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

Appaji Panchangam, **Dennis R. Claflin**, Mark L. Palmer, John A. Faulkner.
University of Michigan, Ann Arbor, MI, USA.

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 % s^{-1}; temperature, 15°C). During steady-state activation prior to lengthening, fibers produced an isometric stress of 133 ± 29 kPa at a mean L_s of 2.54 ± 0.16 μm. The lengthening contractions resulted in a 19 ± 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

Giovanni Cecchi[1], Barbara Colombini[1], Marta Nocella[2], Giulia Benelli[2], Maria Angela Bagni[1].
[1]Interuniversity Institute of Myology, Dept. Scienze Fisiologiche, University of Florence, Firenze, Italy, [2]Dept. Scienze Fisiologiche, University of Florence, Firenze, Italy.

Fast stretches (~25 nmhs^{-1} amplitude and ~400 μs 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 (~50 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

Tim R. Leonard.
University of Calgary, Calgary, AB, Canada.

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.

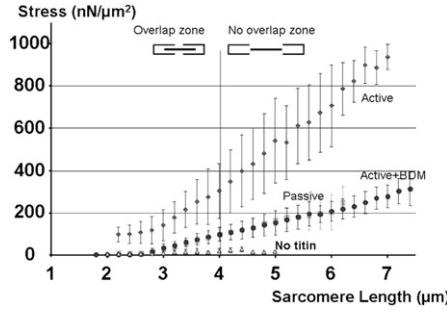

Figure 1. Actively stretched myofibrils show greater force beyond myofilament overlap than either passively stretched, or BDM actively stretched myofibrils. This suggests that passive titin forces increase with active force production and/or cross-bridge attachment but not with calcium activation.

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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

Luca Fusi, Elisabetta Brunello, Massimo Reconditi, Vincenzo Lombardi, Gabriella Piazzesi.
University of Florence, Sesto Fiorentino, Italy.

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 μm 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 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 >5nm 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 (~10^4 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

Luca Fusi[1], Elisabetta Brunello[1], Marco Linari[1], Massimo Reconditi[1], Thomas Irving[2], David Gore[2], **Gabriella Piazzesi**[1], Malcolm Irving[3], Vincenzo Lombardi[1].
[1]University of Florence, Firenze, Italy, [2]Illinois Institute of Technology, Chicago, IL, USA, [3]King's College London, London, United Kingdom.

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 ~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

increases faster than SM3 during force development (Brunello *et al.*, *J. Physiol.* 577:971, 2006) and the M6 intensity at T_0 is much higher than expected from the myosin motor conformation (Huxley *et al. J. Mol. Biol.*, 363:743, 2006), suggesting that the M6 mainly originates from other filament components. To better understand these structural changes, we recorded X-ray patterns from intact fibers of frog skeletal muscle (~2.15 μm sarcomere length, 4°C) during tetanic contraction under full force control. Force was held near zero for 50 ms after the start of stimulation, increased within 5 ms to T_0 and held there for 230 ms, then returned to zero. During the initial fiber shortening at zero force SM3 was almost constant but SM6 increased by 0.6%. SM3 and SM6 increased to the tetanus plateau values listed above within 10 ms of the force step to T_0, and decreased to steady values of 14.38 nm and 7.24 nm within 20 ms of the force step to zero. These results show that *i*, the full increase in SM3 and SM6 on activation is triggered by the force increase; *ii,* activation at zero force produces a partial (0.6%) increase in the periodicity of the structure responsible for the M6 reflection. Supported by NIH (5R01AR49033), MiUR and CNISM (Italy) and MRC (UK).

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Effects of Length Changes on Force Produced by Ca^{2+} and ADP-Activated Myofibrils along the Ascending Limb of the Force-Length Relation

Clara Pun, Ali Syed, Dilson E. Rassier.
McGill University, Montreal, QC, Canada.

