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.

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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).

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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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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 $cTnI_{147\text{-}163}$.

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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