P1-01

Functional Cardiac Fibroblasts Derived from Human Pluripotent Stem Cells via Second Heart Field Progenitors

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Cardiac fibroblasts (CFs) play critical roles in heart development, homeostasis, and disease. The limited availability of human CFs from native heart impedes investigations of CF biology and applications in cardiac regeneration. Human pluripotent stem cells (hPSCs) provide an unlimited cell source, but effective methods to generate CFs from hPSCs have not been described. Here, we show differentiation of hPSCs using sequential modulation of Wnt and FGF signaling to generate second heart field progenitors that efficiently give rise to hPSC-CFs. Confluent monolayer hPSCs were treated with GSK3β inhibitor (CHIR) followed by treatment with bFGF in a defined medium for 20 days of differentiation. Flow cytometry and qRT-PCR showed sequential upregulation of markers for mesoderm (*T*), cardiac mesoderm (*MESP1*) and SHF progenitors including *GATA4*, *ISL1*, *TBX1* and *HAND2* during day 1-6 differentiation. Continuous treatment with bFGF after day 6 further promoted fibroblasts differentiation. Flow cytometry for fibroblast markers showed ~77% of the cells were fibroblasts after 20 days of differentiation. The hPSC-derived CFs resemble native heart CFs in overall gene expression demonstrated by RNA-seq. Moreover, the hPSC-CFs express key cardiac transcription factors including *BMP4, GATA4, HAND2, HEY1, ISL1, NKX2-5, SOX17 and WT1*. The hPSC-CFs produced abundant extracellular matrix (ECM) when seeded at a high density, forming a unique 3D ECM scaffold composed of collagen and fibronectin. Furthermore, treatment of hPSC-CFs with TGF β1 caused myofibroblast differentiation demonstrated by upregulation of α-smooth muscle actin (SMA) measured by flow cytometry and immunolabeling. Co-culture of hPSC-CFs with hPSC-derived cardiomyocytes alters the electrophysiological properties of the cardiomyocytes based on optical mapping of membrane potential. We conclude that CFs can be efficiently differentiated from hPSCs via SHF progenitors in high yield and purity. The hPSC-CFs provide a powerful cell source for tissue engineering, disease modeling, drug discovery, and therapeutic applications in cardiac regeneration.

P1-02

Cardiac Progenitor Cell Fate in Embryonic and Neonatal Environments

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Introduction: Multipotent c-Kit$^+$ cardiac progenitor cells (CPCs) commit to three distinct cardiac lineages: endothelial cells, smooth muscle cells, and cardiomyocytes. However, adult CPC differentiation and regenerative potential is hindered by poor long-term cell retention following adoptive transfer, perhaps due to hostile recipient environment in infarcted or ischemic hearts that presents a challenge to CPCs survival or differentiation. The prenatal heart possesses highly regulated spatiotemporal coordination allowing for cell lineage diversification, presumably a highly permissive environment for maximizing CPC multipotent potential.

Hypothesis: Embryonic and neonatal cardiac microenvironments provide optimal spatiotemporal conditions to promote CPCs persistence, survival and preservation of multipotent potential.

Methods: CPCs isolated from adult mouse hearts were expanded, fluorescence-tagged, and injected into blastocysts at E3.5, developing embryos at E15.5, and neonatal hearts at P3. Injected embryos or neonatal hearts were analyzed by immunofluorescence for presence of CPC-derived tissues to assess CPCs phenotypic properties *in situ*.

Results and Conclusions: CPCs delivered into P3 hearts demonstrate persistence and engraftment for up to 24 days post injection (dpi). Adoptively transferred CPCs stably engrafted into left ventricular myocardium and coupled with neighboring cardiomyocytes by 14dpi (n=4). Prenatal injection revealed that at E15.5, CPCs predominantly home towards perivascular regions (n=4). At blastocyst stage, donor CPCs anchor in blastocoel and integrate in the trophoblast layer at 2dpi. Interestingly, CPCs integrated into amniochorionic sac when injected at blastocyst and embryonic stage, *revealing a novel extraembryonic fate of CPCs*. In conclusion, this study provides vivid evidence of adoptively transferred CPC adopting different cell fates *in vivo determined by multiple recipient microenvironments. Future studies will focus upon delineating characteristics of* permissive environmental conditions *to provide fundamental insights on CPCs lineage potential and how they interact with the surrounding microenvironment.*

P1-03

Cardiomyocyte Biology Revealed by Fluorescence Ubiquitination-based Cell Cycle Indicators (FUCCI)

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Introduction: Cell-cycle progression critical for *de novo* cardiomyocyte formation is rare and poorly characterized in postnatal mammalian heart. Novel insights of cardiomyocyte cell-cycle dynamics can be revealed using Fluorescence Ubiquitination-based Cell-Cycle Indicator (FUCCI) system, showing inverse oscillation of cell-cycle fusion proteins AzGr-hGeminin (AzGr) and mKO2-hCdt1 (mKO). FUCCI was specifically expressed via tissue-specific transgenesis to reveal cardiomyocyte cell cycle status and define mitotic progression.

Hypothesis: Cardiomyocytes increasingly withdraw cell-cycle progression throughout postnatal (PN) development with limited ability to overcome arrest following acute myocardial infarction (MI).

Methods: A novel cardiac-specific mouse model, aMHC-FUCCI, was created enabling observation of cardiomyocyte cell-cycle progression. FUCCI hearts were harvested from birth through postnatal development, and at 3, 7, 10, 14 and 21 days post MI (dpi) with concomitant daily BrdU injection. Samples were analyzed for FUCCI dynamics using microscopic and biochemical molecular analyses.

Results and Conclusions: Individual mKO (G1) and AzGr (S/G2/M) fluorescence (17.9% combined) peaks at PN2 and decreases over time in cardiomyocytes co-labeled with BrdU and/or mitotic marker phospho-histone H3 indicating cardiomyocytes are active in cell-cycle. Mitotic activity persists throughout PN14 as evidenced by $AzGr^+/pHH3^+$ cardiomyocytes. However, dual-labeled $mKO^+/AzGr^+$ (G1/S) cardiomyocytes comprise over 95% of myocardium by PN30, indicating cardiomyocytes cycle up to two weeks after birth, arresting in a G1/S transition phase as opposed to a mitotic exit (G0). MI injury prompts $BrdU^+$ interstitial cell labeling in the border zone through 14dpi. $BrdU^+$ cardiomyocytes, expressing mKO and/or AzGr detected at 21dpi, indicate a lag in cell-cycle re-entry after MI. Results show cardiomyocytes retain limited ability to re-enter cell-cycle at 21dpi. FUCCI will be employed in future studies advancing resolution of cardiomyocyte cell-cycle dynamics under normal and pathological states and assess strategies used to enhance cardiomyocyte proliferation.

P1-04

Combined methylglyoxal scavenger and collagen hydrogel therapy improves function of the infarcted heart

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Background. Myocardial infarction (MI) stimulates production of the toxic metabolite methylglyoxal (MG), and its accumulation post-MI contributes to reduced vascularization and cardiac dysfunction. We hypothesized that using a collagen hydrogel to deliver fisetin, a methylglyoxal scavenger, would improve post-MI vascularity, morphology and function.

Methods. Endothelial cells were exposed to various concentrations of MG *in vitro* and their viability and angiogenic potential evaluated. Fisetin was loaded into a collagen hydrogel and its release profile determined. *In vivo*, mice were treated at 3h post-MI with intramyocardial injection of either: 1) PBS; 2) fisetin; 3) collagen hydrogel; or 4) fisetin-loaded collagen hydrogel.

Results. *In vitro*, MG between 10 to 100μM reduced endothelial cell viability by up to 68.3% compared to control group ($p \leq 0.002$). MG-exposed cells used in an *in vitro* angiogenesis assay had up to 58.5% less total network length compared to unexposed cells ($p \leq 0.0002$), which was rescued by treatment with 10μM fisetin ($p < 0.001$). Fisetin (80μM) loaded into a collagen hydrogel is released steadily into media *in vitro* over a period of 24h. In MI mice, treatment with the hydrogel ± fisetin reduced the final scar size by about 50% at 5wk post-treatment ($p < 0.05$). An improvement in LVEF from baseline to 5wk was observed only in the hearts treated with fisetin-loaded hydrogels (+4.3%) compared to the PBS group, which experienced a loss-of-function (-7.2%; $p = 0.035$). Arteriole density was increased (100%; $p = 0.045$) and the number of $CD68^+$ macrophages was reduced (27%; $p = 0.05$) in fisetin-hydrogel treated hearts compared to PBS treatment.

Conclusion. Fisetin delivered in a collagen hydrogel reduced chronic inflammation, enhanced vascularity and improved cardiac function post-MI. These results suggest that limiting MG effects in acute MI could be a promising approach to prevent damage and preserve function of the infarcted heart.

P1-05

An injectable CCN1-collagen matrix for cardiac cell support and treatment of myocardial infarction

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Background. Biomaterials have emerged as a promising approach to treat myocardial infarction (MI). We hypothesized that a collagen matrix loaded with the pro-angiogenic, anti-fibrotic matricellular protein CCN1 would positively regulate the function of different cardiac cells and that its delivery into the mouse MI heart would limit adverse remodeling and dysfunction.

Methods. *In vitro*, the effect of CCN1-matrix on the function of heart-derived fibroblasts, cardiomyocytes and bone marrow macrophages was examined. *In vivo*, MI was induced in mice and at 1wk post-MI, mice received intramyocardial injections of: 1) PBS; 2) matrix; or 3) CCN1-matrix.

Results. *In vitro* studies revealed that CCN1-matrix reduced fibroblast proliferation by 29% (p=0.02), and TGF-β-induced expression of the myofibroblast marker α-SMA by 42.1% (p=0.014), compared to matrix culture. Furthermore, fibroblasts on CCN1-matrix had a 31% increase in senescence-associated β-galactosidase activity (p=0.041) and a 12% reduction in collagenase activity compared to matrix-cultured cells (p=0.036). For cardiomyocytes, CCN1-matrix had a protective effect as there was a 39% increase in survival under stress conditions compared to matrix-cultured cells (p=0.03). Also, CCN1-matrix culture of macrophages resulted in a 4.2- and 2.2-fold increase in the M2 surface marker CD206 compared to TCPS and matrix, respectively (p≤0.03). *In vivo*, LVEF was greater for CCN1-matrix treated mice (49.6%) at 4wk post-MI compared to matrix (38.7%) and PBS (28.9%) groups (p≤0.01). Scar size of the CCN1-matrix treated hearts (10.1%) was reduced compared to matrix (15.4%) and PBS (21.4%; p≤0.017) treatments, while vascular density was 23% and 41% greater for the CCN1-matrix treatment group compared to matrix and PBS (p≤0.035).

Conclusion. The addition of CCN1 improves the therapeutic potency of our collagen biomaterial. The combination of matricellular proteins and biomaterials is a novel approach to preserve myocardial integrity and cardiac function post-MI.

P1-06

CardioClusters: Enhancing Stem Cell Engraftment and Myocardial Repair

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Introduction: Stem cells are routinely utilized to blunt damage and enhance repair following pathologic injury to the heart, but marginal engraftment, persistence, and cell communication remain significant challenges. Combinatorial stem cell delivery to enhance repair only partially addresses these issues. A novel approach has been developed by creating "CardioClusters": three dimensional microenvironments consisting of three distinct types of stem cells isolated from the human heart and tested for intramyocardial injection into infarcted myocardium.

Hypothesis: CardioClusters enhance cellular engraftment, persistence, and communication resulting in enhancement of cardiac repair and regeneration following myocardial infarction (MI).

Methods: CardioClusters consist of three defined cell populations from the human heart: c-kit cardiac progenitor cells (CPCs), CD90/CD105 mesenchymal stem cells (MSCs) and CD133 endothelial progenitor cells (EPCs). CardioCluster size can be controlled by the quantity of cells used to create the cluster, allowing for intramyocardial delivery without dispersion into single cell suspensions. MI was induced in mice by left anterior descending coronary artery ligation prior to cell delivery. A total of 200 CardioClusters comprised of $1x10^5$ single cells were injected into the border zone (3 injections/heart). Immunohistochemistry, echocardiogram and flow cytometry were performed to assess cell retention and cardiac function.

Results and Conclusions: CardioClusters maintain three dimensional structural integrity following intramyocardial injection with superior persistence confirmed by immunohistochemistry; CardioClusters were readily detected in mouse hearts at 3 days post injection (n=9). Pilot study results demonstrate functional improvement in mice receiving CardioClusters at 2 weeks compared to MI alone for both ejection fraction and fractional shortening, 11% and 9% respectively (n=4 sham; n=10 MI; n=9 CardioCluster). All cell types within the CardioCluster persist and remain in close proximity creating a niche-like environment at two-weeks post-injection. Future directions will be to perform a large-scale mouse study using *Prkdc*^{scid} mice to assess long-term engraftment and regenerative potential.

P1-07

Regulation of transplanted cell homing by FGF-1 and PDGF-B after doxorubicin myocardial injury

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We have previously shown that ventricular cells from 11-day old embryonic mice are more effective in cardiac regeneration than those from 14-day old embryos (Zhang et al., 2015. Am J Physiol Cell Physiol. 308:C220-8). In the current study, we used a doxorubicin (Dox) cardiac injury model to examine the mechanisms underlying the greater regenerative potential of these younger cells and explored how those mechanisms could be utilized in myocardial repair. Our results indicate that infused E11.5 ventricular cells are more efficient at homing into the injured adult myocardium, and are more angiogenic, than E14.5 ventricular cells. The E11.5 cells were shown to mitigate the cardiomyopathic effects of Dox, as the Dox-injured animals infused with E11.5 cells had their heart rate, QRS interval, ejection fraction and percentage of fractional shortening restored to those of the non-Dox controls. *In vitro*, E11.5 cells were more migratory than the E14.5 cells and expressed higher levels of mRNA for the cytokine receptors Fgfr1, Fgfr2, Pdgfra, Pdgfrb and Kit. The mRNA levels for those cytokines were also significantly elevated in the Dox-injured adult heart, as were the FGF-1 and PDGF-B protein levels. Moreover, exogenous FGF-1 and PDGF-B were both able to enhance E11.5 ventricular cell migration *in vitro*. Whereas neutralizing antibodies for FGF-1 and PDGF-B decreased cell migration, exogenous FGF-1 and PDGF-B prevented the inhibitory effects of the neutralizing antibodies. Our results therefore suggest that the high level of chemotactic cytokine receptors present in E11.5 ventricular cells may play a significant role in myocardial repair and functional restoration after Dox-induced cardiac injury. In addition, our results also indicate that therapies raising the levels of FGF-1 and PDGF-B in other types of cardiac injury could improve cell-based myocardial repair.

P1-08

ARA290, a small non-hematopoietic peptide derived from erythropoietin, prolongs healthspan and attenuates age-associated declines in cardiac function

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Objective: To determine whether a novel anti-inflammatory, erythropoietin-derived synthetic peptide, ARA290, can improve healthspan and delay deteriorations in heart function.

Methods: FBN male rats (n = 50) received bi-weekly injections of ARA290 or saline. Body weight (BW) was recorded every 2 weeks until death. Echocardiograms (ECHO) and electrocardiograms (ECG) were performed at 18, 22, 26, 30, and 33 months, when a frailty index (FI), scored 0 to 1 (least to most frail), assessed health deficits in various body systems. Linear mixed effects and joint models predicted risks of death based on longitudinal and survival data.

Results: ARA290 slowed the decline in basal heart rate (BHR) with age ($p < 0.03$). Intrinsic HR (IHR) declined non-linearly with no treatment differences ($p < 0.003$). IHR subtracted from BHR (ΔHR), an index of autonomic modulation on HR, initially declined in both groups but was stable after 22 months in ARA290 ($p < 0.02$). Ejection fraction (EF) declined 0.469% per month slower in ARA290 ($p < 0.005$). At 33 months EF was 7.5% greater in ARA290 ($p < 0.004$). BW declined 0.04 grams/mo^2 slower in ARA290 ($p < 0.02$). FI score was lower in ARA290 (0.22) than the control (0.30) ($p < 0.001$). The risk of death is 1.2% higher for a 1 BPM decrease in BHR ($p < 0.0001$), 1.5% higher for a 1 BPM decrease in IHR ($p < 0.0001$), 1.1% higher for a 1 BPM decrease in ΔHR ($p < 0.12$), and 11.9% higher for a 1% decrease in EF ($p < 0.0001$).

Conclusions: Reduced frailty of ARA290 is consistent with improved healthspan. Concurrently, ARA290 preserves autonomic modulation of HR and EF with age. Because decreases in these markers predicted significantly higher risks for mortality, ARA290's impact on cardiac function with age may contribute to the improvement in healthspan.

P1-09

Binding of calcium and magnesium to cardiac Troponin C assessed through Isothermal Titration Calorimetry (ITC)

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Cardiac Troponin C (cTnC) is the calcium (Ca^{2+}) sensing component of the myofilament. Ca^{2+} or magnesium (Mg^{2+}) binding to sites III and IV tethers cTnC to the rest of the troponin complex (cTn) and the thin filament (TF). With a dissociation constant (K_d) of 10^{-5} M, the N-terminal site II is unbound at diastolic [Ca^{2+}] and subsequently bound at systolic [Ca^{2+}]. This interaction acts as a switch, initiating conformational changes that culminate in myocardial force production.

ITC has been used to thermodynamically quantify the Ca^{2+}-cTnC interaction (K_d = 15.2 ± 0.4 µM). Contrary to expectation, the data suggests that physiologically relevant concentrations of Mg^{2+} bind site II (K_d = 649.4 ± 25.5 µM). Both cations appear to interact with the same site as pre-incubation with Ca^{2+} significantly decreased Mg^{2+} binding and a similar effect was seen for pre-incubation with Mg^{2+} ($p<0.0001$). Moreover, D67A and D73A mutations in site II lowered Ca^{2+} affinity 11-fold (K_d = 170.9 ± 41.5 µM) and Mg^{2+} affinity 1.7-fold (K_d = 1114.8 ± 227.0 µM). It must be noted that while these parameters can be compared between conditions, they may not translate in absolute terms when the cTnC is incorporated into a more complex system such as the cTn or TF.

Normally, ~95% of cellular Mg^{2+} is buffered, largely to ATP to yield a free cytosolic [Mg^{2+}] of ~1 mM. Although [Mg^{2+}] likely remains constant under normal physiological conditions, we posit that certain stressors such as ischemia may significantly deplete ATP, increasing [Mg^{2+}] and allowing for binding to site II. Pending further studies, our findings suggest that Mg^{2+} may impact Ca^{2+} binding to site II of cTnC thus affecting regulation of heart contractility.

P1-10

The Arrhythmogenic Impact of the Familial Hypertrophic Cardiomyopathy-related Cardiac Troponin T mutation I79N

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Cardiac troponin (cTn) is a heterotrimeric complex that plays an essential role in cardiac contractility. Each complex is composed of a highly conserved Ca^{2+} binding subunit (cTnC), an inhibitory subunit (cTnI), and a tropomyosin binding subunit (cTnT). Familial hypertrophic cardiomyopathy (FHC) is the most common inherited cardiomyopathy, and 7% of FHC is associated with mutations found in the cTnT gene. Unlike the general anatomical abnormalities found in FHC patients, hearts from patients harbouring cTnT mutations show less ventricular hypertrophy but significant arrhythmogenesis. This study focuses on the I79N cTnT mutation that is morphologically asymptomatic but associated with a high incidence of sudden cardiac death.

The biophysical properties of the I79N mutation were investigated in reconstituted thin filaments (RTF) comprised of human recombinant proteins and skinned cardiomyocytes containing this mutation. At the RTF level, the mutation significantly slows the Ca^{2+} dissociation rate (80 s^{-1}) compared to the WT (102 s^{-1}) ($p<0.05$). In addition, higher myofilament Ca^{2+} sensitivity was observed for the cardiomyocytes reconstituted with human I79N cTn as demonstrated through a leftward shift of the pCa curve with ΔpCa of 0.65 ($p<0.05$). This is in agreement with the literature, in which the I79N cTnT mutation increases myofilament Ca^{2+} sensitivity and causes higher susceptibility to cardiac arrhythmia in transgenic mice.

The I79N TnT mutation was also incorporated into hiPSC-CM by genome editing, and the cells were characterized by optical mapping. Simultaneous voltage and Ca^{2+} recordings, using potentiometric (RH-237) and Ca^{2+} indicator (Rhod-2) dyes, were used to quantify the rates of spontaneous activity, action-potential profiles, and Ca^{2+} transient dynamics. The mutant hiPSC-CMs exhibited AP remodeling and triangulation which is considered a predictor of arrhythmogenicity. However, we did not observe significant Ca^{2+} transient prolongation in I79N *TNNT*$^{+/-}$ compared to WT hiPSC-CMs.

P1-11

Molecular defects in cardiac myofilament Ca^{2+}- regulation due to cardiomyopathy-linked mutations can be reversed by small molecules binding to troponin

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The inherited cardiomyopathies, hypertrophic cardiomyopathy (HCM) and dilated cardiomyopathy (DCM) are relatively common, potentially life-threatening and currently untreatable. Mutations are often in the contractile proteins of cardiac muscle and cause abnormal Ca^{2+} regulation *via* troponin. HCM is usually linked to higher myofilament Ca^{2+}-sensitivity whilst in both HCM and DCM mutant tissue there is often an uncoupling of the relationship between troponin I (TnI) phosphorylation by PKA and modulation of myofilament Ca^{2+}-sensitivity, essential for normal responses to adrenaline. The adrenergic response is blunted, and this may predispose the heart to failure under stress.

At present there are no compounds or interventions that can prevent or treat sarcomeric cardiomyopathies. There is a need for novel therapies that act at a more fundamental level to affect the disease process. We demonstrated that epigallocatechin-3 gallate (EGCG) was found to be capable of restoring the coupled relationship between Ca^{2+}-sensitivity and TnI phosphorylation in

mutant thin filaments to normal *in vitro,* independent of the mutation (15 mutations tested). We have labelled this property "re-coupling". The action of EGCG *in vitro* to reverse the abnormality caused by myopathic mutations would appear to be an ideal pharmaceutical profile for treatment of inherited HCM and DCM but EGCG is known to be promiscuous *in vivo* and is thus unsuitable as therapeutic drug. We therefore investigated whether other structurally related compounds can re-couple myofilaments without these off-target effects.

We used the quantitative *in vitro* motility assay to screen 40 compounds, related to C-terminal Hsp90 inhibitors, and found 23 that can re-couple mutant myofilaments. There is no correlation between re-couplers and Hsp90 inhibitors. The Ca^{2+}-sensitivity shift due to TnI phosphorylation was restored to 2.2±0.01 –fold (n=19) compared to 2.0±.24 fold (n=7) in wild-type thin filaments. Many of these compounds were either pure re-couplers or pure desensitisers, indicating these properties are independent; moreover, re-coupling ability could be lost with small changes of compound structure, indicating the possibility of specificity. Small molecules that can re-couple may have therapeutic potential.

P1-12

Physical contact between vascular endothelial and smooth muscle cells mediates increase in intracellular calcium.

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The interaction between vascular endothelial cells (VECs) and vascular smooth muscle cells (VSMCs) plays an important role in the modulation of vascular tone. This dialogue between the two cell types can be mediated by factors released by VECs and/or VSMCs, or by a direct physical communication via gap junctions. The objective of our study was to test the hypotheses that physical contact between VECs and VSMCs modulates intracellular Ca^{2+} ($[Ca]_i$) of these cells via communication through gap junctions. Using the quantitative 3D confocal microscopy technique our results showed that in co-cultures of hVECs and hVSMCs, the physical contact between the two cell types induced a significant increase of nuclear calcium of hVECs without affecting the basal level of $[Ca]_i$ of hVSMCs. In addition, using the indirect immunofluorescence technique, our results demonstrated that this effect on $[Ca]_i$ is mediated mainly via the presence of gap junctions formed mainly of Cx40. Our results suggest that the high level of $[Ca]_i$ observed in co-cultures of hVECs-hVSMCs can be modulated by the formation of gap junctions between the two cell types, a phenomenon that may have important implications in terms of vascular diseases such as atherosclerosis. This work was supported by the Canadian Institutes of Health Research (CIHR).

P1-14

Mechanically-Induced Ventricular Arrhythmias during Acute Regional Ischemia

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Introduction: Ischemia-induced arrhythmias due to coronary artery occlusion constitute a major risk for sudden cardiac death. Arrhythmias usually occur in two phases: an initial wave during the first ~15min of ischemia (Phase 1a), followed by a more lethal period during the next ~15-45min (Phase 1b). Phase 1b arrhythmias have been linked to changes in tissue mechanics, arising from regions of tissue stretch at the ischemic border, however the underlying mechanisms are unknown. This study aimed to investigate the mechanical contribution to ventricular arrhythmias during acute regional ischemia.

