difference is greatly accentuated during acidosis, which is often associated with ischemia of myocardial infarction. *In vitro* and *in vivo* functional studies have shown that replacement of cTnI with ssTnI results in a marked enhancement of myofilament $Ca^{2+}$-sensitivity at acidic pH conditions. Recent reports have indicated that this effect can be ascribed to amino acid sequence differences in ssTnI and cTnI and in particular to a critical A162H substitution in the switch region. In this study, we have used NMR spectroscopy to examine the binding of the switch regions of ssTnI ($sTnI_{115-131}$) and cTnI ($cTnI_{147-163}$) to the N-domain of cardiac troponin-C (cNTnC) at physiological and acidic pH conditions. The results show that the affinity of $sTnI_{115-131}$ for cNTnC•$Ca^{2+}$ ($K_D$ ~50uM) is ~3-fold stronger than that of $cTnI_{147-163}$ ($K_D$ ~150uM), but neither are affected by a pH change from 7 to 6. The pKa of H130 in $sTnI_{115-131}$ is 6.2 when free and 6.7 when bound to cNTnC•$Ca^{2+}$. We have also used {$^1$H, $^{15}$N}-HSQC NMR spectroscopy to monitor the pKa changes of cNTnC•$Ca^{2+}$ from peptide free to peptide bound states. The implications of these results will be discussed in the context of structure and function of myofilament protein interactions.

**763-Pos**

**Structure and Dynamics of Cardiac Troponin C using Paramagnetic Relaxation Enhancement Derived Distances**

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We have prepared four spin-labeled single cysteine mutants of isolated cardiac TnC (cTnC) in order to examine the conformation of the $Ca^{2+}$-loaded *N*-domain and the interdomain dynamics using PRE-NMR. The long-range PRE distances (10 - 30 Å) measured within the regulatory *N*-domain were compared to TnC structures in the Protein Data Bank. Q-factor statistics were used to rank all available TnC PDB structures according to their agreement with our experimentally derived distances with scores ranging from 0.16 (best) to 0.30 (worst). The energy minimized solution structure of isolated human cTnC (1AJ4, Sia *et al*, JBC 1997) demonstrated the best correlation with our PRE data for the *N*-domain. Interdomain dynamics of our isolated cTnC were also examined by comparing PRE distances to available ensembles of TnC structures. Our results indicate that isolated cTnC is more compact than the skeletal TnC isoform, and that the central domain linker is highly flexible with a defined range of relative domain orientations. We also present a simple approach for modeling of the spin label position and mobility using PRE distances which is applicable to all spin-labeled systems for which there are existing structural models.

**764-Pos**

**Protein Kinase a Phosphorylation of Cardiac Troponin I Prevents Cardiac Hypertrophy in Mice**

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R21C is the only FHC-associated mutation located in the N-terminal domain of cardiac TnI (cTnI) and is within the consensus sequence for PKA phosphorylation ($RR_{21}$RSS). We have developed an R21C cTnI knock-in (KI) mouse model and have evaluated the mouse hearts for biochemical, biophysical, structural and functional changes. Our results show that the R21C KI mice (heterozygous;R21C+/- and homozygous;R21C+/+) developed the FHC phenotype as evidenced by the presence of hypertrophy and fibrosis. Some hypertrophic markers such as, ANP, BNP and β-MHC were found elevated at a late age (18 months). The R21C+/- and R21C+/+ mice had decreased phosphorylation at Ser23/24 (~18% and 90%, respectively) compared to WT mice. Top down mass spectrometry of cTnI from the R21C+/- mice demonstrated a molar ratio of 1:4 R21C:WT in the hearts. Using three different methods to sacrifice the WT and mutant mice, we did not find any significant decrease in the $Ca^{2+}$ sensitivity of force upon PKA treatment. Western blot analysis of these mice showed that the endogenous cTnI in the WT and R21C+/- mice is completely phosphorylated at Ser23/24. However, when mice were treated with propanolol (β-adrenergic receptor antagonist) before sacrifice, the $Ca^{2+}$ sensitivity was decreased after PKA treatment of the WT (0.25 pCa) and R21C+/- mice (0.14 pCa). In contrast the R21C+/+ mice did not show a significant decrease in $Ca^{2+}$ sensitivity after PKA treatment. No significant changes were found in the maximal force in all three mice, before and after PKA treatment. Our results suggest that the primary mechanism for producing hypertrophy in the R21C mice results from the impaired ability of the myofilament to respond to the desensitizing effects of PKA phosphorylation. Supported by NIH grant HL042325

