mutations have been characterized in the context of cardiomyopathy pathogenesis, the precise role of individual proteins in regulating length dependence of force remains unclear. Here, we used previously characterized point mutations of regulatory proteins to probe the thin filament and elucidate the role of tropomyosin in modulating the length dependence of cardiac contractility. Twitch amplitude was measured at short (~2.0μm) and long (~2.3μm) sarcomere lengths (SL) of intact cardiac trabeculae from hearts of a transgenic murine model containing a dilated cardiomyopathy-associated Tpm mutation (D230N; denoted Tpm^{D230N}). Trabeculae were mounted between a force transducer and length-controlling motor, perfused with oxygenated physiological solution (30°C), and electrically stimulated at 1 Hz. At short SL, twitch force between wild-type (WT) and Tpm^{D230N} trabeculae were not significantly different (24 ± 4 versus 16 ± 3 mN/mm^2, respectively). At long SL, WT trabeculae produced significantly higher twitch force compared to Tpm^{D230N} (51 ± 6 versus 27 ± 3 mN/mm^2, respectively), demonstrating reduced length-dependent augmentation of contractility in Tpm^{D230N} trabeculae. We hypothesized that this is due to reduced azimuthal displacement of Tpm^{D230N} along the thin filament, limiting effects of cross-bridge-mediated cooperative activation at longer SL. Thus, we incorporated an engineered calcium-sensitizing troponin C mutation (L48Q, denoted TnC^{L48Q}) to aid thin filament activation by developing a Tpm^{D230N}/TnC^{L48Q} double mutant murine model. Twitch forces in intact trabeculae from Tpm^{D230N}/TnC^{L48Q} mice were increased compared to Tpm^{D230N} and were not significantly different than WT at short and long SL (30 ± 4 and 46 ± 6 mN/mm^2, respectively). Our results suggest that tropomyosin plays a unique role in modulating the length dependence of contractility in cardiac muscle.

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The Off State of the Thick Filament of Cardiac Muscle is Not Affected by Inotropic Interventions Like the Increase in Diastolic Sarcomere Length or the Addition of a Beta-Adrenergic Effector

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We study the regulation of cardiac contractility by using SAXS-USAXS at the ID02-beamline of the European Synchrotron (ESRF, Grenoble, France) on intact trabeculae isolated from the rat ventricle to record both the nanometer-scale X-ray signals from the contractile proteins along the thin (actin) and thick (myosin) filaments and the changes of the sarcomere length (SL). Previously we demonstrated that in diastole (external [Ca^{2+}] 2.5 mM, 27 °C) most of the myosin motors are in the off-state (unavailable for actin binding and ATP hydrolysis), packed into helical tracks with 43-nm periodicity on the surface of the thick filament, and that the fraction of myosin motors leaving the off-state during the twitch depends on the load through a rapid positive feedback based on thick-filament mechano-sensing (Reconditi *et al. PNAS* 114:3240, 2017). This regulatory mechanism occurs downstream with respect to the Ca^{2+}-dependent thin-filament activation which controls cardiac contractility via the intracellular [Ca^{2+}] and the Ca^{2+}-sensitivity of the filaments. Here we tested the interdependency of the two regulatory mechanisms by recording the X-ray signals that mark the state of the thick filament during two inotropic interventions that double the twitch force at SL 2.0 μm and external [Ca^{2+}] 1 mM: either SL increase to 2.25 μm or addition to the solution of the β-adrenergic effector isoprenaline (10^{-7} M). In diastole none of the signals related to the off-state of the thick filament was affected by either intervention. The results indicate that Ca^{2+}-dependent thin-filament activation and thick-filament mechano-sensing act independently, further clarifying the role of the thick-filament stress-sensitivity as an energetically well-suited downstream mechanism. Supported by Ente Cassa di Risparmio di Firenze 2016-2018.