Methods: Experiments were performed in Langendorff-perfused isolated rabbit hearts, with an antero-apical ischemic region induced by ligation of the anterior branch of the left circumflex coronary artery for 60 min. Rate was maintained at 4 Hz by right atrial pacing, electrical activity monitored by electrocardiogram, and left ventricular loading controlled by intraventricular balloon. Three groups were tested, with a physiologically-loaded, unloaded, or non-contracting (excitation-contraction uncoupled by blebbistatin) left ventricle (*n*=10 for each). Regional contraction was assessed by speckle-tracking echocardiography in a subset of loaded hearts (*n*=6). Mechanisms of arrhythmias were investigated with calcium buffering (0.1 µM BAPTA, *n*=10), ryanodine receptor stabilisation (1 µM dantrolene, *n*=13), and dual voltage-calcium optical mapping (*n*=10).

Results: In loaded hearts, artery ligation resulted in progressively increasing stretch of tissue at the ischemic border and two peaks of arrhythmias (at 15 and 30 min), which were absent in unloaded and non-contracting hearts. Calcium buffering and ryanodine receptor stabilisation reduced the incidence of these arrhythmias. Optical mapping revealed a spatio-temporal difference in the change of action potential and calcium transient duration in ischemic tissue, resulting in a vulnerable window (between 10-30 min) in which calcium-induced after-depolarisations may occur.

Conclusion: Mechanical effects contribute to ventricular arrhythmias during acute regional ischemia through a

calcium-driven mechanism, suggesting a novel target for anti-arrhythmic therapy.

P1-15

A thermoneutral environment abolishes age-associated reduction in heart rate variability in mice

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Objective: To unravel the impact of autonomic input on age-associated changes in heart rate (HR) and heart rate variability (HRV) over a range of ambient temperatures.

Methods: HR was measured in young (3 mo) and aged (30 mo) male C57/BL6 telemetry-implanted mice via in-vivo ECG after temperature acclimatization. Data was analyzed using linear mixed effects models. The double interaction term (temperature and age) in the models was used to evaluate effect of temperature on HR and HRV by age group while correcting biases from repeated measurements and uneven group sizes.

Results: Changing the ambient temperature altered mean HR of both age groups and several HRV indices in the aged mice. Mean HR of both young and aged mice were markedly decreased at 30ºC ($p < 0.0001$). Coefficient of variance (CV), an indicator of overall time-domain HRV, was significantly reduced in old mice than in young mice. This difference disappeared at 30ºC because the CV of the aged mice increased ($p < 0.0001$). Detrended fluctuation analysis (DFA), a measure of correlation within a time series, was significantly lower in the old mice at 30 than 20ºC ($p < 0.008$). The changes in CV and DFA suggest a restoration in complexity in the old mice at 30ºC. Aged mice also showed a significant increase in the high frequency power spectral density compared at 30ºC to 20ºC, indicating increased parasympathetic tone at NT_A ($p < 0.0001$).

Conclusion: Rodents are studied in cold-stressed conditions at standard laboratory ambient temperature (LT_a) and increased sympathetic stimulation distorts the correct view of autonomic balance. An age-associated decline in HRV observed at LT_A was ameliorated when mice were studied at their thermoneutral ambient temperature (NT_A). Thus, a true elucidation of autonomic neurotransmitter modulation of cardiovascular function alterations in advanced age require mammals to be at their NT_A.

P1-16

Dual optical mapping of the innervated mouse heart reveals unique electrophysiological responses during fight-or-flight

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Background: The mouse is a mainstay in cardiovascular research, yet the detailed electrophysiological and Ca^{2+} handling changes in the intact mouse heart in response to physiological sympathetic activity have never been investigated. In larger mammals, sympathetic activation typically results in increased heart rate (HR), accompanied by shortening of action potential duration (APD) to accommodate shorter cycle lengths and ensure adequate diastolic filling time. However, data on the effects of sympathetic activation on the rodent APD are somewhat inconsistent, with both shortening and prolongation reported. Here, for the first time, we systematically investigated the integrated effects of adrenergic activation and dynamic changes in HR in the mouse heart.

Methods and Results: The heart and posterior thoracic cavity from mice (C57Bl6, N=12) were dissected and perfused through the descending aorta for dual optical mapping of transmembrane potential and intracellular Ca^{2+} transients (CaT) to study the effects of sympathetic nerve stimulation (SNS). SNS was performed via spinal cord stimulation at T1-T3. As expected, SNS (60sec, 10Hz) caused a monotonic increase in HR and CaT amplitude. Interestingly, APD showed a biphasic response, with initial prolongation (50.9±5.1ms at t=0sec to 60.6±4.1ms at t=20sec, $p<0.05$) followed by shortening (46.5±9.1ms at t=60sec). Mathematical modeling revealed that the initial APD prolongation during SNS was necessary to allow for optimal increases in CaT amplitude and positive inotropy and lusitropy. Further, experiments demonstrated a similar biphasic response when the SNS-mediated HR increase was mimicked with pacing (56.7±1.0ms at t=0sec, 66.0±1.0ms at t=10sec, 53.8±3.3ms at t=30sec), suggesting HR increase as a primary contributor to APD changes during SNS. When HR was held constant, SNS had more modest effects on APD, with a slight monotonic increase.

Conclusions: The mouse heart displays unique electrophysiological responses to physiological sympathetic activation that may be essential for the optimal fight-or-flight response in rodent hearts.

P1-17

Regional cardiac sympathetic denervation produces supersensitivity of action potential and Ca^{2+} handling properties to β–adrenergic stimulation

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Rationale: Myocardial infarction (MI) can result in chronic loss of sympathetic nerve fibers in the infarct region. Heterogeneity of sympathetic transmission combined with ischemic damage and fibrosis are all likely contributors to post-MI arrhythmogenesis. However, the precise contribution of denervation to electrophysiological remodeling – independent from ischemic damage – has not been systematically investigated.

Objective: To create a novel mouse model of regional cardiac sympathetic denervation and measure resulting electrophysiological and Ca^{2+} handling dynamics.

Methods and Results: A targeted toxin (anti-dopamine beta hydroxylase [DBH] conjugated to saporin) was applied to the mid/apical region of the anterior left ventricle in a survival surgery. Untargeted anti-IgG-saporin was applied as a control. Five days post-surgery, sympathetic nerve fiber density was reduced in the anterior portion of the denervated hearts compared to control hearts (2.976%±0.549% vs. 1.482±0.055%; $p<0.05$), while posterior sections were not different. Action potential and Ca^{2+} handling parameters were measured in Langendorff-perfused hearts using optical mapping with voltage- and Ca^{2+}-sensitive indicators at baseline and with isoproterenol (ISO, 1μm) challenge. Without ISO, the mean action potential duration (APD_{80}) was similar between control and denervated hearts. With ISO, significant shortening of apical APD in the denervated hearts was observed (Control BL: 55.55±1.83ms vs. ISO: 41.01±8.71ms, p=ns; Denervated BL: 50.86±5.38ms vs. ISO: 27.95±9.68ms, $p<0.01$). Additionally, ISO produced spontaneous diastolic Ca^{2+} elevation in the denervated hearts, which was not observed in control hearts (Control: 3.78±2.43% *vs.* Denervated: 14.06±4.39% Ca^{2+} elevation, $p=<0.0001$).

Conclusion: At 5 days post-denervation, a reduction of sympathetic nerve fibers is observed along with supersensitivity to β–AR stimulation in the denervated region, leading to spontaneous Ca^{2+} elevation and dramatic regional APD shortening. Together, these changes may lead to triggered activity and APD dispersion necessary to initiate and sustain arrhythmia.

P1-18

Age-related changes in sympathetic responsiveness and cardiac electrophysiology

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Introduction: Aging is associated with chronic increases in sympathetic activity, yet neurodegeneration also occurs, leading to a loss of functional sympathetic fibers in the heart. How these age-related changes in sympathetic structure and function directly impact cardiac electrophysiology and intracellular Ca^{2+} handling have not been systematically characterized.

Methods: Fully innervated hearts from young (3 – 4 months, YWT, n=10) and aged (20 – 24 months, AGED, n=11) female mice (C57Bl6) were optically mapped using voltage-sensitive (V_m, Rh237) and calcium-sensitive (Ca^{2+}, Rhod2-AM) indicators. Sympathetic nerve stimulation (SNS) was performed via a stimulation electrode inserted into the spinal canal (T1-T3). After SNS, hearts were perfused with isoproterenol (1mM, ISO) to assess β-adrenergic responsiveness. Hearts were then preserved for immunohistochemistry and HPLC analysis of norepinephrine (NE) content.

Results: Stimulation thresholds necessary to produce a defined increase in heart rate (HR) with SNS were higher in AGED vs. YWT hearts (5.36±0.37 vs. 3.81±0.44 Hz, $p<0.05$). Maximal HR increase with supra-threshold SNS was lower in AGED compared to YWT (20.5±3.41 vs. 73.0±7.63 %, $p<0.05$). Sympathetic nerve density and NE content were decreased in AGED compared to YWT. AGED hearts also had decreased β-adrenergic responsiveness (measured as % increase in HR with ISO: 75.3±22.5 vs. 148.5±19.8%, $p<0.05$). Despite an increased susceptibility to ventricular arrhythmias and Ca^{2+} alternans in AGED, there were no significant differences in action potential or Ca^{2+} transient duration between groups. SNS significantly increased action potential duration in YWT but not AGED.

Conclusions: Stimulation thresholds for SNS-induced changes in HR were increased in AGED and supra-threshold SNS resulted in minimal changes in HR, action potential, and Ca^{2+} handling properties compared to YWT responses. This is the result of decreased nerve density and function, as well as decreased β-adrenergic responsiveness.

P1-19

The role of Natriuretic Peptide Receptor C in atrial electrophysiological remodelling in hypertensive heart disease

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Atrial fibrillation (AF) is prevalent in hypertension in association with alterations in angiotensin II (Ang II) signaling. Natriuretic peptides (NPs) are a family of hormones with potent effects on atrial electrophysiology mediated in part through the NPR-C receptor. The goal of this study was to determine the role of NPR-C in atrial electrophysiological remodelling in Ang II treated mice (3 mg/kg/day for 3 weeks). Ang II treatment increased the susceptibility to AF in WT ($P = 0.001$) and was more severe in NPR-C$^{-/-}$ ($P = 0.03$) mice vs. saline controls. Action potential (AP) upstroke velocity (V_{max}; $P = 0.01$) and peak Na^+ current (I_{Na}; $P < 0.001$) were reduced in left atrial (LA) myocytes from Ang II treated WT mice, which was attributed to enhanced PKCα signalling. Ang II differentially prolonged the AP in right atrial (RA; APD_{50}; $P < 0.001$) and LA ($P < 0.001$) myocytes. In addition, Ang II reduced I_{to} in RA myocytes ($P < 0.001$) and to a greater extent in LA myocytes ($P = 0.045$). In the RA of NPR-C$^{-/-}$ mice, Ang II prolonged ($P < 0.05$) APD_{50} to a similar extent vs. WT. In contrast, V_{max} was reduced and APD_{50} was prolonged by Ang II to a greater extent ($P < 0.05$) in the LA of NPR-C$^{-/-}$ vs. WT mice. I_{to} was similarly reduced ($P<0.05$) in the RA and LA of Ang II treated WT and NPR-C$^{-/-}$ mice. Co-treatment with cANF (NPR-C agonist) reduced the susceptibility to AF ($P = 0.04$) in WT mice vs. Ang II alone. Strikingly, APD_{50} was reduced ($P = 0.01$) and I_{to} was increased ($P = 0.001$) in the RA, but not LA, by cANF co-treatment. Collectively, these data demonstrate that NPR-C plays a critical role in modulating disease progression in the atria elicited by Ang II treatment.

P1-20

Pathophysiology of R222Q mutant SCN5a channels

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Consistent with the role of sodium channels in initiating action potentials throughout myocardial tissue, mutations in SCN5a are associated with arrhythmias and cardiomyopathy. The present studies aim to advance understanding of the mechanism underlying the arrhythmias and dilated cardiomyopathy (DCM) observed in patents with an SCN5a mutation (c.665G>A/R222Q) located in one of the voltage sensor regions. Induced pluripotent stem cells (iPSCs) were generated from a patient heterozygous for the R222Q mutation. Isogenic controls were generated by corrected the mutation. Directed differentiation techniques were used to produce ventricular CMs characterized by the expression of cTnT (Control: 85.10±2.90%, Mutant: 83.14±4.46%) and ventricular-specific MLC2v (Control: 45.62±7.02%, Mutant: 43.92±3.86%). The SCN5a gene undergoes extensive alternative splicing, which is developmentally regulated, resulting in the R222Q loci being expressed exclusively in adult SCN5a mRNA. Consistent with an immature phenotype in our iPSC-derived CMs, about half of the SCN5a mRNA is the adult isoform (Control: 54.40±9.29%, Mutant: 48.45±6.21%, n=4), indicating that ~25% of the SCN5a channels expressed in mutant CMs contain the R222Q mutation. Optical mapping of our 2-dimentional tissue model (monolayers) reveal slower conduction velocities in mutant compared to control monolayers (13.14±0.74cm/s, n=10, vs. 25.09±1.99cm/s, n=7, p<0.05). Treatment with flecainide (10 μM), a sodium channel blocker associated with arrhythmia reduction and DCM reversal in R222Q patients results in conduction slowing (Control: 25.09±1.99cm/s to 18.47±3.20cm/s, n=7, p<0.05, Mutant: 13.14±0.74cm/s to 8.92±0.64cm/s, n=10, p<0.05) and a reduction in maximum capture rates (Control: 3.22±0.14Hz to 1.42±0.13Hz, n=5, p<0.05, Mutant: 3.43±0.18 to 2.38±0.22Hz, n=10, p<0.05). We are currently performing electrophysiological studies to further assess the mechanisms underlying R222Q-associated cardiomyopathy and arrhythmias and advancement of therapeutic options for R222Q patients.

P1-21

Atrial arrhythmias and adverse atrial remodeling induced by exercise requires soluble tumor necrosis factor alpha (TNFα) derived from atrial myocardium

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Background: Intense endurance exercise is linked to atrial fibrillation (AF). We previously identified the pro-inflammatory cytokine, TNFα, as a key mediator of exercise-induced atrial remodeling and AF vulnerability in mice. However, the nature of TNFα-dependent cardiac signaling and the cardiac source of TNFα mediating exercise-induced AF remain elusive.

Purpose: To determine whether the TNFα-dependent atrial changes induced by exercise requires soluble TNFα derived from atrial myocardial.

Methods and Results: Adverse atrial remodeling, characterized by atrial fibrosis and inflammatory cell infiltrates, as well as increased AF susceptibility induced by six weeks of intense swim exercise were prevented when the TNFα gene was selectively ablated in the atrial myocardium of mice with floxed TNFα genes and with cre-recombinase expression under the control of the atrial-specific NPPA promoter. On the other hand, reductions in heart rate, increased vagal tone and enhanced ventricle function were unaffected by disruption of TNFα in the atrial myocardium. To determine whether the exercise-induced atrial changes involves signaling through TNFα receptors via enzymatically liberated soluble TNFα (solTNF) versus via membrane bound TNFα, we treated mice with XPRO^R, a selective dominant-negative inhibitor of solTNF. XPRO^R also largely prevented the adverse atrial changes induced by exercise, independent of beneficial physiological changes.

Conclusions: Our results establish that exercise-induced atrial remodeling and AF vulnerability requires soluble TNFα originating from the atrial myocardium.

P1-22

Effects of ageing on cardiac function in zebrafish

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Introduction: Age-associated alterations in cardiac structure and function have been observed from the molecular to whole organ level in humans and mammalian models. Understanding the mechanisms involved is important for explaining the development of cardiac disease with age and developing novel strategies for its treatment and prevention. The zebrafish represents a potentially powerful model for ageing studies, as it can model various cardiac pathologies, it is easily genetically modified, and it is a relatively low cost and high-throughput option. In aged zebrafish, myocyte hypertrophy, ventricular density and fibrosis, and valvular lesions, as well as reductions in coronary vasculature have been described. The functional consequences of these structural changes, however, have not been well described. In the current study, we investigated age-related changes in cardiac function in the zebrafish.
Methods: Hearts isolated from 3-24 month old wild-type zebrafish were monitored by ECG. HR, HR stability, rates of contraction and relaxation, and sinus node recovery time (SNRT) following burst pacing were assessed. Action potential and calcium transient morphology were measured by fluorescence imaging. Response to autonomic input was evaluated by vagal nerve stimulation (VNS). Intracardiac innervation was visualized *via* acetylated tubulin, human neuronal protein C/D, and choline acetyltransferase immunohistochemistry.
Results: Basal HR and rates of contraction and relaxation did not change with age. HR and APD_{80} and CaT_{80} were less stable in young animals and SNRT increased in the 24-month group. HR response to VNS was reduced in the 12- and 24-month groups. Innervation of the heart showed significant changes in both total neuron number and the proportion of cholinergic neurons with age.
Conclusion: These results suggest that age-related changes in both myocyte and intracardiac neural structure and function exist in the zebrafish heart, offering a new model for studies of cardiac ageing.

P1-23

Mechanical Determinants of the Chronotropic Response to Sinoatrial Stretch

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The sinoatrial node (SAN) adapts its firing to changes in mechanical load, with stretch increasing heart rate (HR). This response is important for matching cardiac output to venous return and may contribute to SAN dysfunction in disease. Surprisingly, in mouse stretch decreases HR, reducing its utility for exploring underlying mechanisms. The zebrafish represents an attractive alternative model, as its cardiac electrophysiology is similar to human and it is easily genetically modified. We have demonstrated that stretch of zebrafish SAN increases HR in a magnitude-dependent manner similar to mammals, such as rabbit. Yet molecular mechanisms and mechanical determinants of this chronotropic response remain unknown. Our aim is to determine factors underlying the SAN response to stretch using zebrafish and rabbit.

The SAN was isolated from hearts of adult zebrafish (n=10) and rabbits (n=8). The zebrafish SAN (an oval-shaped ring) had a pair of custom micro-sized glass hooks (coupled to a piezo-translator and force transducer) inserted into its opening and 10-50% strain was applied from the pre-stretched state. The rabbit SAN was attached at either end

to clips (coupled to a linear servomotor and force transducer) and 10-50% strain was applied from a baseline force of 0.5g. HR was measured by local electrocardiogram. For the zebrafish SAN it was found that the increase in HR was correlated with pre-stretch force, rather than applied force, suggesting the need for a consistent baseline force. For the rabbit SAN, the stretch-induced increase in HR (from a similar baseline force) was instead correlated with applied force but interestingly, inversely correlated with tissue stiffness. Similar experiments are now being performed in zebrafish.

Results confirm that in both zebrafish and rabbits the chronotropic response to SAN stretch is magnitude-dependent but is also influenced by tissue mechanics. Molecular mechanisms are now being explored using pharmacologic, genetic, and optogenetic interventions.

P1-24

Deficiency of miR-1954 promotes cardiac fibrosis

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Cardiac remodeling due to hemodynamic overload is associated with significant morbidity and mortality. In response to stress, cardiomyocyte (CM) become hypertrophied whereas cardiac fibroblasts convert into myofibroblasts. The phenomenon leads to the development of cardiac hypertrophy, fibrosis and impair cardiac function. Previously, we have shown the pivotal role of miRNA (a new class of post-transcriptional regulators) in cardiac remodeling, but, loss of miRNA contributing the onset of cardiac remodeling remains elusive. Using next generation miRNA sequencing, we discovered a panel of novel dysregulated miRNAs from read-data, secondary structure and miRPara classification score analyses in wild-type mice (WT) infused with Angiotensin II (Ang-II). Among them, one was identified as miR-1954, a novel miRNA which was significantly reduced in Ang II-infusion. Following an unbiased approach, we confirmed that Sp1-Gata4-Col I-Tsp1-axis is the bona-fide targets. Our hypothesis is that deficiency of miR-1954 exacerbates cardiac remodeling leading to hypertrophy and fibrosis and overexpression of miR-1954 mitigates the cardiac damage and abrogates remodeling by modulating Sp1-Gata4-Col I-Tsp1-axis. Data demonstrated that depletion of miR-1954 in CM triggers hypertrophic response by modulating Sp1 and Gata4; releases soluble factors (Tgfb1) that triggers cardiac fibroblasts proliferation; upregulation of thrombospondin 1 (Tsp1) and collagen I (Col I). Overexpression of miR-1954 in CM reverses the process implicated a cellular cross-talk. Cardiac-specific overexpression of pre-miR-1954 transgenic mice (miR-1954 Tg) showed reduced cardiac mass and improved function compared to WT littermate after Ang-II treatment. Along the line, data showed significant reduction of hypertrophy marker genes, fibrotic genes, inflammatory genes and restoration of SERCA2. Inhibition of miR-1954 by locked nucleic acid of anti-miR-1954 exacerbates cardiac hypertrophy and fibrosis. Our findings provide evidence that loss of miR-1954 promotes cardiac remodeling and overexpression of miR-1954 reverses the process. We conclude that miR-1954 is a critical regulator in cardiac remodeling and may be useful for therapeutic benefit.

P1-25

Control of cardiac fatty acid metabolism in infants with hypoplastic left heart syndrome

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Introduction: Following birth there is a rapid increase in cardiac fatty acid oxidation, which becomes the main source of energy for the newborn heart. However, the presence of hypertrophy, which occurs in many forms of congenital heart disease (CHD), can delay this maturation of fatty acid oxidation, resulting in a decreased capacity of the heart to produce energy. Hypoplastic left heart syndrome (HLHS) is a severe CHD consisting of underdevelopment of the left-side of the heart, resulting in right ventricular hypertrophy. We determined if the maturation of cardiac fatty acid oxidation was impaired in infants with HLHS. **Methods:**Right ventricular biopsies were collected from CHD patients (grouped as HLHS or non-HLHS patients) during corrective heart surgery. Expression of key metabolic enzymes controlling fatty acid and glucose metabolism were examined. **Results:**Expression of the fatty acid β-oxidation enzymes β-hydroxyacyl CoA dehydrogenase and long chain acyl CoA dehydrogenase were not significantly different between groups. Acetylation of these enzymes, which normally increases following birth to increase enzyme activity, was also not different, nor were the expression of the enzymes controlling mitochondrial acetylation (GCN5L1) or deacetylation (SIRT3). Phosphorylation of cardiac AMPK, which increases fatty acid oxidation rates, was increased in HLHS patients versus non-HLHS patients, as was the expression of PGC-1α, a regulator of mitochondrial biogenesis. No changes in cardiac expression were seen in the glycolytic regulator HIF-1α, or in the glucose oxidation enzyme pyruvate dehydrogenase (PDH), its kinases and proteins regulating PDH kinase

expression (E2F1, p-Rb, p-Cyclin and CDK4). Regulators of triacylglycerol and ceramide synthesis, DGAT2/ATGL and SPT1/SPT2, respectively, were also not significantly different between non-HLHS and HLHS patients. **Conclusion:**Control of fatty acid oxidation was not impaired in hearts of HLHS infants, and maturation of fatty acid oxidation may have been facilitated by an increase in AMPK and PGC-1α activity.

P1-26

Activation of Ca^{2+}-dependent signaling causes postpartum cardiac hypertrophy in rats with gestational diabetes

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The incidence of gestational diabetes mellitus is growing due to increasing rates of metabolic syndrome and obesity in the general population and more advanced maternal age. GDM heightens the risk of developing cardiac hypertrophy and dysfunction later in life, but the underlying mechanisms are largely unknown. Here we tested the role of Ca^{2+}-dependent hypertrophy signaling in the structural remodeling of the heart following a GDM-complicated pregnancy. Female rats expressing the human isoform of the pancreatic hormone amylin (HIP rats) were used as a GDM model and WT littermates served as controls. In both groups, glucose tolerance decreased during pregnancy and recovered after giving birth, with HIP females remaining glucose intolerant compared to the WT throughout the study. Cardiac hypertrophy, assessed from heart weight-to-body weight ratio, heart weight-to-tibia length ratio and echocardiographic measurements of the left-ventricular wall, occurred in both HIP and WT females during pregnancy. By two months postpartum, heart size returned to the pre-pregnancy level in the WT but remained significantly larger in HIP females. The activity of calcineurin/ NFAT hypertrophy pathway, assessed from the nuclear-to-cytosolic localization of NFATc4, was reduced during late pregnancy in both groups. In WT females, this pathway returned to its baseline activation level within two months postpartum. However, in postpartum HIP females the ratio of nuclear-to-cytosolic NFATc4 was significantly larger than at baseline, indicating activation of this hypertrophy pathway. In contrast, the CaMKII/HDAC hypertrophy signaling was strongly activated in late pregnancy and returned to baseline postpartum in both HIP and WT females. Ca^{2+} transient decay was slower in myocytes from postpartum HIP females *vs*. baseline, while no differences occurred in the WT. In summary, two months after a GDM-complicated pregnancy, female rats show cardiac hypertrophy that is likely caused by activation of calcineurin/NFAT hypertrophy pathway.

