**2557-Pos Board B527**

**Actin Sliding Velocities Are Influenced By The Chemical Driving Force Of Actin-myosin Binding**

**Josh E. Baker**, Ryan D. Smith, Steven F. Shannon, Travis J. Stewart, Olivia N. John.
University Nevada Reno, Reno, NV, USA.

It is widely assumed that muscle shortening velocity, *V*, is solely limited by actin-myosin detachment kinetics; however, it was recently shown (Hooft et al., 2007) that *V* is influenced by a driving force modulated by actin-myosin attachment kinetics and energetics. To further test this hypothesis, we have developed a novel *in vitro* motility and force assay that allows us to correlate changes in internal driving force with changes in *V*. We alter the driving force by varying inorganic phosphate, $P_i$, and blebbistatin concentrations using an *in vitro* motility assay to measure corresponding changes in *V*. We estimate changes in driving force by calculating the rate, $k_{break}$, at which actin filaments break during this assay. We observe that at 30 μM ATP, both *V* and $k_{break}$ decrease by approximately 50% (*V* from 1.5 to 0.88 $\mu m \cdot sec^{-1}$ and $k_{break}$ from 0.04 to 0.02 $sec^{-1}$), demonstrating that actin-myosin driving forces in an *in vitro* motility assay decrease with *V* upon addition of $P_i$. Similarly upon addition of 50 μM blebbistatin, a small molecule known to decrease actin-myosin mechanics by inhibiting actin-myosin binding kinetics, both *V* and $k_{break}$ decrease by approximately 50% (*V* from 1.5 to 0.62 and $k_{break}$ from 0.04 to 0.02 $sec^{-1}$). These results support the hypothesis that in an *in vitro* motility assay internal forces modulated by actin-myosin binding energetics influence actin sliding velocities, supporting a new paradigm for the mechanism of muscle shortening.

**2558-Pos Board B528**

**Flexibility of Actin Filaments During Myosin Induced Sliding**

**Petr G. Vikhorev**, Natalia N. Vikhoreva, Alf Månsson.
University of Kalmar, Kalmar, Sweden.

The bending flexibility of actin filaments, freely suspended in solution or propelled by heavy meromyosin (HMM) in the *in vitro* motility assay, was studied under different experimental conditions using both phalloidin-stabilized and phalloidin-free filaments (visualized by N-Hydroxysuccinimide-Rhodamine). For actin filaments propelled by HMM, the persistence length (inversely related to the bending flexibility) was obtained from exponential fits to the cosine correlation function for sliding paths. In solution (in the absence of HMM) both phalloidin free and phalloidin stabilized filaments exhibited a nearly rectilinear increase (slope ≈ 0.046 μm/mM) in persistence length upon increased ionic strength of the observation solution (from 40 to 130 mM). For phalloidin-free and phalloidin-stabilized actin filaments (80 mM ionic strength), $L_P$ in solution was 9.2 ± 0.8 μm (mean ± 95 % confidence interval) and 15.7 ± 1.1 μm, respectively (> 80 filaments). For phalloidin free filaments, the persistence length was similar whether the filaments were propelled by HMM or freely suspended in solution in the absence of HMM. In contrast, the persistence length for phalloidin stabilized actin filaments was reduced to the same level as for phalloidin free filaments during HMM induced sliding. The results accord with the idea that a high flexibility structural state of the actin filament is a perquisite for actomyosin motility. However, the results do not support ideas that a low-to-high flexibility transition of the filament should be an important component of the force-generating step *per se*. Finally, our results challenge the general view that phalloidin stabilized filaments behave as native actin filaments in their interaction with myosin.

**2559-Pos Board B529**

**The Molecular Effects of Skeletal Muscle Fatigue on Myosin Mechanics and Kinetics**

**Michael J. Greenberg**[1], Tanya R. Mealy[1], Michelle Jones[2], Danuta Szczesna-Cordary[2], Jeffrey R. Moore[1].
[1]Boston University School of Medicine, Boston, MA, USA, [2]University of Miami Miller School of Medicine, Miami, FL, USA.

