Ca-activated SPOC conditions: (1) MYBPC3 mutants display depressed contractility; (2) this effect is independent of the gene variant but is consistent with a mechanism of haplotype insufficiency (rather than ''poison peptide'' effect); and (3) HCM mutations display different phenotypes when examined under conditions of low strain.

**1755-Pos Board B485**

**The Role of Cardiac Myosin Light Chain 2V Phosphorylation in the Healthy and Failing Myocardium**

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While a number of factors underlie cardiac dysfunction in HF, recent attention has fallen on phosphorylation changes in myofilament proteins, notably the regulatory proteins. Cardiac myosin regulatory light chain (cMLC2) is a 166 amino acid protein located on the hinge region of myosin heavy chain and optimally positioned to modulate contraction. Recent studies have shown that cMLC2v contributes to regulating cardiac pump function and allows for beat-to-beat tuning of the myosin motor arm. Humans, as well as large rodents such as rabbits, have high sequence homology and a single phosphorylation site at ser15. We hypothesize that human MLC2v phosphorylation is a critical regulator of myocyte contraction that is dysregulated in HF and that increasing MLC2v phosphorylation with myosin light chain kinase (MLCK) can partially reverse the dysfunction seen with HF. Human donor or HF permeabilized cardiac tissue were incubated with either alkaline phosphatase to reduce phosphorylation or PKA to induce maximal phosphorylation of the other myofilament proteins. Maximal cMLC2v phosphorylation was then achieved by incubation with MLCK. Force-calcium relationship, rate of tension redevelopment, tension cost and length dependent activation was determined. To assess the role of cMLC2v phosphorylation on dynamic regulation of length dependent activation, intact rabbit cardiomyocytes were isolated from rabbit hearts and transfected with MLCK to provide maximal cMLC2v phosphorylation. Cells were then attached using a force transducer and length controller and a simulated length-force relationship, which approximate the pressure-volume relationship in vivo, was evaluated at different diastolic sarcomere lengths. We show that MLC2v phosphorylation is a critical regulator of myocyte contraction that is dysregulated in the failing heart and treatment with MLCK can partially reverse the dysfunction seen with HF.

**1756-Pos Board B486**

**Existence of Length-Dependent Modulation of Cross-Bridge Cycling Kinetics in Right Ventricles of Non-Failing and Failing Human Myocardium**

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Heart failure affects more than 5 million patients in the United States. The mechanisms by which the heart regulates cross-bridge cycling rate and if and how such regulation is altered in heart failure are poorly understood. One possible modulator of this cycling rate is muscle length. In a previously published study, we showed that the rate of tension redevelopment ($k_{tr}$) can be reproducibly measured in intact cardiac trabeculae using a novel approach. Furthermore, we showed that slacking trabeculae from $L_{opt}$ corresponding to end-diastolic volume *in vivo*) to $L_{90}$ (corresponding to end-systolic volume *in vivo*) increases $k_{tr}$ by about 60%. While this regulation is present in a small animal model, its role in human myocardium in both health and disease remains unanswered. Therefore, we extended our studies using our novel method to right ventricular trabeculae isolated from non-failing donor and patients with heart failure. The failing samples had preserved length-tension relationship, reduced force-frequency relationship, and β-adrenergic desensitization as compared to non-failing donors. Contractile and relaxation kinetics of twitch of both non-failing and failing trabeculae were slowed down with increasing muscle length from $L_{90}$ to $L_{opt}$. We were able to reproducibly measure $k_{tr}$ in intact human cardiac trabeculae; $k_{tr}$ during maximal myofilament activation in both non-failing and failing samples decreased with increasing muscle length. This effect of muscle length on $k_{tr}$ was also present at submaximal activation. These effects can be potentially due to post-translational modifications. We have preliminary $k_{tr}$ data after rapid length changes during maximal activation and PKA/PKCBII inhibition at maximal and sub-maximal activation levels. The overall data provides evidence that muscle length can regulate cross-bridge cycling kinetics in both non-failing and failing human hearts.