P1-27

Successful Identification of Cardiac Troponin Calcium Sensitizers Using a Combination of Virtual Screening and ROC Analysis of Known Troponin C Binders

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Calcium dependent cardiac muscle contraction is regulated by the protein complex troponin. Calcium binds to the N-terminal domain of troponin C (cNTnC) which initiates the process of contraction. Heart failure is a consequence of a disruption of this process. With the prevalence of this condition, a strong need exists to find novel compounds to increase the calcium sensitivity of cNTnC. Desirable are small chemical molecules that bind to the interface between cTnC and the cTnI switch peptide and exhibit calcium sensitizing properties by possibly stabilizing cTnC in an open conformation. To identify novel drug candidates, we employed a structure-based drug discovery protocol that incorporated the use of a molecular dynamics simuations. In preparation for the virtual screening, cNTnC conformations were identified based on their ability to correctly predict known cNTnC binders using a receiver operating characteristics analysis. Following a virtual screen of the National Cancer Institute's Developmental Therapeutic Program database, a small number of molecules were experimentally tested using stopped-flow kinetics and steady-state fluorescence titrations. We identified two novel compounds that show increased calcium sensitivity of cTnC in the presence of the regulatory domain of cTnI. The effects of those compounds on the calcium dissociation rate was stronger than that of the known calcium sensitizer bepridil. Additionally, we identified a 3-phenylchromane group as a possible key pharmacophore in the sensitization of cardiac muscle contraction. Finally, we are also developing new computational techniques to quantitatively assess the impact of cardiomyopathy mutations and small molecules on troponin function.

P1-28

Inhibition of inflammatory serine proteases promotes vascularization and enhances cardiac repair after myocardial infarction

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Introduction: Vascularization after myocardial infarction (MI) is critical to maintain cardiac function. Evidence show the role of inflammation in new blood vessel formation post-MI. However, the molecular pathways that control vessel growth after MI remain unclear. We elucidated whether inflammatory serine proteases (ISPs) contribute to blood vessel growth and cardiac repair after MI by using mice lacking dipeptidyl peptidase I (DPPI), a lysosomal enzyme involved in the activation of major ISPs.

Methods and results: Here we report that DPPI expression and activity were increased early after MI and remained elevated up to 4 weeks post-MI. DPPI deficient mice show markedly reduced activity of neutrophil- and mast cell-derived serine proteases after MI compared to WT mice, along with an improvement in cardiac contractile function. DPPI deficiency also increased the number of capillaries and mature vessels in infarcted hearts by upregulating the expression of angiogenic cytokines such as vascular endothelial growth factor (VEGF) A and B. Investigation of the mechanisms involved show reduced levels of soluble VEGF receptor 1 (sVEGFR1) accumulation in DPPI KO infarct together with increased phosphorylation of VEGFR2. The negative role of DPPI on angiogenesis was further demonstrated *in vitro* in tube formation assay. Treatment of human umbilical vein endothelial cells with the neutrophil-derived serine protease cathepsin G led to up-regulation of sVEGFR1 and its interaction with VEGFA, which blocked VEGFA-mediated VEGFR2 signaling activation and tube formation. In contrast, treatment of endothelial cells with neutralizing anti-sVEGFR1 antibodies attenuated cathepsin G-induced endothelial cell tube disorganization, improved VEGFR2 signaling and preserved new vessels formation.

Conclusions: These findings identify DPPI and ISPs as important regulators of blood vessel growth post-MI and point to DPPI inhibition as a potential therapeutic target for stimulation of angiogenesis and the maintenance of cardiac function post-MI.

P1-29

PTP1B regulates the thyroid hormone responsiveness of β-MHC expression in cardiac hypertrophy

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Cardiac hypertrophy and heart failure are associated with increased reactive oxygen species (ROS) production. However, pathological redox signaling events responsible for the detrimental effects of ROS remain largely undefined in the heart. We investigated the redox-regulated molecular mechanisms underlying changes in microRNA (miRNA)-mediated gene silencing in adult cardiac remodeling. We identified protein tyrosine phosphatase 1B (PTP1B) as a target of ROS signaling in pressure overload-induced hypertrophy, and showed that disruption of PTP1B function in cardiomyocytes was an important checkpoint involved in pathological remodeling *in vivo*. Inactivation of PTP1B led to increased phosphorylation of its substrate, argonaute 2 (AGO2) at tyrosine 393, an inactivating phosphorylation regulated by EGFR and PTP1B. We previously reported that phosphorylation of AGO2 at tyrosine 393 prevents the loading of miRNA onto AGO2 and uncouples miRNA expression from gene silencing. We found that post-transcriptional regulation of MED13 mRNA and the loading of miR-208b onto AGO2 were compromised as a consequence of PTP1B inactivation in pressure-overload induced hypertrophy. Furthermore, the repression of β-Myosin Heavy Chain (MHC) transcription by MED13 caused cardiac dysfunction in a thyroid hormone-dependent manner in pressure-overload induced hypertrophy. Our results clearly demonstrate that PTP1B links redox signaling and gene silencing to the thyroid hormone responsiveness of β-MHC expression in pathological cardiac hypertrophy.

P1-30

Forkhead box protein O1 (FoxO1) is required for exercise-induced, but not PI3K-induced, physiological cardiac hypertrophy

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Background: Physiological cardiac hypertrophy is induced by exercise and is mediated by phosphoinositide 3-kinase (PI3K) / Akt signalling. The transcription factor forkhead box protein O1 (FoxO1) is an Akt substrate that regulates cardiomyocyte hypertrophy *in vitro*, however its role in physiological hypertrophy is unknown. **Aim:** To determine whether FoxO1 is a critical regulator of physiological heart growth. **Methods:** Cardiomyocyte-specific FoxO1 heterozygous (HET) and knockout (KO) mice were generated by Cre-*loxP*-mediated recombination. Cre-positive *Foxo1*$^{+/+}$ and Cre-negative *Foxo1*$^{+/+}$, *Foxo1*$^{+/loxP}$ and *Foxo1*$^{loxP/loxP}$ littermates served as controls (CON). Basal phenotype was assessed (3 and 8 months) by echocardiography, gravimetry and molecular analyses (Study 1). Adult female mice underwent twice-daily swim training for four weeks (Study 2). Mice were mated with cardiomyocyte-specific constitutively active (ca) PI3K transgenic mice (Study 3). **Results:** FoxO1 protein expression was reduced ~33% and ~50% in ventricular lysates from HET and KO mice, respectively. FoxO3a and FoxO4 levels were unchanged. Study 1: Left ventricular (LV) internal dimensions were increased in male KO mice

(*P*<0.05, n=8-14/group). LV dilatation was not accompanied by changes in LV wall thicknesses, systolic function, heart or atria weights. Female HET and KO mice were indistinguishable from CON (*n*=6-13/group). Study 2: Exercised CON mice displayed a ~22% increase in heart weight (HW) normalized to tibia length (TL; *P*<0.05, *n*=12/group). Exercise-induced hypertrophy was blunted in HET and KO mice (no significant increases in HW/TL in exercised vs sedentary mice, *n*=6-13/group). Study 3: Male and female mice expressing caPI3K displayed ~16% and ~22% increases in HW/TL vs CON, respectively (*P*<0.05, *n*=5-8/group). FoxO1 deletion had no effect on the degree of hypertrophy in caPI3K transgenic mice. **Conclusion:** FoxO1 regulates the hypertrophic response to exercise but is not required for PI3K-induced physiological hypertrophy.

P1-31

Psychosocial stress unmasks latent doxorubicin-induced cardiotoxicity

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Background: Childhood Cancer Survivors (CCSs) have about 15 times higher risk of heart failure than their siblings, mainly due to cardiotoxicity of chemotherapy received during cancer treatment. It is estimated that 50% of pediatric cancer patients receive anthracyclines such as doxorubicin (DOX), which are known to cause cardiotoxicity. Psychosocial stress is a significant risk factor for cardiovascular diseases in the general population. Nevertheless, the role of psychosocial stress in the pathogenesis of chemotherapy-induced cardiomyopathy in CCSs is not known, despite the enormous burden of psychosocial stress in CCSs. In this study, we utilized a novel mouse model of latent juvenile DOX-induced cardiotoxicity to determine the cardiac effects of psychosocial stress in adult mice pre-exposed to DOX as juveniles.

Methods: Five week old male C57Bl/6N mice were administered DOX (4 mg/kg/week for three weeks) to induce latent cardiac injury. At the age of 12 weeks, a cohort of DOX-exposed mice was subjected to chronic psychosocial stress (14-day sensory contact with a dominant animal, and daily 5-minute defeat episodes). On the 15th day, mice were sacrificed, and hearts were harvested for histopathology analysis and molecular biology work.

Results: DOX-exposed mice manifested exaggerated myocardial fibrosis and increased gene expression of pro-inflammatory (e.g. interleukin-6, IL-6, and cyclooxygenase-2, Cox-2) and pro-fibrotic cytokines (e.g. transforming growth factor-β, TGF-β), in response to chronic psychosocial stress. Neither DOX exposure nor psychosocial stress alone was sufficient to cause significant myocardial fibrosis.

Conclusion: Exposure to psychosocial stress exacerbated DOX-induced cardiac damage and precipitated significant cardiac fibrosis in adult mice pre-exposed to DOX as juveniles. These findings underscore psychosocial stress as a risk factor in the transition from latent cardiotoxicity to overt cardiomyopathy in CCSs and offer a preclinical model to elucidate the molecular determinants of this transition.

P1-32

hRelaxin-2 fusion protein treatment prevents isoproterenol-induced hypertrophy and fibrosis

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Cardiac hypertrophy accompanies many forms of heart disease, including ischemic disease, hypertension, and heart failure. Anti-fibrotic therapy is promising for prevention and treatment of hypertrophic cardiomyopathy as the disease is associated with interstitial fibrosis. The search for new drugs for symptom relief and to improve long-term outcomes in heart failure has led to development of serelaxin, a recombinant human relaxin-2 hormone, and hRelaxin-2 fusion protein (hRLX-2). Comparatively, hRLX-2 has an extended half-life, potential superior efficacy, and convenient dosing, which suggests an improved safety profile. The purpose of this study was to test whether hRLX-2 is protective in cardiac hypertrophy and fibrosis. Isoproterenol was used to induce cardiac hypertrophy model in C57BL6J mice. Vehicle or isoproterenol (15 mg/kg/day) was delivered via Alzet minipumps for 14 days, and hRLX-2 (30 mg/kg) was given twice by subcutaneous injection at day 0 and 7. For comparison, a group with serelaxin (500 mg/kg/day, un-modified recombinant human relaxin-2) or enalapril (2.5 mg/kg/day, an ACE inhibitor) was co-infused with isoproterenol. Isoproterenol increased cardiac hypertrophy (as measured by heart weight/body weight, heart weight/tibial length, and echocardiography) and fibrosis (collagen content and histological assessment) compared to vehicle. H-RLX-2, enalapril or serelaxin treatments attenuated the isoproterenol induced cardiac hypertrophy and fibrosis. Isoproterenol-induced hypertrophy significantly increased TGFβ1/Smad-induced fibrotic signaling, which was attenuated by hRLXn-2. Consistent with a previous study using seralaxin, we found

that hRLX-2 significantly increased AKT/eNOS signaling. In addition, hRLX-2 treatment increased protein S-nitrosylation, which has been shown in other cardioprotective models. These findings support a potential role for hRLX-2 in the treatment of hypertrophic myocardiopathy.

P1-34

Pregnancy-Induced Remodeling of Heart Valves

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Although many cardiovascular tissues have been shown to remodel when exposed to chronic changes in hemodynamic stresses, little is known about the capacity of heart valves to adapt in a non-pathological state such as pregnancy. Pregnancy is a volume-overload state that triggers enlargement of the heart and valve orifices, thereby elevating valve leaflet stresses. The aim of this work was to (i) investigate pregnancy-induced alterations in valve leaflet biomechanics, (ii) perform structural studies defining the material basis for these changes, and (iii) link the observed structural and mechanical information using a structural constitutive model. Valve leaflets were harvested from non-pregnant heifers and pregnant cows. Gross leaflet structure was characterized by leaflet dimensions, and small-angle light scattering was used to assess changes in internal collagen fiber architecture. Tissue composition was determined using standard biochemical assays. Histological studies assessed architectural changes in cellular and matrix components. Leaflet mechanical properties were assessed using equibiaxial mechanical testing. Collagen thermal stability and crosslinking state was assessed using denaturation and hydrothermal isometric tension tests. We observed rapid and extensive heart valve remodeling in pregnancy, with alterations to leaflet geometry, fiber architecture, composition, biomechanics, and cellularity. All the valves expanded in pregnancy, via an increase in the production of collagen, with associated changes in tissue composition and structure of the collagen network (i.e. loss of crimp, crosslink maturation, and reduction in thermal stability). Our structural constitutive model suggests that there is an initial period of permanent set-like deformation where no remodeling occurs, followed by a remodeling phase that results in near-complete restoration of homeostatic tissue-level stresses and mechanical properties. Understanding the valvular adaptations to pregnancy may be fundamental both to developing interventions and treatments for valve disease and heart failure, as well as recognizing the implications of pregnancies on maternal long-term vascular risk.

P1-35

Prevalence of High Blood Pressure among Civil Servants in the City Of Winnipeg

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A mobile hypertension awareness campaign was created to: 1) determine the prevalence of hypertension in Winnipeg, Manitoba, Canada, 2) increase hypertension awareness, 3) identify reasons for lack of therapy adherence . The team participated in a wellness fair organized by the City of Winnipeg Occupational Health from September 2017 to March 2018 in the City of Winnipeg . 437 (310 males and 127 female civil servants participated from different departments including Fire Paramedic, Administration, Public Works and Transit. Blood pressure (BP) readings (systolic/diastolic) were classified as: Normotensive (90-120)/(60-80); Pre-Hypertensive (121-139)/(81-89) ; Stage 1 Hypertensive (140-159)/(90-99); and Stage 2 Hypertensive (>160/>100). Forty percent (175) of the participants presented with elevated BP. Additionally, 11% (46 of the participants, average age 53yrs) reported currently taking anti- hypertensive medications. However, of these 37% were pre hypertensive and 45% exhibited uncontrolled BP (> 140/90 mmHg). The remaining 137 participants were not taking hypertensive medication. Two out of 5 subjects with BP reading of 180/100 (Stage 2) had not been diagnosed previously with hypertension. Four out of 43 subjects in the Stage 1 group were previously diagnosed with high BP but had stopped taking their medications for the last two years. The reasons for lack of therapy adherence included denial, and being unaware of the health consequences of improper management of hypertension. The prevalence of high BP and particularly hypertensive emergencies was higher than expected. A public mobile hypertension clinic may provide a strategy for increasing the awareness of impending medical need. These hypertension awareness clinics should be integrated into a strategic plan to prevent the impact of hypertension on our society. Supported by CIHR, Western Grain Research Foundation, ARDI, SaskFlax and St Boniface Foundation.

P1-36

Sex-dependent regulation of autophagy by midkine in pediatric dilated cardiomyopathy

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Background: Sex hormones are thought to influence sex-based clinical and molecular differences in adult patients with dilated cardiomyopathy (DCM). Interestingly, sex differences are also observed in pre-pubertal pediatric DCM patients, as girls with DCM have worse outcomes than boys. This suggests that sex-hormone-independent differences also play a role in cardiac pathology. One potential factor which may regulate sex differences in pediatric cardiac function is midkine (MDK), a protein that is highly elevated in the serum of pediatric DCM patients. While MDK's role in cardiac dysfunction is unclear, in cancer models, MDK has been found to inhibit autophagy. The objective of this study was to determine if high exogenous MDK leads to sex differences in autophagy and cardiac function.

Methods: Young male and female mice were treated for 7 days with 3mg/kg/week recombinant human MDK. Cardiac pathology was assessed by analysis of gene expression and protein changes, echocardiogram, and sarcomeric contractility and relaxation measured through myofibril mechanics.

Results: Female mice treated with MDK demonstrated upregulation of genes involved in the fetal gene program. Further, there were sex-specific differences in whole heart and subcellular function in MDK treated mice. Fractional shortening and rate of activation of contraction were decreased in female mice treated with MDK. In addition, myofibril relaxation mechanics were altered in female mice treated with MDK. Finally, analysis of mediators of autophagy indicate that MDK in female mice leads to inhibition of autophagy in the heart.

Conclusions: Elevated exogenous MDK in mice leads to sex-specific differences in cardiac function, sarcomeric mechanics, and alterations in autophagy. These findings suggest that MDK may contribute to worsened outcomes in pediatric girls with DCM.

P1-37

Phosphodiesterase-5 is Elevated in Failing Single Ventricle Myocardium and Affects Cardiomyocyte Remodeling *in vitro*

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Background: Single ventricle congenital heart disease (SV) is universally fatal without intervention, and eventual heart failure (HF) is a major cause of morbidity and mortality in this population. While there are no proven medical therapies for the treatment or prevention of HF in SV patients, phosphodiesterase-5 inhibitors (PDE5i), such as sildenafil, are increasingly utilized. While the pulmonary vasculature is the primary target of PDE5i therapy in patients with SV, the effects of PDE5i on the SV myocardium remain largely unknown. We sought to determine PDE5 expression and activity in the single right ventricle (RV) of SV patients relative to non-failing (NF) controls, and to determine if PDE5 impacts cardiomyocyte remodeling using a novel serum based *in vitro* model.

Methods and Results: PDE5 expression (n=9 NF, n=7 SV), activity (n=8 NF, n=9 SV) and localization (n=3 SV) were determined in explanted human RV myocardium. PDE5 is expressed in SV cardiomyocytes and PDE5 protein expression and activity are increased in SV RV compared to NF RV. Isolated neonatal rat ventricular myocytes (NRVMs) were treated for 72 hours with NF or SV patient serum ± sildenafil. RT-qPCR (n=5 NF, n=12 SV) and RNAseq (n=3 NF, n=3 SV) were performed on serum-treated NRVMs and demonstrated that treatment with SV sera results in pathological gene expression changes which are attenuated with PDE5i.

Conclusions: Findings from this study suggest that elevated PDE5 in failing SV myocardium may contribute to adverse myocardial remodeling, and in addition to effects on the pulmonary vasculature, PDE5i may provide direct myocardial benefit in this population. These results underscore the importance of pediatric-specific investigations and justify attempts to improve the understanding of SV-specific molecular mechanisms leading to HF.

P1-38

Loss of obscurin/Obsl1 results in diastolic heart-failure

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Background

Muscle proteins of the obscurin protein family were shown to play roles for sarcomere organization, sarcoplasmic reticulum (SR) and T-tubule architecture and function.

However, their precise biological roles, functional redundancies between members of this protein family, and involvement in cardiac diseases remain to be fully understood.

Methods

We set out to investigate the role that obscurin and its close homologue obscurin-like 1 (Obsl1) play for cardiac development and function. We generated and analyzed cardiac functions of obscurin, Obsl1 single, as well as obscurin/Obsl1 double-knockout mice using trans-thoracic echocardiography and hemodynamics studies. Changes to SR structure, protein content and ultrastructure were investigated by immunofluorescence, immunoblot and serial-blockface electron microscopy analyses. SR-dependent changes to cellular calcium cycling were studied by imaging neonatal cardiomyocytes.

Results

Mice lacking obscurin and Obsl1 develop normally, but show dramatic changes to SR architecture and function on the microscopic and biochemical level. While obscurin has been shown to be important for SR structure, our data reveal for the first time that Obsl1 has similar functions. Alterations to SR structure are also reflected in dramatically altered calcium cycling. Neonatal cardiomyocytes from obscurin or Obsl1 single knockouts displayed reduced calcium amplitude (calcium release) and prolonged calcium re-uptake (tau-values), which became more pronounced in double-knockout cells.

On the physiology level, loss of obscurin and Obsl1 results in a profound relaxation defect associated with heart-failure in double-knockout mice after 1 year of age. Intriguingly, the diastolic dysfunction is not accompanied by hypertrophy or increased fibrosis, but changes on the metabolic level.

Taken together, our data indicate that obscurin and Obsl1 are crucial for SR structure, calcium storage and re-uptake. We propose that double-knockout mice may serve as a new model to investigate age-dependent diastolic heart-failure.

P1-40

Oxidized phosphatidylcholine induces cardiomyocyte cell death in ischemia/reperfusion injury through ferroptosis

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Introduction: Reperfusion represents the major therapeutic aim for the treatment of a myocardial infarction. However, it is followed by a large production of reactive oxygen species (ROS) that can lead to a generation of oxidized phosphatidylcholines (OxPCs). Two of the fragmented OxPCs: POVPC (1-palmitoyl-2-(5'-oxo-valeroyl)-*sn*-glycero-3-phosphocholine) and PONPC (1-palmitoyl-2-(9'-oxo-nonanoyl)-*sn*-glycero-3-phosphocholine) are not only the most abundant ones produced during I/R but showed significant cardiotoxic effects. The aim of this study was to identify the mechanism of OxPC action in I/R injury in order to find a potential target for therapy for reperfusion injury.

Methods: Adult rat ventricular cardiomyocytes were exposed to increasing concentrations of POVPC and PONPC. Cell viability was determined using Live/Dead™ assay. The form of cell death induced by those compounds was investigated by Western blot analyses for cleaved caspase 3 as a marker for apoptosis and HMGB1 as a marker for necrosis. The role of ferroptosis was investigated by addition of ferrostatin -1 together with POVPC. To investigate the protective role of ferrostatin-1 and E06 in I/R, cardiomyocytes were exposed to simulated ischemia followed by reperfusion and ferrostatin-1 or E06 were added at the time of reperfusion.

Results: POVPC and PONPC induced a cardiotoxic effect in a concentration dependent manner. OxPCs did not activate caspase 3 in cells. Treatment of cardiomyocytes with ferrostatin-1 and POVPC attenuated the cardiotoxic effect of POVPC. Ferrostatin or E06 also protected cardiomyocytes exposed to I/R challenge.

Conclusion: OxPCs induce cardiomyocyte cell death in ischemia/reperfusion injury. The mechanism of their action is through the ferroptotic pathway. These results suggest a novel pathway of cardiomyocyte cell death in I/R injury as well as a novel therapeutic approach for I/R injury.

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P1-41

The effects of dietary flaxseed on cardiac function in rats after myocardial infarction

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Background: Flaxseed is the richest source of n-3 fatty acids and is reported to have significant antihypertensive, hypolipidemic, antiatherogenic, antioxidant, and anti-inflammatory activities. However, no studies have investigated the effect on ventricular remodeling after a myocardial infarction (MI). The aim of the study was to examine the effects of dietary flaxseed on heart function and arrhythmias that develop after an MI.

Methods: The left anterior descending coronary artery was ligated in Wistar rats to induce the MI. Rats were randomized into six dietary groups: sham MI with normal chow, MI with normal chow, MI with 10% milled flaxseed supplementation, MI with 4.4% supplemented flax oil rich in alpha-linolenic acid (ALA), MI with flax lignan secoisolariciresinol (SDG) supplementation (0.44%), and MI with diet supplemented with 7% partially defatted flaxseed meal (PDFM). Animals were fed for two weeks before surgery and for 8 weeks after surgery with the standard rat chow or the different components of flax. Echocardiography and continuous ECG recordings were obtained after ligation to confirm the induction of the infarction, to check for arrhythmias and to assess cardiac function.

Results and conclusion: No significant changes were observed in echocardiographic measures of systolic and diastolic function. However, hearts obtained from animals fed the flax and flax oil supplementation exhibited the smallest infarct size. In addition, dietary supplementation with flax oil prevented the development of arrhythmic events by 94%. These results suggest dietary flaxseed and especially flax oil may have a beneficial impact on cardiac recovery post-MI.