Isometric forces produced by skeletal muscles are higher after stretch and smaller after shortening. A few studies investigating these length-dependent changes in force were conducted on the ascending limb of the force-length (FL) relation, showing conflicting results with elusive mechanisms. The purposes of this study were: (i) to evaluate the effects of muscle stretching and shortening on forces along the ascending limb of the FL relation, (ii) to evaluate if sarcomere length dispersion changes after the imposed length changes, and (iii) to assess if cross-bridges play a role in the length-induced force changes. Rabbit psoas myofibrils were attached between two pre-calibrated micro-needles, and their images were projected into a photodiode array for measurements of individual sarcomere length (SL). Myofibrils were activated by Ca^{2+} or ADP - the later induces cross-bridge attachment to actin independently of Ca^{2+}. After activation myofibrils were subjected to three stretches or shortenings (~4%SL), with isometric periods allowed between length changes so that force would achieve a steady-state. Forces of ADP-activated myofibrils were greater (7-8%) than those of Ca^{2+}-activated myofibrils at corresponding SL_s (range: 2.2-2.4μm) after shortening, but forces were similar after stretch. Forces were greater (26% with ADP and 15% with Ca^{2+}, SL: 2.2μm) after stretch than after shortening. Sarcomere dispersion was similar after stretch or shortening in Ca^{2+} and ADP-activated myofibrils. The results suggest that stretching and shortening affects isometric forces on the ascending limb of the FL relation through different mechanisms, and are not associated with SL dispersion. While cross-bridges seem to be involved in force depression, they are likely not involved in force enhancement.

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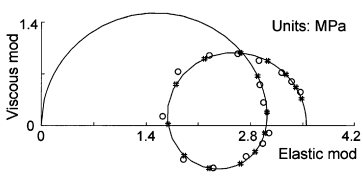
Cross-Bridge Kinetics Studied in Single Myofibrils by Sinusoidal Length Alterations during Maximal Activation

Masataka Kawai[1], Bogdan Iorga[2], Gabriele Pfitzer[3], Robert Stehle[3].
[1]University of Iowa, Iowa City, IA, USA, [2]Universities of Cologne and Bucharest, Cologne and Bucharest (Romania), Germany, [3]University of Cologne, Cologne, Germany.

Single myofibrils are the desirable system to study cross-bridge kinetics, because (1) little diffusion delay due to consumption/generation of ligands (ATP, ADP, Pi); (2) easy monitoring of sarcomere length during activation; and (3) solution change can be achieved in <10msec. Sarcomere length was set to 2.5μm, myofibrils (diameter 2-3μm, length ~60μm) were maximally activated with a solution switched from relaxing to activating solution (6mM CaEGTA, 5mM MgATP, 8mM Pi, 15mM CP, 200mM ionic strength, pCa=4.66, pH=7.0) at 15°C, changed the myofibril length sinusoidally in 15 frequencies ranging 1Hz and 350Hz at a low amplitude (~0.2%). We then characterized concomitant tension transients in terms of three exponential processes A, B and C, and results were compared to those obtained from single muscle fibers under the same activating conditions. 2πa (rate constant of low frequency exponential advance) $=2.5s^{-1}$, and 0.3x of that in fibers. 2πb (medium frequency exponential delay) $=94s^{-1}$, and 2x of fibers. 2πc (high frequency exponential advance) $=310s^{-1}$, and 0.7x of fibers. There was no sarcomere inhomogeneity developed during activation and/or oscillation. These results indicate that cross-bridge kinetics can be studied in single myofibrils using sinusoidal analysis.



Nyquist plot of activated myofibrils at 8mM Pi. Average of 12 preps. (O) are experimental points, solid curve and (#) are theoretical projections based on 3 exp processes.

3188-Pos Board B235

Mechanical Properties of Individual Sarcomeres Isolated From Skeletal Muscles

Ivan Pavlov, Dilson E. Rassier.
McGill University, Montreal, QC, Canada.

