

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  $45,348 \pm 2373 \mu\text{m}^3$ ,  $n=47$  vs. OV 6 weeks  $48,785 \pm 2237 \mu\text{m}^3$ ,  $n=60$  vs. OV 10 weeks  $56,066 \pm 3091 \mu\text{m}^3$ ,  $n=42$ ;  $p<0.05$ ) 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  $1.69 \pm 0.1 \times 10^7$ ,  $n=44$  vs. OV 10 weeks  $9.45 \pm 1.0 \times 10^6$ ,  $n=33$ ;  $p<0.001$ ), despite preserved  $\text{Ca}^{2+}$  transients. Unloading decreased cardiomyocyte size and induced time-dependent  $\text{Ca}^{2+}$  transient changes.  $\text{Ca}^{2+}$  release synchronicity (measured by the variance of the time-to-peak of the  $\text{Ca}^{2+}$  transient) was disrupted at 8 but not 4 weeks (Control  $269.6 \pm 17.13 \text{ms}^2$ ,  $n=106$  vs. UN 4 weeks  $331.6 \pm 23.67 \text{ms}^2$ ,  $n=46$  vs. UN 8 weeks  $403.5 \pm 35.4 \text{ms}^2$ ,  $n=50$ ;  $p<0.01$ ), as was the t-tubule regularity (Control  $1.59 \pm 0.01 \times 10^7$ ,  $n=107$  vs. UN 4 weeks  $1.75 \pm 0.1 \times 10^7$ ,  $n=53$  vs. UN 8 weeks  $1.1 \pm 0.0007 \times 10^7$ ,  $n=82$ ;  $p<0.01$ ).

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

David Crossman<sup>1</sup>, Peter Ruygrok<sup>2</sup>, Alistair Young<sup>1</sup>, Christian Soeller<sup>1</sup>, Mark Cannell<sup>3</sup>.

<sup>1</sup>The University of Auckland, Auckland, New Zealand, <sup>2</sup>Auckland City Hospital, Auckland, New Zealand, <sup>3</sup>University of Bristol, Bristol, United Kingdom.

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

Frank R. Heinzel<sup>1</sup>, Felix Hohendanner<sup>1</sup>, Niall Macquaide<sup>2</sup>, Michael Sacherer<sup>1</sup>, Gudrun Antoon<sup>1</sup>, Karin Sipido<sup>2</sup>, Burkert Pieske<sup>1</sup>.

<sup>1</sup>Medical University of Graz, Graz, Austria, <sup>2</sup>Katholieke Universiteit Leuven, Leuven, Belgium.

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, Fluoro-4 AM) were recorded using confocal microscopy in ventricular cardiomyocytes. Time constant of cytosolic [Ca] decay ( $\tau_{\text{loc}}$ ) was quantified at 1  $\mu\text{m}$  intervals across the cell. In murine cardiomyocytes  $\tau_{\text{loc}}$  distribution was inhomogeneous, with a maximal difference between cell regions of  $237 \pm 9$  ms and a variation coefficient (CV $_{\tau}$ ) of  $14 \pm 2\%$  (mean  $\pm$  S.E.M.,  $n=10$  cells).  $\tau_{\text{loc}}$  was not related to the local amplitude or release kinetics of Ca. Coherent regions of fast (fastCaR,  $\tau_{\text{loc}} < \text{mean } \tau_{\text{loc}}$  of whole cell) and slow (slowCaR) Ca removal had similar widths ( $4.6 \pm 0.2$  vs.  $5.1 \pm 0.5$   $\mu\text{m}$ ). 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 $\pm$  knock-out mice, suggesting no contribution of NCX to dyssynchrony in cytosolic Ca removal.  $\tau_{\text{loc}}$  distribution was also inhomogeneous 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 $_{\tau}$   $24 \pm 1\%$  vs.  $20 \pm 1\%$ ,  $n=57$  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  $\tau_{\text{loc}}$  more in fastCaR (to  $126 \pm 12\%$  vs.  $112 \pm 10\%$  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

David Baddeley<sup>1</sup>, Isuru D. Jayasinghe<sup>1,2</sup>, Cherie H.T. Kong<sup>1,3</sup>, Xander H.T. Wehrens<sup>4</sup>, Mark B. Cannell<sup>1,3</sup>, Christian Soeller<sup>1</sup>.

<sup>1</sup>University of Auckland, Auckland, New Zealand, <sup>2</sup>University of Queensland, Brisbane, Australia, <sup>3</sup>University of Bristol, Bristol, United Kingdom, <sup>4</sup>Baylor College of Medicine, Houston, TX, USA.

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  $\sim 89\%$  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

Jose L. Puglisi, Leighton Izu, Ye Chen-Izu, Donald M. Bers.

University of California, Davis, Davis, CA, USA.

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-Weser & Blinks in 1963 to the most sophisticated ones of present days. This property of the cardiac muscle is amplified by the  $\beta$  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  $\beta$ -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