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

understood. Accordingly, we studied myofilament function in an experimental model of DHF. DHF was induced by chronic Angiotensin II infusion via surgically implanted infusion pumps (400ng/kg/min)/saline pumps (0.9%) in female Dunkin Hartley Guinea pigs (400g). Following eight weeks of treatment, LV samples were snap frozen in liquid N2. Skinned myocyte fragments were prepared by mechanical dissociation and subsequently glued to a force transducer and motor attached to micropipettes that were positioned on the stage of an inverted microscope. Preliminary data indicate that myocyte myofilament function is depressed in the DHF group in terms of maximum Ca2+ saturated force development (15.8 ± 0.9 vs. 28.1 ± 0.9 mN/mm2) and cooperativity (Hill coefficient; 2.8 ± 0.1 vs. 3.4 ± 0.6), but not Ca2+ sensitivity (EC50; 2.21 ± 0.06 vs. 2.23 ± 0.13 μM). In addition, 2-D DIGE gel analysis revealed shifts in the phosphorylation profiles of the contractile proteins MyBP-C and Troponin I. We conclude that myofilament dysfunction underlies, in part, the decreased pump function that is seen in this guinea-pig experimental model of DHF and that this phenomenon may be caused by maladaptive contractile protein phosphorylation.

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Guinea-pig model of Diastolic Heart Failure characterized at three different pathophysiological states

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Diastolic heart failure (DHF) is a recently recognized syndrome defined as heart failure with preserved systolic cardiac pump function. We developed a minimally invasive, physiologically relevant, gradual pressure-overload experimental model of DHF in Guinea Pigs (GP). GP were divided into two groups - control and treatment. Based on a dose-response curve and time period study, we established the pressure overload model by surgically implanting Angiotensin II pumps (400ng/kg/min)/saline pumps (0.9%) in female Dunkin Hartley Guinea pigs (400g); up to 12 weeks. At different time points three stages were identified in this model 1) initial hypertensive, 2) compensatory DHF, and 3) decompensated diastolic/systolic heart failure as based on invasive hemodynamic and M-mode echocardiography analyses at 4, 8, and 12 weeks of Angiotensin II treatment. Thus, maximum positive dP/dt increased ~50% at stage 2 and decreased ~55% at stage 3; LV hypertrophy was ~10% at stage 2, and ~55% at stage 3. We conclude that chronic treatment with Angiotensin II is a useful experimental model of compensated and decompensated diastolic HF in the guinea-pig.

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Failure of the Frank-Starling Relationship in Infarcted Hearts is Correlated with Infarct Size

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Heart failure has been associated with a depression or loss of the ability of the heart to increase cardiac output in response to increased ventricular filling (i.e., the Frank-Starling Relationship). This loss is often seen as a long-term effect of conditions such as congestive heart failure, but the response of the heart to more acute pathological conditions such as following a myocardial infarction (MI) is less well known. Experiments here were designed to test the hypotheses that responsiveness to preload is reduced within 3 weeks following MI, and that the relative loss of function will correlate with the size of the infarct. MI was induced with permanent ligation of the left ascending coronary artery and cardiac function was monitored every week using echocardiography to calculate fractional shortening (FS). After three weeks, heart function was assessed using a modified whole working heart preparation with precise control of preload, afterload, and heart rate. The hearts were then vibratomed in 1mm thick cross-sections from apex to base and infarct size was calculated using Image-J on bright field microscopy images. FS was decreased over sham-operated control, and correlated well with infarct size (2%, 6%, and 10% infarct size presented with 50%, 25%, and 20% FS, respectively). Interestingly, the 2% infarct working heart had a nearly normal response to increases in preload from 7.5 to 25 cm H2O, while the 6% infarct response was blunted above 12.5 cm H2O and the 10% infarct was completely unresponsive to changes in filling pressure. These data imply that the Frank-Starling relationship is impaired following MI in an infarct size dependent manner. Future studies will focus on whether this can be reversed with cellular or genetic therapies. Support: NIH R24 HL64387 (MR, CEM).