Key words: Flaxseed, alpha-linolenic acid, arrhythmias, echocardiography, myocardial infarction

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P1-42

A knock-in mutation at a site of S-nitrosylation on TRIM72 is cardioprotective and improves insulin sensitivity

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TRIM72 is a membrane repair protein, involved in many pathways including cardiac hypertrophy, insulin sensitivity, and ischemia reperfusion (I/R) injury. Our lab has previously identified Cysteine 144 (C144) on TRIM72 as a site of S-nitrosylation. Experiments suggested that S-nitrosylation of C144 of TRIM72 is protective against I/R injury. To further study the importance of C144, we generated a knock-in mouse with C144 mutated to a serine (TRIM72 C144S). TRIM72 C144S knock-in hearts were protected against I/R injury. After 20 min of equilibrium perfusion, Langendorff perfused hearts were subjected to 20 min of global ischemia followed by 90 min of reperfusion. Post-ischemic recovery of rate pressure product was better in the TRIM72 C144S heart compared to wild-type (61.9±6.1% vs 37.4±2.9%, n=5). Further the infarct size was smaller in the TRIM72 C144S compared to wild-type (27.2±4.0% vs 54.2±4.4%, n=5). To test whether this beneficial effect was due to improved stability of TRIM72 protein, we subjected a second set of wild-type (WT) and TRIM72 C144S hearts to either perfusion, just ischemia, or the full I/R protocol. However, TRIM72 levels did not appear to explain the beneficial effect of the mutation. Since TRIM72 has also been implicated in metabolism and insulin sensitivity we also examined whole body insulin sensitivity. Male TRIM72 C144S mice had significantly better glucose tolerance than WT mice. These data are consistent with the hypothesis that the improved insulin sensitivity contributes to the cardioprotective effects of TRIM72 C144S. In conclusion, these data suggest that post translational modification of this C144 is important in the regulation of TRIM72 function and regulation of insulin sensitivity and I/R injury.

P1-43

Pharmacologic ATF6 Activation Confers Global Protection in Widespread Disease Models by Reprogramming Cellular Proteostasis

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Pharmacologic activation of stress-responsive signaling pathways provides a promising approach for ameliorating imbalances in proteostasis associated with diverse diseases. However, this approach has not been employed *in vivo*. Here, using a mouse model of myocardial ischemia/reperfusion, we showed that selective pharmacologic activation of the ATF6 arm of the unfolded protein response (UPR) during reperfusion, a typical

clinical intervention point after myocardial infarction, transcriptionally reprograms proteostasis, ameliorates damage and preserves heart function. These effects were lost upon cardiac myocyte-specific *Atf6* deletion in the heart, demonstrating the critical role played by ATF6 in mediating pharmacologically activated proteostasis-based protection of the heart. Pharmacological activation of ATF6 was also protective in renal and cerebral ischemia/reperfusion models, demonstrating its widespread utility. Thus, pharmacologic activation of ATF6 represents a first-in-class proteostasis-based therapeutic strategy for ameliorating ischemia/reperfusion damage, underscoring its unique translational potential for treating a wide range of pathologies caused by imbalanced proteostasis.

P1-44

Matricellular proteins Nov and Wisp1 in aging and myocardial infarction

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Background. CCN matricellular proteins are signaling molecules in the extracellular space, which can have pro-angiogenic, anti-inflammatory and anti-fibrotic properties. Their role in repair and remodeling post-myocardial infarction (MI) remains to be better elucidated. In this study, the age-associated expression of Nov (CCN3) and Wisp1 (CCN4) were examined post-MI in mice. **Methods and Results.** *In vivo*, MI was induced in young (6wk) and old (12-14 months) mice. Echocardiography showed that left ventricular ejection fraction was reduced in old mice (33.9%) at 14 days post-MI compared to young mice (43.9%; p=0.002). RT-qPCR analysis of myocardial tissue revealed that mRNA expression of several matricellular proteins in healthy tissues was decreased by 3- to 15-fold in old compared to young mice. Post-MI, mRNA expression of Nov was reduced in the infarct (by 9-fold) and border (by 21-fold) zones ($p\leq$0.01) in old vs. young mice. Nov and Wisp1 protein expression was also reduced in old compared to young mice in the infarct and border zones; specifically for Nov in the infarct zone at 2 days post-MI (p=0.003) and 14 days post-MI (p=0.008), and for Wisp1 at 7d post-MI in the border zone (p=0.003). *In vitro*, the expression of Nov (2.7-fold) and Wisp1 (2.3-fold) protein was increased in TGF-β stimulated cardiac fibroblasts after 48h, as did the expression of the myofibroblast marker α-SMA (1.7-fold; p=0.02). Cardiac fibroblasts treated with Nov+TGF-β exhibited greater proliferation (by 29%; p<0.01), as did those treated with Wisp1+TGF-β (by 16%; p<0.05). **Summary.** There is an age-associated difference in the expression of matricellular proteins Nov and Wisp1 in both healthy and MI mice. A better understanding of Nov and Wisp1 function in aging and post-MI repair may help identify novel therapeutic targets for limiting damage post-MI and improving regeneration and heart function.

P1-45

Sex-specific acute beneficial effects of an estrogen receptor agonist added to cardioplegic solution in adult and aging mouse hearts

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Objective: Low estrogen levels alter myocyte calcium homeostasis and promote ischemia and reperfusion (I/R) injury. As I/R injury occurs during cardiac surgery, we propose to determine whether the G-protein coupled estrogen receptor (GPER) agonist, G1, enhances the benefits of cardioplegia.

Methods: Hearts were isolated from adult (6-9 mos) and aged (22-24 mos) mice of both sexes (n=44) for Langendorff perfusion and perfused with Krebs-Henseleit buffer (15min). Perfusion was interrupted and St. Thomas'2 cardioplegia was delivered either with G1 (500 nM), G1 (500 nM)+G15 (GPER antagonist; 1 μM) or with vehicle alone for 6min, followed by 90min global ischemia and 30min reperfusion. Cardiac troponin-I (cTnI) release was measured with ELISA and hearts were perfused with triphenyltetrazolium chloride following reperfusion, to differentiate infarcted tissue.

Results: Post-ischemic functional recovery in hearts perfused with cardioplegia+G1 was significantly better than cardioplegia alone in females regardless of age. In adult females, left ventricular developed pressure (LVDP) recovered to only 46.9±11.1% in control but recovered to 76.1±5.3% when G1 was used (p<0.05). G1 also improved the rates of pressure development (+dp/dt) and decay (-dp/dt). These effects of G1 were blocked by G15 (LVDP recovered to 59.0±1.8%). Similar beneficial effects were seen in hearts from old females. G1 also reduced cTnI release in adult females (9.0±2.0 ng/ml and 6.5±3.0 ng/ml for control and G1, respectively) and reduced infarct sizes (20.0±3.5% and 15.3±3.6% for control and G1, respectively). By contrast, no significant beneficial effects of G1 were observed in hearts from male mice of any age.

Conclusion: The addition of G1 enhances the cardioprotective properties of cardioplegia and improves functional recovery after ischemia across the lifespan, but only in female hearts.

P1-46

Remote ischemic preconditioning via incomplete vascular occlusion

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Background: Remote ischemic preconditioning (RIPC) involves episodes of vascular occlusion to a limb or distant tissue which induces cardioprotection. A variety of methods have shown promising pre-clinical results, however none have proven feasible or beneficial clinically. Most pre-clinical models are either surgical and are not feasible in patients and/or involve total occlusion of an artery. Therefore, we propose a more practical preclinical method that can be easily translated clinically. The objective of this study was to test the hypothesis that incomplete vascular occlusion induces RIPC.

Methods: C57/BL6 mice were used to establish the timing of preconditioning and its effect on infarct size and cardiac function in the setting of a myocardial infarction (MI). RIPC was induced by applying an elastic material of consistent diameter to the left forelimb of the mice and verifying capillary refill after placement. Mice were randomized into four groups (n=4 per group): Chronic treatment for 1wk prior to MI, Acute treatment on the day of MI, Chronic + Acute, or no treatment. Cardiac function was assessed at baseline and 24 hours post-MI via echocardiography. Hearts were stained with triphenyltetrazolium chloride to measure infarct size.

Results: Control group had an infarct size of 63.4±4.0%, while the Acute and Chronic + Acute groups were smaller at 34.7±2.6% and 40.0±2.5%, respectively. This effect was not seen in the Chronic group (53.5±4.5%). Similarly, cardiac function via echocardiography demonstrated a decreased ejection fraction in the control group 38.7±3.7%, while the Acute and Chronic + Acute groups were higher, closer to normal function (46.1±2.9% and 49.9±2.8%, respectively). This cardioprotective effect was not seen in the Chronic group (43.3±3.0%).

Conclusion: A pre-clinical murine model including incomplete occlusion of an extremity elicits an RIPC effect.

P1-47

Mitochondrial DNA (mtDNA) damage in diabetic heart: 4-hydroxy-2-nonenal (4HNE) inhibits mtDNA repair enzyme, 8-oxoguanine glycosylase 1

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Background: Diabetes mellitus (DM) affects a variety of organs including myocardium. 8-oxoguanine glycosylase 1 (OGG-1) repairs mitochondrial DNA (mtDNA) by base excision repair process. We hypothesize that DM mediated 4-hydroxy-2-nonenal (4HNE) contributes to mtDNA damage by forming adducts with mtOGG-1 and thus inhibiting its activity and thereby contributing to cardiac dysfunction in the diabetic heart.

Methods and results: First of all, we treated 4HNE (1, 10 and 100 μM) directly to recombinant OGG-1, analyzed the 4HNE adduction on specific amino acids in OGG-1 and measured its activity, in vitro. 4HNE dose-dependently inhibited the activity of OGG-1 by forming adducts. We identified that several amino acids on OGG-1 such as Cys241, His237, Lys238, Cys163, His282, and Lys249 were 4HNE adducted. A type-2 diabetic model, db/db mice were sacrificed at six months when they exhibit cardiac dysfunction. We found a decrease in myocardial OGG-1 activity in db/db mouse hearts compared to db/dm hearts. We observed an increase in 4HNE adducts on OGG-1 in db/db mouse hearts. We also found increased mtDNA damage. The activity of aldehyde dehydrogenase (ALDH) 2 which detoxifies 4HNE was decreased in db/db mouse hearts which may have led to increases in the 4HNE levels. Before the 4HNE challenge, pre-incubation with recombinant ALDH2 decreased the 4HNE-mediated reduction in OGG-1 activity, in vitro. Therefore, we treated db/db mice with Alda-1, an ALDH2 activator and found a decrease in 4HNE adducts and thus decreased mtDNA damage along with improved cardiac function.

Conclusion: Increased 4HNE formed adducts with OGG-1 and inhibited its activity and thus led to mtDNA damage in a type-2 diabetic heart. ALDH2 decreased the 4HNE adduction on OGG-1 and thereby improved mtDNA repair and cardiac function

P1-48

Magnesium Supplementation Improves Cardiac Mitochondrial and Diastolic Function

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Rationale: In congestive heart failure and type 2 diabetes mellitus (DM), hypomagnesemia has been found in the majority of patients, and supplementation of Mg has improved heart function and insulin resistance, respectively. Recently, we have shown that diabetes can cause cardiac diastolic dysfunction. Therefore, we hypothesized that Mg supplementation would benefit diastolic function.

Methods and Results: High fat diet (HFD)-induced DM mouse hearts showed cardiac diastolic dysfunction (DD) and hypertrophy. DD was manifested with increased E/e' in echocardiograph (45±2 in DM mice vs. 32±2 in control mice), left ventricular diastolic volume (LVDV, 80±1 µL of DM vs. 61±4 µL of control), and incidence of DD (9 of 10 mice in DM vs. 1 of 10 mice in control). Hypertrophy was shown with increased left ventricular posterior wall thickness (LVPWT, 0.93±0.02 mm in DM vs. 0.77±0.04 mm in control) and heart weight/tibia length (HW/TL, 72±2 mg/cm in DM vs. 60±1 mg/cm in control; P<0.05 for all). DM mice also showed hypomagnesemia (plasma Mg concentration: 0.80±0.04 mmol/L in DM vs. 0.98±0.03 mmol/L in control, P<0.05). Ventricular cardiomyocytes isolated from DM mice exhibited decreased mitochondrial ATP production (75±11% of control), a 1.7±0.2-fold increase of mitochondrial reactive oxygen species (mitoROS), significantly depolarized mitochondrial membrane potential, and mitochondrial Ca^{2+} overload (3.7±1.3-fold; P<0.05 for all). Dietary Mg administration (50 mg/mL, ≈6-8 g/kg/day) for 6 weeks increased plasma Mg concentration (1.5±0.1 mmol/L, P<0.001 vs. DM), improved cardiac function (E/e' 37±1; LVDV 54±5 µL; LVPWT 0.77±0.02 mm; the incidence of DD, 2 of 10 mice; HW/HL 64±1 mg/cm; P<0.05 vs. DM for all). Mitochondrial function was improved significantly by Mg with increased ATP production, decreased mitoROS, repolarized mitochondrial membrane potential, and decreased mitochondrial Ca^{2+} overload in DM mice.
Conclusion: These results indicate that Mg supplementation improved mitochondrial function, reduced oxidative stress, and prevented diastolic dysfunction in DM.

P1-49

Caloric restriction limits fatty acid oxidation and improves cardiac function in heart failure associated with obesity

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Background: The general consensus is that heart switches from fatty acid oxidation (FAO) to glucose metabolism in heart failure. However, what happens to FAO in heart failure associated with obesity, a condition known to increase cardiac FAO, is not known. We therefore, investigated what effect obesity has on cardiac FAO and function in the failing heart, and what effect weight loss due to caloric restriction has on these parameters.

Methods and Results: Male C57BI/6J mice were fed with either a high fat (HF) diet or low fat (LF) diet for 4 weeks. Mice in each group were then randomised to undergo either sham operation or a transverse aortic constriction (TAC) and continued on their respective diets for a further 6 weeks, where they developed obesity and heart failure (HF-TAC-mice showed a significant reduction in % ejection fraction (%EF) (44.2±3.0 vs 56.5±2.8 HF-sham-mice, p<0.01). HF-TAC-mice were then randomized to either continue on a HF diet or subjected to caloric restriction (CR) (40% reduction in the HF diet) for a further 8 weeks. By 18-weeks there was further decrease in %EF in the HF-TAC-mice to (26.4±1.4), whereas %EF remained at (39.8±1.9) in the CR mice. FAO and glucose oxidation measured in isolated working hearts at 18-week showed that FAO was very high and dominated as a source of ATP production in the HF-TAC hearts (providing 93.3% of ATP production). CR decreased the contribution of FAO to 50.3% of ATP production and increased glucose oxidation rates (to 363±32 $nmol{\cdot}g\ dry\ wt^{-1}{\cdot}min^{-1}$ compared to 117±8 $nmol{\cdot}g\ dry\ wt^{-1}{\cdot}min^{-1}$ in HF-TAC, p<0.05).

Conclusions: Weight loss by CR improves cardiac function in obese mice with heart failure. This is associated with a reduction in FAO and an improvement in glucose oxidation, suggesting that reducing FAO is beneficial in heart failure with obesity.

P1-50

Epigenetic regulation of cardiometabolic disease by HDAC-BET association

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Little is known about the biological function of histone deacetylase 11 (HDAC11), which is the lone class IV HDAC. Here, we demonstrate that deletion of HDAC11 in mice stimulates brown adipose tissue (BAT) formation and beiging of white adipose tissue (WAT). Consequently, HDAC11-deficient mice exhibit dramatically enhanced thermogenic potential and, in response to high-fat feeding, attenuated obesity, insulin resistance, and hepatic steatosis. *Ex vivo* and cell-based assays revealed that HDAC11 catalytic activity suppresses the BAT transcriptional program, in both the basal state and in

response to β-adrenergic receptor signaling, through a mechanism that is dependent on physical association with BRD2, a bromodomain and extraterminal (BET) acetyl-histone binding protein. At the level of the heart, we provide evidence to support a role for HDAC11 as a negative regulator of physiological hypertrophy. These findings define a novel epigenetic pathway for the regulation of energy homeostasis and cardiac growth, and suggest a potential for HDAC11-selective inhibitors for the treatment of obesity, diabetes, and associated cardiac disease.

P1-51

G Protein-coupled receptor kinase 2 impairs fatty acid metabolism in the failing heart through novel mechanisms

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Compromised contractility and energetics are hallmarks of heart failure. Increased G protein-coupled receptor kinase (GRK)2 is central to HF pathogenesis, via desensitization of beta-adrenergic receptors and, therefore, loss of contractile reserve. We, and others, have reported that GRK2 also has non-canonical activity that contributes to its pathological functionality in HF. For example, GRK2 can compromise fatty acid (FA) metabolism in cardiomyocytes, the mechanism of which remains unknown. Our aim is to investigate the role of GRK2 in FA metabolism and bioenergetics in the heart. To do this, we measured FA uptake, cluster of differentiation (CD)36 expression, phosphorylation, and ubiquitination, and FA-driven bioenergetics in mice with cardiac-specific overexpression of GRK2 (TgGRK2) or a GRK2 inhibitory peptide (TgβARKct) or global heterozygous GRK2 knockout ($GRK2^{+/-}$) mice. Additionally, we determined CD36 expression and phosphorylation following transverse aortic constriction (TAC). The results show a significant reduction in FA uptake rates (0.3-fold) and CD36 protein (0.8-fold), which is associated with an increase in its phospho-serine/threonine (0.7-fold increase) and ubiquitin-lysine (0.7-fold) content in TgGRK2 mice. These parameters were unchanged in TgβARKct or $GRK2^{+/-}$ mice. A reduction in CD36 mRNA (0.7-fold) and protein levels (0.2-fold) were detected in post-TAC, failing hearts, which was associated with an increase is its phosphorylation (0.5-fold). Notably, normalization of CD36 protein levels is observed in $GRK2^{+/-}$ mice post-TAC. Maximal respiratory capacity was also enhanced in TgβARKct (0.6-fold) and $GRK2^{+/-}$ cardiomyocytes (0.8-fold). Together, our results show that up-regulation of GRK2 induces CD36 phosphorylation, ubiquitination, and downregulation, which is associated with reduced FA uptake. Thus, we propose that the increase in GRK2 observed during HF is, at least partly, responsible for reduced FA uptake and oxidation and may be an important nodal switch in substrate utilization during HF.

P1-52

MCL-1 Couples Mitochondrial Dynamic Machinery to Mitochondrial Quality Control in Response to Stress

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Myeloid Cell Leukemia-1 (MCL-1) is an anti-apoptotic BCL-2 family protein that plays a crucial role in maintaining cardiac homeostasis in the adult heart. Our lab previously showed that cardiac-specific ablation of MCL-1 in mice led to severe contractile dysfunction and mitochondrial deterioration accompanied by signs of necrotic, rather than apoptotic, cell death. This indicates that MCL-1 has an additional role in maintaining mitochondrial function in cardiac myocytes. MCL-1 localizes to two distinct mitochondrial locations in myocytes: one form exists on the outer mitochondrial membrane ($MCL\text{-}1_{OM}$) and a shorter cleaved form resides in the mitochondrial matrix ($MCL\text{-}1_{Matrix}$). Interestingly, overexpression of $MCL\text{-}1_{WT}$ or $MCL\text{-}1_{OM}$, but not $MCL\text{-}1_{Matrix}$, induces fragmentation and perinuclear aggregation of the mitochondria. This effect is abrogated upon mutation of MCL-1's BH3 domain responsible for its anti-apoptotic function, indicating that MCL-1's pro-fission function may be coupled with its anti-apoptotic function. We also found that MCL-1 interacts with the fission protein Drp1 in response to stress induced by treatment with the chemical uncoupler FCCP. As mitochondrial fission has been shown to precede mitophagy, we found that overexpression of MCL-1 also promotes mitochondrial clearance in response to FCCP treatment. Furthermore, we confirmed that MCL-1 interacts with LC3 II on the autophagosome membrane upon treatment with FCCP and are currently investigating the role of MCL-1's several putative LC3-Interacting Region (LIR) motifs. Interestingly, MCL-1-mediated mitophagy is abrogated in autophagy-deficient $Atg5^{-/-}$ MEFs, further confirming that it is occurring via the autophagy pathway. Surprisingly, other milder stimuli, such as glucose deprivation or hypoxia, did not induce an interaction between MCL-1 and LC3, suggesting that MCL-1 selectively promotes clearance of depolarized mitochondria. Thus, our data suggest that upon mitochondrial damage and depolarization, $MCL\text{-}1_{OM}$ acts as a mitophagy receptor to promote efficient removal of the mitochondria by coupling Drp1-mediated fission and clearance.

P1-53

The E3 Ubiquitin Ligase Parkin Regulates Mitophagy from the Nucleus

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Parkin is known for facilitating clearance of damaged mitochondria. We have previously shown that Parkin is important for adaptation to myocardial infarction (MI) and that loss of Parkin leads to accumulation of dysfunctional mitochondria. Parkin enables mitophagy by translocating to depolarized mitochondria; however, it is unclear whether Parkin also functions in other subcellular compartments. Here, we found that wild type (WT) Parkin overexpressed in neonatal myocytes localized to both the cytosol and the nucleus, whereas the pathogenic mutant ParkinR42P was excluded from the nucleus. Western blotting of subcellular fractions from WT mouse hearts confirmed the presence of endogenous Parkin in the nucleus. Additionally, hearts from cardiac-specific Parkin transgenic mice had elevated nuclear Parkin, which correlated with increased ubiquitination of nuclear proteins. Interestingly, WT mice subjected to MI displayed an increase in both Parkin and ubiquitinated proteins in the nuclear fraction of border zone myocytes. To further examine conditions that induce Parkin nuclear translocation, we subjected HeLa cells stably expressing YFP-Parkin to starvation or hypoxia. Under nutrient-limiting conditions, Parkin rapidly exited the nucleus and accumulated in the cytosol. Conversely, exposure to hypoxia caused Parkin to translocate to the nucleus. To evaluate the function of Parkin in the nucleus and its effect on mitophagy, we generated nuclear- and mitochondrial-targeted constructs: NLS-Parkin and Mito-Parkin. Mito-Parkin displayed accelerated mitochondrial clearance compared to WT Parkin in response to treatment with mitochondrial uncoupler FCCP. Unexpectedly, NLS-Parkin also induced mitochondrial clearance, albeit at a slower rate, suggesting that Parkin may regulate mitophagy transcriptionally. In addition to mitochondrial-Parkin, receptors on the outer mitochondrial membrane can also initiate mitophagy. We therefore examined transcription of various mitophagy receptors and found that cells overexpressing Parkin exhibited increased transcript levels of BNIP3 and NIX. These data indicate that Parkin facilitates mitophagy from multiple subcellular locations to ensure efficient mitochondrial clearance.

P1-54

Protein S-nitrosylation inhibits respiration in the failing heart

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Introduction: Heart failure is associated with alterations in cardiac nitric oxide production. Following ischemia the electron transport chain is inhibited by S-nitrosylation, however relatively little is understood about the role of this modification in chronic heart failure. Therefore the aim of this study was i) to characterise the S-nitrosylation profile in heart failure and ii) to determine the functional implications of S-nitrosylation on respiration.

Methods: Tachypacing was used to induce heart failure in sheep. Following euthanasia, tissue from the left ventricular posterior free wall was snap frozen for mass spectrometry. Protein S-nitrosylation was enriched using resin assisted capture. Mitochondria were isolated from the same region for functional oxygraph experiments. Data presented as mean ± standard error.

Results: The overall number of S-nitrosylated proteins identified increased in heart failure (232±18 in control to 314±28 in heart failure, p=0.02, n=6). Within the electron transport chain several complex subunits had increased levels of S-nitrosylation in the disease state (eg. NDUFS1 ↑2.9 fold, SDHA ↑1.2 fold, UQCRH ↑1.1 fold, n=6). Functionally, a mitochondrial targeted nitic oxide donor (mitoSNO) inhibited pyruvate, malate and glutamate driven state 3 respiration in control mitochondria (to 10±0.02% of baseline, p<0.01, n=16). MitoSNO inhibition was reversed minimally by oxyhaemoglobin (3±0.003 with mitoSNO to 8±0.01% of baseline with oxyhaemoglobin, experiment performed at <70nmol/ml O_2, p<0.01, n=9) but to a much greater extent by DTT (10±0.03 with mitoSNO to 58±0.02% of baseline with 1mM DTT, p<0.01, n=11) suggesting that this was an effect of S-nitrosylation.

Conclusions: This study demonstrates there is a gross increase in myocardial and mitochondrial S-nitrosylation in heart failure and that this leads to inhibition of respiration in the disease state.

