translates to changes in contractility and calcium transients in isolated adult rat cardiac myocytes. One day after cell isolation, we found an acute, dose-dependent decrease in the peak height of contraction with tamoxifen concentrations ranging from 1-10μM. This was accompanied by diminished calcium transient amplitude. Additionally, the percentage of rod-shaped cells that visibly contract dose-dependently decreased over the course of one hour of pacing with tamoxifen. Raloxifene, also in the SERM class of drugs, had a pattern of effects similar to tamoxifen. In conclusion, the acute tamoxifen and raloxifene-induced inhibition of cardiac myocyte contractility may contribute to the transient cardiomyopathy seen in MCM transgenic mice. The results of this study emphasize the importance of using the minimum dose of tamoxifen required for gene excision in MCM transgenic mice, as well as incorporating appropriate controls to address tamoxifen-mediated acute cardiomyopathy.

1800-Pos Board B570

Myofilament Dysfunction in the Infarct Border Zone

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Introduction: Soon after myocardial infarction, a poorly contracting border zone forms adjacent to the infarct. Myocardium in the infarct border zone remains normally perfused with blood, and the cause of border zone dysfunction is unclear. **Goal:** Investigate the role of myofilament dysfunction in the impaired contraction of the infarct border zone. **Methods:** We studied sheep hearts, 2 or 8 weeks after infarction of the apex of the heart and non-operated controls. Myofilament contraction was assessed using in-vitro contractions of skinned cardiac muscle fibers. Muscle fiber bundles were dissected from the infarct border zone and from multiple regions ranging up to 6 cm from the infarct. Skinned fiber force development was referenced to several measures of the content of contractile material within the samples. **Results:** In the border zone immediately adjacent to the infarct, maximal force development (Fmax) was reduced by 38 ± 2% (n=7, $P<0.001$) compared with Fmax of myocardium remote from the infarct. Fmax for remote zone myocardium was similar to that for uninjured myocardium (~90 mN/mm^2). The width of the border zone was defined by a decreased Fmax that extended up to 5-6 cm away from the infarct. Fmax rose in a linear gradient between the infarct and the remote zone. There were no differences between border zone and remote zone in: histological staining for collagen; the area fraction of myocardium occupied by myofilbrils (or mitochondria); or in the abundance of myosin. Therefore, depressed border zone Fmax was not explained by decreased content of contractile material. Moreover, treatment of skinned fibers with protein phosphatase 1 did not affect border zone Fmax, suggesting that myofilament protein phosphorylation was not involved in border zone dysfunction. **Conclusions:** Myofilament dysfunction contributes to impaired contraction in the infarct border zone.

1801-Pos Board B571

Dynamic Perturbations within the Ubiquitin Proteasome System in Diabetic Cardiomyopathy Associated with Type 1 Diabetes Mellitus

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Patients with type 1 diabetes mellitus (T1DM) typically have Diabetic Cardiomyopathy (DCM) characterized by diastolic dysfunction and cardiac remodeling. The ubiquitin proteasome system (UPS) is the primary proteolytic system active in cardiac muscle protein degradation and has been shown to be activated by hypoinsulinemia. The Akita mouse model offers a whole organism model of T1DM which develops hypoinsulinemia and hyperglycemia in a timeline that is analogous to the human disease. Cardiac tissue from mice that were pre-diabetic (2 weeks old), recently diabetic (5 weeks old) and diabetic (12 weeks old) was analyzed. At two weeks of age, with no hyperglycemia or cardiac atrophy, proteasome caspase-like, trypsin-like and chymotrypsin-like activities were not altered. At five weeks of age, mice were hyperglycemic with decreased cardiac mass and had caspase-like and trypsin-like 26S activities that were both suppressed approximately 15%. Interestingly, protein levels of the RPT1 19S proteasome subunit were also decreased. At 12 weeks of age, the 20S caspase-like and trypsin-like activities were both increased by over 20%. Lysosomal proteases showed a marked attenuation of activity at this time point. Caspase-3 and calpain activity levels were not altered at any time. Immunoblotting of the 20S subunit PSMA6 suggests an increase in the amount of 20S proteasomes in diabetic hearts at this age. The inducible proteasome subunit, MECL-1 also increased at this time point ($P<0.06$). RT-PCR showed that the mRNA levels of the proteasome activator, PA28 alpha, and one 20S catalytic subunit, PSMB5, were not changed at 12 weeks. These results suggest that perturbations within cardiac proteolytic systems during T1DM are dynamic with disease progression and the status of the UPS in this disease is complex, likely involving multiple levels of regulation.

