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.

3196-Pos Board B243

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.

3197-Pos Board B244

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.

3200-Pos Board B247

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

100%O_2 (Normo) or 100%N_2 (Hypo). We recorded XRD patterns and LV pressure (LVP) at end-diastolic LVP (EDP) of 0 and 20 mmHg. **Results**: Under Normo, increasing EDP significantly increased the developed LVP (EDP0: 104 ± 26mmHg vs. EDP20: 141 ± 25mmHg, $p<0.01$). Under Hypo, developed LVP and its duration did not show significant differences compared with those in Normo. However, increasing EDP under Hypo significantly decreased developed LVP (72 ± 13mmHg vs. 96 ± 16mmHg, $p<0.01$). The minimum value of the (1,0)/(1,1) intensity ratio (I_{min}) provided by the XRD analysis was used as an index of AMI. I_{min} showed a significantly negative correlation with developed LVP regardless of Normo or Hypo. The diastolic myosin filament lattice spacing (MFL) calculated from the diffraction angle of the (1,0) equatorial reflection would be reduced by increasing EDP. In contrast to a significantly positive MFL-I_{min} correlation under Normo, we observed the significantly negative MFL-I_{min} correlation under Hypo. We confirmed that the duration of Ca^{2+} transient was slightly longer but the amplitude of Ca^{2+} transient was unchanged under Hypo. **Conclusion**: These novel findings suggest that under Hypo the probability of AMI decreases even though the MFL was reduced with increasing preload. This is an underlying mechanism for reduced cardiac contractile performance under hypoxia.

3201-Pos Board B248

Influence Of Acidic pH On The Rate Of Force Development In Cardiac Muscle

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Cellular acidosis, a consequence of myocardial ischemia, reduces the Ca^{2+} sensitivity of cardiac contraction and maximal Ca^{2+} activated force in cardiac muscle. These effects are similar to that seen in fast (psoas) and slow (soleus) skeletal muscle with reduced pH. Previous studies have also demonstrated no effect of low pH on the rate of force redevelopment (k_{tr}) at maximal Ca^{2+} activation in slow and fast muscle fibers, but that k_{tr} is slowed at submaximal Ca^{2+} activation. However, it is unknown whether low pH affects calcium dependence of k_{tr} in cardiac muscle. To characterize the influence of acidic pH on k_{tr} we have measured Ca^{2+} activation of skinned cardiac trabeculae at pH 7.0 and 6.5. As in skeletal muscle, reduced pH significantly decreased isometric force in cardiac muscle at all levels of Ca^{2+} activation ($\Delta pCa_{50} = 0.73$). Interestingly, in contrast to skeletal muscle, k_{tr} at low pH in cardiac trabeculae was significantly faster at both maximal (pH 7 = 5.1 ± 0.5 s^{-1}, pH 6.5 = 6.9 ± 0.3 s^{-1}) and half-maximal (pH 7 = 3.0 ± 0.3 s^{-1}, pH 6.5 = 6.6 ± 0.2 s^{-1}) Ca^{2+} activation. This is consistent with previous studies showing increased force redevelopment in cardiac muscle when force is inhibited with phosphate, vanadate, or reduced sarcomere length. Our results support the idea that k_{tr} is negatively correlated to the size of the cross-bridge pool available for recruitment to cooperative activation of the thin filament. Force inhibition such as that seen with lower pH may reduce the cross-bridges available for recruitment, which would reduce this slowing effect and speed force redevelopment.. Supported by NIH R01 HL 65497 (MR), T32 HL07828 (FSK) and NSERC Discovery (TEG).

3202-Pos Board B249

Role of Strongly-Bound Crossbridges in Cooperative Cardiac Thin Filament Activation

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Experimental evidence indicates that activation of cardiac thin filaments is enhanced by strongly-bound myosin crossbridges and that crossbridge binding is in turn cooperatively regulated by end-to-end interactions between adjacent tropomyosins. We examined the impact of crossbridge binding and nearest-neighbor tropomyosin interactions on thin filament activation using a computational model.

We represented individual thin filament regulatory units (RUs) with the model of McKillop and Geeves [Biophys J, 1993 65(2)] wherein RUs are found in blocked (B, non-permissive), closed (C, permissive), and open (M, permissive with crossbridge) states. The B to C transition was assumed to depend upon Ca2+ concentration. Nearest-neighbor RU interactions were represented by causing transitions of the individual RU model to depend on the status of neighbors. Ensembles of N interacting RUs were modeled as Markov networks generated by considering all possible unique configurations of individual RUs (B, C, or M) within a chain.