P1-55

Coupling to Gq Signaling Is Required for Cardioprotection by an Alpha-1A-Adrenergic Receptor Agonist

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Rationale: Gq signaling in cardiac myocytes is classically considered toxic in heart disease. Targeting Gq directly to test this is not definitive, because cardiac myocytes have many Gq-coupled receptors.
Objective: Test whether Gq coupling is required for

cardioprotective effects of an alpha-1A-adrenergic receptor (AR) agonist.
Methods and Results: We studied a knock-in mouse with a 6-residue substitution in the third intracellular loop of the mouse alpha-1A-AR. In recombinant cells, this mutated receptor does not couple to Gq signaling. In the knock-in mouse, heart alpha-1A receptor levels were at least as high as in wild type (WT), and receptor affinity for antagonists was normal. In WT cardiac myocytes, the selective alpha-1A agonist A61603 stimulated phosphoinositide phospholipase C and myocyte contraction. In Gq mutant knock-in myocytes, both A61603 effects were absent, indicating that Gq coupling was absent. Surprisingly, A61603 activation of cardioprotective ERK was markedly impaired in Gq mutant myocytes, and A61603 did not protect the cells from doxorubicin toxicity in vitro. Similarly, A61603 did not rescue cardiac function after transverse aortic constriction in mice with the Gq coupling-defective alpha-1A receptor. **Conclusion:** Gq coupling is required for cardioprotection by an alpha-1A-AR agonist. Gq signaling is not invariably cardiotoxic.

P1-56

Exogenous CXCL4 Infusion Inhibits Macrophage Phagocytosis by Limiting CD36 Signaling to Enhance Post-myocardial Infarction Cardiac Dilation

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Aims: Macrophage phagocytosis of dead cells and debris is a prerequisite for inflammation resolution. Because CXCL4 induces macrophage phagocytosis *in vitro*, we examined the hypothesis that a translational gain-of-function approach would dissect the impact of exogenous CXCL4 infusion on cardiac wound healing following myocardial infarction (MI). **Methods and Results:** CXCL4 expression significantly increased in the infarct region beginning at day 3 post-MI, and macrophages were the predominant source. Adult male C57BL/6J mice were subjected to coronary artery occlusion, and MI mice were randomly infused with recombinant mouse CXCL4 or saline beginning at 24 h post-MI by mini-pump infusion. Compared to saline controls, CXCL4 infusion dramatically reduced 7 day post-MI survival (10% (3/30) for CXCL4 vs. 47% (7/15) for saline, $p<0.05$). By echocardiography, CXCL4 significantly increased LV volumes and dimensions at day 5 post-MI (all $p<0.05$), despite similar infarct areas compared with saline controls. While macrophage numbers were similar at day 5 post-MI, CXCL4 infusion increased Ccr4 and Itgb4 and decreased Adamts8 gene levels in the infarct region, all of which linked to CXCL4-mediated cardiac dilation. Isolated day 5 post-MI macrophages exhibited comparable levels of pro-inflammatory markers between saline and CXCL4 groups. Interestingly, by both e*x vivo* and *in vitro* phagocytosis assays, CXCL4 reduced macrophage phagocytic capacity, which was connected to decreased mRNA and protein levels of the phagocytosis receptor CD36, and not CD163, LRP1, MERTK, MRC1, or MSR1. CXCL4 infusion significantly elevated infarct matrix metalloproteinase (MMP)-9 levels at day 5 post-MI, and MMP-9 can cleave CD36 as a downregulation mechanism. **Conclusion:** CXCL4 infusion impaired macrophage phagocytic capacity by inhibiting CD36 expression through signaling dependent and independent of MMP-9, leading to adverse post-MI wound healing.

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P1-57

Epicardium-derived resident mesenchymal cells promote cardiac fibrosis

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Rationale: The epicardium is the primary source of cardiac fibroblasts, which secrete scar tissue following a myocardial infarction (MI). Serum response factor (SRF) and the SRF co-activators myocardin-related transcription factors (MRTFs) are required for epicardium-derived progenitor cell (EPDC) mobilization during development. However, the description of key transcriptional programs that regulate epicardium-derived fibrosis in adult cardiac disease requires further investigation.

Objective: To evaluate the consequence of disrupting the SRF-MRTF transcriptional axis in the epicardium on the development of fibrosis and pathological cardiac remodeling.

Methods and Results: Using fluorescence based genetic lineage tracing of Wilm's Tumor 1 (Wt1)-positive cells, we show that EPDCs significantly contribute to necrotic tissue replacement and fibrosis following MI, a process that is inhibited upon epicardial specific deletion of *Srf*. Furthermore, we investigated mice lacking expression of MRTFs (MRTF-A and -B; called $MRTF^{epiDKO}$) by utilizing a $Wt1^{cre}$ driver to delete floxed alleles of *Mrtf-b* in the epicardium of a mouse with a global knockout of *Mrtf-a*. In $MRTF^{epiDKO}$ hearts, *Mrtf-a* is deleted in all cardiomyogenic cell populations, while *Mrtf-b* deletion is observed only in cardiac interstitial cells. $MRTF^{epiDKO}$ mice display reduced pathological remodeling post-MI leading to preserved cardiac function and physiology up to 14 days after injury. Left ventricular collagen deposition and myofibroblast activation is significantly attenuated in $MRTF^{epiDKO}$ hearts after MI, correlating with a decrease in circulating cytokines related to the promotion of fibrosis and inflammation. Interestingly, while young MRTF wild-type (WT) and $MRTF^{epiDKO}$ hearts display normal cardiac physiology, aged $MRTF^{epiDKO}$ mice are protected from age-associated diastolic dysfunction and display decreased deposition of interstitial collagen.

Conclusions: Our findings indicate MRTF and SRF expression in adult EPDCs is critical for cardiac interstitial cell function and implicate EPDCs to drive post-MI cardiac remodeling and diastolic dysfunction in aging.

P1-58

Endoplasmic Reticulum Stress Promotes Inflammation Through iNOS-Regulated Toll-Like Receptor 2 (TLR 2) in Doxorubicin-Induced Cardiomyopathy

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The deleterious side effect of doxorubicin (Dox) in causing cardiomyopathy (DIC), limits its usage in treating cancer patients. Subcellular signaling pathways involved in DIC are largely affected by oxidative stress leading to heart failure. Dox causes dilation of the endoplasmic reticulum (ER) in cardiomyocytes and could be an important factor in DIC. Here, we demonstrate that during DIC, ER stress promotes inflammation through iNOS-induced TLR 2 activation. Male Wistar rats were given six equal injections of Dox (each 2.5mg/kg ip) or saline (control) over three weeks for a cumulative dose of 15mg/kg body wt. Two weeks after the last treatment, Dox did not effect unfolded protein response (UPR)-specific proapoptotic C/EBP-homologues protein, and its upstream transcription factors inositol requiring enzyme 1 (IRE1) and protein kinase RNA-like ER kinase (PERK). However, there was increased expression of the ER chaperone binding immunoglobulin proteins (Bip) suggesting ER stress. There was interaction of Bip with ATF6 which further suggested an increased UPR contributing to ER stress. This increase in ER stress promoted iNOS activity. The latter was associated with increased expression of TLR2 and TRAF2 together with NFκB105/50 expression suggesting that ER-stress promotes inflammatory response in the myocardium. These data indicate ER-stress mediated inflammation in DIC. Mitigation of this complex signaling pathway may be of therapeutic benefit in the prevention of cardiomyopathy due to Dox. (Supported by CIHR and Research Manitoba)

P1-59

Antioxidant Enzymes Exhibit Resistance Against Oxidative Stress Regulation

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The functional roles of antioxidant enzymes in regulating cellular oxidative stress and maintaining redox balance are well defined in a variety of model systems. Namely, there are 7 key enzymes responsible for antioxidant generation and ROS detoxification (i.e., Glutamate cysteine ligase [Gcl], NAD(P)H dehydrogenase [quinone] 1 [Nqo1], mitochondrial Glutathione reductase [Gsr], Catalase [Cat], Glutathione S-transferase [Gst], Glutathione peroxidase [GPx], and Superoxide dismutase [Sod]). However, the underlying molecular mechanism for how they themselves are modulated under oxidative stress remains largely unknown. Using a quantitative proteomics approach, we performed D2O labeling and computational modeling to examine the oxidative stress-sensitive regulation of these proteins in 6 mouse strains treated with isoproterenol (ISO). We identified 12 protein isoforms of these 7 enzymes in cardiac tissue (2 Gcl isoforms, 3 Gst, 2 Gpx, and

2 Sod). We examined their expression on 7 time points over 2wks and observed stable expression. Their turnover rates were also largely unchanged, with the exception of Gsta4. Furthermore, we examined the oxidative stress-sensitive post-translational modifications (O-PTMs) for all 12 enzymes. We identified 2 distinct groups of O-PTM regulation responders: Group1 showed oxidative stress resistance (Gclm, Nqo1, Gsta4, GPx3, and Sod1), and they bore no O-PTMs despite carrying potential amino acid residues. Group2 showed oxidative stress sensitivity (Gclc, Gsr, Cat, Gstu1, Gstu2, GPx1, and Sod2). In Group2, a total of 23 OPTM sites were identified in controls, including methionine sulfoxidation, cysteine carbonylation, cysteine sulfonylation, proline carbonylation, and lysine carbonylation, whereas ISO stimulation triggered significant alterations in their O-PTM landscape (modification sites, types, occupancies), leaving only 17 O-PTMs on 4 (Cat, Gstu1, Gstu2 and Sod2) of the 7 isoforms with altered occupancies. These findings unveil molecular fingerprints of oxidative stress regulation of redox family of proteins, providing novel insights to our understanding of cellular redox balance and regulation.

P2-01

iPSC BASED MULTISYSTEMIC-DISEASE MODELS FROM PATIENTS OF MITOCHONDRIAL DISORDER DISPLAY VARIED DISEASE PROGRESSION IN DIFFERENT CELL TYPES

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Introduction: Treatment modalities for individuals suffering from mitochondrial disorders are unreliable and without consensus, owing to a huge amount of variability in clinical expression and complexity in the genotype-phenotype. This has led to a paucity of models- either cell/animal. This study investigates the feasibility of employing induced pluripotent stem cells (iPSC) to provide stable *in vitro* models that directly mimic the multisystemic genotypic, phenotypic and physiological characteristics found in the patient. **Methods and Results:** Blood was collected from two patients – a girl with the autosomal recessive mitochondrial disorder Leigh Syndrome and a boy with autosomal recessive mutations in mitochondrial fatty acid β-oxidation pathway; as well as from age and sex matched controls. Peripheral-blood mononuclear cells were reprogrammed using Sendai-Virus and differentiated into fibroblasts, neural progenitor cells (NPCs) and cardiomyocytes (CMs). Lactate levels in the patient iPS cells were elevated whereas patient iPSC-fibroblasts did not show any difference compared to control. Conversely, the population doubling of fibroblasts and the NPCs was markedly reduced but unaffected in the iPSCs. ATP levels in all the cell types showed reduction in comparison to control. Creatine-kinase activities in the patients' blood, as well as in iPSC and derived cells were similar to that of control. Patient-CMs showed non-significant changes in the beat period, but presented with aberrant beating patterns when challenged with β-agonist and calcium channel blockers. Seahorse analysis revealed the accumulated lactate in the patient-iPSCs was associated with its dependence on glycolysis while the marked decrease in ATP production in the patient-fibroblasts accompanied the decreased OCR in comparison to control. **Conclusion:** iPSC establishment provided a stable long-term *invitro* model of the biochemical, molecular and physiologic abnormalities seen in the patients replete with variable disease progression in different cell types, hinting at a safe patient- and organ-specific therapeutics development before patient administration.

P2-02

Mitochondrial Dysfunction and Senescence of Human Cardiac Progenitor Cells Are Prevented by Hypoxic Culture

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Rationale: Therapeutic potential of c-Kit$^+$ cardiac progenitor cells (CPCs) is modest in autologous cell therapy clinical trials and could benefit from optimization to maximize functional activity. Early proliferation arrest limits expansion potential of human CPCs in vitro. In contrast, self-renewal is preserved and mitochondrial reactive oxidative species (mtROS) minimized within the CPC hypoxic niche in vivo. Expansion under oxygen tension comparable to the hypoxic niche may therefore improve CPC reparative function.

Hypothesis: Senescence of CPCs derived from heart failure patients is suppressed by dampening mtROS through long-term hypoxic culture.

Methods and Results: Environmental oxygen was maintained with constant hypoxia (1% O2; H-CPCs) or atmospheric oxygen tension (20 - 21% O2; N-CPCs) for isolation and expansion of CPCs from patients undergoing left ventricular assist device implantation. A 4-fold increase in clonogenesis and 73% fewer senescence-

associated β-Gal-positive cells were found in H-CPCs. Oxidative stress and DNA damage were also reduced with 80% lower malondialdehyde content and significantly fewer nuclear γ-H2AX foci in H-CPCs. Accumulation of dysfunctional mitochondria was revealed in N-CPCs through functional metabolic analyses. Basal (−59%), maximal (−50%), and ATP production-coupled (−62%) oxygen consumption rates were significantly lower in N-CPCs, despite upregulation of electron transport chain subunits and 72% greater mitochondrial DNA content than H-CPCs. MtROS was elevated, and large mitochondria with swollen morphology were found by TEM in N-CPCs, confirming mitochondrial damage. Key indicators of mitochondrial function including NAD^+/NADH ratio and autophagic flux were preserved in H-CPCs.
Conclusions: Development of mitochondrial dysfunction and senescence are consequences of normoxic CPC expansion. CPC senescence is delayed by preserving mitochondrial health and limiting oxidative damage through hypoxic culture.

P2-03

Non-toxic chemically crosslinked collagen hydrogels for cell delivery

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Background. Biomimetic hydrogels can be used as therapy for post-myocardial infarction repair by improving the delivery of therapeutic cells or drugs and/or by providing a supportive environment. Most hydrogels for cardiac repair need to be prepared fresh just prior to their application, which can be time-consuming and impose additional considerations for the surgeon. Furthermore, many chemically crosslinked hydrogels make use of crosslinking agents that have an associated degree of toxicity concern, thus potentially minimizing their efficacy for cell delivery. To avoid such limitations, we designed a collagen-based hydrogel crosslinked using a semi-bioorthogonal “click” chemistry reaction, which can be carried out in the presence of cells. **Methods.** To this end, collagen was modified to introduce thiol groups and was then mixed with multi-arm PEG-maleimide to produce a chemically crosslinked hydrogel. The physical properties of the hydrogels were tested to evaluate their potential for cardiac repair. **Results.** The hydrogels were stable within physiological pH and temperature range. The mechanical properties could be tuned by adjusting constituent composition and collagen modification parameters. The scaffolds exhibited shear-thinning and self-healing behavior, allowing for injection and the ability to be dispersed within the cardiac muscle instead of forming a bolus. Fibroblasts and human umbilical vein endothelial cells were entrapped in the scaffold *in vitro* and live/dead staining was used to assess cell viability. The fibroblasts showed viability of ≥80% and the endothelial cells ~60% for up to 4 days in 3D culture. **Conclusion.** In summary, we designed a simple two-component chemically crosslinked injectable collagen hydrogel which could be crosslinked *in situ* and in the presence of cells. As such, it shows promise as a hydrogel suitable for cell delivery post-myocardial infarction.

P2-04

Injection of a recombinant human collagen hydrogel improves cardiac function and reduces pathological remodeling post myocardial infarction

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Introduction: Pathological remodeling of the cardiac extracellular matrix (ECM) post-myocardial infarction (MI) is a key contributor to heart failure (HF). Injectable biomaterials are of clinical interest as they can limit remodeling and stimulate infarct repair. Our objective was to test the ability of the first injectable hydrogel formulations of recombinant human collagen type I (rHCI) and type III (rHCIII) to improve cardiac function post-MI.

Methods: One week after an MI was induced by ligation of the LAD (baseline), mice received echo-guided intramyocardial injection of: PBS (n=11), rHCI (n=15) or rHCIII (n=13). Cardiac function was monitored by echocardiography from baseline to follow up 28 days post-treatment.

Results: Comparing cardiac function at baseline vs. follow-up: rHCI injection increased LVEF (from 34.6±1.6% to 44.2±1.9%; p=0.0006), rHCIII-treated hearts exhibited no change (36.7±2.2% vs. 41.4±2.4%; p=0.3), and PBS treated mice got worse (37.9±2.2% vs. 28.2±1.7%; p=0.003). Histological analysis at follow up showed that rHCI (33.7±8.6%; p=0.004) and rHCIII (36.0±6.4%; p=0.023) treated mice had smaller scar size as compared to PBS mice (52.4±9.4%). The remote wall of rHCI treated mice was also thicker (1.28±0.05 mm; p=0.03) compared to PBS mice (0.96±0.10 mm). Confirming this finding rHCI mice had a 22% increase in cardiac troponin postive area in the borderzone as compared to PBS (p=0.008). The infarcts of rHCI treated mice also showed a 1.6-fold increase in $CD206^+$ cell density at 28 days post-injection compared to PBS (p=0.03). Finally, there was an increase in borderzone capillary density for both rHCI and rHCIII treated hearts

compared to PBS hearts (p=0.006 and p=0.047, respectively).

Conclusion: These results establish that rHCI injection into the infarcted mouse heart restored heart function and stimulated infarct repair.

P2-05

Therapeutic modulation of heme metabolism *post*-myocardial infarction improves cardiac remodeling, ventricular function and survival

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INTRODUCTION

Current approaches to preventing heart failure following myocardial infarction are limited. Here we introduce a novel interventional strategy targeting heme metabolism and the cytoprotective enzyme heme oxygenase-1, HMOX1, capable of improving cardiac remodeling, function and survival even when administered days after myocardial infarction.

HMOX1 is an inducible enzyme responsible for the natural breakdown of heme into biliverdin, carbon monoxide and iron. These products synergistically exert robust anti-oxidant, anti-inflammatory and vasodilatory effects, however HMOX1-targeted therapeutics have yet to be translated to the clinic. To do so, the bioavailability of heme and its regulatory enzymes, in clinically and temporally relevant models, require consideration.

Hemin is both a potent inducer and substrate of HMOX1. Thus, hemin reduces the risk of heme-depletion, unlike HMOX1-only therapies, while still conferring its cytoprotective benefits. As an FDA-approved heme surrogate, hemin is used in the treatment of porphyria, however it has yet to be tested for myocardial infarction.

HYPOTHESIS

Administration of hemin *post*-myocardial infarction is an effective strategy for reducing pathological remodeling leading to improved survival and cardiac function.

METHODS

Hemin's ability to confer cytoprotection against oxidative stress (H_2O_2) was first investigated in H9C2 rat cardiomyotubules. Hemin pharmacodynamics/kinetics were measured in healthy CD-1 mice via protein quantification. Acute myocardial infarction was modeled through permanent ligation of the left anterior descending coronary artery. Hemodynamic, histological and morphometric analyses were performed following intraperitoneal hemin administration post-acute myocardial infarction.

RESULTS

In vitro, hemin administration prior to oxidative injury improved cell survival. *In vivo*, administration of hemin pre- and post-myocardial infarction (2h pre- or post-myocardial infarction or, with notably improved outcomes, 5-28d post-myocardial infarction) significantly reduced infarct size, improved left ventricular function and improved survival.

CONCLUSIONS

Here we have demonstrated for the first time a novel drug intervention capable of rescuing cardiac function after myocardial infarction.

P2-06

Intrinsic Functional 3D Micro- and Macrovascular Structures for Cadiovascular Tissue Engineering

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Introduction: The most fundamental problem facing cardiac therapy, unlike vascular grafts and heart valves, is to repair and/or regenerate the damaged myocardium. Restricted myocardial regeneration after tissue damage and shortage of donor organs for cardiac transplantation are the major constraints of conventional therapies. The most daunting task in the field of cardiovascular tissue engineering is the creation and/or regeneration of an *in vitro* engineered cardiac muscle; tissue engineering is associated with two common underlying concerns for clinical applicability, viz., contractility and thickness. However, both the thickness and the contractility of the derived cardiac tissue are dependent on the vascularity of the construct.

Hypothesis: Functional vascularized cardiac tissue can be generated by the interaction of human induced pluripotent stem cell-derived embryonic cardiac myocytes (hiPSC-ECMs) and human multipotent mesenchymal stem cells (hMSCs) on a 3D prevascularized collagen cell carrier (CCC) scaffold.

Methods and Results: First, to generate the prevascularized scaffold, human cardiac microvascular endothelial cells (hCMVECs) and hMSCs were co-cultured onto a 3D CCC for 7 days under vasculogenic culture conditions, hCMVECs/hMSCs underwent maturation, differentiation, and morphogenesis characteristic of micro- and macrovascular structures, and formed dense vascular networks. Next, the hiPSC-ECMs and hMSCs were co-cultured onto these generated prevascularized CCCs for further 7 or 14 days in myogenic culture conditions. Lastly, expression and functional analyses of the differentiated progenies revealed neo-cardiomyogenesis and neo-vasculogenesis. In this milieu, not only were hMSCs able to

couple electromechanically with developing hiPSC-ECMs, but also able to contribute to the developing vasculature as mural cells, respectively.

Conclusions: Thus, our unique 3D co-culture system provided us the apt in vitro functioning prevascularized 3D cardiac graft that can be utilized for cellular cardiomyoplasty.

P2-07

Age-specific changes in myofibril mechanics in pediatric dilated cardiomyopathy

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Background: Pediatric dilated cardiomyopathy (DCM) is a poorly understood disease with most clinical treatment paradigms extrapolated from the adult population. We have shown that pediatric DCM patients have a distinctive gene expression profile. In this study, we demonstrate that the differences between pediatric and adult DCM patients extends to the most fundamental contractile unit (the myofibril).

Methods: Six parameters of sarcomere contractility and relaxation were assessed in myofibrils isolated from adult non-failing (NF; n = 11) and DCM (n = 13), and pediatric NF (n = 9) and DCM (n = 12) left ventricle tissue. Gene expression changes were evaluated to identify differences in sarcomeric proteins between pediatric and adult NF and DCM patients.

Results: Compared to NF adults, myofibrils from NF pediatric hearts held higher resting tension and slower relaxation kinetics. In response to the stress of heart failure, myofibrils from adults with DCM increased kinetics of tension generation and developed more maximal tension; these contractile parameters were unchanged in children with DCM. Instead, myofibrils from pediatric DCM patients displayed shorter relaxation duration and faster relaxation rate constant. These data indicate that adults and children respond to heart failure by altering myofibril mechanics in fundamentally different directions. Analysis of sarcomeric gene expression changes suggest that transcriptional regulation between children and adults are also fundamentally different. In particular, pediatric DCM patients have upregulation of key contractile proteins of the thin filament that are critical to regulating cardiac relaxation such as the trimeric troponin complex and tropomyosin.

Conclusions: Our results show that myofibrils from children and adult are fundamentally different in mechanical properties at baseline. In response to heart failure, myofibrils from adult hearts demonstrated augmented contractility, while myofibrils from children demonstrate altered relaxation. The differential responses of myofibrils from adults and children are correlated by distinct gene expression profile.

P2-08

Isoflurane Anesthesia Masks Elevated Filling Pressures in Hypertensive Rats with Diastolic Dysfunction and Preserved Ejection Fraction: Comparison to Ketamine/Xylaxine Sedation

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Introduction: Elevated left ventricular (LV) end-diastolic pressures (EDP) carry important prognostic value in patients with cardiomyopathies. Echocardiographic tools permit the non-invasive evaluation of EDP, however, in the laboratory they may be confounded by the need for sedation. In particular, inhaled agents have potent vascular effects which may mask changes in EDP. This study assessed the cardiovascular effects of isoflurane (ISO) and Ketamine/Xylazine (KX) sedation in rats with elevated EDP.

Methods: Obese/hypertensive rats (ZSF1) and age-matched controls (CTRL) were instrumented for chronic left-ventricular pressure assessments (via telemetry). Rats were studied in a two way crossover design with a minimum of 48 hours of recovery: under ISO (3%), and under KX (60:6 mg/kg IP, with 0.5% ISO). In both arms LV pressures were recorded in the conscious state prior to echocardiographic evaluation.

Results: Conscious ZSF1 rats were hypertensive (137±3* vs. 112±5 mmHg), had elevated EDP (9±1* vs. 5±1 mmHg), and slightly enhanced systolic function (CI: 145±3* vs. 128±1 1/s). In both ZSF1 and CTRL, ISO treatment resulted in lower end-diastolic dimensions (7.3±3* vs. 8.0±0.3 mm), faster heart rates, and lower EDP (4±2* vs. 8±1 mmHg); ISO decreased EDP -63±15%* vs. conscious values. Both ISO and KX arms lead to comparable systolic indices (EF: 77±2% in ISO vs. 77±3%) and reductions from conscious values (CI: -18±3 vs. -17±3%). Under ISO, both tau and isovolumic relaxation time tended to be shorter, presenting lower E/e' (25±2 vs. 35±1) and faster e' (-41±6 vs. -27±3 mm/s).

