2466-Pos Board B452
Length Dependence of Activation (LDA) Studies in Mice Deficient in the N2B Element of Titin
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The increase in Ca^{2+} sensitivity that occurs when sarcomere length (SL) is increased (LDA), is considered the cellular basis of the Frank-Starling law of the heart. Recent findings show increased LDA in a mouse model in which the N2B spring element has been excised (N2B KO) (Lee *et al*., 2010). Here we investigated LDA in the presence of 3% Dextran (Dex), the effect of PKA phosphorylation on LDA, and structural changes of myofilaments by using low-angle X-ray diffraction. Fiber bundles of skinned papillary muscle from left ventricular of N2B KO and WT mice were used. The passive tension, SL and X-ray diffraction patterns were collected at various SLs from 1.95 to 2.3μm. From X-ray diffraction patterns, myofilament lattice spacing (LS; d_{10}) and the position of the myosin head relative to the thin filaments (intensity ratio; I_{11}/I_{10}) were obtained. We also performed mechanical studies to characterize Ca^{2+} sensitivity and LDA. For X-ray diffraction study, d_{10} was significantly decreased with SL regardless of genotype and Dex (p-values $<.05$). The slope of this negative correlation was greater in KO compared to WT, however, statistical significance was found only in Dex ($p<.01$). Passive tension at SL 2.3μm was correlated with changes in d_{10} ($p<.01$) in KO and WT mice. I_{11}/I_{10} was decreased proportionally to SL, however, no significant difference was found on the slope of correlation between genotype. Significantly greater LDA was observed in KO compared to WT (0.16 and 0.07, respectively; $p<.01$) in Dex. PKA phosphorylation increased LDA in both KO and WT mice by 0.12 in Dex and 0.06 in normal solutions, on average. These results indicate that in N2B KO myocardium, LDA is increased more than in WT and that d_{10} is decreased but not I_{11}/I_{10}.

2467-Pos Board B453
Quantification of Titin Based Viscosity: Temperature, Lattice Compression and Integrative Physiology
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Viscosity is proposed to modulate diastolic function, but there is only limited understanding of the source(s) of viscosity. In-vitro experiments have shown that the proline-glutamic acid-valine-lysine (PEVK) rich element of titin binds actin, causing a viscous force in the sarcomere. It is unknown whether this mechanism contributes to viscosity in-vivo. Therefore, we sought to test, via an integrative physiological study on a unique PEVK-knockout (KO) mouse, the hypothesis that PEVK-actin interaction causes significant cardiac viscosity and is important in-vivo.
METHODS AND RESULTS: Both skinned cardiomyocytes and papillary muscle fibers were isolated from wildtype (WT) and PEVK KO mice and viscosity was examined using stretch-hold-release and sinusoidal analysis. We previously found that viscosity is reduced by ~60% in KO myocytes and ~50% muscle fibers at room temperature (24°C). Inhibition by blebbistatin reveals that actomyosin interaction is not present at room temperature but contributes to viscosity at physiologic temperature (37°C). We also examined the passive contribution of lattice compression and temperature while inhibiting actomyosin interactions with blebbistatin. Lattice compression using the osmotic agent Dextran T500 enhances viscosity in WT but not KO tissues but increasing temperature alone does not significantly increase viscosity. We also studied intact isolated hearts via a Langendorff perfused volume-controlled system. Stretch-hold protocols and sinusoidal frequency protocols indicated that KO hearts have a ~30% reduction in viscosity. Finally, transmitral Doppler echocardiography and kinematic modeling was utilized to examine left ventricular viscosity in-vivo. Quantifying viscosity with both traditional and kinematic echocardiographic measurements suggest a ~40% decrease in viscosity in the KO left ventricle.
CONCLUSIONS: This integrative study is the first to quantify the consequences of a specific molecular (PEVK-actin) viscosity in-vivo and physiologic modulation of this passive viscosity by lattice spacing.