The model-generated steady-state force-pCa curve (N=6) possessed a Hill coefficient of 3.0. Hill coefficients fit separately to portions of the curve below and above half activation were 3.2 and 2.4, respectively. Rate of force redevelopment following rapid slack/restretch (ktr) showed strong dependence on activation level (ktr=2.6 s-1 at pCa 6.0 vs. 9.3 s-1 at pCa 4.3). Increasing the cross-bridge duty cycle in the model increased myofilament Ca2+ sensitivity but had an opposite effect on Ca2+ sensitivity of ktr. Simultaneous matching of reported force and ktr sensitivities required a duty cycle of 30%. Increasing N toward 26 (a realistic filament length) tended to improve the fit with experiments. These results suggest that cycling crossbridges act through nearest-neighbor interactions along the thin filament to 1) increase myofilament Ca2+ sensitivity, 2) cooperatively enhance activation, and 3) slow the rate of force redevelopment at low levels of activation.

3203-Pos Board B250

Kinetics of ADP Release From Cycling Cross Bridges In Contracting Skinned Cardiac Muscle Monitored With A Fluorescent Probe

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Kinetics of ADP release from cycling cross bridges were studied in Ca activated skinned papillary muscle by displacement of fluorescent ADP bound to the cross bridges in AM*ADP state(s) by non-fluorescent ADP photogenerated from caged ADP. A strip of glycerinated papillary muscle (100um, 2mm) from guinea pig left ventricular in Ca-free solution was loaded with a mixture of non-fluorescent ATP (1mM), fluorescent 3′-amino-deoxy ATP (aminoATP) (50μM) and 5 mM caged ADP in the presence of an ATP back-up system. At the plateau of force at pCa5.8 the muscle was rapidly transferred into the photolysis trough filled with silicone oil and irradiated by a 437 nm laser pulse. Alternatively, a muscle loaded with only fluorescent amino ATP (50μM) was allowed to contract at pCa4.5 in oil and develop rigor with amino ADP bound to the cross bridges. Following photolysis of caged ADP the kinetics of force and fluorescent transients were found markedly different in contracting and rigor muscles. In contracting muscle the force and fluorescence both increased following caged ADP photolysis, while in rigor muscle the photolysis induced an increase in fluorescence, but decrease in force. Kinetics of ADP release estimated by the rate of fluorescence increase was significantly slower in contracting muscle than that in rigor: 2-4 s-1 vs 18-20s-1, suggesting that at least two different AM*ADP states exist during ATP hydrolysis by cycling cross bridge in contracting papillary muscle. Supported by NIH grant R03 AR05 2885 for A.K.

3204-Pos Board B251

Mechanoenergetics of Actomyosin Interaction Analyzed by Cross-Bridge Model

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We present a mathematical model of actomyosin interaction, as a further development of actomyosin model that links mechanical contraction with energetics (Vendelin et al, Annals of Biomedical Engineering: 28, 2000). The new model is a three-state Huxley-type model, with two strong binding states and one weak binding state, for cross-bridge interaction and a model of calcium induced activation. The force produced by the attached cross-bridge in strong binding state is assumed to be elastic and depends linearly on the axial distance z along the myosin and actin filaments between the equilibrium position of the myosin head and the nearest actin binding site. The model is self-consistent and is based on T. Hill formalism linking free energy profile of reactions and mechanical force.

In several experimental studies it has been shown that the dependency between oxygen consumption and stress-strain area is linear. Additionally, the relation between stress-strain area and oxygen consumption is the same for isometric and shortening contractions. In this work, we analyzed free energy profiles of actomyosin interaction by changing free energies of intermediate states and free energies of activation of different reactions.

In our simulations we replicated the linear dependence between oxygen consumption and stress-strain area together with other important mechanical properties of cardiac muscle such as developed stress dependence on the sarcomere length and force-velocity relationship.

3205-Pos Board B252

Increasing Heart Rate Decreased Actin-Myosin Interaction in Isolated Beating Rat Whole Heart

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Background: Heart rate (HR) is one of the determinant factors of cardiac performance. Failing human myocardium shows negative force frequency relation, whereas normal one shows positive relation. **Purpose**: To test the effect of HR on actin-myosin interaction (AMI) in beating rat hearts those have negative