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).

**3186-Pos Board B233**

**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.

**3187-Pos Board B234**

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

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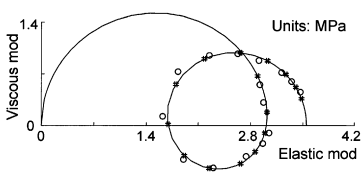
Single myofibrils are the desirable system to study cross-bridge kinetics, because (1) little diffusion delay due to consumption/generation of ligands (ATP, ADP, Pi); (2) easy monitoring of sarcomere length during activation; and (3) solution change can be achieved in <10msec. Sarcomere length was set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

**3188-Pos Board B235**

**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