the cMyBP-CDDD group. Similar results were obtained for maximum force development. As a consequence, LDA was blunted (~40%) in cMyBP-CAAA myocardium. There were no differences in the level of cooperativety as indexed by the Hill coefficient in any group.
Conclusion: Phosphorylated cMyBP-C has been shown to contribute to regulation of cardiac sarcomere function via modulation of the cMyBP-C-actin interaction as well as the disposition of the cross-bridges in relation to the thin filament. Moreover removal of cMyBP-C results in blunted LDA, and a cardiac dysfunction that can be prevented by cMyBP-CDDD but not cMyBP-CAAA. Our data showed that lack of cMyBP-C phosphorylation results in blunted LDA, similar to that found previously in the absence cMyBP-C. We conclude that cMyBP-C phosphorylation modulates myofilament length dependent activation, possibly via modulation of the cMyBP-C interaction with actin.

3912-Pos Board B640
Effect of a High-Salt Diet on the Mechano-Energetics of Left Ventricular Trabeculae Isolated from Dahl Salt-Sensitive Rats
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Dahl salt-sensitive (SS) rats were weaned at 3 weeks of age onto either a high salt diet (4% NaCl) or a normal diet (0.4% NaCl) until termination at 12 weeks of age. Blood pressure measurements, recorded using implanted telemeters, showed a hypertensive response in the high-salt group (MAP = 137 ± 6 mmHg) compared to the normal diet group (MAP = 119 ± 1 mmHg). Compensated hypertrophy was seen in the high-salt group where the wet weights of the hearts were heavier (1.88 ± 0.03 g versus 1.50 ± 0.05 g) and the LV walls were thicker (5.56 ± 0.14 mm versus 4.48 ± 0.12 mm). To investigate the effects of the high salt diet on the mechano-energetics of the heart tissue, trabeculae from the left ventricle were isolated and transferred to a work-loop calorimeter where force production, length change and heat output were simultaneously measured. The experiments were performed at 32°C and the trabeculae were stimulated at 3 Hz. Preliminary results show that there are no statistically significant differences in the peak active stress (62 ± 6 kPa versus 52 ± 6 kPa), peak work (1.67 ± 0.21 kJ m^{-3} versus 1.32 ± 0.16 kJ m^{-3}) or peak mechanical efficiency (16.2 ± 1.1% versus 13.7 ± 1.0%) between the high-salt and normal diet groups, respectively. Hence, despite evidence of hypertension and hypertrophy in the hearts of SS rats fed a high-salt diet, there appear to be no significant differences in the mechano-energetic performance at the tissue (trabecula) level. The data from our experiments are used to parameterize thermodynamically constrained models of cross-bridge kinetics and whole-cell bioenergetics to determine the relationships between model parameters and key experimentally-determined outputs such as work and efficiency.

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Oxygen Consumption of Brown Adipose Tissue (BAT) and Skeletal Muscle is Inversely Related
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During exercise, the muscles' energy demand increases with increasing work load. In humans, the relationship between work load and oxygen uptake is linear until the maximal oxygen uptake (VO_{2max}) is reached and higher exercise intensity requires additional anaerobic energy supply. VO_{2max} is thought to reflect the maximum oxygen transport capacity of the cardiovascular system.
We show that at room temperature normal mice could triple running speed at 25% inclination after reaching VO_{2max} in spite of very modest increase of anaerobic muscle metabolism. In mice with cardiac dysfunction due to cardiac disruption of the *Serca2* gene (S2KO), VO_{2max} was reduced from week4 to week6 after gene disruption in parallel with progression of cardiac dysfunction. However, S2KO mice maintained maximal running speed at the same the level as the controls. Thus, paradoxically, running economy was better in S2KO than in controls. In S2KO, blood lactate was almost double of that of controls and respiratory exchange ratio was near 1, indicating greater reliance on anaerobic metabolism. However, heat production was lower in S2KO than in controls as reflected by tail temperature. Activity of BAT measured by fluorodeoxyglucose using PET was reduced by 60 ± 7% during running in controls and by 82 ± 3% in running S2KO mice.
In mice, the oxidative metabolism in non-muscle tissue, mainly in BAT, is reduced during exercise to provide more oxygen to the working muscles. This redistribution of oxygen delivery leaves the total VO_2 unchanged over a wide range of exercise intensities. When cardiac output and VO_{2max} are abnormally low, exercise intensity can be maintained since muscles can utilize the oxygen normally used by non-muscle tissue such as BAT. We conclude that oxygen consumption of skeletal muscle and BAT is regulated in a reciprocal way.

