responses detected in non-lipid raft domains were more sensitive to direct stimulation of AC activity with forskolin. Computational modeling suggests that AC activity may play a more important role in explaining the compartmentalized responses observed in HEK293 cells, while PDE activity may be more important in cardiac myocytes.

3916-Pos Board B644
Upreregulation of α1-Adrenergic Inotropy in Failing Right Ventricle (RV) is Mediated by the α1A Subtype
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In non-failing mouse RV, α1-adrenergic receptors (α1-ARs) mediate a negative inotropic effect (NIE). We reported that α1-AR inotropy in heart failure was dramatically switched to a positive inotropic effect (PIE). The two predominant α1-AR subtypes in heart are the α1A and α1B. However, their inotropic roles in heart failure are unclear.

Goal: Determine the roles of α1A and α1B subtypes in upregulation of α1-AR inotropy in failing RV.

Methods: We used a mouse model of bleomycin-induced RV failure. Bleomycin or saline was instilled into the trachea. Using RV cardiac trabeculae, we assessed contractility by the TRIC. α1-AR subtype-specific agonist phenylephrine (PE, stimulates α1A and α1B subtypes), or subtype-selective agonist A61603 (stimulates only α1A subtype).

Results: Two wk after bleomycin, there was pulmonary fibrosis, pulmonary hypertension and RV failure. For non-failing RV, A61603 caused a NIE (force decreased 48 ± 10%, n=3) similar to the NIE mediated by PE (force decreased 52 ± 12%, n=3). Thus, stimulation of the α1A subtype singly, or together with the α1B subtype, produced a similar NIE. In contrast, for failing RV, stimulation with A61603 caused a switch to a PIE (force increased 13±36%, n=7; P<0.05). However, the PIE mediated by PE was much lower (force increased 34 ±14%, n=7; P<0.05). Thus, stimulation of the α1A subtype singly produced a much greater inotropic response versus stimulation of both α1A and α1B subtypes. This suggests that upregulation of α1-AR inotropy in the failing RV was mediated by the α1A subtype, but opposed by the α1B subtype. Preliminary studies suggest a role for myosin light chain kinase in upregulation of α1-AR inotropy in failing RV.

Conclusion: The switch to a PIE induced by α1-ARs in failing RV is mediated by the α1A subtype, not the α1B subtype.

3917-Pos Board B645
Cardiac-Specific Overexpression of FOXO Affords Protection Against Age-Associated Decline in Cardiac Performance in the Drosophila Model
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Normal cardiac function declines with age, and a major contributor to heart failure is diastolic dysfunction. The causes of diastolic dysfunction are not yet fully known, although perturbations that affect cardiomyocyte passive mechanics, or stiffness, and calcium handling have been implicated. Here, we studied the effect of aging on cardiac dysfunction and myocardial stiffening in Drosophila melanogaster, an ideal model for studying senescence due to its short lifespan and ease of genetic manipulation. The transcription factor FOXO, a member of the insulin signaling pathway family, has been shown to mediate an extensive variety of cellular responses in humans, has also been shown to promote general muscle proteostasis and, more specifically, improve cardiac performance following pacing-induced stress in Drosophila. In this study, using high-speed video microscopy and motion analysis, we measured a significant decrease in heart rate and diastolic diameter and increased arhythmic beating patterns in control fly hearts with age. These changes suggest senescence-related decreases in cardiac output. Furthermore, using an atomic force microscopy-based nanoinodendration approach, we determined that control hearts underwent age-related transverse stiffening. Overexpression of FOXO in a heart-specific manner ameliorated these effects as indicated by a lower incidence of arrhythmias, elevated heart rate and increased diastolic diameter with age as well as by affording protection against age-related changes in transverse myocardial stiffness. These data support the hypothesis that increased FOXO activity helps maintain muscle proteostasis in aging hearts. Because aberrant cardiac homeostasis in cardiomyocytes may contribute to diastolic dysfunction, we are also interested in evaluating possible cardioprotective effects of FOXO overexpression on calcium handling in aging fly hearts.