3198-Pos Board B245

Reduced Expression of Alpha MHC in Failing, Pre-LVAD Human Myocardium Contributes to Depressed Rates of ATP Utilization

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The ventricles of human myocardium normally express low levels of α myosin heavy chain (MHC) on a predominately β MHC background. However, in heart failure the distribution changes to ~100% β MHC with virtually undetectable levels of α MHC. While it has been known for some time that α MHC exhibits greater rates of ATP utilization and maximal shortening velocity (V_{max}), we have recently shown that the low level of α MHC normally present in the ventricles of larger mammals increases the rate of rise of force compared to myocardium expressing 100% β MHC. Here, we tested the hypothesis that the loss of α MHC in human heart failure impairs contraction kinetics and contributes to mechanical dysfunction by measuring the rate of ATP utilization and isometric force in normal donor hearts and in failing myocardium excised from patients prior to the implantation of a left ventricular assist device (LVAD). Permeabilized multicellular preparations from normal myocardium yielded maximal rates of ATP turnover approximately 3-fold greater than in pre-LVAD failing myocardium, while maximal isometric force between the two groups was similar. This equates to a nearly 3-fold greater tension cost in normal human myocardium, and it is possible that the lower tension cost observed in failing myocardium would enhance the efficiency of contraction under conditions of impaired energetics. Furthermore, SDS-PAGE indicated a reduction in α MHC content in pre-LVAD, failing myocardium compared to normal myocardium. These results suggest that a loss of α MHC in human heart failure would be at least partly responsible for the decrease in contractile function and would contribute to lower rates of pressure development (dP/dt) *in vivo*, ultimately impairing both systole and diastole. This work supported by NIH RO1-HL61635 (RLM).

3199-Pos Board B246

The Positive Force-Frequency Relationship Is Maintained In Absence Of Sarcoplasmic Reticulum Function In Rabbit, But Not In Rat Myocardium

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Myocardial calcium handling differs between species, mainly in the relative contribution between the sources for activator calcium. To investigate the role of the myofilaments and intracellular calcium decline in governing the relaxation phase of cardiac muscle, and to elucidate additional determinants of relaxation other than the sarcoplasmic reticulum (SR) at various frequencies within the *in vivo* range, the present study was performed by altering the calcium handling in rat and rabbit. Trabeculae at optimal preload and at 37 °C were iontophoretically loaded with bis-fura-2 to monitor cytoplasmic calcium levels before being subjected to ryanodine and cyclopiazonic acid to inhibit SR function. Simultaneous force and $[Ca^{2+}]_i$ measurements were obtained at 1-4 Hz in rabbit and at 4-8 Hz in rat before and after SR inhibition. Inhibition of SR function resulted in increased diastolic and peak calcium levels. Developed force increased with frequency in rabbit but decreased in rat after inhibition of SR function, despite that both species normally exhibit a positive force-frequency relationship. Calcium transient amplitude decreased in rat, but increased in rabbit after SR inhibition. Time to peak tension, RT_{50}, time to peak calcium, and time from peak calcium to 50% calcium decline were all prolonged. Results suggest that L-type calcium channel current is responsible for increases in calcium with increasing frequency, and that the SR amplifies this effect in response to increased L-type current. The response of the myofilaments to alterations in calcium handling plays a critical role in the final determination of force, and may differ between species. These results imply the balance between force relaxation and calcium decline is significantly different in larger mammals, necessitating a critical re-evaluation of how myocardial relaxation is governed, specifically regarding frequency-dependent activation.

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Increasing Preload Reduced Actin-Myosin Interaction in Isolated Beating Rat Whole Heart Under Hypoxia

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Background: Hypoxia reduces cardiac contractile performance. However, there is no direct observation on how preload affects the actin-myosin-interaction (AMI) in beating hearts during hypoxia. **Purpose**: The aim of this study is to investigate this theme using X-ray-diffraction (XRD) analysis at a third-generation synchrotron radiation facility. **Methods**: Eight isolated isovolumically contracting rat hearts were paced at 120 bpm after complete heart block, mounted so that the X-ray beam (15.0 keV) passed the deeper layer of left ventricular (LV) free wall, and perfused with Tyrode solution bubbled with