determine whether myofilaments activated with ADP (without Ca) are sensitive to kinase treatment.
Results: We show that ADP, in the absence of Ca, accelerates cross-bridge cycling and force development in cardiomyocytes from HF patients, which was sensitive to PKA-mediated phosphorylation (i.e. decreased sensitivity to ADP). Ca-sensitivity increased in the presence of increasing [ADP] and was accompanied by significant slowing cross-bridge cycling kinetics. This was correlated with significant increases in residual force enhancement (i.e. high initial tension recovery).
Conclusions: The current data show that high [ADP] reduces the ability to desensitize myofilaments to Ca, which likely compromises restoration of end-diastolic length. High ADP increased cross-bridge strain (i.e. diastolic dysfunction) and depressed myofilament cycling kinetics, which may limit muscle shortening (i.e. systolic dysfunction). The present study suggests that inability to lower myocardial ADP levels can be a primary determinant of contractile dysfunction and disease progression in human HF.

1002-Plat
Myocardial Strain Rate Modulates the Speed of Relaxation in Dynamically Loaded Twitch Contractions
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Slow myocardial relaxation is an important clinical problem in about 50% of patients who have heart failure. Prior experiments had suggested that the slow relaxation might be a consequence of high afterload (hypertension) but large clinical trials testing this hypothesis have failed; lowering blood pressure in patients who have heart failure with preserved ejection fraction does not help clinical outcomes. We performed new experiments using mouse, rat, and human trabeculae (Chung et al., Biophys J. 106,564a, 2014) and showed that it is not afterload but the strain rate at end systole that determines the subsequent speed of relaxation. To investigate the molecular mechanisms that drive this behavior, we ran simulations of our mechanical experiments using the freely available software MyoSim (http://www.myosim.org). This software simulates the mechanical properties of dynamically activated half-sarcomeres by extending A.F.Huxley's cross-bridge distribution technique with calcium activation and cooperative effects. We discovered that our experimental data could be reproduced using a relatively simple framework consisting of a single half-sarcomere pulling against a series elastic spring. Further analysis of the simulations suggested that quick stretches speed myocardial relaxation by detaching myosin heads and thereby disrupting the cooperative mechanisms that would otherwise prolong thin filament activation. The simulations therefore identify myofilament kinetics and tissue strain rate as potential therapeutic targets for heart failure attributed to slow relaxation.

1003-Plat
Myosin MgADP Release Rate Decreases at Longer Sarcomere Length to Prolong Myosin Attachment in Skinned Rat Myocardial Strips
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Cardiac contractility increases as sarcomere length (SL) increases, suggesting that intrinsic molecular mechanisms underlie the Frank-Starling relationship to confer increased cardiac output with greater ventricular filling. Myosin's capacity to generate force is Ca^{2+}-regulated by thin-filament proteins and sarcomere length, which dictates the number of potential actin-myosin cross-bridge interactions. One mechanism underlying greater cardiac contractility at longer SL could involve longer myosin attachment duration (t_{on}). To test this idea, we used stochastic length-perturbation analysis in skinned rat papillary muscle strips to measure t_{on} as [MgATP] varied (0.05-5 mM) at 1.9 and 2.2 μm SL. From this t_{on}-MgATP relationship, we calculated cross-bridge MgADP release rate (k_{-ADP}) and MgATP binding rate (k_{+ATP}). As MgATP increased t_{on} decreased hyperbolically for both SL, but t_{on} was roughly 50% longer for 2.2 vs. 1.9 μm SL at each [MgATP] (25 ± 3 vs. 16 ± 1 ms at 5 mM MgATP, 17° C, $p<0.05$). These t_{on} differences arose from slower k_{-ADP} at 2.2 μm SL (42 ± 3 vs. 74 ± 8 s^{-1}, $p<0.001$), as MgATP binding rates did not differ with SL (281 ± 56 vs. 327 ± 93 mM^{-1} s^{-1}). Absolute tension values were greater at 2.2 vs. 1.9 μm SL for relaxed (4.4 ± 0.7 vs. 0.8 ± 0.2 kPa at pCa 8.0, $p<0.001$) and maximally activated (20.0 ± 1.4 vs. 14.2 ± 1.6 kPa at pCa 4.8, $p<0.001$) conditions, and the force-pCa relationship was more sensitive to Ca^{2+} at 2.2 μm SL ($pCa_{50}=5.45 \pm 0.01$ vs. 5.36 ± 0.01, $p<0.05$). These increased tension values suggest that cross-bridges may bear greater loads at longer SL, which diminishes MgADP release to prolong t_{on} and amplify cooperative cross-bridge contributions to thin filament activation. Therefore, load-dependent rates of the actomyosin cross-bridge cycle may vary with SL to contribute, in part, to the Frank-Starling relationship in the heart.

