using thoracic aortic constriction. Left ventricular cardiomyocytes were isolated enzymatically and studied using confocal microscopy.
Chronic overloading increased cardiomyocyte volume at 10 but not 6 weeks (Control 45,348 ± 2373μm^3, n=47 vs. OV 6 weeks 48,785 ± 2237μm^3, n=60 vs. OV 10 weeks 56,066 ± 3091μm^3, n=42; p<0.05) and disrupted t-tubule regularity, which was measured as the power of the dominant frequency of the Fourier transform of Di-8-ANEPPS images (Control 1.69 ± 0.1x10^7, n=44 vs. OV 10 weeks 9.45 ± 1.0x10^6, n=33; p<0.001), despite preserved Ca^{2+} transients. Unloading decreased cardiomyocyte size and induced time-dependent Ca^{2+} transient changes. Ca^{2+} release synchronicity (measured by the variance of the time-to-peak of the Ca^{2+} transient) was disrupted at 8 but not 4 weeks (Control 269.6 ± 17.13ms^2, n=106 vs. UN 4 weeks 331.6 ± 23.67ms^2, n=46 vs. UN 8 weeks 403.5 ± 35.4ms^2, n=50; p<0.01), as was the t-tubule regularity (Control 1.59 ± 0.01x10^7, n=107 vs. UN 4 weeks 1.75 ± 0.1x10^7, n=53 vs. UN 8 weeks 1.1 ± 0.0007x10^7, n=82; p<0.01).
We demonstrate that an increase or decrease in load results in time-dependent disrupted t-tubule morphology. This can provide a mechanism for inefficient CICR observed in chronic heart failure and the functional deterioration after prolonged unloading of the heart using left ventricular assist devices.

2806-Pos Board B576
Transverse Tubule Structure is Related to Contractile Function in Human Heart Failure
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The transverse tubules (t-tubules) of cardiac myocytes facilitate a rapid and synchronous contraction. Loss of t-tubule structure has been reported in several animal models of cardiac failure and also in human heart failure. The loss of t-tubule structure is thought to disrupt calcium induced calcium release and contribute to impaired cardiac contraction. We sought to determine if a relationship between contractile performance and t-tubule structure may contribute to human heart failure. To test this idea we exploited the regional heterogeneity in contractility found within the failing human heart. MRI analysis was performed on patients with non-ischemic dilated cardiomyopathy awaiting a cardiac transplant. Tagged MRI was used to locate regions of different fractional shortening (circumferential strain) within the same heart. A model of the diseased heart was then created using MRI images from which a sampling map was created and used to sample tissue regions from the patient's heart (at transplantation). Using this methodology we were able obtain 14 tissue samples from 5 diseased hearts with corresponding contractility data ranging from poor (%S_c 2) to near normal function (%S_c 15). Tissue sections from these regions were labelled with fluorescent wheat germ agglutinin (WGA) and imaged using confocal microscopy. Fourier analysis of WGA labelled t-tubule images was used to assess t-tubule integrity of the cardiac myocytes using a modification of an established technique. Briefly, the height of the peak in the power spectrum corresponding to sarcomere spacing of t-tubules provided a metric of integrity (TT power). The mean TT power showed a strong positive relationship with fractional shortening data (R^2=0.61 p<0.001) suggesting that loss of t-tubule structure plays a role in reduced contractile function in human heart failure.