Skeletal muscle, during periods of exertion, experiences several different fatigue based changes in contraction including reductions in force, velocity, power output, and energy usage. The physiological bases of fatigue induced changes in contractility stem from many different factors including changes in neuronal activity, excitation-contraction coupling, and actomyosin contraction. The direct changes in the actomyosin contractile complex have previously been shown to be the result of alterations in the levels of metabolites, oxidative damage, and an increase in myosin regulatory light chain phosphorylation. Here, we measured the direct molecular effects of fatigue-like conditions on actomyosin velocity and force generation using the in vitro motility assay. We examined how changes in ATP, ADP, Pi, and pH affect the ability of the myosin to translocate actin. We show that fatigue-induced increases in metabolites decrease unloaded shortening velocity, with ADP and pH contributing the most to the observed changes in velocity. We also examined whether myosin regulatory light chain phosphorylation alters the sensitivity of the myosin to fatigue-like conditions. We show that under fatigue-like conditions, phosphorylation of the myosin regulatory light chian enhances force production and reduces actin sliding velocity, similar to the effects of phosphorylation under fatigue-like conditions observed in both muscle fiber and in vitro motility studies. Furthermore, we found that force production by dephosphorylated myosin is very sensitive to fatigue-like conditions whereas force production by phosphorylated myosin is rather fatigue insensitive. These results suggest that phosphorylation of the myosin regulatory light chain in skeletal muscle may serve as a protective mechanism against fatigue. Supported by NIH-HL077280, AHA-0435434T (J.R.M.), AHA-0815704D (to M.J.G.), and NIH-HL071778 (D.S-C.).

**2560-Pos Board B530**

**Different Molecular Mechanisms of Force Enhancement by Myosin Head Domain Mutations R723G and R453C**

Dejan List[1], Benjamin Seebohm[1], Faramarz Matinmehr[1], William J. McKenna[2], Antonio Francino[3], Francisco Navarro-Lopez[3], Bernhard Brenner[1], **Theresia Kraft**[1].
[1]Medical School Hannover, Hannover, Germany, [2]The Heart Hospital, UCL, London, United Kingdom, [3]Hospital Clinic, Univ. of Barcelona, Barcelona, Spain.

The myosin head transforms chemical energy from ATP hydrolysis into mechanical work to generate isometric force or to drive muscle shortening. During the power stroke, small changes in the catalytic ATPase site of the actin-bound myosin head are coupled to tilting of the light-chain binding domain relative to the catalytic domain. In this mechanism distortion of an elastic element within the actomyosin complex is essential for strain to develop prior to movement.

We have studied missense mutations naturally occurring in Familial Hypertrophic Cardiomyopathy (FHC) to test molecular mechanisms and functional roles of specific domains of the myosin head. Slow soleus muscle fibers (expressing the ventricular myosin isoform) of FHC-patients were used for these studies. Previously we found that mutation R723G which is located in the converter domain of the myosin head increases active force and resistance to elastic distortion (fiber stiffness) during contraction, relaxation, and rigor, while cross-bridge cycling kinetics were unchanged. This indicated that the converter is the part of the actomyosin complex where most of the elastic distortion occurs.

We now included mutation R453C located near the nucleotide binding pocket of myosin in our functional studies. This mutation was found to also increase active force, however, without affecting fiber stiffness. Instead, mutation R453C increased fiber ATPase activity and the rate constant of force redevelopment (kredev) significantly, which were unchanged by mutation R723G. Thus, both FHC mutations cause force enhancement in muscle fibers from FHC patients, however, by distinctly different mechanisms. R723G affects resistance to elastic distortion of the myosin head while R453C alters cross-bridge cycling kinetics. The data underline the different functional roles of the domains within the myosin head and point to an involvement of residue R453 in human ventricular myosin ATPase activity.

**2561-Pos Board B531**

**Kinetics Of Two Single Point Mutants Of Drosophila Myosin S1**

**Marieke J. Bloemink**[1], Corey M. Dambacher[2,3], Girish Melkani[2], Michael A. Geeves[1], Sanford I. Bernstein[2].
[1]Department of Biosciences, University of Kent, Canterbury, United Kingdom, [2]Department of Biology, Molecular Biology Institute, and SDSU Heart Institute at San Diego State University, San Diego, CA, USA, [3]Scripps Research Institute, Dept of Chemistry and Skaggs Institute for Chemical Biology, San Diego, CA, USA.

We investigated the kinetic properties of two homozygous-viable *Drosophila* myosin mutants, *D45* ($A^{261}T$) and *Mhc*$^5$ ($G^{200}D$). The *D45* mutation is located near β7, while the *Mhc*$^5$ mutation is located at the N-terminal side of loop 1. *D45* was originally identified as a suppressor of the troponin I (TnI) *hdp*$^2$ mutation, which causes muscle hypercontraction, resulting in a wings-up phenotype. The *Mhc*$^5$ mutation is lethal when co-expressed with *hdp*$^2$, presumably enhancing the hypercontraction phenotype. *Drosophila* hearts, carrying either the *D45* or *Mhc5* mutation, demonstrated depressed or enhanced motor function and this evoked a dilatory or restrictive response, similar to that seen with vertebrate dilated or restricted cardiomyopathy myosin mutations. Using flash photolysis, our transient kinetics results suggest that suppression of the TnI hypercontraction phenotype is accomplished by expression of a less-active myosin (*D45*), whereas enhancement of muscle hypercontraction can be achieved with the expression of an overactive myosin isoform (*Mhc5*). Both

mutations affect the rate of nucleotide exchange from the nucleotide-binding pocket and show altered ATP-binding and ADP release rates. Homology models of myosin S1, either *D45* or *Mhc5*, suggest a possible mechanism by which the single point mutation can alter the kinetic properties of the myosin head.