**765-Pos**

**Structure and Dynamics of the Mobile Domain of Troponin I by SDSL-EPR**

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The Troponin (Tn) molecular switch contains highly dynamic regions which allow for $Ca^{2+}$ induced conformational changes to be propagated through to the thin filament. The Mobile domain (Md) of TnI, a secondary thin filament binding domain, is a key player in this process. The functional importance of the Md is also highlighted through the clustering of cardiomyopathy mutations. Structural elucidation of this region by traditional methods is often limited by the absence of key thin filament binding partners. Current Md models describe a highly dynamic region with either a nascent or a well-defined structure. We have utilized Site-Directed Spin Labeling Electron Paramagnetic Resonance (SDSL-EPR) to elucidate the structure of the Md upon interaction with the thin filament. EPR mobility measurements from cysteine scanning of the Md (res. 175-206) in the reconstituted thin filament show a highly dynamic domain in the $+Ca^{2+}$ (ON) state. A decrease in the mobility occurs in the $-Ca^{2+}$ (OFF) state, indicating interaction with the thin filament. Further, trends in the mobility of the EPR label reveal two helical structural components within the Md (res. 175-179 & 192-202). Conventional EPR methods were used to measure three interspin distances (176/178, 176/179 & 176/180) which further confirm this assignment. Double Electron-Electron Resonance (DEER) was used to measure the longer interspin distance (178/206) and found that the Md exists in an extended conformation ($34 \pm 26$Å). An extended helical structural model for the interaction of the Md with the thin filament through electrostatic bonding is proposed. Residues involved in cardiomyopathy are found clustered at the interacting interface.

**766-Pos**

**Desensitizing Effect of N-terminal Truncated cTnI In RCM Myofibrils**

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Cardiac TnI (cTnI) mutations have been associated with the development of restrictive cardiomyopathy (RCM) characterized by a $Ca^{2+}$ hypersensitivity and diastolic dysfunction in cardiac myofibrils. Whereas cTnI N-terminal deletion (cTnI-ND) caused by restricted proteolysis in cardiac adaptation to stress manifests a lower left ventricular end diastolic pressure and an enhanced ventricular diastolic function. By crossing the RCM cTnI R193H transgenic mice ($cTnI^{193His}$) with cTnI-ND transgenic mice (cTnI-ND) that contain 100% cTnI-ND in the heart, we have obtained double TG mice containing both the cTnI R193H mutant and cTnI-ND. In this study, by using these TG mouse lines, we have investigated the desensitizing effect of cTnI-ND on the RCM cTnI mutant mice and myofibrils. Our survival data for these mice indicated that cTnI-ND greatly reduced the mortality of the RCM $cTnI^{193His}$ mice. $Ca^{2+}$ sensitivity measured in skinned myofibrils confirmed that increased myofibril $Ca^{2+}$ sensitivity was the major mechanism that resulted in impaired relaxation and diastolic dysfunction in RCM $cTnI^{193His}$ mice and that cTnI-ND could reverse the cellular dysfunction by desensitizing the myofibrils to $Ca^{2+}$. The PKA stimulation assays showed that $cTnI^{193His}$ myofibrils were able to respond to PKA activation, resulting in a right-shift of pCa curve after PKA treatment. However, since the myofibrils from cTnI-ND hearts lacked Ser residues 23 and 24, they had no response to PKA stimulation, showing a similar pCa curve before or after PKA. Our data have, for the first time, demonstrated a desensitizing effect by an endogenous myofibril protein proteolysis without the intervention of β-adrenergic stimulation mediated cTnI phosphorylation. The desensitizing function in cTnI-ND hearts indicates that the removal of cTnI N-terminal extension by restricted proteolysis represents a novel mechanism to improve myofibril relaxation and cardiac diastolic function in cardiac adaptation to hemodynamic and inotropic stresses.

**767-Pos**

**Effects of Pseudo-Phosphorylation of cTnI by $P^{21}$ Activated Kinase-3 (PAK3) on Structure and Kinetics of $Ca^{2+}$-Induced Cardiac Thin Filament Regulation**

