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

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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

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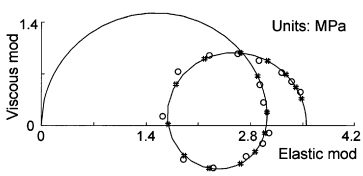
Single myofibrils are the desirable system to study cross-bridge kinetics, because (1) little diffusion delay due to consumption/generation of ligands (ATP, ADP, Pi); (2) easy monitoring of sarcomere length during activation; and (3) solution change can be achieved in <10msec. Sarcomere length was set 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.

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Mechanical Properties of Individual Sarcomeres Isolated From Skeletal Muscles

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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

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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

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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

bag3 deficient mice develop severe myopathy and die before 4 weeks after birth. Pathological pattern of the myopathy indicated myofibrillar degeneration with Z-disk disruption and is categorized in myofibrillar myopathy. Recent genetic analysis of myofibrillar myopathy cases revealed mutations in various heterogeneous genes, which encode proteins connecting to or existing on Z-disc, and supporting its structure. To understand the molecular mechanism of myofibrillar degeneration observed in bag3 deficient muscle, we used primary culture of rat neonatal cardiomyocytes with shRNA mediated gene knockdown and addressed the effect of mechanical stretch on Z-disc and myofibrillar structure. Equibiaxial strain was applied to cardiomyocytes, which were infected with adenovirus carrying siRNA of bag3. Interestingly, in bag3 knockdown cardiomyocytes, mechanical stretch rapidly disrupted both F-actin and Z-disc structures. Ex-vivo contracture experiments of papillary muscle strips of bag3 null mice indicated a rapid reduction of both active and passive tension. We will discuss potential molecular mechanism of BAG3 for maintenance of myofibrillar structure under the mechanical stress. This work is supported by NIH AR052925.

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Modeling The Membrane-Costamere-Myofibril Complex from Normal and Desmin or Dystrophin Mice as a Distributed Elastic System

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We studied the stiffness (**k**) of the membrane-costamere-myofibril complex and of the sarcolemma alone in myofibers from control and desmin-null or dystrophin-null (mdx) mice. Negative pressure (**P**) was applied with an elastimeter through a pipette to the sarcolemma of myofibers, isolated from murine extensor digitorum longus muscles, to form blebs. We analyzed the results using a distributed spring model, based on the presumptive organization of the proteins in the extended complex. The model was solved as a lumped system. From the model, we computed **k**. We estimated **k** of the complex from 1450 to 2600, from 1100 to 1600 and from 900 to 1300 dyne/cm for control, desmin null, and dystrophin null myofibers, respectively. Values of **k** for the sarcolemma alone varied from 1000 to 1900, 700 to 1400 and 700 to 1000 dyne/cm for the same groups., The controls are therefore stiffer than either of the null mutants, and the dystrophin-null is more compliant than either controls or desmin-nulls. We compare the experimental values of **k** for the complex in control and mutant muscles to the theoretical values obtained by the iteration of **k** for each protein. Normalizing the experimental **k** values for control myofibers as 1.00, we found values of 0.73 and 0.52 for the desmin- and dystrophin-null muscles, respectively. Computed theoretical values were 1.0, 0.72 and 0.53, in good agreement with our experimental results. We conclude that the complex of proteins that link myofibrils to the sarcolemma at costameres can be modeled as a distributed, lumped spring system, in which each protein has a different **k**. As a result, the, absence of desmin or dystrophin affects the mechanical properties of the complex differently.
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Modeling the Response of Airway Smooth Muscle to Cyclic Loading

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Airway smooth muscle (ASM) exhibits complex contractile dynamics and has a highly disordered structure. This contrasts with skeletal muscle which contains ordered arrays of contractile filaments aligned with the long axes of the cells. Models of ASM, however, are often based on Huxley's cross-bridge model, which was developed for skeletal muscle and does not take into account the rheological properties of the non-contractile components of the tissue. Here we use a modeling approach to investigate the relative contributions of tissue viscoelasticity and crossbridge kinetics to the mechanical response of ASM to cyclic loading.

