

of 50 Hz, 400 ms duration) at a length either 3.6mm shorter or 3.6mm longer than the reference length. During the repetitive contractions, muscle length was changed periodically to the other length to observe 1-2 contractions, then returned to the test length. Initial active force was 2.53 ± 0.4 (mean \pm SD) and 6.26 ± 1.2 N at short and long lengths respectively. Active force at the long length would be similar to that of the short length if active force was calculated in the traditional manner. Active force decreased to 1.90 ± 0.5 and 1.8 ± 1.0 N at the short and long lengths respectively. During repetitive contractions at the short length, active force was 3.6 ± 1.1 N, when measured at the long length. During repetitive contractions at the long length, active force was 0.67 ± 0.4 N, when measured at the short length. Clearly, the long length resulted in substantially greater fatigue than the short length. There would be no explanation for this if active force was calculated in the traditional manner. The higher real active force and therefore metabolic demand of contractions at the long length can explain the greater fatigue.

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Lengthening Contractions Produce Strain-Dependent Regional Changes in the Passive Length-Tension Properties of Permeabilized Single Fibers

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During forced lengthening of an activated skeletal muscle fiber (a "lengthening contraction"), the applied strain is not distributed uniformly along the length of the fiber. Instead, regions having the longest sarcomere lengths (L_s) prior to the stretch are strained the most during the stretch (Panchangam et al. Biophys. J. 95:1890-1901, 2008). These differences in regional strain result in differences in strain history that could influence the subsequent resting L_s of the regions. We hypothesized that the change in resting L_s of a region following a lengthening contraction correlates positively with the strain of the region during the lengthening contraction. This hypothesis was tested on permeabilized fibers ($n=15$) obtained from *soleus* muscles of adult rats (8-9 mo, $n=5$). A laser diffraction technique was used to make rapid measurements (500 s^{-1}) of the L_s in 20 contiguous regions of fibers before, during, and after a single lengthening contraction (strain, 27 %; strain rate, $54 \% \text{ s}^{-1}$; temperature, 15°C). During steady-state activation prior to lengthening, fibers produced an isometric stress of $133 \pm 29 \text{ kPa}$ at a mean L_s of $2.54 \pm 0.16 \mu\text{m}$. The lengthening contractions resulted in a $19 \pm 9 \%$ loss in isometric stress. For each of the 20 contiguous regions, the difference between the resting L_s 5 min before and 10 min after the lengthening contraction was plotted as a function of the increase in L_s at the peak of the lengthening contraction. The increase in resting L_s correlated positively ($r=0.71$) with the increase in L_s during lengthening contractions. We conclude that lengthening contractions produce regional changes in the passive length-tension properties of permeabilized single fibers and that these changes can be attributed to the recent strain history of the fiber regions. Support: NIH AG-13283; AG-015434.

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Crossbridge Properties During The Quick Force Recovery In Single Frog Muscle Fibers

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Fast stretches ($\sim 25 \text{ nmhs}^{-1}$ amplitude and $\sim 400 \mu\text{s}$ duration) which induced the forced rupture of the crossbridge ensemble were applied to intact muscle fibers to investigate the actomyosin bond properties during the force recovery following a step length change (release or stretch of 2 or 4 nm amplitude). Force and sarcomere length were measured with a fast force transducer ($\sim 50 \text{ kHz}$ natural frequency) and a striation follower device. To reduce fiber damaging by the stretches and to reduce the influence of myofilament compliance on the measurements, experiments were made on the tetanus rise at tension of about 0.5 the maximum plateau tension. Fast stretches were applied before and at progressively increasing times (up to 20 ms) after the step length change. The rupture force of the crossbridge ensemble (P_c) and the sarcomere elongation at P_c (L_c) were measured. In contrast with the data obtained previously on the tetanus rise (Bagni et al. J. Physiol., 2005; 565). The results showed that: (1) P_c was almost independent of the tension developed by the fiber and (2), L_c was not constant but increased immediately after the release and decreased after the stretch. These changes were still present 2 ms later when the quick recovery was almost complete and disappeared completely within 15-20 ms. Data analysis suggests that: 1) crossbridge number remains almost constant during the quick force recovery; 2) crossbridge detachment by the fast stretch is preceded by the reversal of the myosin head power stroke and, 3) the extent of the power stroke can be measured by the changes in L_c occurring during the quick recovery.

