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^{o}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