2468-Pos Board B454
Comparison of Human and Mouse Recombinant Titin N2B Fragment as a Substrate for PKA and PKG
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Phosphorylation of cardiac titin spring elements by serine/threonine (S/T) protein kinases affects the diastolic function of the heart. The detection and identification of these phosphorylation sites in different species is critical to our understanding of this important biological process. β-adrenergic and cGMP activation of PKA and PKG, respectively, plays critical roles in the functional regulation of the heart and cardiovascular system; the PKA/PKG target domain is the N2B spring element. The current aim is to determine differences in N2B phosphorylation between mouse and human using autoradiography and mass spectrometry. For autoradiography, mouse and human N2B recombinant fragments were incubated with PKA or PKG, separated by SDS-PAGE (4-20% gradient), and Coomassie blue stained. Autoradiography results indicate that human N2B is a stronger substrate for PKA and PKG than mouse N2B. To understand the molecular basis of these differences we carried out alignment analysis of human and mouse N2B sequences (53% homology) and determined phosphorylation sites of the human N2B by MS. For mass spectrometry, the human N2B protein band was excised, digested with trypsin, and the products analyzed by nano-LC-MS/MS. The MS analysis of human N2B detected 10 residues phosphorylated by PKA (2S/8T) and 7 by PKG (6S/1T). Comparing MS data alignment of the mouse and human N2B sequences showed that mouse N2B has less possible phosphorylation sites for PKA/PKG compared to human N2B; only S478, phosphorylated by both PKA and PKG in human, is present in mouse N2B. Also, S348 in human is substituted by a T in mouse. In conclusion, the data suggests that β-adrenergic and cGMP activation may be evolutionarily less important in mouse heart function than in human.

2469-Pos Board B455
Characterization of Titin Following Transverse Aortic Constriction in Mice
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Heart Failure is characterized by an impaired ability of the left ventricle to fill or eject blood and is typically accompanied with cardiac hypertrophy. Furthermore, sustained cardiac hypertrophy transitions into maladaptive hypertrophy, with left ventricular dilation, decrease contractile properties, increased myocardial stiffness (through alterations in titin and collagen), and reduced cardiac output. The current aim was to investigate alterations in heart function, titin isoform shifts, and myocardial stiffness following transverse aortic constriction-induced heart failure in mouse. Echocardiology showed increased aortic velocity (0.5 vs 4.5m/s), diastolic (55 vs 90ul) and systolic (20 vs 53ul) ventricular volume, LV mass (133 vs 220mg) and decreased ejection fraction (65% vs 35%) and mitral valve deceleration time (23 vs 18ms) (indicating a significant increase in myocardial stiffness). Additionally, E/A ratios dramatically increased from 1.5 to 5, signifying extensive heart failure. In order to further characterize myocardial stiffness, skinned LV muscle bundle fibers were mechanically stretched and titin and collagen forces were measured. At sarcomere length 2.15, data showed an increase in total passive tension (35%), total collagen force (120%), and total titin force (15%). Protein gels were ran to help identify the source of increased titin tension. Gels showed an increase in the long N2BA as related to the short N2B titin isoform (0.20 vs 0.37) indicating that titin passive tension should actually decrease, not increase. However, western blots using phospho-specific antibodies for titin's S11878 and S12022 (phosphorylated by PKCα resulting in increased passive tension) indicate a 2.5 fold increase in phosphorylation in the end-stage heart failure mice. In conclusion, TAC-induced heart failure in mice resulted in an increased diastolic stiffness partially through PKCα phosphorylation-mediated increases in titin-based tension.

2470-Pos Board B456
The Role of Titin's N2B Element in Limiting Energy Loss of Mouse Myocardium During Repeated Loading Cycles
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Utilizing a N2B knockout (KO) mouse model in which the exon that encodes the cardiac-specific N2B element (exon 49) has been deleted, we investigated the mechanical role of the N2B element, one of the three extensible regions of titin. It has been proposed that the extensibility provided by the N2B element limits unfolding of titin's Ig domains that reside in series with the N2B element, thereby reducing energy loss during stretch and shortening (i.e., during diastole and systole) cycles of the beating heart. We were able to show significant increases in hysteresis, a measure of energy loss determined from the area between the stretch and release force-SL curves, through triangular stretch/release protocols using a range of velocities (10, 100 and 1000 % /s) and three amplitudes (0.2, 0.3, and 0.4 μm/sarcomere). In order to more closely simulate physiological conditions, triangular stretch/release traces were concatenated in excess of 1000 cycles to properly precondition the tissue. Results showed that hysteresis decayed over preconditioning cycles (30% of initial hysteresis at 200 preconditioning cycles) but held the significance increase in KO compared to wildtype (WT) mice. (Hysteresis was $650 \pm 90 pJ/mm^2$/sarcomere vs. $300 \pm 50 pJ/mm^2$/sarcomere, $p<0.005$, at 200 preconditioning cycles) Using skinned muscle fibers, it is known that they exhibit expanded sarcomere lattice spacing, in order to eliminate this difference we ran this preconditioning protocol in 3% dextran at 37.5oC. Returning the lattice spacing to physiological conditions increased the amount of hysteresis in both WT and KO mice, this increase was concentration dependent though. At 3% dextran the KO held