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Active And Passive Myofibrils Lengthened Beyond Acto-myosin Filament Overlap Produce Different Forces

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We stretched myofibrils actively and passively beyond myofilament overlap and measured forces. We expected active myofibrils stretched beyond myofilament overlap to produce the same force as equally long passively stretched myofibrils. Actively stretched myofibrils produced approximately four times more force than passively stretched myofibrils (Figure 1). Titin deletion with active and passive stretching resulted in complete force loss suggesting titin plays a crucial role in active and passive force production. Calcium activation and force inhibition through BDM reproduced the passive force curve, suggesting that titin and active force and not just Ca^{2+} activation was required for the large force of actively stretched myofibrils at lengths beyond myofilament overlap. Based on these results, we suggest that titin is a molecular spring whose stiffness is regulated by changes in effective length which in turn are controlled by force-dependent actin-titin interactions.

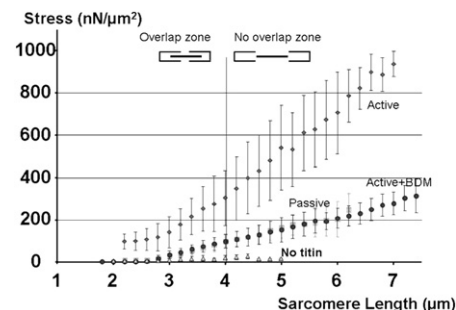


Figure 1. Actively stretched myofibrils show greater force beyond myofilament overlap than either passively stretched, or BDM actively stretched myofibrils. This suggests that passive titin forces increase with active force production and/or cross-bridge attachment but not with calcium activation.

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The Extent And Speed Of The Myosin Motor Recruitment Following 1-5 Nm Stretch Per Half-sarcomere Of Single Frog Muscle Fibers

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The contracting muscle exhibits a quite high resistance to sudden increase in load up to twice the isometric force (Katz, *J. Physiol.* 96:45, 1939). The increase in half-sarcomere (hs) stiffness and the changes in the x-ray interference of myosin-based reflections during muscle stretch indicate that the second motor domain of the myosin molecules with the first motor domain already attached to actin in the isometric contraction attaches within 2 ms following the stretch (Brunello et al., *Proc. Natl. Acad. Sci. USA*, 104:20114, 2007). The mechanism is further investigated here by using single frog fiber mechanics (*Rana esculenta*, 4°C , $2.1 \mu\text{m}$ sarcomere length). Stretches between 2 and 8 nm hs^{-1} , complete within 100 μs , were applied at the tetanus plateau (T_0) and the fraction of new motors relative to the isometric number (f) was determined either at the peak of the force response to stretch (T_1) or at the end of the quick phase of force recovery, 2 ms after the stretch (T_2). We show that: 1) for stretches $< 5 \text{ nm}$, independently of the phase of the force transient elicited by the stretch, f depends solely on the size of the axial distortion (Δz) of the attached motors; 2) for stretches $> 5 \text{ nm}$ at T_1 f reaches a maximum value of 0.3, while at T_2 f reaches a maximum value of 1. These results support the idea that the distortion of the attached motor domain of one myosin molecule promotes the attachment of the partner motor domain and indicate an upper limit ($\sim 10^4 \text{ s}^{-1}$) for the rate of the recruitment process. Supported by NIH (Grant no. 5R01AR49033-4) and MiUR, Italy.

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Load Dependence of Structural Changes in the Myosin Filament during Muscle Activation

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The M3 and M6 X-ray reflections from the myosin filaments of skeletal muscle correspond to axial periodicities SM3 and SM6 which are 14.34 and 7.19 nm at rest and 14.58 and 7.31 nm at the tetanus plateau (force T_0). This $\sim 1.5\%$ periodicity increase is much larger than the instantaneous filament compliance, and is probably due to an activation-dependent change in filament structure. SM6