**1757-Pos Board B487**

**Increased CAMKII Activity Impairs Contractile Function in Human HCM Myocardium**

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**Background and Rationale:** we have previously shown that the myocardium of patients with Hypertrophic Cardiomyopathy (HCM) shows increased CaMKII activity with respect to non-HCM non-failing surgical patients (controls). We found higher phosphorylation levels of several tested CaMKII targets, such as L-Type $Ca^{2+}$ channels, $Na^{+}$ channels and phospholamban (Coppini et al., Circulation 2013). Such changes are likely to determine significant deleterious effects on the electrical and mechanical function of HCM myocardium.

**Methods:** we assessed the effects of a selective inhibitor of CaMKII, the cell-permeant Autocamtide-related Inhibitory Peptide II (AIP-II), on force and intracellular $Ca^{2+}$ in intact trabeculae and cardiomyocytes isolated from surgical septal samples of HCM patients who underwent myectomy.

**Results:** HCM trabeculae display an increased diastolic tension and a slower kinetics of isometric force generation when compared to control trabeculae. AIP-II determines a reduction of diastolic tension, with larger effects at fast pacing rates ($-13 \pm 3$ % at 2Hz). Additionally, AIP-II accelerated the kinetics of force generation in HCM muscles (time to peak contraction at 1Hz: $255 \pm 17$ ms at baseline vs. $232 \pm 10$ ms with AIP-II, $p<0.05$). Accordingly, in HCM cardiomyocytes AIP-II lowered intracellular diastolic $Ca^{2+}$ levels (from 278 ± 40 nM to 159 ± 28 nM, $p<0.01$) and reduced the frequency-dependent increase of diastolic $[Ca^{2+}]$. The kinetics of $Ca^{2+}$ transients, however, were not significantly affected by AIP-II. This suggests that reduced CaMKII phosphorylation of myofilament proteins may underlie the accelerated contractile kinetics observed in HCM trabeculae following AIP-II exposure.

**Discussion:** The results indicate that increased activation of CaMKII plays a significant role in determining the abnormalities of myocardial contraction in HCM. Pharmacological inhibition of CaMKII may represent a viable option to reduce diastolic dysfunction in HCM patients.

**1758-Pos Board B488**

**The Cardiac Troponin T R92L Hcm Mutation Alters Cardiac Troponin I Dynamics and PKA Phosphorylation Potential**

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Mutations in cardiac troponin T (cTnT) have been linked to hypertrophic cardiomyopathy (HCM). Previous experiments in our lab utilizing a novel cTnT R92L transgenic mouse model revealed an allosterically mediated decrease in the PKA phosphorylation potential of cardiac troponin I (cTnI). We hypothesized the cTnT R92L mutation causes a change in the dynamics of the N-terminal extension, hindering the availability of PKA substrates S23/S24 on cTnI. In the current study this hypothesis was tested both *in silico* and *in vitro*. First, molecular dynamics (MD) simulations using our all-atom thin filament model in the $Ca^{2+}$ saturated state were performed. MD predicted an approximately 7Å narrowing between the N-terminal extension of cTnI and cardiac troponin C (cTnC). To experimentally evaluate the in-silico predictions, Förster Resonance Energy Transfer (FRET) experiments were conducted on fully reconstituted thin filaments. The cTnI was labeled with the FRET donor AEDANS at three sites on the N-terminal extension of cTnI. The acceptor DDPM, was labeled on six sites on cTnC. Steady state and time resolved FRET experiments were performed in the presence and absence of the TnT R92L mutation. Both theoretical and experimental approaches revealed that in the presence of the cTnT R92L mutation the dynamics of cTnI was allosterically altered. The N-terminal extension of cardiac troponin I moved closer to the N-domain of cTnC. This anharmonic fluctuation likely hinders the availability of the PKA substrate sites and leads to the observed blunting of the beta-adrenergic response in animal models and human patients.