of cardiac troponin C's defunct Ca^{2+}-binding site (site 1). In this study, we combined in vitro and in situ structural and functional techniques to elucidate the role this mutation may play in the modulation of troponin's function. We used Nuclear Magnetic Resonance to solve the structure and characterize the backbone dynamics of the regulatory lobe of troponin C with this mutation. The overall structure and dynamics of troponin C was not significantly altered by L29Q; however there was a slight rearrangement of site 1 making it more similar to trout cardiac troponin C, which also has a glutamine at position at residue 29 and displays increased Ca^{2+} sensitivity. Backbone dynamics measurements indicated that Q29 was more flexible than L29. The structure and function of L29Q was also assessed in demembranated ventricular trabeculae using Fluorescence for In Situ Structure. The structure and/or orientation of the regulatory lobe of troponin C was slightly perturbed by L29Q in relaxing conditions and was unaffected at activating Ca^{2+} concentrations. The Ca^{2+} sensitivity of the structural change and contractility were both unaltered by the L29Q mutation, suggesting that while this may cause a small change in the structure of troponin C, this does not translate to a large functional effect in cardiac muscle.

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Troponin I Ser-150 Phosphorylation Sustains Troponin Ca^{2+} Sensitivity in an Acidic Environment

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A hallmark of cardiac ischemia is decreased intracellular pH which can affect a number of cellular processes. Such an acidic environment alters cardiac troponin (Tn) myofilament regulation to decrease Ca^{2+} sensitive force production. Tn also undergoes cardiac ischemia-induced AMPK troponin I (TnI) Ser-150 phosphorylation. We recently characterized the effects of TnI Ser-150 phosphorylation demonstrating that it blunted the functional effects of canonical TnI Ser-23/24 phosphorylation; however, the role of Ser-150 phosphorylation in ischemia remains unknown. As an initial step, we sought to investigate the effect of acidic pH on myofilament regulation in the presence of TnI Ser-150 phosphorylation alone and in combination with Ser-23/24 phosphorylation. We first investigated the effect of in vivo cardiac ischemia on levels of TnI Ser-150 and Ser-23/24 phosphorylation. Exposure to 30 minutes of regional ischemia resulted in elevation of both TnI Ser-150 and Ser-23/24 phosphorylation. Next we determined the effects of TnI Ser-150 pseudo-phosphorylation (S150D) on the myofilament by measuring troponin C (TnC) Ca^{2+} binding properties at normal and acidic pH. Results demonstrate acidic pH decreases steady-state Ca^{2+} binding to TnC in reconstituted thin filaments across all Tn (WT, S150D, S23/24D, and S23/24/150D) such that TnI S150D Ca^{2+} sensitivity at pH 6.5 is similar to WT at pH 7. Decreasing the pH had no effect on Ca^{2+} dissociation such that compared to WT, S23/24/150D remained fast while S150D was slowed. We conclude that TnI Ser-150 phosphorylation imparts resistance to acidic pH-induced myofilament Ca^{2+} desensitization while retaining increased Tn Ca^{2+} dissociation when in combination with Ser-23/24 phosphorylation suggesting the potential for an increase in force while maintaining accelerated Ca^{2+} dissociation. Future investigations are aimed at examining the effect of TnI Ser-150 and Ser-23/24 phosphorylation on protease cleavage of TnI.

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Deficiency of Slow Skeletal Muscle Troponin T Causes Atrophy of Type I Slow Fibers and Decreases Tolerance to Fatigue

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Loss of slow skeletal muscle troponin T (ssTnT) due to a nonsense mutation at codon Glu180 in exon 11 of the TNNT1 gene causes a severe form of recessive nemaline myopathy (Amish nemaline myopathy, ANM). To investigate the pathogenesis and muscle pathophysiology of ANM, we studied the phenotypes of partial and total loss of ssTnT in Tnnt1 gene targeted mice. An insertion of neomycinR cassette in intron 10 of Tnnt1 caused approximately 60% decrease in ssTnT protein expression whereas deletion of exons 11-13 using cre-loxP approach resulted in total loss of ssTnT as that seen in the muscle of ANM patients. In diaphragm and soleus muscles of the knockdown and knockout mouse models, we demonstrated that ssTnT deficiency resulted in significantly decreased levels of other slow fiber-specific myofilament proteins while fast fiber-specific myofilament proteins were increased. Histology studies revealed that ssTnT deficiency caused significant atrophy of type I slow fibers and a hypertrophic growth of type II fast fibers. Along with the slow fiber atrophy and the changes in myofilament protein isoform contents, ssTnT deficiency in soleus muscle shifted the force-frequency relationship toward the fast muscle type and significantly reduced the tolerance to fatigue. ssTnT deficient soleus muscle also exhibited a significant number of smaller size central nuclei type I fibers, indicating an adaptive regeneration. ssTnT deficient mouse soleus muscle contained apparently normal number of spindles, in which intrafusal fibers were positive for type I myosin with a trend of atrophic morphology. The results demonstrate the essential function of ssTnT in skeletal muscle and the causal effect of its loss on the pathology of ANM.