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Residue Ser151 of rat cardiac troponin I (cTnI) can be phosphorylated by P21-activated kinase 3 (PAK3). It has been found that PAK3 phosphorylation of cTnI induces an increase in $Ca^{2+}$ sensitivity of myofilament, but detailed mechanism is unknown. We investigated the structural and kinetic effects of phosphorylation of cTnI PAK3 site Ser151 on the $Ca^{2+}$-induced thin filament regulation. Using

steady-state and time-resolved Förster Resonance Energy Transfer (FRET) measurements and stopped-flow kinetic studies, we measured $Ca^{2+}$-induced changes in FRET distance from the residues 160 and 167 in the regulatory region of cTnI to the residue 89 of cTnC to monitor cTnC and cTnI interactions. The measurements were done with the reconstituted thin filament containing PAK3 pseudo-phosphorylation of cTnI(S151E). We hypothesized that the charge modification at the interface between troponin C (cTnC) and cTnI caused by the phosphorylation at the N-terminus of the regulatory region of cTnI may affect the binding of the regulatory region of cTnI to cTnC. Our results showed that the pseudo-phosphorylation of cTnI(S151E) favors the binding by shortening the distances between the regulatory region of cTnI and cTnC and increasing $Ca^{2+}$ sensitivity of the structural change. Furthermore, the pseudo-phosphorylation showed similar kinetic effects as the strongly-bound crossbridges on the thin filament regulation by significantly slowing down the kinetics of the $Ca^{2+}$ dissociation-induced structural transitions of the regulatory region of cTnI. This is consistent with the decreased tension cost observed in the tension measurements of cardiac muscle fiber bundle reconstituted with the pseudo-phosphorylated cTnI, which suggest a decrease in crossbridge detachment rates. Our results provide novel information on the potential molecular mechanism underlying modulation of cardiac thin filament regulation by PAK3 phosphorylation of cTnI.

**768-Pos**

**Fast-To-Slow Fiber Type Switch Increases Fatigue Resistance as a Compensatory Adaptation In Gsα-Deficient Soleus Muscle**

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Genetically modified mice with Gsα-specific deficiency in skeletal muscle showed reduced glucose tolerance, muscle atrophy and force reduction, along with a fast-to-slow fiber type switch (Chen et al., AJP 296:C930-40, 2009). We further investigated a hypothesis that the switching to more slow fibers is an adaptive response with functional significance. Corresponding to the muscle type switch evident by myosin isotyping, the thin filament regulatory proteins troponin T and troponin I both had significant changes to their slow isoforms in the atrophic soleus muscle of 3-month-old Gsα-deficient mice. This fiber type switching progressed and soleus muscles of one-year-old Gsα-deficient mice expressed only slow isoforms of troponin. Functional characterization of soleus muscle of 3-month-old Gsα-deficient mice showed slower contractile and relaxation velocity in twitch and tetanic contractions than wild type controls. Examination of fatigue tolerance showed that Gsα-deficient soleus muscle was more resistant to intermittent fatigue stimulation with faster and better recovery as compared with wild type controls. Our results suggest that fast-to-slow type switch improves fatigue resistance of skeletal muscle as a compensatory adaptation to muscle glucose intolerance and atrophy in Gsα-deficiency, suggesting a mechanism for improving muscle function in diabetic patients.

**769-Pos**

**Strong Crossbridges are Required to Recapitulate the $Ca^{2+}$ Affinity Changes Produced by HCM-cTnC Mutants in Skinned Fibers**

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This spectroscopic study examines the steady state and kinetic parameters governing the crossbridge effect necessary to increase the $Ca^{2+}$ affinity of hypertrophic cardiomyopathy-cardiac troponin C (HCM-cTnC) mutants to the level seen in skinned fibers. Previously, it was shown by Landstrom, *et al.* (*J. Mol. Cell Card.* 45:281-288; 2008) and Pinto, *et. al.* (*J. Biol. Chem* 284(28): 19090-19100; 2009) that the cTnC mutations A8V, C84Y, E134D and D145E do not increase the apparent $Ca^{2+}$ affinity of isolated cTnC (D145E shows a slight increase) as monitored by 2-(4′-(2″-iodoacetamido)phenyl)aminonaphthalene-6-sulfonic acid (IAANS) fluorescence. Follow-up experiments showed that when cTnC mutants are incorporated into regulated thin filaments (RTF), only the A8V mutant increased the apparent $Ca^{2+}$ affinity. Addition of myosin subfragment-1 (S1) to mutant RTFs (in the absence of ATP) increased the apparent $Ca^{2+}$ affinity to similar levels seen in cTnC mutant reconstituted skinned fibers. Therefore, strong crossbridges were required to fully alter the apparent cTnC $Ca^{2+}$ affinity and recapitulate the changes observed in the $Ca^{2+}$ sensitivity of tension. Stopped flow fluorescence techniques were also used to measure the kinetics of $Ca^{2+}$ binding to troponin complex (cTn) and RTF prepared with IAANS labeled cTnC mutants. At the cTn level, both A8V and D145E cTnC decreased the rate of $Ca^{2+}$ dissociation; while in the RTF, only A8V decreased the rate of $Ca^{2+}$ dissociation. Future experiments will determine the rate of $Ca^{2+}$ dissociation from RTFs in the presence of S1. This study indicates that although these HCM-cTnC mutants display similar phenotypes in skinned fibers, they utilize different molecular mechanisms to alter the $Ca^{2+}$-sensitivity of skinned muscle. Supported by NIH HL-42325 (JDP) and AHA 0825368E (JRP) and AHA 09POST2300030 (MSP).