1802-Pos Board B572

Reduced Efficiency of Intact Papillary Muscles in *ACTC* E99K Transgenic Mouse Heart

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The E99K transgenic (TG) mouse expresses mutant cardiac actin as reported in human patients being diagnosed with hypertrophic cardiomyopathy (HCM) predominantly in the apex. The mouse has recapitulated many phenotypes as in human patients including sudden cardiac death, apical hypertrophy, fibrosis, myocyte disarray and higher Ca^{2+}-sensitivity. We are now reporting a reduced efficiency of the mutant cardiac muscle comparing with their non-transgenic (NTG) litter mates. Intact papillary muscle used in the experiments and the efficiency during contractions was measured with a protocol of 40 twitches in 20s (27°C). The work and the heat production were measured during each set of 40 twitches and during runs with no stimulation as control. To mimic the cardiac cycle, each stimulus was followed by an isometric period (isovolumic contraction) of 0.12s, shortening by 10%L_0, an isometric period (isovolumic relaxation), and lengthening by 10% L_0. Four movement velocities, 0.5, 0.67, 1 and 2 L_0/s were tested. The isovoluminc relaxation periods were adjusted accordingly. Net work was calculated as the integral of active force and length change. Heat production, an index of metabolic cost, was calculated from temperature change measured with a thermopile of constantan-chromel thermocouples. Muscle of TG mouse (n=12) did more work/mg muscle (33 ± 1, mJ/g) than muscles from NTG littermates (19 ± 4, mJ/g, n=11), with disproportionately more total energy, work + heat (263 ± 10, mJ/g) than NTG (114 ± 18, mJ/g). Thus E99K muscle was less efficient (= work/total energy), 0.127 ± 0.005 than NTG muscle, 0.166 ± 0.015. Considering the higher Ca^{2+}-sensitivity detected in myofibril and skinned papillary muscle levels, more energy was likely used for active transport of ions such as Ca^{2+}.

1803-Pos Board B573

Post-Infarction Remodelling of Cardiac Muscle Assessed by Diffusion-Weighted MR Imaging and Histological Methods

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Cardiac magnetic resonance (MR) is a non-invasive imaging method that can be used to assess important alterations in the structure and function of the ischemic heart muscle. In this study, we used a pre-clinical swine model of chronic infarction generated by an occlusion-reperfusion method. The hearts from six animals were explanted at ~5 weeks post-infarction, and imaged on a 1.5T GE SignaExcite magnet using a high-resolution DW-MRI method (voxel size $< 1\text{mm} \perp 3$). In these images we delineated necrotic core scar (CS) areas and peri-infarct (PI) areas, where fractional anisotropy and apparent diffusion coefficient had significantly altered values compared to those in remote, healthy myocardium. Representative samples cut in short-axis, underwent histo-pathological analysis. In order to evaluate the severity of structural changes due to fibrosis, quantitative histological analysis using Sirius Red stain was performed. This demonstrated very good correspondence between the areas scored as dense collagen (>75%) as well as intermediate collagen density (25-75%, where fibrotic zones intermingled with viable myocytes), with the CS areas and PI areas, respectively, identified in MR images. Furthermore, to evaluate the changes in electrical function in the heterogeneously remodelled areas, we employed light micrographs of connexin Cx43 (which is responsible for cell-to-cell electrical coupling) prepared using imunohistochemistry methods, as well as fluorescence micrographs of Cx43. The analysis of fluorescence images demonstrated a disturbed pattern of gap junctions and a reduction of Cx43 density in the PI areas compared to areas selected from healthy myocardial tissue. The structural and functional remodelling of the cardiac muscle in the post-infarction period often underlie malignant arrhythmic events; therefore, the localization (particularly deep in the myocardium) and characterization of the CS and PI areas by means of a non-distructive way (such as DW-MR imaging) is extremely valuable.

