σ is stress, ε is strain, and θ is the phase shift. We found that both the viscous modulus and the elastic modulus were higher in the AI KO at high frequencies. These results suggest that WT passive tension levels rely on an intact A-I junction; the removal of this region results in increased titin stiffness.

1747-Pos Board B477

Myocardial Titin: An Important Modifier of Cardiac Stiffness

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Background: A well-established function of titin is the determination of passive tension ($F_{passive}$) in myocardium. Modifications to the elastic titin region have been suggested to contribute to left ventricular (LV) diastolic dysfunction in heart failure (HF). Titin-based stiffness can be modulated by isoform switch or phosphorylation.

Results: We find that titin-isoform switch accounts for a significant amount of myocardial stiffness modulation, giving rise to increased or reduced $F_{passive}$ in different types of heart failure. In addition, both acute and chronic modulations of cardiomyocyte $F_{passive}$ occur via altered titin phosphorylation. Cyclic AMP-dependent protein kinase-A, cGMP-dependent protein kinase-G, and extracellular signal-regulated kinase-2 phosphorylate titin at a cardiac-specific domain, the N2Bus; this phosphorylation results in a reduction in cardiomyocyte $F_{passive}$ in various species. PKCα phosphorylates the PEVK-domain of titin, which increases $F_{passive}$ of normal mouse cardiomyocytes, but does not significantly alter $F_{passive}$ of cardiomyocytes obtained from a dog HF model. Calcium/calmodulin-dependent protein kinase-II (CaMKII) is the first kinase found to phosphorylate both the N2Bus and the PEVK-domain. This phosphorylation reduces cardiomyocyte $F_{passive}$, as demonstrated in skinned mouse cardiomyocytes incubated with recombinant CaMKIIδ. Moreover, $F_{passive}$ is elevated in cardiomyocytes of CaMKIIγ/δ double knockout mice and reduced in those of CaMKIIδ-overexpressing transgenic mice. In both human and experimental HF, a global titin phosphorylation deficit is observed, but site-specific titin phosphorylation can be increased or decreased in HF, presumably depending on the activity and expression level of the relevant kinases.

Conclusion: Titin phosphorylation may have beneficial effects in the heart via reducing myocardial diastolic stiffness and improving ventricular filling. Altered titin phosphorylation in HF may severely affect $F_{passive}$ and compromise cardiac function. The degree, to which the different protein kinases contribute to alterations in diastolic passive stiffness, needs to be determined.

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A Gain-Of-Function Mutation in Cardiac Myosin Binding Protein-C Increases Viscoelastic Load and Slows Shortening Velocity in Myocytes from Transgenic Mice

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Cardiac myosin binding protein C (cMyBP-C) is a sarcomeric protein involved in the regulation of cardiac muscle contraction. Effects of cMyBP-C on contraction are thought to be mediated in part by limiting the interactions of actin and myosin to slow myocyte shortening velocity and power output. Although interactions with myosin S2 on the thick filament have been proposed as a way in which cMyBP-C could limit shortening velocity (e.g., by creating a drag force on myosin heads), interactions of cMyBP-C with actin could also account for slowed shortening velocity. For instance, cMyBP-C could create a drag that opposes filament sliding by transiently linking thick and thin filaments together. To explore this possibility we created transgenic mice that express a mutant cMyBP-C with a point mutation (L348P) located in a conserved sequence within the regulatory M-domain that increases cMyBP-C binding to actin in vitro (Bezold et al, JBC, 2013). We reasoned that if the mutation also enhanced binding to actin in sarcomeres then shortening velocity would be slowed in myocytes from L348P mice. Results show that transgenic mice expressing the L348P mutation are viable and that L348P cMyBP-C is expressed in sarcomeres. Permeabilized myocytes from transgenic mice showed altered force production including reduced maximal force and enhanced Ca^{2+} sensitivity of tension. Shortening velocity and power output were significantly reduced whereas passive stiffness and myocyte visco-elasticity were significantly increased. Together these data are consistent with the idea that cMyBP-C creates an internal load in the sarcomere by binding to actin. This

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Exercise-Induced Enhancement of Cardiac and Sarcomere Performance is Larger in Male than in Female MYBPC3 Mutation Heterozyous Knock-In Mice

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Hypertrophic cardiomyopathy (HCM) is the most common genetic cardiac disorder. Mutations in the gene (*MYBPC3*) encoding cardiac myosin binding protein C (cMyBP-C) are a frequent cause of HCM. Clinical as well as animal-model studies have reported sex-related differences in HCM disease onset and severity. In addition, it has been established that physiological stimuli such as exercise may elicit a sexually dimorphic cardiac response. However, less attention has been paid to the sex-specific differences in the cellular pathophysiologic mechanisms underlying HCM. Therefore, we studied functional properties of the heart and sarcomeres in male and female sedentary and exercise (exposed to 8 weeks voluntary wheel running) mice.

Echocardiography and isometric force measurements in mechanically isolated left ventricular (LV) membrane-permeabilized cardiomyocytes were performed in Wild-type (WT) and heterozygous (HET) knock-in mice carrying a *Mybpc3* point mutation (G>A transition) associated with HCM.

The LV mass was significantly lower in female WT and HET mice (23% in WT and 25% in HET), compared to corresponding male mice. Isometric force measurements revealed a significant lower maximal generated tension (F_{max}) in HET male (13.0 ± 1.1 kN/m^2), than in females (20.0 ± 2.2 kN/m^2). Exercise induced a higher fractional shortening in HET male mice, which is correlated with an increased F_{max} in exercised HET males. In contrast, LV weight was significantly increased in exercised HET females compared to sedentary females (7% in WT and 15% in HET). Ca^{2+}-sensitivity was increased in exercised male and females WT mice. Similarly, Ca^{2+}-sensitivity was enhanced in HET females, however not in exercised HET mice.

In conclusion, exercise training improved cardiac and myofilament performance particularly in HET male mice, indicating that physiological stimuli may elicit a sexually dimorphic cardiac response in heterozygous *Mybpc3*-targeted knock-in mice.

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Gender Differences in Passive Tension in Hypertrophic Cardiomyopathy Patients

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Background

Hypertrophic cardiomyopathy (HCM) is an inherited cardiac disorder with a prevalence of 1:500. In ~65% of all HCM patients the causative mutation is identified. HCM patients that are sarcomere-mutation negative tend to have a less severe phenotype. Mutations in the *MYBPC3* and *MYH7* genes encoding cardiac myosin-binding protein C (cMyMP-C) and β-myosin heavy chain (MyHC) represent >80% of all genotyped HCM cases. HCM is characterized by asymmetric hypertrophy of the left ventricle and diastolic dysfunction. In the present study we investigated if passive stiffness of the sarcomeres may underlie diastolic dysfunction.

Methods

In-vitro passive tension measurements were done at sarcomere lengths of 1.8 to 2.2μm in cardiomyocytes from 10 sarcomere mutation-negative patients(SMN: 5 male, 5 female), 17 patients carrying a *MYBPC3* mutation(MYBPC3: 10 male, 7 female), and 10 patients carrying a *MYH7* mutation (MYH7: 5 male, 5 female). Tissue was obtained during myectomy surgery from the interventricular septum. Cardiomyocytes were mechanically isolated and Triton-permeabilized.