3918-Pos Board B646
Myosin Storage Myopathy Mutations Disrupt Myofilibrillar Assembly/ Stability and Cause Progressive Muscle Degeneration in a Drosophila Model
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Myosin storage myopathy ( MSM) is a congenital disorder caused by dominant missense mutations in the β-cardiac MHC rod and characterized by subsarcolemmal accumulation of β-cardiac myosin that has a hyaline appearance. These mutations map near to or within the assembly competence domain known to be crucial to filament assembly. The mutations disrupt hydrophapy or charge of residues in the heptad repeat, altering interactions that stabilize myosin coiled-coil dimers and thick filaments. This potentially disrupts ordered myofilibrillar assembly, causing myofibrillar disarray and myosin aggregation. Our Drosophila models for MSM make it possible to examine interactions between wild-type and mutant full-length myosins for pursuing mechanistic investigations. We introduced the R1845W, L1793P or the E1883K mutation into a Drosophila MHC transgene and expressed each in the indirect flight/jump muscles and in the heart. Our studies show a severe reduction in the flight and jump ability of the rate of degradation of the FHC mutant E1010 is currently being carried out with an age-dependent worsening of muscle function. Electron and confocal microscopy of the indirect flight muscles of transgenic lines show myofibrillar disarray with large areas of granular filamentous inclusions similar to hyaline bodies found in affected humans. Semi-automated optical heartbeat analysis of the mutant heterozygotes shows restrictive cardiac physiology and diastolic dysfunction with evidence of worsening cardiac phenotype with age. Lifespans of the MSM mutants are also reduced in comparison to the transgenic control. Future studies will aim at analyzing in vitro filament formation ability of the mutant myosin to determine if defective filament formation and/or instability of the myosin filaments are the basis of MSM. Our model would also potentially help discern if specific chaperones, small molecule chaperone inducers or enhanced autophagy can ameliorate myopathic defects in MSM.

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Effects of FHC-Related Troponin T Mutations on Proteasome Activity and Half-Life of Troponin T
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Familial hypertrophic cardiomyopathy (FHC) is a genetic disease of the heart muscle that can be caused by mutations in sarcomeric proteins such as cardiac troponin T (TnT), a thin filament regulator of muscle contraction. Results from our lab show that the ubiquitin proteasome system (UPS) is affected in TnT-related cardiomyopathies; in FHC mouse expressing the Tn9N or R278C mutant forms of TnT, changes in the protein subunit expression and gene expression of proteins in the ubiquitination pathway, increased levels of oxidized proteins, and decreases in proteasome activity in 3 month old Tn9N mice were observed, suggesting that UPS dysfunction may be an important contributing factor to the pathogenesis of this disease. Mutations in sarcomeric proteins can alter their rates of proteasomal degradation, and increased degradation may lead to proteasome functional insufficiency by competitively inhibiting breakdown of other proteasome substrates. To investigate whether the observed impairment of proteasome function was due to a change in the degradation rate of mutated TnT, the degradation rates of wild-type and mutated (Tn9N or R278C) TnT were determined in CV-1 cells. The half-life of TnT was not affected by either mutation, suggesting that the effects of these mutations on the proteasome are not due to a difference in the degradation rate of TnT. Experiments to determine the rate of degradation of the FHC mutant E1010 are currently being carried out. Overall, our results suggest that the effects of FHC mutations on proteasome function are not due to the mutation directly affecting the proteasome. This work was supported by NIH Grant HL096819.

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Mirna-448 is a Precursor of Ros-Derived Dystrophic Cardiomyopathy
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NAD(P)H oxidases (NOXs) are of one the major sources of ROS in heart. Recently we reported that NOX2-mediated oxidative stress drives the development of cellular phenotype of cardiac dystrophy. Here we investigated the role of miRNAs in upregulation of NOX2 gene expression. Initial screening with a microRNA target prediction on-line database identified several microRNAs that are potential regulators of NOX2 genes. Following qRT-PCR screening of these microRNAs showed a drastic 10-fold down-regulation of