(5.57 ± 0.03). E99K increased T_{LC} (tension at pCa 8.0: 0.22 ± 0.03 T_{HC}) compared to WT (0.10 ± 0.02 T_{HC}), but A331P maintained similar T_{LC} (0.08 ± 0.01 T_{HC}) to WT. Five equilibrium constants of the cross-bridge cycle were deduced using sinusoidal analysis. E99K caused a decrease in both K_0 (ADP dissociation: 16.6 ± 4.6 mM^{-1}) and K_1 (ATP association: 1.51 ± 0.08 mM^{-1}) compared to WT (35.04 ± 6.3 mM^{-1} and 2.1 ± 0.3 mM^{-1}, respectively). A331P and WT showed similar K_0 (31 ± 5.4 mM^{-1} and 35.04 ± 6.3 mM^{-1}), K_1 (1.6 ± 0.2 mM^{-1} and 2.1 ± 0.3 mM^{-1}), K_2 (cross-bridge detachment: 0.69 ± 0.1 and 0.78 ± 0.13), K_4 (force generation: 0.27 ± 0.03 and 0.34 ± 0.03), and K_5 (Pi association: 0.13 ± 0.2 mM^{-1} and 0.12 ± 0.01 mM^{-1}). The cross-bridge distribution was similar among WT, E99K and A331P, indicating that force/cross-bridge is decreased in K99K and A331P. In conclusion, E99K actin causes HCM through impaired relaxation and elevated pCa_{50} (diastolic problem), and lowered force/cross-bridge (systolic problem), whereas A331P actin causes HCM through decreased pCa_{50} and force/cross-bridge (systolic problem).

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Stiffening of Embryonic Myocarium during Development Parallels Myosin and Matrix Expression and Dynamically Matches Cell Function

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Healthy heart function and structure, from the tissue to cellular scale, depends on establishment of proper tissue mechanics. On the cellular scale, late embryonic, neonatal and adult cardiomyocytes exhibit mechanosensitivity to ECM elasticity [1], such that culturing on substrates of physiological stiffness of ~10-20 kPa optimizes function. However, the heart begins functioning long before this mature mechanical environment is established. Here we examine the effects of microenvironment mechanics on chick cardiomyocyte function during early development, embryonic day 2-4 (E2-E4). Micropipette aspiration measurements give an effective Young's modulus of ~1-2 kPa at these earliest stages. Quantitative Mass Spectroscopy of embryonic myocardium identifies abundant structural proteins that most likely contribute to tissue mechanics. In particular, collagen I expression parallels the mechanical stiffening of the heart tissue. Treatment with collagenase or transglutaminase respectively softens or stiffens heart tissue in a dose dependent manner while maintaining functional cellular contractile machinery. Both stiffening and softening whole heart tubes reduces tissue strain during contraction, suggesting an optimum mechanics for myocardial contraction even at the earliest functional stages. Contraction wave velocity, however, changes proportionally to the stiffness of the tissue. E3-4 cardiomyocytes cultured on collagen-coated polyacrylamide gel substrates of different stiffnesses are best able to contract on substrates of ~1.5 kPa, consistent with the physiological stiffness of the tissue from which they were derived. As in the whole heart, the cells were also less able to contract on much softer substrates of 300 Pa, or stiffer substrates of 10 kPa or more. This further points to optimizing mechanics for cardiomyocyte strain that matches the physiological stiffness of myocardium throughout development. Finally, effects environmental mechanics on nuclear proteins during development are being explored.

[1] Majkut SF, Discher DE. (2012). BMMB. Doi: 10.1007/s10237-012-0413-8

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Increased Tension Cost in Human Familial Hypertrophic Cardiomyopathy Caused by the MYH7 Mutation R403Q

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Familial hypertrophic cardiomyopathy (HCM) is frequently caused by mutations in genes encoding sarcomeric proteins. Energy depletion of the heart is thought to initiate and promote HCM disease development. It has been hypothesized that HCM sarcomere gene mutations increase ATP utilization for myofilament contraction and thereby increase energy demand of the heart. Previous studies in single left ventricular myofibrils from myocardium harbouring the first identified causal HCM mutation R403Q in the gene encoding β-myosin heavy chain revealed increased rates of tension generation and relaxation in R403Q compared to myofibrils from healthy control myocardium. The altered kinetics observed in the mutant sample predicted a 3-fold increase in the energy cost of tension generation in R403Q sarcomeres. In the present study we investigated if tension cost was indeed increased in human myocardium harbouring the R403Q mutation.