Experiments were performed using rat trachealis muscle strips. Breathing was mimicked by applying sinusoidal length oscillations (frequency: 2Hz; amplitude: 1-4%). In unstimulated muscle, peak force during length oscillation followed a typical stress relaxation trajectory. In stimulated muscle, peak force decreased dramatically over the first 5-10 cycles to a level close to the isometric force at the mean length. Furthermore, steady-state peak force decreased as loading amplitude increased. 'Sighs' were mimicked by applying a large-amplitude loading cycle (5-25%). Sighs caused a transient but long-lasting reduction in peak force, with the degree of force reduction increasing with sigh amplitude. The response of unstimulated muscle to length oscillation could be reproduced well with a model consisting of a Hill-type contractile element and a parallel elastic element, both in series with a nonlinear Kelvin body (viscoelastic element). In order to reproduce the response of stimulated muscle to length oscillation, cross-bridge kinetics had to be included either using a Huxley-type model or by including first-order cross-bridge attachment and detachment kinetics in the Hill model. The decrement and slow recovery of force after a sigh, however, could not be reproduced by either model, indicating that additional mechanisms are required to explain this phenomenon.

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Changes in Thick Filament Structure of Isolated Intact Rat Cardiac Muscle During Contraction Determined by 2-D X-ray Diffraction Analysis

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A complete understanding of excitation /contraction coupling in cardiac muscle requires knowledge of the sequence of structural changes in the myofilaments in response to the release of calcium from internal stores. We used isolated, membrane intact, electrically stimulated, cardiac trabeculae to obtain improved 2-dimensional X-ray patterns under three conditions: 1) diastolic conditions (no Calcium), 2) at peak calcium response but with 5 mM EGTA to inhibit calcium response and 3) at peak calcium response but where force was inhibited using the myosin ATPase inhibitor Blebbistatin which prevents strong binding of myosin heads to the thin filament. The resulting 2 dimensional X-ray diffraction patterns indicated that with the release of calcium from internal stores, the myosin heads, without generating active force, move towards the thin filaments as evidenced by an inward shift of the first maximum on the unsampled 4th myosin layer line. Surprisingly, the diffraction patterns, in the presence of Blebbistatin and calcium, indicated a more ordered structure, than in its absence, suggesting that the attachment of myosin heads and force development involves transient increases in cross-bridge ordering prior to tension generation. This is in contrast to previous results, from skeletal muscle preparations, that have been interpreted as the process activation inevitably involves a rapid disordering of the thick filament.

Cardiac Muscle II

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Fiber Contractility In An *In Vivo* Model Of Myocardial Ischemia - Reperfusion

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Altered blood flow to the heart, either transient or chronic, underscores the progression towards heart failure. Multiple models have suggested that alterations in Ca^{2+} handling and reduced energy reserves contribute to the reduction in cardiac muscle contractility. However, we have hypothesized that altered blood flow is also responsible for reversible, post-translational modifications to proteins of the contractile filaments, in turn limiting muscle contractility independent of available Ca^{2+} or ATP. Using an *in vivo* rat model, three experimental groups (perfused, ischemic, and reperfused) were established by limiting and re-establishing blood flow through the left anterior descending artery. Thin strips of the anterolateral papillary muscle were recovered and permeabilized with Triton-X100 to measure various contractile parameters. The maximum force and stiffness per cross-section (F_{max} and S_{max}) of fibers from the three conditions were measured in pCa4 solution. The F_{max} and S_{max} were significantly reduced in ischemic fibers (79% and 74% of perfused fibers), but restored to some extent in reperfused fibers (90% and 75% of perfused fibers). However, the Ca^{2+} sensitivity of contraction (EC50) was significantly shifted rightward only in ischemic fibers, with complete recovery in reperfused fibers. The reversible nature of the force decline and change in EC50 during ischemia suggests that the underlying changes in the contractile proteins were reversible, and most likely post-translational in nature. Additional experiments characterizing the altered contractility of ischemic fibers will be presented. Supported by NIH grant HL78845.

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Myofilament Dysfunction in a Guinea-pig model of Diastolic Heart Failure

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Diastolic heart failure (DHF) is characterized as heart failure with preserved systolic function; the mechanisms underlying this syndrome are incompletely