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).

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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).

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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