Maximal force generating capacity (F_{max}) and ATP consumption (ATP_{max}) were simultaneously measured in Triton-permeabilized left ventricular muscle strips (n=8) from a patient carrying the R403Q mutation. Left ventricular muscle strips (n=17) from 5 HCM sarcomere mutation negative patients (HCM_{smn}) and strips (n=6) from 2 patients with secondary hypertrophy due to aortic stenosis (LVH_{ao}) served as a control groups. Economy of myofilament contraction is expressed as tension cost (TC), i.e. amount of ATP used during force development (ATP_{max}/F_{max}). Both F_{max} and ATP_{max} were significantly lower in R403Q compared to HCM_{smn} and LVH_{ao}. TC was significantly increased in R403Q (3.8 ± 0.5 $\mu molL^{-1}s^{-1}/kNm^{-2}$) compared to HCM_{smn} and LVH_{ao} (1.6 ± 0.1 and 1.7 ± 0.1 $\mu molL^{-1}s\text{-}1/kNm^{-2}$, respectively).

Our data provide direct evidence that TC is increased in myocardium harbouring the MYH7 mutation R403Q, indicating that expression of R403Q in the heart impairs economy of myocardial contraction.

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Synthetic Peptides Model Instability of Cardiac Myosin Subfragment-2

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A cluster of cardiomyopathy mutation sites in β-cardiac myosin including a particularly lethal mutation of E930del can be targeted to part of the N-terminal region of myosin subfragment-2. A peptide model of this system was synthesized and labeled with resonance energy transfer probes to detect the stability of coiled coil formation and estimates of the monomer-dimer equilibrium of the peptide. Distance measurements between the donor and acceptor probes were consistent with computational simulations of the labeled peptides in a coiled coil structure which matched simulations of the unlabeled wildtype structure. The addition of a site-specific antibody to this peptide strongly inhibited resonance energy transfer, indicating that the coiled coil structure was destabilized. In contrast, the addition of certain polyamine structures improved the stability of the coiled coil, while other polyamines had little effect despite having significant binding constants to myosin as determined by competitive ELISA assays. The length of the polyamine appears to be important for this ability to stabilize the coiled coil in this region of negatively charged glutamates in the myosin subfragment-2 sequence. Some of the polyamines may be capable of binding both α-helixes of the myosin subfragment-2 simultaneously, thereby stabilizing the coiled coil structure. This synthetic peptide model system holds promise as a test system for small molecule compounds that may counteract the destabilizing effects of some cardiomyopathy mutations on the myosin coiled coil structure. (sponsored by NSF ARRA)

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Regulatory Light Chain Mutants Linked to Heart Disease affect the Cardiac Myosin Lever-Arm

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Myosin is the chemomechanical energy transducer in striated muscle working in the heart to circulate blood. Transduction occurs by cyclical actomyosin interaction causing myosin thick filament sliding relative to the actin thin filament while consuming chemical energy from ATP. Transduction begins in the myosin head domain or cross-bridge with ATP hydrolysis driving rotary movement of a lever-arm converting rotation into linear translation. Myosin regulatory light chain (RLC) binds to the lever-arm and affects its ability to translate actin. Gene sequencing implicated several RLC mutations in human heart disease and three of them are investigated here using single molecule detection of photoactivatable GFP tagged RLC (RLC-PAGFP) exchanged into permeabilized porcine papillary muscle fibers. The RLC-PAGFP detects single lever-arm orientation. Single molecule orientation detection benefits from new analysis tools to deduce PAGFP orientation from the 3 spatial dimensional fluorescence emission pattern. Experiments were performed in a microfluidic chamber designed for isometric contraction, total internal reflection fluorescence single molecule detection, and for 2-photon excitation second harmonic generation to evaluate sarcomere length. The RLC-PAGFP reports discretized lever-arm orientation intermediates in active isometric fibers characteristic to the wild type (WT) RLC where lever-arms occupy intermediates on the free-energy gradient that on average correspond to the cross-bridge stall force. Two disease linked mutants introduced to RLC move state occupancy further down the free energy gradient implying lever-arms rotate more to reach cross-bridge stall force because mutant RLC increases lever-arm shear strain. Lower free energy states could involve strong binding that will inhibit filament sliding in contraction. This research was supported by NIH Institutes: NIAMS R01 AR049277 and NHLBI HL095572 and by the Mayo Foundation.