**2562-Pos Board B532**

**Comparison Of Mechanical Properties Of Single Intact Fibres From Wild-type And Mlc/migf-1 Mouse Muscle**

**Barbara Colombini**[1], Giulia Benelli[2], Marta Nocella[2], Antonio Musarò[3], Giovanni Cecchi[1], Maria Angela Bagni[1].
[1]Interuniversity Institute of Myology, Dept. Scienze Fisiologiche, University of Florence, Firenze, Italy, [2]University of Florence, Firenze, Italy, [3]Interuniversity Institute of Myology, Dept. Istologia ed Embriologia Medica, University of Rome ''La Sapienza'', Rome, Italy.

In this study we compared the mechanical properties of single intact muscle fibres of wild-type (WT) and MLC/mIgf-1 (TG) mice, in which the localized Igf-1 transgene expression sustains hypertrophy (Musarò et al., *Nat. Genet.* 27, 2001). The study has been focussed on ''static stiffness'' (SS), a non cross-bridge calcium-dependent stiffness previously identified in activated frog muscle fibres (Bagni et al., *Biophys. J.* 82, 2002).

Single intact fibres, dissected from the flexor digitorum brevis muscle, were mounted in an experimental chamber (~23°C) between the lever arms of a force transducer and of an electromagnetic motor to apply fast stretches. Sarcomere length was measured by means of a videocamera and with laser diffraction. Tetanic tension and force-velocity relation in WT and TG mice were not significantly different, however, the maximum shortening velocity (Vmax) was faster than previously reported and comparable with frog muscle. Compared to frog fibres, the plateau of length-tension relation shifted according to the different myofilament lengths. TG fibres exhibited an increase in diameter and maximum force, but specific force was the same as for WT fibres. SS was present either in WT or in TG fibres and its time course, independent from isometric tension, was faster than in frog.

A preliminary analysis suggests that the only significant mechanical difference between WT and TG fibres is in the SS properties. This may be related to a different compliance of the structure responsible of the SS that we speculated could be titin.

**2563-Pos Board B533**

**Reactive Oxygen Species Alter Activation Of Cardiac Myofilaments And Modify Sarcomeric Proteins**

**Benjamin S. Avner**, R. John Solaro.
University of Illinois at Chicago, Chicago, IL, USA.

The generation of reactive oxygen species [ROS] such as hydrogen peroxide [$H_2O_2$] is elevated in acute and chronic cardiac pathophysiology. Post-translational oxidative modification of sarcomeric proteins important to cardiac function, such as actin and tropomyosin [Tm], represents a possible mechanism by which ROS may induce changes in cardiac function. Here we present data that test the hypothesis that ROS modify the function of ventricular muscle through oxidation of sarcomeric components. We directly exposed cardiac muscle to oxidation by treatment of detergent-extracted myofibril bundles from murine papillary muscles with 2.5 mM $H_2O_2$. From the same hearts, we prepared homogenates of the ventricular sarcomeric proteins; each sample was divided and processed with and without exposure to $H_2O_2$. We compared the oxidation state of the proteins, employing electrophoresis to analyze the formation of reduction-sensitive disulfides by oxidized cysteine residues. Compared to untreated fiber bundles, those treated with $H_2O_2$ showed significantly blunted cooperative activation in response to strong actin-myosin cross-bridge binding, as measured by addition of N-ethylmaleimide modified myosin sub-fragment 1 [NEM-S1]. Cross-bridge dependent effects are important for full activation of the cardiac thin filament and are believed to control the kinetics of ejection and relaxation. Results from ''diagonal'' gels (SDS-PAGE run successively under non-reducing and reducing conditions) revealed reduction-sensitive products which were more abundant in peroxide-treated compared to untreated tissue samples. Western immuno-blot analysis confirmed that these products contained actin and Tm. Overall, our findings represent evidence for the ROS-induced oxidation of myofibrillar proteins along with impairment in cardiac muscle function. Investigation of whether the endogenously generated ROS observed in pathological settings have similar effects *in vivo* will aid in assessing the significance of these modifications, and may suggest a therapeutic target.

