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.
Supported by MDA to RJB and CONACyT

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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.

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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.

3195-Pos Board B242

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