2807-Pos Board B577
Intracellular Dyssynchrony in Calcium Removal in Ventricular Cardiac Myocytes
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In cardiomyocytes, cytosolic Ca removal is mediated by sarcoplasmic reticulum Ca ATPase (SERCA), sarcolemmal Na/Ca exchanger (NCX), plasmalemmal Ca ATPase and mitochondrial Ca uniporter (MCU). We quantified spatiotemporal inhomogeneities in cytosolic Ca removal in normal and diseased hearts and explored underlying mechanisms. Ca transients (1 Hz, Fluo-4 AM) were recorded using confocal microscopy in ventricular cardiomyocytes. Time constant of cytosolic [Ca] decay (tau_loc) was quantified at 1 μm intervals across the cell. In murine cardiomyocytes tau_loc distribution was inhomogeneous, with a maximal difference between cell regions of 237 ± 9 ms and a variation coefficient (CV_tau) of 14 ± 2% (mean ± S.E.M., n=10 cells). tau_loc was not releated to the local amplitude or release kinetics of Ca. Coherent regions of fast (fastCaR, tau_loc < mean tau of whole cell) and slow (slowCaR) Ca removal had similar widths (4.6 ± 0.2 vs. 5.1 ± 0.5 ìm). Forskolin accelerated, and SERCA inhibitor cyclopiazonic acid (CPA) inhibited Ca removal significantly more in slowCaR than in fastCaR. In contrast, NCX-inhibitor SEA0400 slowed cytosolic Ca removal similarly in slowCaR and fastCaR. Also, CV_tau was unchanged in NCX+/- knock-out mice, suggesting no contribution of NCX to dyssynchrony in cytosolic Ca removal. tau_loc distribution was also inhomogenous in pig cardiomyocytes. Ca removal was more dyssynchronous in cardiomyocytes from chronic (4 weeks) ischemic pig myocardium (peri-infarct zone) vs. sham-operated pigs (CV_tau 24 ± 1%vs. 20 ± 1%, n=57 cells/group, p<0.05).Simultaneous recording of Ca transients and mitochondrial signal (mitotracker) unveiled a significant correlation between FastCaR regions and mitochondria (n=9 cells, p<0.05). Ru360 (MCU-inhibitor) slowed tau_loc more in FastCaR (to 126 ± 12% vs. 112 ± 10% of baseline in SlowCaR;p<0.05, n=5).Conclusion: Cytosolic Ca removal is dyssynchronous in mouse and pig cardiomyocytes. Ca removal in chronically ischemic cardiomyocytes is more dyssynchronous, suggesting a potential new mechanism of cardiomyocyte dysfunction. Mitochondria influence the differences in local Ca reuptake.

2808-Pos Board B578
Using Multi-Color Super-Resolution Microscopy to Probe the Organization of Dyadic Proteins within Rat Cardiac Myocytes
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Cardiac excitation-contraction coupling relies on calcium release from internal stores (the sarcoplasmic reticulum -SR) via ryanodine receptors (RyRs), which are intracellular calcium channels. Most RyRs are organized into clusters within narrow dyadic junctions between sarcolemmal and sarcoplasmic reticulum (SR) membranes. It is still unclear how junctions are formed and maintained. One protein that has been implicated in dyadic junction formation is the cardiac isoform of the junctophilin family, junctophilin-2 (JPH2). With the increased resolution available in multi-color super-resolution microscopy it is now possible to investigate the nanoscale relationship between proteins such as RyRs and JPH2 which are found within the junction. Using single fluorophore localization based super-resolution microscopy we have studied the distribution of a number of such proteins in rat ventricular myocytes.
Analysing the peripheral distributions of the cardiac ryanodine receptor (RyR) and a junctional protein, junctophilin-2 (JPH2), we have found that JPH2 was almost completely associated with RyR clusters. Estimates of co-localization of ~89% between JPH2 and RyRs confirmed near complete colocalization within peripheral couplons. The shape of associated RyR clusters and JPH2 clusters were very similar, suggesting that JPH2 is dispersed throughout RyR clusters and that the packing of JPH2 into junctions and the assembly of RyR clusters are tightly linked. Our data shows, with nanoscale resolution, that junctophilin is not constrained to specific anchoring domains within the junction, but is interspersed throughout the entire area occupied by RyRs.
These results demonstrate the utility of fluorescent multi-colour super-resolution immuno-labeling to investigate protein proximity at the nanometer scale. The ability to resolve the association of different molecules into the same biological structure at the nanoscale (here the dyad junction) should give new insight into the assembly and function of macromolecular signaling complexes.

2809-Pos Board B579
Feed Forward Modeling. Fixing the Force Frequency Relationship
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The force frequency relationship has intrigued researchers since its discovery by Bowditch in 1871. Several attempts were made to construct mathematical descriptions of this phenomenon; from the simple formulation of Koch-Wesser & Blinks in 1963 to the most sophisticated ones of present days. This property of the cardiac muscle is amplified by the β adrenergic stimulation. In a coordinated way the neurohumoral state alters both: frequency (acting on the SA node) and force generation (modifying the ventricular myocytes). This synchronized tuning is needed to meet new metabolic demands. Failure to do so has deleterious consequences. We implemented this physiological coordination in a new version of LabHEART (v5.5) where the cell parameters are updated according to the frequency of stimulation in a sigmoidal fashion (namely: ICaL, IKs, SERCA pump and myofilaments' Ca-sensitivity). This feed forward modeling helps to reproduce a more realistic cell behavior and complement the information obtained by experiments where frequency has been altered but without adding β-adrenergic agonists. Plots like rate-adaptation or APD vs cycle length can, now, be mapped into the neurohumoral state to provide insights about arrhythmias (or antiarrhythmic drugs). Cardiac