Mechanical properties of skeletal muscles have been investigated with muscle cells and myofibrils, preparations in which large sarcomere length non-uniformities are observed. The purpose of this study was to investigate the dependence of force on length of isolated sarcomeres. Myofibrils were dissected from rabbit psoas muscles and one sarcomere was selected for experimentation. Two pre-calibrated micro-needles (stiffness: 200 - 377 nm/μm) controlled by micromanipulators were used to capture the sarcomere, a few nanometers externally adjacent to each Z-line. One micro-needle was attached to a motor that is used for inducing fine, computer-controlled length changes. The sarcomere was set at a length between 1.48 and 3.48μm, and was activated using an automated perfusion system. The force produced by the sarcomeres was determined by the deflection of the micro-needles (force $= K_1d_1 + K_2d_2$, where K = stiffness, d = displacement, 1 and 2 = micro-needles 1 and 2, respectively). During activation, sarcomeres shortened by 0.34 ± 0.01μm (mean ± SEM). The amount of shortening showed a weak dependence of initial length (r^2=0.15). The forces produced by sarcomeres contracting between 2.26 and 2.43μm, the plateau of the theoretical force-length (FL) relation, was 123.07 ± 8.16 nN (mean ± SEM), comparable to previous studies with myofibrils. Forces along the ascending limb (from 1.27 to 2.26 μm) followed the predictions of the theoretical FL relation, but forces along the descending limb (between 2.43 and 3.91μm) were higher than those predicted by the theoretical FL relation, especially at sarcomeres beyond 3.0μm; a result that needs further examination. The single sarcomere technique represents a reliable method to evaluate mechanical properties of striated muscles, and the FL relation may be investigated without confounding effects arising from sarcomere non-uniformity and instability.

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The Mechanical Properties of Drosophila Jump Muscle Expressing Wild-type and an Embryonic Myosin Isoform

Catherine J. Eldred, Dimitre Simeonov, Ryan Koppes, Chaoxing Yang, David Corr, Douglas Swank.
Rensselaer Polytechnic Institute, Troy, NY, USA.

Transgenic *Drosophila* are highly useful for muscle protein structure-function studies, particularly myosin isoform diversity. However, our ability to mechanically analyze mutant proteins in *Drosophila* muscle has been limited to the skinned indirect flight muscle (IFM) preparation. We have developed a new preparation using the *Drosophila* tergal depressor of trochanter muscle (TDT) that increases our experiments to include maximum shortening velocity (V_{max}), force-velocity relations, and steady-state power generation, which are not possible using IFM fibers. As with the IFM, we can replace the native TDT myosin with our myosin of choice. When expressing its native isoform (P2), the TDT is equivalent to a very fast vertebrate muscle, with a V_{max} of 6.1 ± 0.3 muscle lengths/second at 15°C, a steep tension-pCa curve, a Hill coefficient of 11 ± 2, a high active isometric tension of 37 ± 3 mN/mm^2, and maximum power production (P_{max}) at 43% of V_{max} and 42% of maximum tension. Expressing an embryonic myosin isoform (EMB) in the TDT muscle decreased V_{max}, isometric tension and P_{max} by 50%, and the tension-pCa Hill coefficient decreased to 6 ± 2. Varying ATP concentration, while measuring V_{max}, revealed a higher ATP affinity for EMB than P2. Increasing Pi concentration reduced isometric tension of TDT expressing either isoform. A slight decrease in TDT V_{max} with increasing Pi concentration suggests TDT V_{max} may be influenced by Pi release rate. TDT V_{max} was not influenced by [Pi] when expressing EMB. With our advances in the TDT preparation we will now be able to test a wider speed range of myosin isoforms, including the superfast IFM myosin, to test our hypothesis that a step associated with Pi release is rate limiting for V_{max} of very fast myosins, while a step associated with ADP release is limiting for slower isoforms.

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Co-chaperone BAG3 Has Critical Roles For Maintaining Z-disc And Myofibrillar Structure Under Mechanical Stress

Akinori Hishiya, Toshio Kitazawa, Shinichi Takayama.
Boston Biomedical Research Institute, Watertown, MA, USA.

Bcl-2 associated athano gene 3 (BAG3) is a member of the co-chaperone BAG family proteins that bind to and regulate Hsp70 molecular chaperones. The