expressed in both slow skeletal and cardiac muscle. The cTnC HCM mutations A8V, C84Y and D145E increase the Ca^{2+}-sensitivity of contraction in cardiac muscle; however, their functional consequences in slow skeletal muscle regulation are unknown. Here we investigated the Ca^{2+}-sensitivity of TnC-extracted rabbit soleus skinned fibers reconstituted with either WT or HCM mutant cTnC. Surprisingly, the slow skeletal skinned fibers extinguished the Ca^{2+}-sensitization typical of cardiac fibers for A8V (pCa_{50} = 6.01) and D145E (pCa_{50} = 6.05), while for C84Y (pCa_{50} = 6.33) the Ca^{2+}-sensitization was maintained compared to WT (pCa_{50} = 6.00). To determine whether slow skeletal TnI (ssTnI) alone was responsible for the protective effect of incorporating the A8V and D145E mutants into the slow skeletal myofilaments, cardiac skinned fibers were reconstituted with a hybrid cardiac troponin complex containing cTnT, ssTnI and cTnC-WT or -HCM mutants. The presence of ssTnI in cardiac fibers partially protected against Ca^{2+}-sensitization by the cTnC mutants in cardiac skinned fibers, with the exception of C84Y. MgATPase activity of slow or cardiac myofibrils replaced with exogenous HCM cTnC mutants were also affected. The absence of increased Ca^{2+}-sensitivity of contraction arising from the A8V and D145E in the soleus muscle suggests that the aberrant effects of cardiomyopathic cTnCs may be tissue-specific, and ssTnI is an important partner that partially protects the slow muscle from a deleterious effect of the HCM cTnC mutants. Supported by NIH HL103840 (JRP).

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Structural and Functional Changes in Skeletal Muscles in an A8V-Troponin C Hypertrophic Cardiomyopathy Knock-In Mouse Model

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Missense mutations in the *TNNC1* gene encoding cardiac and slow skeletal troponin C (c/ssTnC) are associated with phenotypic outcomes of hypertrophic cardiomyopathy (HCM). The impact of HCM c/ssTnC mutations in skeletal muscle structure and function is unknown; therefore, we examined the effect of an HCM-associated c/ssTnC A8V mutation on myosin heavy chain (MHC) isoform expression, contractile properties of different skeletal muscles, and cardiac autonomic function in a homozygous knock-in mouse (KI-TnC-$A8V^{+/+}$). The distribution of MHC I/II isoforms in various muscles from 3-month old male WT and KI-TnC-$A8V^{+/+}$ mice were analyzed by glycerol SDS-PAGE and the muscle/body weight ratios (M/BW) were recorded. Mechanics of contraction in isolated intact soleus muscle, running/endurance capacity and heart rate variability (HRV) were also measured. In comparison to the WT mice, MHC isoform switching was observed in diaphragm (increased MHCIIb, decreased MHCI) and soleus (decreased MHCIIa, increased MHCI) muscles of KI-TnC-$A8V^{+/+}$ mice; while, the M/BW ratio was unchanged for all tested muscles. The force *vs* frequency relationship at 15Hz and the $t_{1/2}$ of relaxation increased in the intact soleus muscle of KI-TnC $A8V^{+/+}$ mice in comparison to the WT; whereas, no changes in maximal force, time-to-twitch peak, velocity and power output were observed. In endurance and HRV tests, KI-TnC-$A8V^{+/+}$ mice had a lower running distance capacity and their global autonomic, cardiovagal, and baroreflex activities were significantly reduced compared to WT. In spite of the increase in type I fibers in soleus muscle of mice expressing c/ssTnC A8V, they display reduced endurance capacity. These findings suggest that the slow skeletal muscle is trying to compensate for the cardiac dysfunction caused by the c/ssTnC $A8V^{+/+}$ mutation. Supported by NIH HL103840 (JRP).