**770-Pos**

**Changes in the Conformation of Troponin C on Activation of Skeletal Muscle**

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Skeletal muscle contraction is regulated by calcium-dependent changes in the structure and thin-filament location of troponin and tropomyosin. The structural changes in the isolated calcium-binding subunit of troponin (TnC) are well characterized, but those of TnC in the native thin filament are much less clear. We measured the *in situ* orientation of the C-terminal lobe of TnC (CTnC) by polarized fluorescence from bifunctional rhodamine (BR) probes cross-linking pairs of cysteines at TnC residues 96-103, 116-123, 132-139, and 119-135. Each BR-labeled TnC was exchanged into single permeablized fibers from rabbit psoas muscle, and polarized fluorescence from the BR-TnCs was measured during relaxation and maximal calcium activation. The orientation distribution of CTnC with respect to the thin filament axis was calculated by maximum entropy analysis using the *in vitro* structure of CTnC in the troponin core complex (Vinogradova *et al.* (2005) *PNAS***102**:5038-5043). The peak angle between E helix of CTnC and the filament axis was 49° in relaxed muscle and 64° during maximal activation. Comparison with the results of our previous study of the orientation of the N-terminal lobe of TnC (Ferguson *et al.* (2003) *Mol. Cell***11**: 865-874) suggests that the central D/E helix of TnC is bent by about 30° in relaxed muscle and becomes straight during maximal activation.
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**771-Pos**

**The Perturbation of the Open-Closed Transition of Troponin C by the Mutation L48Q Leads to an Enhanced Troponin I Affinity**

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Cardiac muscle contraction is regulated by $Ca^{2+}$ binding to the N-domain of troponin C (cNTnC). Following $Ca^{2+}$ association, the relocation of troponin I from actin to troponin C triggers contraction. In a diseased heart, there is a desensitization of the myocardium for $Ca^{2+}$, and one treatment strategy is to use pharmaceuticals that stabilize the open conformation of cNTnC, and thus enhance its interaction with troponin I ($cTnI_{147\text{-}163}$). Another option would be to engineer variants of troponin C that resemble the drug-induced open state of cNTnC. One possible mutant, L48Q, has been shown to increase thin filament $Ca^{2+}$-sensitivity. L48 is involved in forming crucial hydrophobic interactions with F20 and A23 in both the apo and $Ca^{2+}$-bound forms of cNTnC. The replacement of leucine with glutamine decreases the hydrophobicity in this region, and therefore may destabilize the closed state of cNTnC. We used nuclear magnetic resonance (NMR) to investigate how the L48Q mutation might increase thin filament $Ca^{2+}$-sensitivity. We found that the affinity of L48Q-cNTnC for $cTnI_{147\text{-}163}$ was enhanced by ~3 fold, with a $K_D \sim 50 \in \mu M$ (wt-cNTnC; $K_D \sim 150 \in \mu M$). We have developed a computational method to predict the tertiary structural changes in cNTnC by comparing the $^1H,^{15}N$ - HSQC spectra with control spectra from open and closed forms of cNTnC. The chemical shift patterns of residues in the defunct $Ca^{2+}$-binding site I of L48Q-cNTnC resemble the $cTnI_{147\text{-}163}$-bound form of wt-cNTnC, indicative of a more open state. We conclude that the L48Q mutation disrupts the hydrophobic packing of cNTnC such that it stabilizes a more open state of cNTnC, and it is this structural perturbation that is responsible for the enhanced affinity of L48Q-cNTnC for $cTnI_{147\text{-}163}$.

**772-Pos**

**Effects of Cardiac TnC Variants on cTnC-cTnI Interaction;Solution and Molecular Dynamics Simulation Studies**

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To better understand the complex protein interactions involved in cardiac muscle contractile activation we have developed a series of troponin C (cTnC) variants with increased or decreased Ca2+ binding affinity (in solution) that alter Ca2+ regulation of force development. We have previously reported that increasing or decreasing Ca2+ binding affinity by substitution of glutamine for leucine at residue 48 (L48Q cTnC) or isoleucine at residue 61 (I61Q) increased or decreased (respectively) Ca2+ sensitivity of steady state force in rat skinned