whose mechanism is currently unknown. Experiments here were designed to test the hypothesis that the properties of Tn are important in determining the SL dependence of force in cardiac muscle. We compared trabeculae exchanged with WT cTn vs. cTn containing a mutant (L48Q) cTnC with enhanced Ca^{2+} affinity. L48Q cTnC caused a left-shift in the force-pCa relationship at long (2.3 μm) SL as compared to WT cTnC. Interestingly, L48Q cTnC effectively eliminated SL-dependence of the force-pCa relationship, via a much larger left-shift at short SL, while SL dependence of F_{max} was unaffected. This suggests that SL-dependence of cardiac force development can be greatly influenced by the properties of native cTn, perhaps by limiting crossbridge binding, and that this effect is likely most important at shorter SL. Furthermore, increasing the Ca^{2+} binding and/or cTnC-cTnI interaction properties of cTn (such as with L48Q cTnC) can reduce or eliminate this limitation. Ongoing experiments will determine whether TnI phosphorylation can restore SL dependence by decreasing Tn Ca^{2+} affinity, thereby reducing myosin access to actin binding sites. This will also be tested by exchange with I61Q cTnC-Tn, a mutant with reduced Ca^{2+} affinity. Overall, these results imply that the cardiac length-force relationship is governed, at least in part, by properties of thin filament regulatory units. Support appreciated from NIH R01 HL 65497 (MR) and T32 HL07828 (FSK).

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Effect of pH and Electrostatic Interactions on Myofilament Lattice Volume

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Changes interfilament lattice spacing is a major determinant of force production in striated muscle with reductions in force generation being observed with both expansion and compression of the lattice spacing relative to the normal physiological values. Previous studies have concluded that lattice spacing depends complexly on the balance of outward repulsive forces and inward attractive and compressive forces between thick and thin filaments. Since lattice spacing has important implications on force generation, we examined the effects of alterations in filament charge, induced by changes in intracellular pH (by the rapid application and withdrawal of 30 mM NH4Cl), on lattice spacing in intact twitching cardiac trabeculae. Since we observed changes in sarcomere length in response to changes in intracellular pH and since changes in sarcomere length induce changes in lattice spacing as a result of the isovolumic volume behavior of intact sarcomeres, sarcomere length was maintained at a fixed value (~2.2 μm) during the pH interventions. Lattice spacing increased ($p < 0.05$) following NH4Cl wash in (measured after 5 minutes) when the pH is estimated to increase to ~7.8 (Balnave 2000 J. Physiol.) to 36.9 +/-0.2. Following the washout of NH4Cl (which is estimated to decrease pH to ~6.5 (Swietach 2005 J. Physiol.)) lattice spacing increased to 37.6+/−0.4 elevated above ($P < 0.05$) the space observed at control pH. Since the isoelectric point for myofilaments is ~5 (Naylor, Biophys J, 1985), our findings suggest that, in addition to electrostatic, van der Waals' and osmotic forces, other pH-sensitive forces are also critical determinants of the lattice spacing, possibly the M-protein, myomesin or other M-line proteins which are postulated to hold thick filaments together in the M-band of striated muscle.

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Molecular and Functional Characterization of a Novel Cardiac Specific Human Tropomyosin Isoform

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Previous work using human cardiac RNA identified a novel tropomyosin (TM) isoform designated as TPM1-kappa. We developed a TPM1-kappa specific antibody and quantified the levels of TPM1-kappa protein in the hearts of normal and various cardiomyopathy patients. Our study reveals that TPM1-kappa protein is expressed in the human heart and its level is differentially regulated during cardiomyopathic conditions. To investigate the role of TPM1-kappa in sarcomeric function, we generated several lines of transgenic (TG) mice overexpressing TPM1-kappa in the hearts. Immunohistochemical studies show the incorporation of the TPM1-kappa protein in the myofilaments but no significant pathological alterations are observed in these TG mice. Hearts that express TPM1-kappa protein exhibit significant decreases in rates of contraction and relaxation when assessed by ex vivo work-performing cardiac analyses. Studies on skinned fiber bundles demonstrate decreased myofilament calcium sensitivity with no change in maximum developed tension. Additional biophysical studies, including circular dichroism and in vitro actin-binding assays using recombinant TM proteins demonstrate less stability and weaker actin-binding affinity of TPM1-kappa as compared to TPM1-alpha. Energetic analyses of the structural models of TPM1 kappa and alpha isoforms reveal regions which decrease the overall stability of the TPM1 kappa/kappa and kappa/alpha coiled-coil dimers. This functional analysis of TPM1-kappa provides a possible mechanistic explanation for the isoform switch that is observed in cardiomyopathy patients.

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SPOC: A Functional Assay of Failing and Non-Failing Human Cardiomyocytes

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SPontaneous Oscillatory Contractions, known as SPOCs, are well characterized for skeletal muscle fibers but less so for cardiac muscle. A survey of SPOCs on mammalian hearts from rat to cow showed that, like myosin ATPase activity, SPOC contractions exhibit a linear relationship relative to body size. SPOCs exhibit slow, travelling waves of relaxations that can be induced under precise solvent conditions. These are observed by light microscopy and the time-resolved images are analysed with high spatial and temporal resolution for up to 1 h. Here we examine the functional performance of failing and non-failing human heart samples snap frozen at -200°C (liquid nitrogen) within minutes of clamping the coronary arteries. Thus, it is possible to study the contraction and relaxation of cardiomyocytes from hearts that were also used in transcriptomics studies. With our large bank of frozen human heart tissue we can study the effects of: (1) end-stage heart failure on multiple heart samples from a wide range of patients; and (2) aging on contractile performance using multiple samples of non-failing hearts. We will report preliminary findings from end-stage heart failure patients with familial and non-familial dilated cardiomyopathy, and from un-used donor hearts aged 9-65 years.