiber (Figs. 5 and 6) and sectors exhibit a broad range of normalized change in sarcomere lengths. The range from the sector with the least length change to the sector with the greatest length change was approximately 35% of the mean for cycle 1 and 27% of the mean for cycle 8. Since the change in length of a sector is indicative of the stiffness of that sector, it follows that the stiffness of a sector is also independent of the initial length of the sector. Therefore, these data indicate that, over the range of sarcomere lengths studied, the initial length does not predict “strong” versus “weak” sarcomeres in a relaxed fiber. Our previous report on the response of an activated fiber during stretch indicated that when active and passive contributions are combined, the distribution of strain is predicted by the initial sector length but displays considerable variability (Fig. 8 in Panchangam *et al.*, 2008). The present findings suggest that the passive component may contribute to the considerable variability seen in the relationship between L_s and the relative increase in L_s observed in active fibers (Panchangam *et al.* 2008). In addition, the relative passive stiffness may play an important role in the dynamics of the failure among “weak” sarcomeres.

Cycle-dependent response of sarcomeres

The cycle-dependent response of regional average sarcomere length profiles to repeated stretches was examined by calculating the root mean square deviation (RMSD) between cycles (Fig. 8). The RMSD between cycle 1 and cycle 2 was significantly different from the RMSD between subsequent cycles. This finding indicates that the changes in the lengths of individual sectors from cycle 1 to cycle 2 are much larger than in subsequent cycles. Therefore, the sarcomere length profile for a particular fiber develops a “signature” following cycle 1 that is minimally changed with repeated cycling as is evident in the response of the representative fiber shown in Fig. 5. Examination of the data from all sectors from all fibers in this study as shown in Fig. 7 suggests two effects of repeated cycling on sarcomere lengths. First, the clockwise rotation of the fitted line away from unity (Figs. 7A and 7C), indicates a tendency toward homogenization of sector lengths; the longest and shortest sarcomeres in cycle 1 are closer to the mean in cycle 4. In subsequent cycles, the best fit line is close to unity (Figs. 7B and 7D) indicating that the tendency toward homogenization of sector lengths is no longer present between cycles 4 and 8. The second effect of cycling evident in Fig. 7 is the establishment of a consistent characteristic strain of individual sectors in response to the global strain applied to a fiber. This effect can be seen in Figs. 7B and 7D where the close proximity of all data points to the line of unity indicates very little inter-cycle change in sector sarcomere lengths after cycle 4, in agreement with the RMSD results shown in Fig. 8. The data points in the shaded regions of Figs. 7A and 7C indicate sectors stiffer than the mean in cycle 1 becoming more compliant than the mean in cycle 4 or sectors more compliant than the mean in cycle 1 becoming stiffer than the mean in cycle 4. The absence of data points in the shaded regions in Figs. 7B and 7D indicate that the length response of sarcomeres, which is dependent on their stiffness, becomes more stable and repeatable with increased cycling and that, after a small number of cycles, the short sectors tend to stay short and long sectors tend to stay long. With an increase in the number of preconditioning cycles beyond 4, the mean and standard deviation of the RMSD remains approximately the same for both the onset and the peak of stretch (Fig. 8). Therefore, repetitive cycling of relaxed fibers does not significantly change the spread in the sarcomere lengths. Taken together, this suggests that once the sarcomere length “signature”

has been established for a fiber, the fiber will maintain that profile. Coupling the redistribution of the passive stiffness in a fiber with a passive tension response that is 30% higher in unconditioned fibers, the absence of preconditioning cycles may result in sarcomeres erroneously categorized as “strong” versus “weak”.

Decline in Force with Cycling

A potential cause of the decline in tension observed in Fig. 3 is slippage of the fiber at the sites of attachment to the testing apparatus when the fiber is under tension. Slippage would result in an increase in the number of sarcomeres in series within the scanning region. An increase in the number of sarcomeres would manifest as a decline in the mean L_s for a fiber with increasing cycle number. This possibility was ruled out by the results shown in Fig. 2 indicating that there was no change in the mean and standard deviation of the regional average sarcomere lengths calculated for each sector with increasing cycle number. Other possible causes of the decline in passive stress are the phenomenon of stress relaxation (Bartoo et al. 1997; Minajeva et al. 2001; Mutungi and Ranatunga 1996a, b) and “molecular fatigue” of the titin molecule (Kellermayer et al. 2001), which is largely responsible for the passive elastic response of skeletal muscle fibers (Funatsu et al. 1990; Goldstein et al. 1987; Horowitz 1999; Horowitz and Podolsky 1987; Labeit and Kolmerer 1995; Maruyama et al. 1981; McComas 1996; Wang et al. 1993; Yasuda et al. 1995).