1804-Pos Board B574

A Model of Hypertrophic Cardiomyopathy Induced by a Protein Tyrosine Phosphatase SHP2 Mutation Demonstrates Increased Myofibrillar Function

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In the majority of cases, hypertrophic cardiomyopathy (HCM) is a genetic disease of the sarcomere or sarcomere-related proteins. Many of these mutations have been shown to directly affect sarcomere contractile performance. However, mutations in other, non-sarcomeric proteins can also cause HCM. For

example, in the majority of families with LEOPARD Syndrome-associated HCM, mutations occur in the protein tyrosine phosphatase SHP2. We therefore hypothesized that the sarcomeric mechanical properties are altered by SHP2 mutations. Transgenic mice were generated with cardiac myocyte-specific expression of a loss-of-function mutation of SHP2 (Q510E-SHP2). These mice developed an early-onset form of HCM with increased myocyte size, heart-to-body weight ratios, and interventricular septum thickness. Interestingly, we found that permeabilized cardiac myocyte preparations from 1-month old Q510E-SHP2 mice displayed greater maximal Ca^{2+}-activated tension (SHP2 = 84 ± 2 kN/m^2; Wt = 45 ± 14 kN/m^2) and power generating capacity (SHP2 = 10 ± 2 μW/mg; Wt = 5 ± 1 μW/mg) compared to myocytes from littermate wildtype (Wt) mice. We also found PKA-mediated phosphorylation of both myosin binding protein-C (MyBP-C) and cardiac troponin I (cTnI) was increased in cardiac myofibrils from 1 month old Q510E-SHP2 mice. Taken together, these results implicate a compensatory increase in the contractile state of cardiac myofibrils in response to loss of SHP2 function.

1805-Pos Board B575

DCM-Linked D230N Tropomyosin Mutation Results in Early Dilatation and Systolic Dysfunction in Mice

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Recently, a study in two large multi-generational families described a familial dilated cardiomyopathy (DCM) caused by a single amino acid substitution Asp230Asn (D230N) in tropomyosin. These families demonstrated a unique bimodal disease distribution in which infants presented with a severe form of DCM, while adults presented with a mild to moderate clinical phenotype. To determine the biophysical consequences of this mutation on tropomyosin and its effects on regulatory function in the sarcomere, we employed circular dichroism and the regulated in vitro motility assay. We found that while this mutation does not affect overall thermal stability of tropomyosin, it has a profound effect on regulatory function. As previously shown in solution, the presence of the D230N mutation decreases the maximal velocity of filament sliding and calcium sensitivity of thin filament activation compared to wild type filaments. Additionally, the D230N mutation increases the cooperativity of myofilament activation. In order to further explore our biophysical observations and the physiologic effects of the D230N mutation, we created a transgenic murine model. In mice carrying the D230N tropomyosin mutation we found evidence of early dilatation and systolic dysfunction by echocardiogram in the absence of histological changes such as fibrosis or inflammatory cell invasion. Ultrastructural analysis of transgenic left ventricular tissue demonstrated z-disk alterations. Finally, preliminary studies on isolated myocytes from transgenic mice loaded with fura-2AM demonstrate no discernible differences in calcium transients compared to non-transgenic siblings suggesting that functional impairments are not due to calcium handling defects. Collectively, these studies suggest that the D230N mutation in tropomyosin is responsible for alterations in structure and function of the thin filament that result in a primary dilatation of the cardiac left ventricle. *This work is supported by funding from the Children's Cardiomyopathy Foundation.*

Muscle: Fiber & Molecular Mechanics & Structure II

1806-Pos Board B576

Geometric Changes of Transverse Tubules in Rabbit Cardiac Myocytes during Contraction

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Invaginations of the sarcolemma, called 'transverse tubules' (t-tubules), allow for rapid communication of electrical activation deep into the interior of ventricular cardiomyocytes. Given the length and radius of t-tubules, the rate of diffusion alone appears to be insufficient for homeostasis of the t-tubular content, especially during exercise [1]. Previously, we reported in rabbit ventricular myocytes that positive longitudinal strain modulates geometrical features of t-tubules [2] and their mouth-region [3] in a way that could support convective re-distribution of t-tubular content. Here, we test the hypothesis that cell contraction also affects t-tubular volume.