1004-Plat
Inherent Force-Dependent Properties of β Cardiac Myosin Contribute to the Force-Velocity Relationship of Cardiac Muscle
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The cardiac cycle is a tightly regulated process in which the heart generates power during systole and relaxes during diastole. Appropriate power must be generated to effectively pump blood against cardiac afterload. Dysfunction of this cycle has devastating consequences for affected individuals. Cardiac power output is regulated by several feedback mechanisms (e.g. neuronal, hormonal, mechanical) which ultimately lead to changes in the force and power output of the molecular motor, β-cardiac myosin (βCM). Despite its importance in driving and regulating cardiac power output, the effect of force on the contractility of a single βCM has not been measured at physiological [ATP]. Using optical trapping techniques, we found that similar to some other myosins, βCM has a two-substep working stroke where the second mechanical substep is associated with ADP release. At saturating [ATP] (4 mM), forces that resist the power stroke slow myosin-driven contraction, suggesting that the inherent properties of myosin contribute to the force-velocity relationship in muscle and play an important role in the regulation of cardiac power output. Based on our results and kinetic modeling, we propose that force inhibits the mechanical transition associated with ADP release, leading to slowing of the rate of ADP release, the same kinetic step that limits muscle shortening. These results have important implications for cardiac diseases which affect power output, such as heart failure and cardiomyopathies. This work was supported by the American Heart Association (14SDG18850009 to M.J.G.) and National Institutes of Health (R01GM057247 to E.M.O. and K99HL123623 to M.J.G.).

1005-Plat
Effect of Mutations in cMyBP-C on Sarcomere Mechanical Function
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A significant number of cardiac myopathies in children and adults are caused by mutations in the cMyBP-C gene. In disease, cardiac output is compromised by altered cardiac muscle fiber contractility due to modulated interactions between cMyBP with actin or with S2. Comparative measurements in WT and knockout mice (cMyBP-$C^{-/-}$) muscle fibers have showed increased isotonic shortening velocity, power output and rate of force redevelopment in absence cMyBP-C. Thus, change of only a few amino acids in mutant cMyBP-C, especially in regions rich with phosphorylation sites, may cause significant change in dynamics of muscle contractility. Comparison of measured sliding velocities of actin filaments over the regions of myosin filament with and without cMyBP-C in motility assays have provided molecular insight how these structural changes alter the kinetics of the interactions of cMyBP-C with myosin and actin filaments. We used a multi-scale, computational modelling platform, **MUSICO,** (**MU**scle **SI**mulation **CO**de) to assess the effect of cMyBP-C mutations on sarcomere contraction. This platform includes explicit 3-D sarcomere structures, extensible actin and myosin filaments, various models for the acto-myosin cycles, thin filament regulation via a continuous flexible chain (CFC) model and now cMyBP-C using the kinetic parameters for dynamically forming and disrupting connections between cMyBP-C and actin, derived from the motility studies. We compared the model predictions between different mutations and the corresponding mechanical experiments. The predictions from cMyBP-C sarcomeric model showed significant differences between the mutants, and closely followed observations. This results allow the quantitative evaluation of the role of cMyBP-C in the regulation of sarcomere structure and function, the development of a multi-scale myoarchitectural representation of disease phenotype and the creation of a novel diagnostic and prognostic methodology for tracking disease progression in patients.
Supported by: R01s AR048776 and DC 011528

1006-Plat
The A31P Hcm Mutation in cMyBP-C Disrupts the Structure of the C0 Domain But Does Not Cause Haploinsufficiency in a Population of Older Cats Heterozygous for the A31P Allele
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