**2564-Pos Board B534**

**Effects Of Blebbistatin And BDM (2,3-Butanedione Monoxime) On The Short-range Mechanical Properties Of Murine Diaphragm Muscle Fibers**

**Mihail I. Mitov**, Kenneth S. Campbell.
Department of Physiology, University of Kentucky, Lexington, KY, USA.

Blebbistatin (BLEB) and 2,3-butanedione monoxime (BDM) are well-know inhibitors of myofilament force production and useful tools in structural and functional studies of cell motility and muscle contraction. In this study, we investigated the effects of BLEB and BDM on the short-range mechanical properties of single chemically permeabilized murine diaphragm fibers. BLEB and BDM were used in separate sets of experiments to reduce isometric force in saturating Ca2+ solution to approximately 50% of the control value. Muscle fibers were subjected to repeated triangular length changes (paired ramp stretches/releases, 0.04 $l_0$, 0.33 $l_0$ $s^{-1}$) imposed under fiber length control in solutions with free $Ca^{2+}$ concentrations ranging from pCa 9.0 to pCa 4.5. Short-range stiffness values were calculated from the slopes of regression lines fitted to the first 15 ms of XY plots of force against muscle length for each stretch response and expressed as Young's Moduli. Analysis of results obtained in control Ca2+ solutions (without BLEB or BDM) showed that short-range stiffness increased proportionately with the level of isometric force. Experiments performed with BLEB showed that short-range stiffness declined in proportion with the reduction in isometric force (no change in the stiffness/force ratio). In contrast, BDM produced a disproportionately large decrease in isometric force (that is, the stiffness/force ratio increased in the presence of BDM, ANACOVA test, $p<0.001$). These results support the hypothesis that BLEB and BDM reduce isometric force in skeletal muscles by different mechanisms. BLEB seems to prevent myosin heads from attaching to the thin filament while BDM probably reduces force by decreasing the rate at which myosin heads undergo tension-generating biochemical transitions.

## Cardiac Muscle I

**2565-Pos Board B535**

**Stretching Cardiac Trabeculae Increases the Force by Decreasing the Cross-bridge Weakening Rate in a Velocity Dependent Manner**

Moran Yadid, **Amir Landesberg**.
Technion - Israel Institute of Technology, Haifa, Israel.

**Background:** Stretch increases the force and decreases energy consumption in skeletal muscle. However, the underlying mechanisms and the effects of stretching cardiac muscle remain elusive. We hypothesized that stretch increases the force by modulating the cross-bridge (XB) cycling rates. **Methods:** Trabeculae (n=*6*) were isolated from rat right ventricles. Sarcomere length was measured by laser diffraction and controlled by a fast servomotor. The number of strong XBs ($N_{XB}$) was quantified by measuring the dynamic stiffness. Ramp stretches (n=42) at different velocities and onset times were imposed on sarcomere isometric twitches. Normalized stress (stiffness) enhancement, $\sigma_E$ ($K_E$), was defined as the increase in the stress (stiffness) during stretch normalized by the instantaneous isometric stress (stiffness). **Results:** Stretches yielded identical increases in $\sigma_E$ and $K_E$, implying that the stretch increases force by increasing $N_{XB}$. A unique linear relationship was observed between the instantaneous normalized stress and stiffness, for all the stretch velocities ($1.03 \pm 0.078$, $R^2=0.99 \pm 0.026$), suggesting that the unitary force per XB is constant for all stretch velocities (in contrast, a velocity dependent decrease in the force per XB was obtained during sarcomere shortening, in congruent with previous publication). The rate of $\sigma_E$ development depended linearly on the stretch velocity ($7.35 \pm 1.07$ [1/μm]). Interestingly, the rate of $\sigma_E$ development was independent of the stretch onset time, indicating that it is not dominated by changes in XB recruitment, but is an inherent property of the strong XB, since the population of available XB varies during the twitch. **Conclusions:** Constant force per XB, independence on the recruitment rate, and the linear dependence of $\sigma_E$ on the stretch velocity, strongly suggest that stretch decreases the rate of XB turnover from strong to weak conformation in a velocity dependent manner.

**2566-Pos Board B536**

**Microfabricated Post Array Detectors to assess cardiomyocyte forces induced on their environment via focal adhesions**

**Anthony G. Rodriguez**, Sangyoon Han, Michael Regnier, Nathan Sniadecki.
U. of Washington, Seattle, WA, USA.

There is vast potential in regenerative medicine to improve cardiac muscular dysfunction, but difficulties arise in functionally integrating implanted cells