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Attentuating the Depressive Effect of Acidosis with Mutations in Troponin and with 2-Deoxy-Atp

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Repeated, intense contractile activity compromises the ability of skeletal muscle to generate force and velocity, which defines fatigue. The decrease in velocity is thought to be due, in part, to the intracellular build-up of acidosis inhibiting the function of the contractile proteins myosin and troponin; however, the underlying molecular basis of this process remains unclear. We sought to gain novel insight into the decrease in velocity by determining if the depressive effect of acidosis could be altered by 1) introducing Ca^{++}-sensitizing mutations into troponin (Tn) or 2) by agents that directly affect myosin function, including inorganic phosphate (P_i) and 2-deoxy-ATP (dATP) in an *in vitro* motility assay. Acidosis reduced regulated thin filament velocity (V_{RTF}) at both maximal and sub-maximal Ca^{++} levels in a pH-dependent manner. A truncated construct of the inhibitory subunit of Tn, R156, and a Ca^{++}-sensitizing mutation in the Ca^{++}-binding subunit of Tn, V43Q, increased V_{RTF} at sub-maximal Ca^{++} under acidic conditions, but had no effect on V_{RTF} at maximal Ca^{++} levels. In contrast, both 15mM P_i and replacement of ATP with dATP reversed much of the acidosis-induced depression of V_{RTF} at saturating Ca^{++} (0.7 ± 0.1 control, 2.0 ± 0.3 with P_i, 1.8 ± 0.3 with dATP, 3.8 ± 0.1 with both P_i and dATP), with the combined effect fully restoring the V_{RTF} to the value under control conditions. Interestingly, despite producing similar magnitude increases in V_{RTF}, the combined effects of P_i and dATP were additive, suggesting different underlying mechanisms of action. These results suggest that the major mechanism by which acidosis slows V_{RTF} is through directly slowing myosin's rate of detachment from actin.

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The Effect of Truncated Troponin Components on Activation of Lethocerus Flight Muscle

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Indirect flight muscle (IFM) of *Lethocerus* is activated by periodic stretches at a constant priming concentration of calcium. The muscle is unusually stiff and stress is transmitted to the thick and thin filaments by kettin, which reinforces links between both filaments and the Z-disc. The activating effect of stress on thin filaments is likely to affect troponin. The isoforms of troponin in IFM differ from those in other muscles. TnT has a C-terminal extension not present in vertebrate TnT; TnH is an isoform of TnI with a C-terminal extension rich in Pro and Ala; TnC is present in two isoforms: F1 binds a single calcium in the C-lobe and is needed for stretch-activation; F2 binds one calcium in both N- and C-lobes and is needed for isometric force. Under conditions of low ionic strength, native fibres have a force-pCa curve that shows high calcium-sensitivity and low cooperativity ($pCa_{50} = 6.2$, $n_H = 1.3$). Fibres with F2 alone have a pCa curve similar to that of cardiac muscle, ($pCa_{50} = 5.8$, $n_H = 3.2$). A fragment of F1 without the N-lobe (F1-Ct) inhibits stretch-activation; therefore the N-lobe of F1 is necessary, although it does not bind calcium or TnH. F1-Ct is displaced by F2 and isometric force is restored, but not stretch-activation. We hope to show the effect of replacing endogenous troponin in fibres with a complex containing TnT truncated at the C-terminus, TnH with TnI sequence but without the Pro-Ala extension, and either F1 or F2. This will show how important the IFM isoforms of troponin are to the stretch-activation response.

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Changes in the Orientation of the Myosin Light Chain Domain (LCD) Associated with Thick Filament-Based Regulation of Skeletal Muscle

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The dependence of myosin LCD orientation on temperature, myofilament lattice spacing and sarcomere length was determined using fluorescence