3914-Pos Board B642
Optogenetic G_S Activation in Cardiomyocytes Enhances Pacemaker Activity
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Stimulation of G_S-protein coupled receptors leads to an increase of the second messenger cyclic adenosine monophosphate (cAMP). In cardiomyocytes the G_S-signaling cascade is involved in positive regulation of chronotropy and contractility but chronic G_S stimulation can also induce cardiac hypertrophy or arrhythmia.
Experimentally, the G_S-signaling cascade can be activated by ß-receptor agonists but diffusion of drugs does not allow the precise control of location and timing. To overcome these limitations we used the optogenetic protein JellyOp, a directly G_S-coupled, light-sensitive receptor (Bailes et al. PloS One, 2012) to activate G_S-signaling by light.
Illumination of JellyOp expressing HEK 293 cells resulted in elevation of cAMP levels without detectable dark activity. Cardiomyocytes were differentiated within embryoid bodies (EBs) from transgenic mouse embryonic stem cells that express JellyOp under control of the ubiquitous chicken ß-actin promoter. Spontaneously beating EBs were analyzed at day 13 of differentiation by infrared video microscopy. Brief illumination (20 sec, 470 nm, 166.7 nW/mm^2) increased beating frequency to 1239 ± 349% of baseline (n=3) which returned to baseline after termination of illumination. The lowest effective light intensity was of 9.1 nW/mm^2 resulting in frequency acceleration to 428 ± 131% of baseline and the shortest effective illumination was 1 sec. Similar to dose-response-curves of receptor agonists, light-induced frequency acceleration showed a sigmoid dependence on light-intensity with a half maximal light intensity of 33.5 nW/mm^2. Direct comparison showed that the rate of frequency increment was much faster using illumination (13.9 ± 3.1%/s) than using perfusion with the ß-receptor agonist isoprenaline (2.7 ± 1.5%/s) but both stimulations led to a similar response in frequency elevation.
In summary optogenetic JellyOp activation in cardiomyocytes enables the stimulation of the G_S-signaling pathway with high temporal precision and will be useful to investigate temporal and site-specific effects of physiological and pathophysiological G_S-activation.

3915-Pos Board B643
Compartmentation of Camp Signaling in Complex and Simple Cells
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Differences in compartmentation of cAMP signaling were compared in adult rat cardiac ventricular myocytes and HEK293 cells. The freely diffusible Epac2-camps FRET-based biosensor was used to monitor cAMP responses in the bulk cytoplasmic compartment of these cells, while Epac2-MyrPalm and Epac2-CAAX versions of the probe were used to measure subcellular cAMP responses associated with lipid raft and non-lipid raft domains of the plasma membrane, respectively. Stimulating raft associated beta-adrenergic receptors (βAR) or non-raft associated E-type prostaglandin receptors (EPR) elicited markedly different cAMP responses in the two cell types. In HEK293 cells, maximal βAR or EPR stimulation produced saturating cAMP responses in all three domains. In cardiac myocytes, maximal βAR stimulation produced non-saturating responses in all three domains. However, EPR stimulation produced responses that were smaller, transient, and more consistently observed in non-lipid raft and bulk cytoplasmic domains. There were also significant differences in the pattern of basal cAMP activity associated with the different microdomains of the two cell types. Direct inhibition of adenylyl cyclase (AC) activity with MDL12330A (MDL) only produced a decrease in basal cAMP activity in non-lipid raft domains of HEK293 cells. However, MDL inhibited basal cAMP activity in non-lipid raft domains as well in the bulk cytoplasmic compartment of cardiac myocytes. In cardiac myocytes, responses detected in all three locations were significantly more sensitive to inhibition of phosphodiesterase (PDE) activity. However, in HEK293 cells,