Isolated ventricular cardiomyocytes from adult New Zealand white (NZW; n=23) rabbits were imaged using an inverted confocal microscope [2] either at slack length or during negative strain to ~85%, caused by exposure to superfusate-induced tonic contracture. Image stacks of cell segments were deconvolved and t-tubules segmented. In addition, ventricular tissue from NZW rabbit hearts, fixed either at zero intra-ventricular pressure (n=2) or during contracture (n=2), was studied using transmission electron microscopy (TEM; [3]). T-tubular length and volume were assessed in confocal images, while ellipticity and orientation were explored both in confocal and TEM data. Statistical significance was determined using a two-tailed t-test with $p<0.05$ considered significant.

T-tubular cross-section changed, reducing ellipticity in contractured myocytes (compared to control), while apparent length and total volume of t-tubules increased. This suggests that both passive distension and active contraction may give rise to a convective component of fluid transport, mixing, and exchange of t-tubular content.

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1807-Pos Board B577

Mechanoregulation of Delayed Stretch Activation

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Delayed stretch activation (SA) is a prominent feature in the function of the cardiac myocyte and plays an important role in regulating cardiac output. A mechanistic understanding of SA is essential for the development of models that quantitatively and causally connect molecular defects to global cardiac function. We propose a novel mechanism that defines how mechanical forces imposed by stretch affect troponin-actin and myosin-actin bonds and thereby modify calcium-modulated thin filament regulation. Tropomyosin molecules are assumed to form two continuous flexible chains (CFC) along each actin filament; tropomyosin movements are restricted by bound troponins and myosin heads bound to actin. Crossbridges transmit sarcomere forces to the thin and the thick filaments. A stretch applied on a sarcomere extends the thin filaments and associated CFCs imposing additional strain (via the CFC) on the TnI-actin and myosin-actin bonds. The spatial positions of these bonds were calculated using the computational platform, MUSICO (**MU**scle **SI**mulation **CO**de) and, hence, the forces acting on TnI-actin and myosin-actin bonds before and after stretch at different Ca^{2+} concentrations. These forces were assessed from finite element analysis of CFCs weakly interacting with the actin surface and strongly interacting with actin via Tn attachments to actin. An imposed stretch leading to sarcomere forces of ~50% of the maximum isometric force increased the forces on the bonds by more than 10 pN, sufficient to strongly tilt the energy landscapes and accelerate the rate of detachment of Tn from actin, even without Ca^{2+} bound to TnC. The maximum effect of this behavior is observed in muscle fibers at submaximal activation (pCa ~ 6). This analysis suggests a mechanism for observed modulation of cardiac myocyte contractility by SA based on altered mechanochemistry of thin filaments regulation via CFC. Supported by NIH R01 AR048776 and R01 DC 011528.

1808-Pos Board B578

A Model for Ca(2+)-Dependent Cooperative Activation in the Cardiac Thin Filament that Allows for Crossbridge Cycle Feedback

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The mechanism underlying the apparent cooperativity of the cardiac thin filament was investigated by using FRET to follow the N-domain opening of recombinant cardiac troponin C (N-cTnC opening) passively exchanged into rat myocardial fiber bundles and tested under a variety of experimental conditions. Calcium titrations conducted in the presence of crossbridge cycle modulators and tension recovery experiments revealed: 1) N-cTnC opening occurred just as ''cooperatively'' with or without crossbridge cycle activity; 2) the calcium sensitivity of N-cTnC opening is enhanced by crossbridge cycling; 3) the rigor state results in no cooperativity and 10% of the cTnC ensemble being apparently open under resting conditions; 4) the breaking of crossbridges preceding a tension recovery phase results in a slight relaxation of the FRET distance associated with N-cTnC opening, which recovers during tension recovery. In light of this and other evidence from the literature, a model of apparent cooperativity based on the three state model is proposed wherein the steep force-Ca^{2+} relationship ultimately arises from a tunable cTnC binding affinity for Ca^{2+}. Activation may be mathematically modeled by a sum of weighted Hill-equation fractions (i.e. $[L]^n / (K_d + [L]^n)$) with each fraction governed by a hill coefficient of n=1 and representing a particular cTnC conformation with a unique affinity for Ca^{2+}, or ''affinity-state''. The affinity-state of an individual cTnC is influenced by the activation state of its neighboring