Conclusions: When compared to KX, isoflurane reduced LV preload masking diastolic impairments and elevated EDP.

P2-09

Phosphorylation of cMyBP-C modulates the activation-dependence of unloaded shortening velocity at low levels of Ca^{2+} activation

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Background: At low levels of Ca^{2+} activation, unloaded shortening velocity (V_o) in cardiac muscle is comprised of an initial high-velocity phase and a subsequent low-velocity phase. The velocities in both the fast and slow phases are known to scale with the level of activation, culminating in a single high-velocity phase at saturating Ca^{2+} (i.e., V_{max}).

Objective: The molecular mechanism underlying the activation-dependence of V_o most likely involves a shortening-induced cooperative inactivation of the thin filament that results in reduced numbers of strongly-bound cross-bridges, giving rise to the slow phase of unloaded shortening. Phosphorylation of cardiac myosin binding protein-C (cMyBP-C) is postulated to modulate the cooperative activation of the thin filament by binding to myosin and/or actin and thereby regulating the probability of cross-bridge binding to actin.

Methods and Results: To test the idea that cMyBP-C phosphorylation contributes, at least in part, to the activation-dependence of unloaded shortening velocity, we measured V_o in skinned trabeculae isolated from mouse models expressing either wild-type cMyBP-C (*tWT*), phosphorylation-deficient cMyBP-C (Ser273, 282, 302Ala; *t3SA*) or phosphomimetic cMyBP-C (Ser273, 282, 302Asp; *t3SD*). During maximal Ca^{2+} activation, V_{max} was monophasic and not significantly different among the three groups. However, while biphasic shortening was observed in all three groups at low levels of Ca^{2+} activation, the respective high- and low-velocity phases were significantly different between the three groups, i.e., faster in the *t3SD* and slower in the *t3SA*, compared to values obtained in *tWT* myocardium.

Conclusion: The results can be explained in terms of a model in which the level of cMyBP-C phosphorylation regulates the degree of cooperative spread along the thin filament following Ca^{2+} binding to troponin C.

P2-10

HCM-related W792R and T1075fs mutations of cMyBP-C accelerate cross-bridge cycling kinetics in murine skinned myocardium

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Background: Cardiac myosin binding protein-C (cMyBP-C) plays a critical role in determining the force and kinetics of contraction, primarily by modulating the probability of cross-bridge binding to actin. (46 words)

Objective: To examine the effects of two hypertrophic cardiomyopathic (HCM) mutations of cMyBP-C (W792R and $T1075_{fs/5}$) on steady-state force development and cross-bridge cycling kinetics in murine myocardium. (27 words)

Methods and Results: Cardiac-specific transgenic mouse models were developed using a Tet-off inducible system to permit the controlled expression of wild-type (WT), W792R and $T1075_{fs/5}$ cMyBP-C on a *MYBPC3 null* background. The Ca^{2+} sensitivity of force (pCa_{50}), the steepness of the force-pCa relationship (n_H) and the activation-dependence of the rate of force redevelopment (k_{tr}) were measured in skinned right ventricular trabeculae isolated from WT, W792R and $T1075_{fs/5}$ mice. While pCa_{50} and n_H did not differ between WT and $T1075_{fs/5}$, we observed a significant increase in pCa_{50} (5.85 ± 0.02 v. 5.78 ± 0.02) and decrease in n_H (2.8 ± 0.1 v. 3.4 ± 0.1) in W792R myocardium. At intermediate and maximal levels of Ca^{2+} activation, W792R and $T1075_{fs/5}$ trabeculae redeveloped forces at a significantly faster rate than WT myocardium. In addition, k_{tr} was significantly faster at low levels of Ca^{2+} activation in the $T1075_{fs/5}$ myocardium, which markedly reduced the overall activation-dependence of the rate of force redevelopment. (162 words)

Conclusion: HCM-related mutations located within either the mid-region (i.e., W792R) or the C-terminus (i.e., $T1075_{fs/5}$) of cMyBP-C can disrupt the dynamic regulation of myosin cross-bridges and actin, leading to altered steady-state force development and cross-bridge cycling kinetics.

P2-11

MYH7 R403Q Mutation in Pigs: Altered Myofilament Dynamics, Hyper-Contractility, and Impaired Function *In Vivo*

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Introduction: Hypertrophic cardiomyopathy is a familial disease characterized by hyper-contractility and impaired diastole that affects 1 in 500 people. The molecular basis for the HCM pathophysiology remains poorly understood, in part due to the limited availability of pre-clinical models

with sarcomere mutations. These *in vivo* and *ex vivo* studies leveraged a novel large-animal model with the R403Q mutation in myosin heavy chain (MYH7) to determine the association between myofilament abnormalities and the resulting *in vivo* HCM phenotype.

Methods: Yucatan mini-pigs with a heterozygous knock-in of the R403Q *MYH7* mutation and age-matched wildtype (WT) herd-mates were studied. *Ex vivo* biochemical and biomechanical studies in fibers as well as *in vivo* assessments were performed. Twitch mechanics (*ex vivo*), as well as load-independent systolic and diastolic function (via LV pressure-volume relationships), were assessed.

Results: At physiological Ca2+ levels, fibers from MYH7 R403Q hearts had increased Ca2+ sensitivity, showing markedly reduced peak tension and minimal decreases in ATPase rates. Mutant myofibrils also showed reduced myosin head population with ultra-slow ATP hydrolysis rates. In vivo, mutant pigs were hyper-contractile (e.g., LVEF: +23 ± 8% and PRSW: +31 ± 5%, vs. WT) and exhibited diastolic impairments with both markedly elevated LV end-diastolic filling pressures and stiff ventricles.

Conclusions: *MYH7* R403Q mutant mini-pigs showed myofilament abnormalities *ex vivo* and a corresponding hyper-contractile phenotype *in vivo*. This translational dataset strengthens the mechanistic link between *in vivo* HCM phenotypes, biophysical twitch mechanics in fibers, and ATPase rates in myofibril preparations.

P2-12

Role of the intracardiac nervous system in stress-induced arrhythmias with Popdc1 gene mutation

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Introduction: Sinoatrial node (SAN) pacemaker cells generate electrical pulses that determine heart rhythm. The SAN is highly innervated by the intracardiac nervous system (ICNS), and neuronal modulation of its firing is essential for the maintenance of normal rhythm and responses to (patho)physiological changes. The mechanisms of age-related SAN dysfunction are not well understood, though changes in ion channel expression and cell degeneration have been implicated. The popeye domain containing (*popdc*) genes encode a family of cAMP-binding proteins expressed in SAN cells and neurons. Though it is known that *popdc* mutations result in age-dependent SAN dysfunction and stress-induced arrhythmias (through autonomic stimulation), the role of the ICNS in this process is unknown. In the current study, we show that ICNS organization and function is variably affected in zebrafish expressing the *popdc1*^{S191F} mutation, homologous to the *POPDC1*^{S201F} mutation found in humans.

Methods: Hearts isolated from adult WT and popdc1$^{S191F/S191F}$ zebrafish were monitored by ECG. Autonomic agents were superfused or cardiac vagal nerves were stimulated with bipolar electrodes. ICNS innervation was visualized using antibodies against acetylated tubulin and human neuronal protein C/D and SAN pacemaker cells with HCN4 channel antibodies.

Results: ECG recordings revealed no basal differences in rhythm between AB zebrafish and popdc1$^{S191F/S191F}$ mutants. Isoproterenol and nerve stimulation evoked rhythm destabilization in popdc1$^{S191F/S191F}$ mutants, including periods of sinus pauses and arrhythmia; neither were observed in the hearts of AB zebrafish. Rhythm destabilization was prevented by pharmacological cholinergic activation. Post-hoc analysis of the ICNS of *popdc1*$^{S191F/S191F}$ mutants revealed that the severity of ICNS abnormalities correlated with the level of rhythm destabilization.

Conclusion: Our results point to changes in ICNS function as a potential mechanism underlying *popdc*-mediated arrhythmias. Further investigation will provide insight into interactions underlying SAN dysfunction, and ultimately to novel therapeutic strategies targeting the ICNS.

P2-13

The role of estrogens in pregnancy-induced increased heart rate

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Pregnancy is associated with an elevated resting heart rate (HR) which is a risk factor for arrhythmias. Significant hormonal changes occur during pregnancy. Notably, major increase in 17β-estradiol (E_2) coincide with HR acceleration. However, no direct evidence is available to support this association. Accordingly, we aimed to determine the role of E_2 on the increased HR and cardiac automaticity during pregnancy, and to delineate the involvement of the estrogen receptor isoforms (ERα and ERβ).

E_2 administration (30 µg twice daily for 4 days) to non-pregnant female mice lacking ERα (ERKOα) or ERβ (ERKOβ) and wildtypes (WT) significantly increased plasma E_2 concentrations, reaching pregnancy levels (18-19 gestational days) (23.3±5nM). Surface ECG data showed an important acceleration of HR in WT (520±15bpm; +E_2=571±16bpm; n=8; p=0.001) and ERKOβ (511±15bpm; +E_2=580±10bpm; n=10; p<0.001) mice following E_2 administration. However, the HR remained unchanged in ERKOα mice (520±16bpm; +E_2=530±21bpm; n=7). Furthermore, nodal-like human-induced pluripotent stem

cell-derived cardiomyocytes (N-hiPSC-CM) were treated with E_2 (100nM for 72h). Spontaneous action potential rate from control and E_2-treated cells showed that E_2 increased the automaticity of the N-hiPSC-CM (79.0±2.2bpm, n=11; +E_2=99.6±5.5bpm, n=8; p<0.05). Additionally, the diastolic depolarization rate of the spontaneous action potential was also increased in E_2-treated cells (27.3±0.9mV/s, +E_2=53.1±4.2mV/s, p<0.05).

In summary, administration of E_2 to both WT mice and N-hiPSC-CM recapitulates the pregnancy phenotype and accelerates automaticity. In addition, the N-hiPSC-CM results support a direct effect of E_2 on cellular automaticity and demonstrate human applicability of these findings. Finally, our results showing that E_2 administration to ERKOβ but not ERKOα mice significantly accelerates HR implicate the E_2-ERα pathway as a major contributor to pregnancy-induced increased HR.

P2-14

Novel role of the protein phosphatase 1 regulatory subunit PPP1R3A in atrial fibrillation

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Background: Atrial fibrillation (AF) is the most common sustained arrhythmia and is associated with dysregulation of the ryanodine receptor (RyR2) channel and abnormal Ca^{2+} release from the sarcoplasmic reticulum. Prior work found that increased RyR2 phosphorylation enhances the susceptibility to AF. Protein phosphatases help maintain steady-state phosphorylation of RyR2, however little is known about their potential contribution to AF pathogenesis. We recently observed that a PP1 regulatory subunit, PPP1R3A (R3A) is downregulated in atrial samples of chronic AF patients. Proteomics and mass spectrometry suggest that R3A binds to RyR2. Thus, we hypothesized that R3A contributes to AF pathogenesis by modulating PP1 binding to RyR2.

Methodology: R3A-/- mice were generated by CRISPR-Cas9 deletion. Co-immunoprecipitation (Co-IP) was used to evaluate the interaction between R3A, PP1 and RyR2. Western blotting was used to determine changes in calcium handling proteins in human or mouse atrial tissues. Electrophysiology studies and Ca^{2+} imaging were conducted to determine AF susceptibility and SR Ca^{2+} handling, respectively, in R3A-/- and WT littermates.

Results: IP-MS revealed R3A is a novel binding partner of RyR2, and Co-IP confirmed R3A binds PP1 to RyR2 in mouse heart. R3A protein was down-regulated in AF patient tissues by 20% (vs sinus rhythm controls, P<0.05). R3A-/- mice showed enhanced susceptibility to pacing-induced AF (40%, n=10) compared to WT (0%, n=12, P<0.05). Binding of PP1 to RyR2 was reduced in R3A-/- mice and this was associated with a 30% increase in RyR2 phosphorylation at S2808 (P<0.05). Atrial myocytes isolated from R3A-/- mice exhibited increased Ca^{2+} sparks (10.2 vs 6.4 sparks/100mm/s in WT, P<0.05) indicating enhanced RyR2 activity.

Conclusions: We demonstrate for the first time that R3A modulates PP1-mediated dephosphorylation of RyR2. Further, deficiency of R3A plays a role in AF pathogenesis by promoting RyR2-mediated Ca^{2+} leak.

P2-15

Understanding the Loss of Capture During Mechanical Pacing of the Heart

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Introduction: Mechanical stimulation by external percussion, high-intensity focused-ultrasound, or implanted microparticles can cause excitation, however, is unsustainable. The cause for the loss of capture during mechanical pacing is unknown but may relate to stimulation-induced changes in tissue mechanics or mechano-sensitive ion channel activity. The goal of the present study was to investigate potential causes for the loss of mechanical pacing capture.

Methods: Trains of local mechanical stimuli were applied to the left ventricular free-wall of isolated rabbit hearts at 1.5× and 2× threshold deformation. Stimulation frequency was increased from 2.5-6.0Hz. The effects of changes in tissue mechanics were tested by application of blebbistatin (8μM, to eliminate active force generation; *n*=10) or paclitaxel (5μM, to increase passive stiffness;

n=7). The effects of changes in tissue electrophysiology were tested by application of no-flow ischemia (*n*=7) or carbenoxolone (25μM, to cause cellular uncoupling; *n*=8). The time for recovery of sustainability (*n*=19) and effects of subthreshold stimulation (*n*=7) were also assessed.

Results: Increasing mechanical stimulus intensity from 1.5× to 2× increased the number of sustained stimuli. Blebbistatin eliminated active force generation, while paclitaxel increased tissue stiffness and decreased the threshold for mechanically-induced excitation, but neither altered pacing sustainability. ECG and optical mapping analysis revealed a continuously increasing delay between mechanical stimulation and excitation with each paced beat. Moderate cellular-uncoupling with carbenoxolone caused increased sustainability, while greater uncoupling and ischemia caused a progressive reduction until complete loss of capture. Recovery time was biphasic, with a reduction in captured beats when pacing was re-initiated after 1-2min, and a further reduction below 10s. Sub-threshold stimulation prior to pacing also reduced the number of captured beats.

Conclusion: The run-down of mechanically-induced current appears responsible for the loss of mechanical pacing capture. Mechanical stimulation is not a clinically viable method for sustained cardiac pacing.

P2-16

Inflammaging does not contribute to age-related changes in cardiac electrical activity

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BACKGROUND: Advanced age is a risk factor for cardiovascular disease (CVD). One type of CVD that is prominent in older adults is cardiac arrhythmias. Currently, it is not clear how aging contributes to the development of arrhythmias. It is possible that age-related mechanisms alter cardiac electrical activity, increasing the risk of cardiac arrhythmias. Specifically, chronic low levels of systemic inflammation (inflammaging) are thought to play a major role in the aging process. Inflammation also can affect cardiac electrical activity. Thus, inflammaging could lead to alterations in cardiac electrical activity, which increases the risk of developing cardiac arrhythmias. **PURPOSE:** To characterize the relationship between age-related changes in cardiac electrical activity and serum inflammatory markers during aging. **METHODS:** Female C57Bl/6J mice were split into two groups: young (10 weeks old; n=16) and aged (22 months old; n=6). Heart rate (HR), PR interval, QRS duration, and QT intervals were measured in conscious non-restrained mice to assess cardiac electrical activity. Serum levels of 23 inflammatory markers were quantified using a multiplex assay. **RESULTS:** ECG analysis found no significant difference in HR or ventricular depolarization and repolarization (QRS duration and QT intervals). However, atrial depolarization duration (PR intervals) decreased with age (26.45±2.78 to 20.31±1.5 ms). Serum levels of IL-2, IL-4, MCP-1, and TNF-α significantly increased with age. Coefficients of determination (R^2) between PR intervals and these cytokines were: IL-2=0.20; IL-4=0.23, MCP-1=0.29, & TNF-α=0.25. **CONCLUSIONS:** In contrast to previous findings, this study showed that aging does not impact ventricular electrical activity. However, aging was associated with significant changes in atrial electrical activity. Although, there was evidence of inflammaging, the lack of a significant relationship between proinflammatory cytokines and PR interval suggests that inflammation is not a major contributor to age-related changes in atrial electrical activity.

P2-17

Identifying the Novel Role of a Presenilin-2 Mutation in Arrhythmogenicity using Patient Specific Induced Pluripotent Stem Cells Derived Cardiomyocytes

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Arrhythmia is a major cause of sudden cardiac death and affects more than 14 million Americans. In familial cases, disease-causing mutations are expected to be found in genes encoding proteins that regulate membrane potential or calcium kinetics. Through genetic testing, we identified a ventricular fibrillation patient with family history of cardiovascular diseases that does not carry any disease-causing mutation in the arrhythmia-related genes. This patient, however, carries a previously reported dilated cardiomyopathy mutation (S130L) in presenilin-2 (PSEN2). To understand if this mutation can contribute to arrhythmia, the beating regularity and action potential morphology of cardiomyocytes derived from the patient-specific induced pluripotent stem cells (hiPSC-CMs) were assessed by fluorescence based membrane potential imaging. Up to 30% of these hiPSC-CMs demonstrated delayed after-depolarizations (DAD) and irregular beating pattern, which were prevented by correcting this PSEN2 mutation through CRISPR/Cas9 genome editing. Interestingly, we were unable to recapitulate the arrhythmic propensity by introducing this mutation into two healthy control hiPSC-CM lines, until we inserted another modulator mutation in histidine-rich calcium binding protein (HRC) that was also found in the patient, suggesting PSEN2 mutation is providing the substrate for arrhythmia induction. Mechanistically, compromised intracellular calcium removal was detected in S130L-PSEN2

hiPSC-CMs, which was concordant with a reduction in SERCA protein expression. Compromised calcium removal also led to elevated diastolic calcium and activated calcium/calmodulin-dependent protein kinase II (CAMKII), indicated by its enhanced phosphorylation. As a result, ryanodine receptor was hyper-phosphorylated at the CAMKII site (ser2814), which could facilitate calcium leakage from the ryanodine receptor and contribute to the occurrence of DAD. Collectively, our findings reveal a previously unknown function of PSEN2 in cardiomyocyte function and suggest that this PSEN2 mutation can compromise normal intracellular calcium cycling and contribute to arrhythmia through activating CAMKII.

P2-18

Role of connexin 40 in male predisposition to atrial fibrillation

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Atrial fibrillation (AF) is the most common cardiac arrhythmia. Although men are twice as likely to suffer from AF as women do, the mechanisms leading to the male higher AF vulnerability remain unknown. We hypothesize that androgens regulate atrial electrical activity and contribute to the sex difference in AF. Accordingly, here we aim to identify sex differences in electrophysiological and structural AF substrates and to explore the contribution of androgens in these differences.

Using electrical programmed stimulations (EPS), AF susceptibility was compared between male and female CD-1 mice (4-5 months). EPS results revealed that AF was induced in 52% (11/21) of males and 25% (6/24) of females. The atrial weight-to-tibial length ratio was 60% larger in males (0.24±0.01mg/mm, n=15) compared to females (0.15±0.01mg/mm, n=7). Using voltage-clamp techniques, major ionic currents were recorded in left atrial myocytes and no differences that could account for the male predisposition to AF were observed. Consistent with these electrophysiological data, qPCR analysis revealed that atrial mRNA expression of the underlying ion channels was comparable between groups. However, mRNA levels of connexin 40 (Cx40) was 40% lower in males, whereas Cx43 expression was comparable (n=4/group). To explore the role of androgens, orchiectomized mice were studied. Their AF susceptibility was lower (38% n=16) than males and their Cx40 mRNA expression was similar to females.

In conclusion, these findings show that the incidence of AF is higher in male than female mice, consistent with clinical data. This difference is associated with larger atria size and lower Cx40 expression in male left atria, which could delay atrial conduction and promote AF. Additionally, the sex difference observed in AF susceptibility and Cx40 expression were both reversed by orchiectomy, supporting a major role of androgens in AF pathophysiology.

P2-19

The Zebrafish as an Experimental Model for Studies of Sinoatrial Node Function

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Background: The zebrafish is an increasingly popular model for the study of cardiac electrophysiology due to its functional similarities to mammals, potential for genetic manipulation, and the ability for *in vivo* observation. We have been using the zebrafish to study intrinsic regulation (*via* stretch and intracardiac nervous system control) of the sinoatrial node (SAN). Yet, mechanisms underlying SAN automaticity in zebrafish are poorly defined. The aim of this study was to determine which components of the 'membrane-/calcium-clock system' found in mammalian SAN contribute to pacemaking in zebrafish, to better define its utility as an alternative experimental model for studies of SAN function.

Methods: Pharmacological agents known to affect elements of the membrane-/calcium-clock in mammals were applied to isolated zebrafish hearts in an environmentally-controlled chamber to assess their contribution to SAN activity. Heart rate (HR) was recorded by electrocardiogram before and after drug application, which was compared by paired Student's t-test.

Results: 'Funny' current (passing through HCN channels) was blocked by ivabradine (3µm, acting intracellularly; *n*=7) or cesium (3mM, acting extracellularly; *n*=6), which decreased HR by 86±3% and 73±4%, respectively. Spontaneous calcium release from the sarcoplasmic reticulum (through ryanodine receptors) was blocked by ryanodine (1µm; *n*=3) or buffered by BAPTA (5µm; *n*=7), which decreased HR by 45±3% and 26±8%. T-type calcium current (Cav1.2) was blocked by nickel (165µm; *n*=7), which decreased HR by 10±3%, while block of L-type calcium current (Cav3.1 and Cav3.2) by nifedipine (2µm; *n*=6) had no effect.

Conclusion: The principal mechanisms of SAN automaticity in mammals are also active in zebrafish, although their relative importance for pacemaking may differ. Future experiments will utilize *in vivo* optical mapping, *in situ* microelectrode recordings, and single cell studies to further investigate mechanisms of SAN function and its intrinsic regulation using zebrafish.

P2-20

Investigating Cardiac Subtype-Specific Pharmacology Using Atrial-like Cardiomyocytes Derived from Human Induced Pluripotent Stem Cells (hiPSCs) and Optical Mapping

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Background: Atrial fibrillation (AF) is expected to increase in prevalence and imposes a larger burden on the healthcare system. Thus, it is imperative to find novel treatments for better management of the disease. An important aspect of AF drug treatment lies in the ability to have atrial-selective effects with minimal risk of ventricular proarrhythmia. As such, the ability to generate hiPSC-derived atrial-like and ventricular-like cardiomyocytes (hiPSC-ACMs and hiPSC-VCMs, respectively) has given the opportunity for a human-specific physiological system and preclinical screening of AF treatments *in vitro*. Here, we present a platform using hiPSC-ACMs and optical mapping for screening of atrial-selective pharmacology.

Methods: We first differentiated subtype specific hiPSC-CMs by modulating the retinoic signaling pathway. The voltage (Vm) and Ca^{2+} transients (CaT) of the two cardiac subtypes were then characterized by a medium-throughput optical mapping system using potentiometric dye RH-237 and Ca^{2+} fluorescence probe Rhod-2AM. Additionally, both hiPSC-CMs subtypes were electrically paced to measure the effective refractory period (ERP), and to interrogate electrical restitution at baseline and post-drug conditions.

Results: hiPSC-ACMs possess shorter action potential (AP) durations at 30% and 50% (APD_{30} and APD_{50}) than their ventricular counterpart, while possessing a triangulated AP morphology. Interestingly, CaT are comparable between the two cell types. Furthermore, electrical restitution dynamics were also different between the two cardiac subtypes as described by the slopes at maximal captured rates. In response to the multi-ion channel blocker vernakalant, hiPSC-ACMs experienced a greater elongation in ERP than hiPSC-VCMs. Additionally, we intend to use selective pharmacology using the compounds AVE0118, carbachol, and UCL1684 to demonstrate that hiPSC-ACMs possess the atrial-specific currents I_{Kur}, $I_{K,Ach}$, and I_{SK}, respectively.

Overall, hiPSC-ACMs coupled with sophisticated optical mapping techniques constitute a comprehensive *in vitro* assay system for future drug development and personalized medical treatment of AF.

P2-21

Single cell investigation of mechanically-induced arrhythmias during acute ischemia

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Introduction: Ischemia-induced ventricular arrhythmias are a major cause of sudden death. We have demonstrated a contribution of altered mechanics to ischemia-induced arrhythmias, which appear to be calcium-driven and facilitated by a 'vulnerable window' (VW) created by spatio-temporal differences in voltage-calcium dynamics. The aim of our current work is to test the hypothesis that mechanically-induced arrhythmias during acute ischemia are indeed calcium-driven, due to distinct changes in action potential (APD) and calcium transient (CaTD) duration.