Conclusion

We used a technique for rapidly measuring sarcomere lengths along a permeabilized single fiber to demonstrate that stretches of relaxed fibers are distributed non-uniformly along the fiber length. The tension response and regional average sarcomere length profile of the fibers changed substantially in response to the first stretch-release cycle. Both the regional average sarcomere length profile and the passive tension response for a particular fiber stabilized with additional stretches, becoming repeatable after the fourth stretch-release cycle, however, significant heterogeneity remained. The regional average sarcomere length profile for each fiber is a unique “signature” that remains stable with repeated cycles. These findings support the hypothesis that, during stretch, strain is distributed non-uniformly along the length of a relaxed fiber and that the sarcomere length distribution is not stable or repeatable without preconditioning. Moreover, variations in sector passive stiffness are sufficient in magnitude to contribute significantly to the dynamic response among “weak” sarcomeres during stretch of an activated fiber.

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Fig. 1.

Experimental protocol. Each fiber was set to a mean initial sarcomere length of $2.7 \mu\text{m}$. (A) Fibers were stretched by $32\% L_f$ at $54\% L \text{ s}^{-1}$ and released at the same speed. The stretch-release cycle was repeated 8 times with 4.5 min elapsing between each successive stretch. Immediately following each stretch-release cycle, a rapid shortening by $22\% L_f$ was applied to determine the force transducer zero. The rapid shortening was followed 40 ms later by a rapid return to the original length. (B) Representative force responses to the length changes shown in A. (C) Details of a typical length record, and (D) corresponding force response. The detailed records are from cycle 3 in panels A and B.

Fig. 2.

Mean sarcomere length versus cycle number. The mean sarcomere length for 180 sectors measured from 9 fibers versus cycle number is plotted for all fibers at the onset of stretch and the peak of stretch. Error bars correspond to the standard deviation (μm). There was no significant difference among the mean sarcomere lengths at the onset of stretch or at the peak of stretch.

Fig. 3. Force response of a representative fiber to passive stretch-release cycles. Force is normalized by the peak isometric force of individual fibers.

Fig. 4.

Normalized force (F/F_0) versus cycle number. For all fibers, the maximum isometric force was measured by activating the fiber after cycle 8. The passive force was normalized by the maximum isometric force. Error bars correspond to the 95% confidence interval for each cycle. Tukey's HSD indicated that at the onset and peak of stretch, Cycles 3 through 8 are significantly different from Cycle 1.

Fig. 5. Results from a representative fiber showing the sarcomere length (μm) for each sector at (A) the onset of stretch and (B) the peak of stretch. To maintain clarity, only the sarcomere length profiles for cycles 1, 4 and 8 are shown. Note the differences between the sarcomere length profile for cycle 1 and the overlapping profiles of cycles 4 and 8.

Fig. 6.

Comparison of the distribution of the normalized change in sarcomere length between cycles 1 and 8. The normalized change in sarcomere length was obtained by dividing the change in sarcomere length for each sector within a fiber by the mean change in sarcomere length for that fiber. The data correspond to all the scanned sectors ($n=180$) from all fibers. (A) Preconditioning results in a change in the range and spread of the normalized changed in sarcomere length. (B) The change in sarcomere length is independent of the initial sarcomere length.

Fig. 7.

Comparisons of relative sarcomere lengths between cycles 1, 4, and 8 at the onset of stretch (A, B) and the peak of stretch (C, D). The data points represent all scanned sectors ($n=180$) from all fibers. The broken lines correspond to the line of identity (slope = 1) and the solid lines represent a least-squares linear fit. The graphs indicate a weak correlation for the sarcomere lengths at the onset of stretch (slope = 0.429; $r^2 = 0.392$; $p < 0.0001$) and peak of stretch (slope = 0.752; $r^2 = 0.539$; $p < 0.0001$) between cycle 1 and cycle 4. A slope less than one indicates that sarcomeres with a pre-stretch L_s less than the mean in cycle 1 tended to increase in length while sarcomeres longer than the mean tended to decrease in length by cycle 4. In contrast there is a strong correlation in sarcomere length at the onset of stretch (slope = 0.967; $r^2 = 0.935$; $p < 0.0001$) and the peak of stretch (slope = 1.06; $r^2 = 0.924$; $p < 0.0001$) between cycle 4 and cycle 8 indicating that beyond cycle 4 the relative sarcomere lengths tended not to change. The shaded quadrants (A, C) contain sectors that reversed length relative the mean.

Fig. 8.

Root mean square deviation (RMSD) of sarcomere lengths (μm). The RMSD is a measure of the change in the sarcomere length profile for a fiber compared to a reference profile. The data presented show the RMSD using the sarcomere length profile from the previous cycle as the reference profile. The change in sarcomere length profiles between cycles for cycles 5 through 8 are significantly different from the previous cycles. In addition, the changes in sarcomere length profiles from cycle 5 through cycle 8 are not significantly different from each other. The mean RMSD at the peak of stretch for cycle 5 to cycle 8 ranges from 0.003 to 0.005 μm representing less than 0.2% of the average sarcomere length for each fiber.