

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

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In this study we compared the mechanical properties of single intact muscle fibres of wild-type (WT) and *MLC/mlgf-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 ( $V_{max}$ ) 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 myofibrillar 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

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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

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Blebbistatin (BLEB) and 2,3-butanedione monoxime (BDM) are well-known 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  $Ca^{2+}$  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  $Ca^{2+}$  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

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**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 ( $7.35 \pm 1.07 [1/\mu m]$ ). 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

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