capable of detecting mechanical separation of the muscle layers, which was found to occur more frequently in w1118 than yw (0.41 ± 0.04 vs. 0.00 ± 0.02, P < 0.05). Detection of mechanical separation between muscle layers via nanoindentation was modeled and verified in a microfabricated polydimethylsiloxane system. This first in situ mechanical analysis of a living myocardium revealed differences in cardiac mechanics due to age and suggest that aspects of the mechanical properties of the aging phenotype differ between Drosophila strains. We investigate on other laboratory Drosophila wildtype strains to assess the impact of diverse genetic backgrounds of mutations on age-related myocardial stiffening and cardiomyopathy.

1782-Pos Board B552
In Situ Mechanical Analysis of Genetic Modification and Aging on Soft, Bilayered Drosophila Myocardium
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Drosophila melanogaster is a genetically malleable organism with a short life span, making it a tractable system in which to study mechanical effects of genetic perturbation and aging on tissues, such as impaired heart function. However, Drosophila heart tube studies can be hampered by its bilayered structure: a ventral muscle layer covers the contractile cardiomyocytes. We have refined an atomic force microscopy-based analysis to measure individual mechanical components of soft composite materials. The technique was validated by its ability to resolve stiffness changes due to cardiac-specific RNA interference to reduce cardiomyocyte myofibrillar assembly or due to aging in Drosophila myocardial layers. Female yellow-white (yw) flies experience decreased diastolic diameter with age (>20%) while cardiomyocytes stiffened more than two-fold with age (1.8 ± 0.1 vs. 3.8 ± 0.3 kPa in 1 and 5 week old flies, respectively) at cell-cell junctions. Cardiac-specific RNA interference against myosin heavy chain severely impaired contraction and reduced stiffness after 1 week (1.0 ± 0.1 vs. 1.8 ± 0.1 kPa) without altering ventral muscle stiffness. This method provides a platform to assess the mechanics of soft biological composite systems and for the first time permits direct measurement of how genetic perturbations, aging, and disease can impact cardiac function in situ.

1783-Pos Board B553
Exploration and Suppression of Cardiac Amyloidosis Induced by Huntington’s Disease-Causing Amyloid in the Drosophila Heart Model
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Accumulation of amyloids is associated with cardiomyopathy; however, the precise mechanism that leads to defective heart structure and function is not known. Amyloid-like inclusions have been detected in patients with Huntington’s disease (HD), which is caused by an expanded polyglutamine (Poly-Q) repeat in the Huntington (HTT) protein. HD patients also demonstrate a greater occurrence of cardiovascular events, presumably as a result of toxic amyloid accumulation due to global protein misfolding and/or oxidative stress. To explore cardiac defects associated with HD-causing amyloid protein, we used the UAS-Gal4 system and a cardiac-specific driver (Hand-Gal4) to express mutant HTT with short (UAS-Httex1-PolyQ25) and disease-causing expanded (UAS-Httex1-PolyQ72) Poly-Q in the Drosophila heart. Expression of disease causing Poly-Q in 1 and 3 week old fly hearts resulted in severe cardiac defects as evidenced by prolonged diastolic and systolic intervals, a significantly increased incidence of arrhythmias and extreme cardiac dilation that was accompanied by a significant decrease in cardiac contractility (reduced fractional shortening). Structural analysis showed myocardial cells with noticeably reduced myofibrillar content, myofibrillar disorganization and the presence of amyloid-aggregates. No such physiological and structural defects were seen upon expression of short Poly-Q under similar conditions. To take advantage of our genetic model and to further explore the mechanism underlying the Poly-Q-induced cardiac defects, we co-expressed expanded Poly-Q with either the antioxidant enzyme superoxide dismutase (SOD) or a chaperone protein UNC-45. Our preliminary results suggest that cardiac dilation is reduced and cardiac performance is enhanced upon co-expression of SOD or UNC-45. Thus we have developed a novel Drosophila model that allows us to explore cardiac defects associated with the accumulation of HD-causing amyloid and to elucidate the mechanisms underlying cardiac failure in HD patients.
Heart failure is a progressive condition in which the ventricles can no longer pump enough blood to meet the body’s basal demands. Our laboratory is investigating whether the transmural variation in cellular contractile properties that occurs in normal hearts (and which is thought to be important for ventricular function) is altered in heart failure. We procured through wall samples of failing left ventricle from patients receiving transplants at the University of Kentucky and non-failing samples from brain dead organ donors. The tissue was divided into epicardial, midmyocardial and endocardial layers and frozen in liquid nitrogen within 30 minutes. Multicellular chemically permeabilized preparations were subsequently obtained from these samples by mechanical homogenization and triton treatment. The samples were attached between a force transducer and a motor and subjected to two mechanical protocols: 1) a stretch-restretch protocol in solutions with different activating Ca$^2+$ concentrations and 2) a force-velocity protocol in which maximally-activated preparations were allowed to shorten against pre-set loads. Parameters including steady-state force, short-range stiffness, short-range force and maximum power output were measured using these two protocols. The results suggested a 30% decrease in maximum power output (p-value = 0.01) and steady-state force (p-value = 0.005) in heart failure patients (n=8, total of 72 preparations) as compared to non-failing (n=4, total of 36 preparations). Short-range stiffness (p-value=0.003) and short-range force (p-value=0.002) also significantly decreased in heart failure vs. non-failing. Transmurally there was a significant difference in maximum power output between the regions (p-value<0.02). The data suggest that mechanical homogenization and triton treatment maybe affected the most in heart failure. Further studies need to be done to understand the protein modifications that may be responsible for these variations.