Methods: Experiments are being performed in single myocytes isolated from the LV freewall of rabbits (maintained at 35ºC and paced at 1Hz). Thus far, cells subjected to normal physiological load by carbon-fibres adhered to either end of the cell have been superfused with pinacidil (50µm) to simulate hypoxia-induced K_{ATP}-channel activation to determine whether this may contribute to the difference in APD and CaTD (VW=CaTD-APD) observed in the whole heart. This was assessed by dual voltage-calcium fluorescence microscopy using fluorescent dyes (di-4-ANBDQPQ and Fluo-5F) and a single camera-optical splitter system.

Results: Application of pinacidil for 10min (n=6 cells, N=3 rabbits) resulted in shortening of both the action potential (APD_{50}: 388±34 to 191±29ms, p=0.003; APD_{80}: 460±15 to 208±19ms; p<0.001) and calcium transient ($CaTD_{50}$: 325±12 to 248±22ms, p=0.047; $CaTD_{80}$: 441±15 to 309±21ms, p=0.013). However, as the degree of APD and CaTD shortening differed, the VW was increased (VW_{50}: -63±36 to 57±22ms, p=0.015; VW_{80}: -19±17 to 101±17ms, p<0.001), such that there was a ~100ms period during which intracellular calcium remained high in repolarized cells.

Conclusion: Activation of K_{ATP}-channels results in a VW for calcium-induced after-depolarizations. Future studies will include application of stretch and simulation of additional components of ischemia to assess arrhythmogenicity.

P2-22

Selective inhibition and activation of retinoid pathways to create chamber spefic cardiac subtypes from human induced pluripotent stem cells (hiPSCs)

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The advent of pluripotent stem cell (PSC) derived cardiomyocytes has revolutionized the field of cardiac research. For the first time, we are able to study human disease in human models while avoiding the challenges of obtaining biopsy tissue. Additionally, we are able to study a patient's disease in a personalized manner by the use the patient-derived induced pluripotent stem cells (iPSCs). Current differentiation protocols result in a mixed cardiac population that consists of mostly ventricular cells. This makes the study of chamber-specific diseases, like atrial fibrillation (AF), difficult. As such, the development of atrial-specific differentiation protocols is vital. Retinoic acid has long been identified as a modulator of cardiac subtypes in animal models. Recent work has shown that the addition of retinoic acid during the cardiac mesoderm phase in embryonic stem cells increased atrial lineage significantly. Here we take lessons learned from previous protocols and attempt to apply them to iPSCs. We hypothesized that the addition of retinoic acid or BMS-189453 during the days in which cells are in the cardiac committed mesoderm phase, days 3-5, will direct them towards an atrial-like or ventricular-like fate, respectively. Cells were then characterized by quantitative real-time PCR (qRT-PCR), flow cytometry and optical mapping.

P2-23

Wnt Signaling Inhibits Calcium Channels in Cardiomyocytes

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Introduction

The Wnt/β-catenin pathway plays a critical role in embryonic heart development but is mostly inactive in adult hearts. Recent studies have shown that the pathway is re-activated in many heart diseases including myocardial infarction, cardiac hypertrophy and heart failure. Previous work from our laboratory demonstrated that activation of the Wnt/β-catenin pathway reduces the voltage-gated Na^+ current (I_{Na}) in neonatal rat ventricular myocytes (NRVMs). In this study, we examined the effects of Wnt signalling on T-type calcium channels ($Ca_v3.1$), which play a key role in the pacemaker function of the sinoatrial node (SAN).

Methods

NRVMs were treated with Wnt3a protein or CHIR-99021, which activates the Wnt/β-catenin pathway by GSK-3β inhibition. PCR array assays, RT-qPCR, and western blot were used to confirm changes in mRNA and protein levels of ion channels. To investigate the impact of ion channel reduction in the SAN, a protocol was developed to isolate SAN tissue from mouse hearts and maintain it ex vivo during ECG recording and drug perfusion.

Results

PCR array assays showed a 72% reduction in mRNA of *Cacna1g* (encoding the Cav3.1 channel) by treatment with Wnt3a protein (130.4±13.2 in control cells vs. 37.4±2.4 in Wnt3a-treated cells, n=4, $p<0.05$). RT-qPCR and western blot analyses showed a dose-dependent reduction ($p<0.05$, n=4) in *Cacna1g* mRNA and Cav3.1 protein levels in NRVMs treated with CHIR-99021. Ex vivo perfusion of mouse SANs with a small molecule inhibitor of I_{Na}, flecainide, led to slowed conduction velocity and uncoupling (between impulse generation and conduction) in a dose-dependent manner ($p<0.05$, n=5).

Conclusion

The Wnt/β-catenin pathway regulates the expression of both T-type Ca^{2+} channels and voltage-gated Na^+ channels, which are important for impulse generation and conduction in the SAN. Thus, the Wnt pathway may play a role in sinoatrial node dysfunction.

P2-24

AMPK activation before left ventricular pressure overload attenuates malapdative remodelling

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Background: Coordination of changes in gene expression and signaling pathways in hearts subjected to left ventricular pressure overload is paramount to maintain cardiac function during stress. We previously showed that loss of steroid receptor coactivator -2 (SRC-2) disrupts stress-induced changes in several signaling pathways, most prominently metabolic and growth signaling, and is associated with increased AMPK activation post-stress. We hypothesized that early metabolic stress in the absence of SRC-2, and either indicated by or driven by AMPK, was leading to blunted hypertrophy and rapid functional loss in SRC-2 knockout (KO) mice. Therefore we investigated the effects of transient pre-activation of AMPK prior to cardiac stress on cardiac hypertrophy and function in the presence and absence of cardiomyocyte SRC-2.

Methods and Results: We used AICAR to activate AMPK transiently prior to transverse aortic constriction (TAC) in wild type (WT) and cardiac-specific SRC-2 knockout (CKO) animals. In unstressed hearts, AMPK activation caused mild activation of protein translation machinery, and in SRC-2 CKO animals, alleviation of a deficit in NAD^+ and increased antioxidant signaling. Upon TAC, WT mice receiving AICAR showed a mild hypertrophic response, decreased fibrosis, and decreased signs of cardiac failure as compared to saline controls. In SRC-2 CKO mice, AICAR treatment before TAC resulted in the same effects as WT mice with additional dramatic improvement in cardiac function in contrast to saline treated SRC-2 CKO animals. Activation of AMPK effectively abrogated the rapid decline in cardiac function and adverse ventricular remodeling in SRC-2 CKO mice.

Conclusions: Our results show that altered molecular signaling prior to stress onset can have extended effects on the sustained cardiac stress responses, and that pre-activation of distinct signaling pathways can prevent stress onset, even when mice are genetically predisposed such as in SRC-2 CKO mice.

P2-25

m6A mRNA methylation drives cardiomyocyte hypertrophy

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m6A methylation is the most prevalent internal post-transcriptional modification in mammalian mRNA, and the role of m6A mRNA methylation in cardiomyocytes is not known. To determine the role of m6A methylation in the development of cardiomyocyte hypertrophy we isolated primary cardiomyocytes and performed m6A immunoprecipitation followed by RNA sequencing. We found abundant m6A methylation on cardiomyocyte mRNA, which is significantly increased in response to hypertrophic stimulation. Analysis of m6A methylation showed significant enrichment in genes that regulate kinases and intracellular signaling pathways. Inhibition of METTL3 completely abrogated the ability of cardiomyocytes to undergo hypertrophy when stimulated to grow, while forced expression of the m6A RNA methylase METTL3 was sufficient to enhance the stability of m6A-targeted mRNAs and promote cardiomyocyte hypertrophy. The ability of METTL3 to promote hypertrophy was confirmed *in vivo* by generating cardiac-restricted transgenic mice overexpressing METTL3. We found that mice with increased expression of METTL3 spontaneously developed compensated hypertrophy at 8 months of age. We have created an *in vivo* model of cardiomyocyte-specific METTL3 knockout to further dissect the role of m6A in cardiac function. We found that METTL3-cardiac knockout mice exhibit morphological and functional signs of heart failure at 8 months of age when compared to control animals.

Our study demonstrates that methylation of mRNA on m6A through the activity of METTL3 is a dynamic modification that accumulates following hypertrophic stimuli and is necessary for the hypertrophic response in cardiomyocytes, where it regulates mRNA stability. Enhanced m6A RNA methylation results in cardiomyocyte hypertrophy whereas diminished m6A prevents hypertrophy and favors eccentric cardiomyocyte remodeling, suggesting the critical importance of this novel stress-response mechanism in the heart.

P2-26

Instability and mutation impact on C-MyBP-C FNIII domain suggests a previously unappreciated functional relevance

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Background: The C6 and C7 domains of cMyBP-C have no defined functional role beyond linking the amino terminal regulatory domains with the C-terminal anchoring domains. Of the 11 domains comprising cMyBP-C, only three, including C6 and C7 are FNIII domains, the remainder are IgG domains. FNIII domains unfold and lengthen with applied tension, and play important roles in the dynamic properties of proteins such as fibronectin and titin. How FNIII domains influence cMyBP-C function in health and disease remains unknown. **Methods:** Wild type (WT) and hypertrophic cardiomyopathy-causing missense mutation containing cMyBP-C fragments C5-C6-C7 and C2-C3-C4 were expressed and purified from bacteria and stability assessed by thermofluor stability assay and tryptic cleavage assay. Mutation containing and WT full-length cMyBP-C proteins were expressed in cMyBP-C null murine cardiomyocytes and protein level and localization confirmed. Contractility was measured using a living engineered cardiac tissue construct. **Results:** WT C5-C6-C7 fragment expression is reduced compared to C2-C3-C4. Mutations in the C6 domain cause a further reduction in both fragment expression and full-length cMyBP-C, reduced thermal stability, and increased sensitivity to tryptic digestion that varied depending on the specific mutation (% folded at 37C: WT 94.4%±5.6; W792R 46.7%±7.45; R820Q 87.2%±4.66; R820W 7.6%±1.04). Mutations in C6 resulted in a gain of function with both increased twitch force and accelerated contraction kinetics. **Conclusion:** The C6 FNIII domain of cMyBP-C is inherently unstable, and HCM-causing missense mutations that do not cause early termination increase this instability

and thus reduce the full-length protein abundance in the sarcomere. How the properties of FNIII domains in the central region influence cMyBP-C's overall function remains to be fully explored.

P2-27

Redox Regulation of Proteotoxic Myocardial Remodeling and Heart Failure in Mouse and Human

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Background: Cardiomyopathy and heart failure (HF) are growing cause of human morbidity/mortality worldwide. Antioxidant-based treatments to detoxify ROS have been largely unsuccessful in protecting humans from cardiovascular diseases. Although transcriptional activation of antioxidants appears to be beneficial, their chronic effects remain elusive. Here, we tested a hypothesis that the sustained activation of Nrf2-antioxidant signaling will promote reductive stress (RS) and lead to hypertrophic cardiomyopathy, and HF.

Methods: Using novel transgenic mouse model expressing Nrf2 in the heart (α-MHC-Nrf2-TG) and their non-transgenic (NTg) littermates, we studied the chronic effects of a hyper-reductive condition on cardiac structure and function (Vevo-2100 echocardiography). Myocardial glutathione redox potential (GSH/GSSG), transcription and translation of antioxidants, and proteotoxic mechanisms for pathological remodeling were assessed at different ages. Furthermore, redox measurements in blood samples of HF patients (n=50) and redox-based stratification were performed.

Results: The Kaplan-Meier survival plots revealed ~40% mortality in TG mice compared to NTg by 60 weeks of age. An abnormally higher GSH redox ratio was preserved along with a significantly diminished ROS in TG vs. NTg mice ($p<0.05$) indicated a hyper-reductive redox state (i.e. RS). This was strongly associated with increased Nrf2 promoter activity and antioxidant genes/proteins in TG vs. NTg mice ($p<0.05$). Echocardiography analyses revealed that progressive cardiac remodeling and impaired cardiac function (EF and M-E/A) in TG mice due to an impaired protein quality control leading to proteotoxicity. Notably, recent clinical observations demonstrated a hyper-reductive (i.e. RS) condition in a sub-set of HF patients, suggesting a role for RS in the development of HF. Continuing studies are focused on examining the molecular mechanisms for RS-based cardiac pathogenesis.

Conclusion: Attenuation of the obligatory oxidative signaling with chronic activation of Nrf2-antioxidants could shift the redox equilibrium to the "reductive" arm and cause proteotoxic cardiac disease.

P2-28

Increasing fatty acid oxidation in the failing heart does not improve cardiac function

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Background: The failing heart has been suggested to be energy-starved due to a decrease in fatty acid oxidation (FAO). Consequently, increasing FAO has been suggested to be an approach to treat heart failure. We therefore assessed what effect increasing FAO has on cardiac function in the failing heart.

Methods: C57BL/6J mice and mice with a cardiac specific deletion of acetyl CoA carboxylase 2 (*Acacb*$^{Cardiac-/-}$) were subjected to transverse aortic constriction (TAC) surgery to induce pressure overload hypertrophy over a 4-week period. Following echocardiography, isolated working hearts from these mice were perfused with appropriately radiolabeled palmitate (0.8 mM), glucose (5 mM), and ß-hydroxybutyrate (0.6 mM) to assess oxidative metabolism and glycolysis.

Results: C57BL/6J TAC mice had impaired *in vivo* and *ex vivo* cardiac function compared to sham mice. However, the 54% decrease in *ex vivo* cardiac work seen in TAC hearts was not accompanied by a decrease in absolute FAO rates. Moreover, the contribution of fatty acids to total ATP production did not decrease in TAC hearts and in fact, FAO per unit work was increased by 95% in the failing hearts compared to sham. *Acacb*$^{Cardiac-/-}$ mice subjected to

TAC had decreased cardiac ACC2 expression and malonyl-CoA levels, and showed a 116% increase in cardiac FAO rates, 56% increase in glucose oxidation, and no change in ketone oxidation or glycolysis compared to control TAC hearts. Furthermore, *in vivo* and *ex vivo* cardiac function was not improved in *Acacb*$^{Cardiac-/-}$ TAC mice compared to control TAC despite having elevated FAO rates.

Conclusions: Cardiac FAO rates are maintained in TAC mice compared to sham mice. Furthermore, decreasing cardiac ACC2 expression and increasing myocardial FAO rates in TAC mice does not improve *in vivo* or *ex vivo* cardiac function. Therefore, increasing FAO may not be an effective approach to treat heart failure.

P2-29

The muscle-specific MuRF1 ubiquitin ligase transcriptionally regulates cardiomyocyte autophagy in a FOXO1/3-dependent manner and protects against cardiac inflammation *in vivo*

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Background. MuRF1 transgenic (Tg+) mice with cardiomyocyte-specific (αMHC-) MuRF1 expression are significantly protected against cardiac I-R injury *in vivo*. When challenged with pressure overload-induced cardiac hypertrophy, MuRF1Tg+ mice exhibit greater susceptibility to heart failure. These findings led us to hypothesize that MuRF1 regulates cardiomyocyte autophagy (i.e., autophagic flux), which would account for the observed protection in cardiac I-R and increased susceptibility to heart failure when challenged with pressure overload. **Results.** We crossed MuRF1Tg+ to LC3-GFP-RFP autophagy reporter mice and assayed for early (LC3-GFP-RFP) and late (LC3-RFP) autophagosome puncta by confocal fluorescence microscopy. MuRF1Tg+//LC3-GFP-RFPTg+ mice exhibited a significant (>2 fold) increase in late autophagosomes (red) compared to wildtype sibling controls. Consistent with an increase in autophagic flux, MuRF1Tg+ hearts also had a significant (>1.5-2.0 fold) increase in VPS34 and P62 protein expression by immunoblot, along with a >300-fold increase in Beclin1 mRNA by RT-qPCR. Since the FOXO1 and FOXO3 transcription factors regulate autophagy (via Beclin1, VPS34, and P62), we hypothesized that MuRF1 enhanced autophagy by enhancing FOXO1/FOXO3 transcriptional activity. Consistent with this, MuRF1Tg+ hearts had significant decreases in phosphorylated (inactive) FOXO3a/total FOXO3a and increasing MuRF1 expression in luciferase reporter assays significantly increased FOXO3a activity. To mechanistically link MuRF1's regulation of autophagy to its effects on FOXO3a, we knocked-down FOXO3a in H9C2 cardiomyocytes to see if it blocked MuRF1's regulation of autophagic flux. Immunoblot analysis of LC3II demonstrated that FOXO3a was required for MuRF1 to increase autophagy. **Discussion.** MuRF1 is a cardiomyocyte-specific regulator of autophagy, which acts through its interaction with FOXO3a to promote its activity, likely by non-canonical ubiquitination, to transcriptionally upregulate autophagy in vivo. These findings demonstrate a novel therapeutic target that could regulate autophagy in a muscle-specific manner in heart failure.

P2-30

A Computational Heart Model for the effects of Pulmonary Arterial Hypertension

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Pulmonary arterial hypertension (PAH) imposes a pressure overload on the right ventricular free wall (RVFW), leading to substantial growth of muscle cells and remodeling in fiber architecture. The effects of these alterations on the biomechanical behavior of the RVFW and the organ-level cardiac function remain largely unexplored. An accurate quantification of the wall stress evolution during the development of PAH is needed to determine the correlation between the wall stress and G&R mechanisms. There is thus a need to develop a detailed computational heart model that can accurately simulate the effect of PAH on right heart, and its connection to wall stress and organ-level cardiac function. We have developed a high-fidelity finite-element (FE) heart model of PAH using extensive time-course datasets from a normal rat heart and from a hypertensive rat heart simulating the pressure overload in the right ventricle (RV). We have implemented a pipeline that integrates a meshed geometry from a high-resolution image of the rat heart, detailed imaging data on the fiber structure of the same heart, and a novel compressible hyperelastic material model accounting for both passive and active behaviors of myocardium. The developed heart model offers a high performance capability for inverse problems such as fine-tuning the active properties of myocardium and characterizing shape change patterns of the RV. We used our model to investigate the correlations between the alterations in the wall stress, the remodeling of the RVFW microstructure, and the shape changes in the RV during the development of PAH. The detailed description of organ-level remodeling patterns can replace the traditional measures of RV dimensions and volume that often lead to gross and limited information on cardiac performance. Ultimately, development and implementation of our model in patient-specific organ-

level simulations will allow investigation of optimal diagnosis and new individualized stem-cell interventions for PAH.

P2-31

Human Antigen R (HuR) as a therapeutic target in pathological cardiac hypertrophy

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RNA binding proteins represent an emerging class of proteins with a role in cardiac dysfunction. Despite being very highly expressed in the heart, little is known about the functional role of the RNA binding protein Human Antigen R (HuR) in the heart. In this work, we show that activation of HuR is increased in the failing human heart. To determine the functional role of HuR in pathological cardiac hypertrophy, we created an inducible cardiomyocyte-specific HuR deletion mouse, and showed that HuR deletion reduces left ventricular hypertrophy, chamber dilation, and fibrosis while preserving cardiac function in a transverse aortic constriction (TAC) model of pressure-overload-induced hypertrophy. Assessment of HuR-dependent changes in global gene expression suggests that the mechanistic basis for this protection occurs through a reduction in fibrotic signaling. Specifically, we show that HuR mediates the expression of TGF-β mRNA in cardiac myocytes. Finally, pharmacological inhibition of HuR at a clinically relevant time point following the initial development of pathological hypertrophy post-TAC also yielded a significant reduction in pathological progression, as marked by a reduction in hypertrophy, chamber dilation, fibrosis, and preserved function. In summary, this study displays a functional role for HuR in the progression of pressure overload-induced cardiac hypertrophy and establishes HuR inhibition a viable therapeutic approach for pathological cardiac hypertrophy and heart failure.

P2-32

Nucleolar Enlargement and Perturbed Ribosome Biogenesis Are Cellular Hallmarks of Cardiac Aging

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Background: Cardiac Aging manifests as structural and functional impairment of the heart and is associated with several changes in the cellular architecture. Of the many alterations in organelle structure and function that occur during aging, changes in the nucleolus and ribosome remain least studied in the cardiac context. The nucleolus functions as a stress sensor and is critical for ribosome biogenesis.

Hypothesis: This study hypothesizes that perturbations in nucleolar architecture and ribosome biogenesis result in accumulation of free ribosomal proteins that stabilize p53 and trigger cardiac aging.

Methods and Results: Using animal models of chronological (CA) and biological aging (BA), we observed that nucleolar size is dramatically increased in senescent myocardial cells (1.75 fold) as well as in aged hypertrophied hearts. Nucleolar enlargement is accompanied by significant increase in pre-ribosomal RNA levels (2 fold, $p<0.01$ in CA hearts; 9 fold in BA hearts) indicating increased ribosome biogenesis. Synthesis of ribosomal proteins (RP) in equimolar amounts to ribosomal RNA that occurs under normal conditions is disrupted in the aged heart, resulting in increased expression of RPL11 (50% in BA heart, 33% in CA heart) that is a component of the 60S large ribosomal subunit. Accumulation of RPL11 coincides with increased p53 expression (2 fold, $p<0.05$ in BA heart; 5.4 fold in CA heart) suggesting a role for RPL11 in mediating p53 stability in the aged heart.

Conclusion: Collectively our data demonstrate nucleolar enlargement as a cellular hallmark of cardiac aging and uncover a molecular mechanism involving perturbed ribosome biogenesis and accumulation of free RPs that contribute to age-associated afflictions in the heart.

P2-33

Human Relaxin-2 Fusion for the Treatment of Heart Failure

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Introduction: Human relaxin-2 (hRelaxin-2), a vasoactive hormone with hemodynamic, anti-fibrotic, and cardioprotective effects, has been pursued as a potential therapy for acute decompensated heart failure. It binds to

RXFP1, its cognate receptor, and to a lesser extent to RXFP2. In clinical trials, intravenous (IV) administration of recombinant hRelaxin-2 (Serelaxin) improved markers of cardiac, renal, and hepatic damage and reduced congestion. However, these effects diminished rapidly upon treatment termination. We hypothesized that the transient treatment benefits could be due to the short half-life ($T_{1/2}$=4.6 hours) of Serelaxin since continuous infusion of hRelaxin-2 using subcutaneous implanted minipump has resulted in long term benefits in animal studies (*Hypertension*. 2014;64:315-322). To test this hypothesis, we constructed a novel hRelaxin-2 fusion (hRLX2) and evaluated its activity in vitro and its pharmacokinetic (PK) profile in vivo.

Methods: The activity and specificity of hRLX2 was evaluated in cAMP production assays using RXFP1, RXFP2, RXFP3 and RXFP4 over-expressing CHO cells, and by monitoring VEGF transcript induction in THP-1 cells. The PK of hRLX2 was evaluated in mouse following IV and subcutaneous (SC) administration and hRLX2 concentration in plasma was measured by ELISA.

Results: The in vitro cAMP assays results showed that hRLX2 signals only through the cognate RXFP1 receptor with an average EC_{50} of 1.62 nM. No activity was detected on cells expressing RXFP2, RXFP3, RXFP4. In addition, we found hRLX2 increased VEGF mRNA levels by 3.5 folds in THP-1 cells. PK evaluation data indicated that hRLX2 has $T_{1/2}$=134+10.3 hours or 169+42 hours for IV or SC administration, respectively.

Conclusion: The hRLX2 molecule has in vitro potency and specificity comparable to hRelaxin-2 with significantly improved PK characteristics. These data supported progression of hRLX2 into *in vivo* efficacy studies. The data from these in vivo efficacy studies will be presented at this meeting as a separate presentation.

P2-34

Remodeling of myocardial extracellular matrix and proteoglycans varies in pediatric versus adult patients with dilated cardiomyopathy

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Background: Idiopathic dilated cardiomyopathy (DCM) is a common cause of heart failure in adult and pediatric patients, but the underlying mechanisms vary since pediatric DCM patients do not respond as well to treatments designed for the adult patient population.

Objective: Determine if differential remodeling of the extracellular matrix (ECM, non-cellular myocardium) contributes to the differences between pediatric and adult DCM hearts.

Methods & Results: Explanted hearts were procured from adult (51-64 years) and pediatric (2-8 years) patients with DCM heart failure (non-failing controls from donors). Fibrillar (collagen, fibrosis) and non-fibrillar (proteoglycans, glycosaminoglycans/GAGs, glycoprotein) ECM, their regulatory enzymes (MMPs, ADAMs, ADAM-TSs) and inhibitors (TIMPs) were assessed (IHC, biochem/activity assays, WB).