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Modelling the Calcium Dependent Actin-Myosin ATP-ase Cycle in Solution

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We have implemented two computation versions of the solution actin-myosin ATPase cycle. A simple numerical model that simulates an eight step ATPase cycle and allows actin, myosin, substrate ATP and product ADP & Pi concentrations to be fixed or varied. This model allows us to rapidly assign realistic rate constants to each step of the cycle for any well-defined myosin isoform. With this set of rate constants in place we can then implement a stochastic version of the eight step ATPase cycle that includes calcium regulation of the actin binding steps according to the McKillop & Geeves Blocked/Closed/Open model of the regulated thin filament. Using this model it is now feasible to model the dependence of the ATPase rate on calcium concentration and predict the occupancy of each state in the cycle for any defined set of protein and substrate concentrations. This allows us, for example, to explore the interplay of calcium and ATP concentrations in the cooperative activation of the ATPase cycle and make predictions for how the system will respond to sudden changes in calcium or ATP concentration. In a further variant of the model the program allows us to predict how the system will respond if variable ratios of fast and slow isoforms of myosin, or variable ratios of proteins carrying cardiomyopathy mutations are present in the system. This will be a useful tool to explore the behaviour of mixed myosin & troponin populations and correlate experimental data with model predictions.
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Effect of Actin and Nucleotide on the Movement of A1-Type Myosin Essential Light Chain, Detected by Time-Resolved FRET

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We have used site-directed time-resolved FRET to detect structural changes of the N-terminal extension on the A1 isoform of ELC, during active interaction with actin. Skeletal muscle myosin subfragment 1 (S1) has two ELC isoforms, A1 and A2, which differ by the presence of 40-45 additional residues at the N-terminus of A1. Removal of ELC from myosin results in a loss of movement of actin filaments, and a reduction in isometric force. It has been proposed that the N-terminal extension of A1 interacts directly with actin to modulate actomyosin kinetics. We have used time-resolved FRET to explore the structural details of this modulation. We recently showed, using probes on actin and ELC, that the amplitude of the myosin power stroke is much greater for A1 than A2. We have now engineered single cysteine (C16, in the N-terminal extension) and double cysteine (C16-C180, in the C-terminal lobe) mutants of A1-ELC and used FRET to determine the distance from C16 to actin 374 or to C180, as affected by the power stroke. Labeled ELCs were exchanged into S1, and the pure isoenzyme (S1A1) was isolated using Talon affinity resin. Labeling and exchange preserved the functional properties of S1A1. Intermolecular FRET between C374 of actin and C16 of A1 showed that the distance increases from 2.9 nm to 3.5 nm upon addition of ATP. Intramolecular FRET between C16 and C180 does not change in the presence of ATP or actin. We conclude that (1) the N-terminus of ELC moves away from actin during the transition between strongly and weakly bound states of acto-S1, and (2) the converter/C-terminal domain of myosin/ELC and the N-terminal extension move as a rigid body during the power stroke.

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Systematic Variations in Fast-Type Myosin Light Chain 1 Sequence Correlate with Species Body Mass

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The N-terminus of vertebrate fast-type myosin light chain 1 (MLC1F) contains a conserved actin-binding domain, a nearby poly-alanine (poly-A) domain, and an adjacent alanine-proline (A-P) repeat domain. The number of residues in the poly-A and A-P repeat domains vary considerably among vertebrate species. We reported a strong correlation between the apparent mass of MLC1F and adult body mass for nineteen mammalian species (Bicer and Reiser, Am. J. Physiol. Regul. Integr. Comp. Physiol., 292:R527-R534, 2007). The MLC1F sequence was then known for five of the nineteen species and the variation in MLC1F mass was determined to be due largely to the variation in the number of residues in the poly-A and the number of A-P repeats among these five species. We have recently examined the reported MLC1F sequence of additional mammalian species and of species of other vertebrate classes to determine the extent of the correlations between the number of poly-A residues and the number of A-P repeats and body mass among vertebrates, with a particular interest in comparing species with different modes of locomotion (e.g., terrestrial walking/running versus swimming). We also examined the sequences of other MLCs for the presence of systematic variations among species and possible correlations with species body mass. The results reveal that the numbers of poly-A residues and A-P repeats increase with species body mass among terrestrial mammals. Furthermore, the MLC1F domains vary most consistently with adult species body mass than any of the other examined MLCs. The results suggest that MLC1F domains are likely involved with modulating the speed and/or economy of locomotion among terrestrial mammals.