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Mechanical and Structural Analysis of Cardiomyopathies at the Single Cell Level

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Inherited cardiomyopathies are the leading cause of sudden death in young people, affecting more than one in five-hundred people, and there is currently no known cure. Two of the most common forms of familial cardiomyopathies, hypertrophic (HCM) and dilated (DCM), show thickening and thinning of the left ventricular wall, respectively. Patients with these diseases frequently experience fibrosis, altered cardiac output, and arrhythmias. These closely related diseases are primarily caused by mutations in sarcomeric proteins that regulate cardiac contractility; however, how these mutations lead to the disease phenotype and to opposite effects on tissue remodeling is unclear. Understanding these diseases will require knowledge of how these mutations affect cardiac contractility across multiple levels of organization. While the disease presentation in intact hearts is well understood, the complexity of the heart makes it difficult to attribute the disease phenotypes to tissue-level reorganization or intrinsic changes in cellular contractility. To study the similarities and differences between these diseases at the cellular level, we have used CRISPR/Cas9 to generate human stem cell lines bearing disease-causing mutations, differentiated these cells to cardiomyocytes, and studied their structural and contractile properties. We focused on two model point mutations in troponin-T that cause either HCM or DCM in patients. Traction force microscopy on patterned substrates with stiffnesses that mimic the human heart was used to study the contractile properties of single cells. Our results indicate features of the disease seen in patients are present at the single cell level. Specifically, the HCM mutant shows hypercontractility and impaired relaxation, while the DCM mutant shows hypocontractility. Additionally, both mutants show altered sarcomeric structure. The development of a cell-based system for these diseases will likely open the door for the development of novel therapeutics for familial cardiomyopathies.

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The Missense E258K-MyBP-C Mutation Increases the Energy Cost of Tension Generation in Both Ventricular and Atrial Tissue from HCM Patients

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Mutations in *MYBPC3*, the gene coding for cardiac myosin-binding protein-C (cMyBP-C) are the most common cause of Hypertrophic CardioMyopathy (HCM). The E258K-MyBP-C is a highly penetrant missense mutation with poorly understood molecular mechanisms. Mechanics and kinetics of contraction as well the energetic cost of tension generation were investigated using left ventricular (LV) and atrial tissue from three E258K HCM patients and compared to those from controls (donor hearts, aortic stenosis patients, and HCM patients negative for sarcomeric protein mutations). Kinetics of tension generation and relaxation were measured in single LV and atrial myofibrils mounted in a force recording apparatus (15 °C), maximally Ca^{2+} -activated (pCa 4.5) and fully relaxed (pCa 9.0) by rapid solution switching (<10 ms). Maximal ATPase and isometric active tension were simultaneously measured in Triton-permeabilized LV and atrial strips. In E258K, maximal tension of both ventricular and atrial myofibrils was reduced compared to controls. The rate of tension generation following maximal Ca^{2+} activation (k_{ACT}) was faster in both ventricular and atrial E258K myofibrils compared to controls. The rate of isometric relaxation (slow k_{REL}) was also faster in E258K myofibrils, suggesting faster cross-bridge detachment and increased energy cost of tension generation. Direct measurements in ventricular and atrial skinned strips confirmed that tension cost was 2-3 fold higher in E258K preparations compared to controls. We conclude that the E258K mutation primarily alters apparent cross-bridge kinetics and impairs sarcomere energetics. In vitro, the mutation induces similar kinetic and energetic effects in both atrial and LV sarcomeres. The smaller impact of the mutation on atrial muscle function compared to LV muscle in vivo is likely due to the different loading conditions of the two chambers.

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Chronic Exercise Increases Compliant Titin and Kettin Isoform Content in Cardiac Muscle of Rat and Drosophila Models

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Reduced cardiac passive stiffness correlates with increased exercise tolerance in mammals. Titin, the giant elastic protein, decreases passive stiffness by expression of larger titin isoforms (increased N2BA:N2B ratio) or altered post-translational modifications (increased PKA, decreased PKC phosphorylation). Short-term and acute exercise has been previously shown to change post-translational modifications but not the N2BA:N2B. Kettin is an elastic protein in invertebrates that is also differentially spliced. Little is known about kettin isoform splicing in response to exercise. The purpose of our study was to evaluate changes in titin and kettin isoform content