Peds-DCM hearts exhibited less fibrosis compared to adults. Total GAGs increased similarly in both DCM groups, but exhibited a significantly lower affinity for TGFβ in adult- vs. peds-DCMs. Consistently, TGFβ activity (p-Smad2/3) was significantly higher in adult-DCMs. Among proteoglycans (versican, biglycan, decorin, osteoglycin, lumican), glycosylated biglycan and versican increased in both DCM. Assessment of glycoproteins (thrombospondin-1, tenascin-X, osteopotin, SPARC, g-sarcoglycan) showed that cleaved thrombospondin-1 increased in both DCMs, while tenascin-X was lower in adult control and DCM. ADAM-TS1 increased in both DCMs, ADAM-TS2 and -7 only in peds-DCM, ADAM-TS4 decreased in adult-DCM, ADAMTS-5 and -9 did not change. ADAM12 and -17 increased more in adult-DCM, ADAM15 decreased in both DCMs, ADAM19 increased in peds-DCM but decreased in adult-DCM. Total MMP activity increased in both DCM groups; MMP9 increased in adult-DCM, while MMP2 increased in peds-DCM hearts. TIMP3 decreased and TIMP2 increased in both DCMs, TIMP1 and TIMP4 were unaltered.

Conclusions: Differential remodeling of GAGs in peds- vs adult-DCMs causes greater TGFβ bioavailabity and more fibrosis in adult-DCM. The divergent remodeling of the fibrillar and non-fibrillar ECM between peds- and adult-DCM hearts highlights the age-dependence of this process.

P2-35

Long-term rescue of a familial hypertrophic cardiomyopathy caused by a mutation in troponin T, via reduced expression of phospholamban

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Background: Hypertrophic cardiomyopathy (HCM), linked to mutations in the sarcomere/cytoskeletal network, is a

common cardiac disorder. HCM is often associated with diastolic dysfunction, increased myofilament Ca^{2+}-sensitivity and a functional inhibition of Ca^{2+}-handling proteins. We hypothesized that promotion of Ca^{2+} fluxes by reduced expression of phospholamban (PLN), the inhibitor of SERCA2a, would improve cardiac morphology and function in transgenic (TG) mice with a mutation in troponin T (TnTR92Q) that is linked to HCM.

Methods: TG and non-transgenic (NTG) mice were cross-bred with PLN knockout (PLN-/-) mice resulting in six groups: both TnTR92Q and NTG mice with expression of either normal level of PLN (PLN+/+), reduced level of PLN (PLN+/-) or no PLN (PLN-/-). Experiments were performed at 4 months and 10-12 months of age. Echocardiography was done using Vevo770 or Vevo2100 systems. Measurements of the pCa-force relation at a sarcomere length of 2.0 μm were performed on detergent-extracted fiber bundles of left ventricular papillary muscles. Histology and hydroxyproline (HOP) assays were done in ventricular tissue to determine cellular disarray and fibrosis. Western blot analysis was used to determine levels of PLN and SERCA2a expression and phosphorylation of PLN.

Results: TG mice displayed severe left atrial enlargement, increased relative wall thickness, diastolic dysfunction, decreased strain and strain rate, increased myofilament Ca^{2+}-sensitivity and increased expression of β-MHC, compared to NTG mice. These parameters were improved to near-NTG levels in TG/PLN+/- and TG/PLN-/- mice up to 1 year of age. Moreover, reduction of PLN expression had no effect on myofilament Ca^{2+}-sensitivity. However, in TG/PLN+/- mice, there was a decrease in PLN phosphorylation at serine 16 compared to TG hearts.

Conclusion: The present study indicates that PLN may be considered as a therapeutic target for HCM linked to mutations in thin filament proteins.

P2-36

Inducing Expression of the Cleaved Form of DJ-1 Attenuates Ischemic-Induced Heart Failure

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Background: DJ-1 is a cytoprotective protein expressed in the heart. It has a range of functions including opposing oxidative and glycative stress. During oxidative stress, DJ-1 is cleaved and converted into an active protease. Currently, it is unknown what role DJ-1 plays in the setting of ischemic-induced heart failure. Thus, the purpose of this study was to investigate how DJ-1 affects post-ischemic cardiac remodeling.

Methods and Results: Initial studies found that DJ-1 deficient mice (DJ-1 KO) displayed enhanced injury following 60 minutes of myocardial ischemia and 4 weeks of reperfusion, as evidenced by increased left ventricular dilatation, hypertrophy, and dysfunction when compared to wild-type control mice. In contrast, overexpressing the cleaved form of DJ-1 using an adenoviral approach (AAV9-CMV-DJ1Δc) attenuated ischemic-induced heart failure when compared to mice administered a control vector (AAV9-CMV-Empty). In an effort to evaluate the potential mechanism(s) responsible for the protective actions of DJ-1, we focused on glycative stress. DJ-1 KO hearts collected at 3 days of reperfusion were found to display enhanced levels of methylglyoxal, advanced glycation end products, carboxymethyllysine, and receptor for advanced glycation end products; indicating that DJ-1 deficiency enhances glycative stress. In contrast, hearts overexpressing the cleaved form of DJ-1 displayed less glycative stress compared to controls. Finally, in an effort to assess the therapeutic potential of DJ-1, mice subjected to 60 minutes of myocardial ischemia and reperfusion were randomly assigned to receive AAV9-CMV-DJ1Δc or AAV9-CMV-Empty at 1 week of reperfusion. At eight weeks post-ischemia, mice administered AAV9-CMV-DJ1Δc displayed significant improvements in heart function compared to mice administered AAV9-CMV-Empty.

Conclusion: These data demonstrate that DJ-1 attenuates ischemic-induced heart failure through an attenuation of glycative stress. Further, this suggests that inducing the expression of DJ-1Dc is a potential new therapeutic strategy for improving cardiac function after an ischemic event.

P2-37

Effects of vitamin D supplementation for six months on cardiac magnetic resonance imaging in mexican patients with heart failure

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A high prevalence of vitamin D deficiency exists among heart failure (HF) patients. Vitamin D deficiency may worsen the inflammatory and pro-thrombotic state of these individuals, which conditions maladaptive cardiac

remodeling. Evidence supports that in its active form, 25-OHD3 plays an active role as an anti-inflammatory agent.

The objective of this single-blind clinical trial is to determine effects of supplementation with vitamin D in HF patients with vitamin D deficiency on cardiac imaging study variables throughout a 12-month follow-up.

Patients were randomized for daily administration of either 5000 UI of 25-OHD3 or placebo (14 and 11 patients respectively). Serum 25-OHD3 was measured by immunoassay every 3 months to assure adherence. Anthropometric measurements were performed and consumption of vitamin D rich foods and vitamins, sun exposure and sunscreen use were assessed monthly through applied questionnaires.

Cardiac magnetic resonance imaging with gadolinium was performed at baseline and 6-months after vitamin or placebo administration.

Median age was 59.8±11; BMI was 28.6±4.8; skin phototypes II-V. Vitamin D significantly increased in the active supplement group (+31.12ng/ml vs.+0.5ng/ml placebo group). Preliminary results from the first follow-up showed no significant differences in cardiac resonance parameters between baseline and after 6 months in the active supplement group nor in the placebo group. There were no significant differences between the supplement group and the placebo group in mean changes from baseline in anthropometric or cardiac imaging variables, including ejection fraction, cardiac output, BSA/EDV. Milk consumption significantly increased in the active supplement group.

Vitamin D administration for 6 months in HF patients did not significantly improve the measured variables in cardiac MRI. Follow-up will be done in the next 6 months to evaluate the long term effects of vitamin D supplementation in heart failure imaging.

P2-38

Chronic treatment with the ACE inhibitor enalapril attenuates the development of frailty, prevents cardiac hypertrophy and increases IL-10 levels in aging male C57BL/6 mice

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Studies on interventions that can delay or treat frailty in humans are limited. There is evidence of beneficial effects of angiotensin converting enzyme (ACE) inhibitors on factors related to frailty, such as physical function, even in those without cardiovascular disease. The aim of this study was to longitudinally investigate the effect of an ACE inhibitor on frailty in aging male mice, and explore potential protective cardiac and inflammatory mechanisms. Frailty was assessed with the mouse clinical frailty index (FI) which quantifies health related deficits. Cardiac outcomes include echocardiographic analysis of heart structure and function and blood pressure assessment. Levels of inflammatory cytokines in serum were measured with multiplex analysis. Chronic treatment with enalapril (30 mg/kg/day in feed) attenuated frailty, without an effect on blood pressure. There was an increase in left ventricle (LV) wall thickness, LV mass and heart weight to tibia length ratio in control mice with increasing age, but these hypertrophic effects were not seen in enalapril-fed mice. Enalapril treatment also resulted in an increase in the anti-inflammatory cytokine interleukin (IL)-10 compared to control treated animals, and prevented the age-related increase in pro-inflammatory cytokines IL-6, IL-12p40 and granulocyte colony stimulating factor (G-CSF). These effects on age-related cardiac remodeling and inflammation may contribute to the protective effects of enalapril against frailty. This is the first study to examine the longitudinal effect of an intervention on the FI in mice, and provides pre-clinical evidence that enalapril may delay the onset of frailty, even when started later in life.

P2-39

Effects of vitamin D supplementation for six months on the cytokine profile in Mexican heart failure patients

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There is a prevalence of 43.6% of vitamin D deficiency among the general Hispanic population. Vitamin D deficiency may worsen the inflammatory and pro-thrombotic state in heart failure (HF) patients, which conditions maladaptive cardiac remodeling. Evidence supports that in its active form, 25-OHD3 plays an active role as an anti-inflammatory agent.

The objective of this single-blind clinical trial is to determine effects of supplementation with vitamin D in HF patients with vitamin D deficiency on the patients' inflammatory status determined by proinflammatory and anti-inflammatory cytokines levels.

Patients were randomized for daily administration of either 5000 UI of 25-OHD3 or placebo (15 and 12 patients respectively). Serum 25-OHD3 was measured by immunoassay every 3 months to assure adherence. Anthropometric measurements were performed and consumption of vitamin D rich foods and vitamins, sun exposure and sunscreen use were assessed monthly through applied questionnaires.

Determination of cytokine profile was performed by using a 13-plex, bead-based immunoassay kit at baseline and 6-months after vitamin or placebo administration.

Median age was 61±10.4; BMI was 29.6±4.7; skin phototypes II-V. Vitamin D significantly increased in the active supplement group (30.02 ng/ml vs. 0.6 ng/ml in the placebo group). Preliminary results from the first follow-up showed no significant differences between the supplement group and the placebo group in mean changes from baseline in anthropometric or left ventricular ejection fraction measured by MRI.

At baseline the highest cytokine levels were MCP-1 and IFN-γ; the lowest were IL-12p70 and IL-10 The 6 months follow up to evaluate the effects of vitamin D supplementation on the inflammatory profile and LVEF in HF patients will be completed shortly.

P2-40

In response to myocardial injury, the Nlrp3 inflammasome-primed neutrophils make a round trip to the bone marrow to amplify granulopoiesis

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Ischemic myocardial damage triggers granulopoiesis resulting in heightened production and recruitment of neutrophils to the ischemic heart. However, little is known about the myocardial molecular drivers that orchestrate with the BM and spleen to stimulate granulopoiesis. To investigate the mechanisms, we first mapped the abundance of different myeloid cell types in mice following myocardial ischemia (MI) and found a dominance of neutrophils in the blood and heart on day 1. This was not due to demargination or apoptosis but rather enhanced proliferation of granulocyte precursor cells in the BM and spleen. Attracted by cellular debris and release of DAMPs (e.g. S100A8/A9) from necrotic cells including the first wave of neutrophils, they rapidly undergo Nlrp3 inflammasome priming orchestrated by S100A8/A9 binding to TLR4 on incoming neutrophils in an autocrine/ paracrine manner. The primed neutrophils fully loaded with pro-IL-1b return to the BM in CXCR4-dependent manner, release IL-1b locally via gasdermin D pore formation in close proximity to IL-1b sensing stem and progenitor cells and stimulate granulopoiesis in a non-cell autonomous manner. Genetic and/ or pharmacological strategies aimed at disruption of S100A8/A9 or its downstream signaling cascade suppressed MI-induced granulopoiesis and improved cardiac remodelling and function. In patients with acute coronary syndrome, higher neutrophil count correlated positively with major adverse cardiovascular disease outcomes. These studies uncovered a rather complex network of signaling pathways harbored by neutrophils that self-govern their production following ischemic myocardial injury.

P2-41

Loss of the Prolyl Hydroxylase OGFOD1 *In Vivo* Leads to Metabolic Alterations

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Prolyl hydroxylation is a post-translational modification that modulates protein stability, turnover, and activity, as well as cell survival. There is a large family of proteins that catalyze proline hydroxylation, including the hypoxia-inducible factor alpha (HIFα) prolyl hydroxylase domain enzymes (HIF-PHDs). Although the HIF-PHDs have been well studied, very little is known about the endogenous function of the prolyl hydroxylase 2-oxoglutarate- and Fe^{2+}-dependent oxygenase 1 (OGFOD1), which in contrast to HIF-PHDs remains active under hypoxic conditions. We investigated whether OGFOD1 has a role in augmenting or suppressing myocardial injury following I/R using OGFOD1 knockout mice, which are viable and fertile. We subjected perfused hearts from 6 wildtype (WT) and 6 OGFOD1 knockout (KO) to ischemia and reperfusion, and found that OGFOD1 loss was protective against I/R injury, with OGFOD1-KO hearts showing a ~33% ($p < 0.01$) reduction in infarct size. To better understand the mechanism responsible for the protection in the OGFOD1-KO hearts, we isolated hearts from 5 OGFOD1-WT mice and 5 OGFOD1-KO mice and used Tandem Mass Tag (TMT) Liquid Chromatography and tandem Mass Spectrometry (LC-MS/MS) to identify proteomic changes. We found that OGFOD1 loss leads to up-regulation of enzymes in fatty acid and ketone oxidation. These results were supported by metabolomics data of cardiac tissue, which showed a significant decrease in the ketone body β-hydroxybutyrate

in OGFOD1-KO hearts, despite comparable levels of β-hydroxybutyrate in serum from OGFOD1-WT and OGFOD1-KO mice. Altogether, these data support the hypothesis that OGFOD1 ablation alters myocardial metabolism, which may contribute to protection against I/R injury.

P2-42

The Oxidized Phospholipids POVPC and PONPC affect Calcium Transients and Contraction in Adult Rat Cardiomyocytes

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Rapidly restoring blood flow to the myocardium after a coronary event using surgical or thrombolytic approaches are effective treatments. However this process of ischemia/reperfusion (IR) activates oxygen-derived free radicals and results in cellular dysfunction by modifying cell membrane phosphatidylcholine (PC) to form oxidized phosphatidylcholines (OxPC). Two pro-apoptotic OxPC species, 1-palmitoyl-2-(5'-oxo-valeroyl)-sn-glycero-3-phosphocholine (POVPC) and 1-palmitoyl-2-(9-oxononanayl)-PC (PONPC), are significantly increased during IR. We hypothesized that direct treatment of cells with OxPC would negatively affect cellular Ca^{2+} and myocyte contraction.

Primary rat cardiomyocytes were first loaded with Fura2 followed by a 1h perfusion with HEPES buffer with or without OxPC's. Ca^{2+} transients and edge detection were measured simultaneously, providing contractile function determination.

When comparing Ca^{2+} transients of myocytes treated +/- OxPC's to control or unoxidized 1-palmitoyl-2-stearoyl-sn-glycero-3-phosphocholine (PSPC), we found there was a significant decrease in the Ca^{2+} transients from 40-60 minutes after exposure to 0.1µM POVPC or 0.1µM PONPC. There were no differences between control and PSPC. There were no significant changes to diastolic Ca^{2+} after treatment with OxPC over 60min. We measured cardiomyocyte net contraction in µm (microns) over a 60min period and began to see significant changes between 30 and 60 min of OxPC treatment compared to control and PSPC. There was no change in cell length with OxPC treatment compared to control or PSPC. Our data suggest that OxPC's have a deleterious effect on the heart by directly affecting how Ca^{2+} is handled within the cardiomyocyte and ultimately contraction.

This work was supported by CIHR.

P2-43

Inorganic Arsenic Exposure and Sex Dependent Susceptibility to Ischemic Heart Injury

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Background: Epidemiological evidence suggests an association between cardiovascular disease and environmental pollutants. Arsenic is a common contaminant in drinking water throughout the world, and recent studies suggest a link between inorganic arsenic (iAS) exposure and ischemic heart disease. Although female hearts exhibit an estrogen-dependent reduction in susceptibility to ischemic injury compared to males, females may be especially vulnerable to iAS exposure due to endocrine disrupting effects. However, iAS exposure and susceptibility to ischemic heart injury have not been examined in mechanistic studies.

Methods: Male and female C57BL/6J mice (aged 8 weeks) were exposed to varying concentrations of sodium arsenite (0ppb, 10ppb, 100ppb, 1000ppb) via drinking water for 4 weeks. Hearts were then excised and subjected to ischemia-reperfusion (I/R) injury via Langendorff perfusion. Pre and post-exposure echocardiography was also conducted, and post-exposure plasma samples were collected for 17b-estradiol measurement.

Results: At 1000ppb dose of iAS, female hearts exhibited modest structural remodeling with no change in heart function, while male hearts showed no change in cardiac morphology or function. Interestingly, we identified substantial sex-dependent changes in I/R susceptibility. iAS-treated female hearts showed a significant decrease in post-ischemic functional recovery and increased infarct size, while iAS-treated males showed significantly enhanced post-ischemic functional recovery and reduced infarct size. Assessment of plasma 17b-estradiol levels revealed a decrease in iAS-treated females (vs. non-treated females), but an unexpected increase in iAS-treated males (vs. non-treated males). eNOS protein levels were also significantly decreased in whole heart homogenates from both iAS-treated male and female hearts. eNOS phosphorylation at Ser1177 was also significantly elevated in iAS-treated male hearts.

Conclusions: These results suggest that an environmental exposure such as iAS, can modulate susceptibility to

ischemic heart disease. Our results further suggest that iAS can modulate myocardial nitric oxide signaling to either increase or decrease susceptibility to ischemic injury.

P2-44

A potential role for human mast cells in the resolution of cardiac inflammation.

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Background: Cardiovascular disease (CVD) is the leading cause of death by non-communicable disease worldwide. After myocardial infarction (MI), improper resolution of inflammation can lead to fibrosis and heart failure. Mast cells (MCs) are immune cells that respond to infection and tissue damage via selective production of granule products, lipid mediators and cytokines. The role of MC in CVD is unclear. IL-33 is crucial to proper healing after MI by preventing cardiomyocyte death. MCs can produce and respond to IL-33, while MC proteases cleave IL-33 to a more bioactive form. We hypothesize that MC contribute to resolution of cardiac inflammation without associated fibrosis by a mechanism which involves IL-33.

Methods: Human cord blood-derived MCs (CBMCs), n=17, were activated *in vitro* with IL-33 at 30 ng/mL. Protein production of selected mediators was evaluated. Human atrial tissue samples, n=84, were obtained from ischemic heart disease patients undergoing cardiac surgery and analyzed for mediator content. MCs were identified via toluidine blue staining. Fibrosis was quantified via Sirius red fast green staining.

Results: CBMCs activated with IL-33 increased production of IL-4 (1.3 fold), IL-13 (3.8 fold), GM-CSF (30.6 fold) and VEGF-A (1.8 fold), but did not produce TGF-β or induce degranulation. In ischemic heart disease patients, serum sST2, the soluble form of the IL-33 receptor, was positively correlated with collagen content in atria at time of surgery ($r^2 = 0.0834$, $p < 0.02$). Finally, patients with higher MC content had lower collagen content in atria at time of surgery ($p < 0.02$), suggesting a beneficial role of MCs in preventing fibrosis.

Conclusion: MCs respond to IL-33 via production of mediators that would induce a beneficial microenvironment in the heart and promote resolution of tissue inflammation without detrimental fibrosis. Data from human atrial tissue supports a role for MCs in reducing the development of heart fibrosis.

P2-45

The Role of Ca^{2+}/calmodulin-dependent kinase II (CaMKII) In Hydrogen Peroxide Mediated Cell Death

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The main effector of ischemia-reperfusion (I/R) injury is the mitochondrial permeability transition pore (PTP). During reperfusion excess mitochondrial calcium uptake and oxidative stress sensitize PTP, leading to cell death. The uptake of calcium into the mitochondrial matrix is facilitated by a channel on the inner mitochondrial membrane (IMM), the mitochondrial calcium uniporter (MCU). However, the signaling pathways that regulate MCU are unknown. Ca^{2+}/Calmodulin Dependent Kinase II (CaMKII) has also been shown to be activated in I/R injury and its inhibition reduces myocardial cell death.

Here we tested whether CaMKII might decrease mitochondrial calcium update through MCU, and thus desensitize PTP in I/R injury. To assess whether CaMKII inhibition protects against oxidative stress we employed an in-vitro model of simulated I/R injury. Wild-type mouse embryonic fibroblasts (WT-MEFs) were exposed to H2O2 while inhibiting CaMKII using KN-93; cell death was measured by propidium iodide. CaMKII inhibition resulted in attenuation of cell death (KN-93: 81.14±0.04% vs DMSO: 100±0.07%, p=0.02, n=8).

To assess whether CaMKII inhibition affected mitochondrial calcium uptake, WT-MEFs were permeabilized, endoplasmic reticulum calcium uptake was blocked with thapsigargin, glutamate/malate was provided for IMM energization, and calcium was administered in 10uM pulses. Extramitochondrial calcium was measured by calcium-green-5N and the rate of calcium uptake was calculated for each pulse. Inhibition of CaMKII resulted in faster mitochondrial calcium uptake (KN-93: 136.70±17.12 vs DMSO: 46.51±4.71 RFU/million cells/sec, p<0.001, n=8).

Consistent with previous studies these data show that inhibition of CaMKII is cardioprotective. The data further suggest that KN-93 can alter increase the rate of mitochondrial Ca^{2+} uptake. Whether this is a direct or indirect effect (e.g. via membrane potential) is under investigation.

P2-46

Methylglyoxal in myocardial infarction and effects on cell function

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Introduction: We previously reported that methylglyoxal (MG), a highly reactive dicarbonyl, accumulates after myocardial infarction (MI), contributing to adverse remodeling and cardiac dysfunction. We hypothesized that MG accumulates post-MI due to reduced expression of Glo1, the MG-metabolizing enzyme, and that MG negatively affects the function of reparative cells in the MI heart (macrophages, fibroblasts, and bone marrow cells (BMCs)). **Methods**: Glo1 expression was determined by qPCR in C57BL/6 mouse hearts at 7d post-MI and compared to healthy hearts. From healthy C57BL/6 mice, the following cells were obtained: BMCs, cardiac fibroblasts, and bone marrow-derived macrophages. Cells were maintained in culture, exposed to MG for 24h, and then used in *in vitro* functional assays. **Results**: Glo1 expression is reduced by 4-fold in the myocardium 7d post-MI, compared to healthy controls; this likely contributes to the observed accumulation of MG. *In vitro*, exposure of macrophages to MG resulted in increased expression of iNOS and CD86 (markers of the M1 inflammatory macrophage phenotype) and reduced Arg-1 expression (marker of M2 wound healing phenotype). For cardiac fibroblasts, MG led to decreased expression of αV, α6 and β1 integrins, which correlated with reduced adhesion to various extracellular matrix proteins. The angiogenic potential of BMCs was reduced by MG treatment as determined by reduced total network length and less cells contributing to capillary-like structures in an *in vitro* angiogenesis assay. Notably, over-expression of GLO1 was able to reduce oxidative stress in MG-treated BMCs and restore their angiogenic potency. **Conclusion**: It appears that reduced GLO1 expression post-MI contributes to MG accumulation, adverse remodeling and loss of cardiac function. Based on the *in vitro* results, this may be due to negative MG effects on macrophages and inflammation, fibroblasts and fibrosis, and BMCs and angiogenesis. This highlights MG as a potential therapeutic target for treating MI.

P2-47

The impact of age and frailty on ventricular function before and after ischemia-reperfusion in C57BL/6 mice.

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Background: Studies show that frail people suffer more disability and death when the heart is exposed to low oxygen conditions, such as during cardiac surgery or myocardial infarction (MI). This suggests the frail heart may be more susceptible to damage following exposure to ischemia and reperfusion (IR). We investigated the influence of frailty on age-dependent ventricular function before and after exposure to IR. **Methods:** Frailty was quantified as deficit accumulation in adult (≈8 mos) and aged (≈27 mos) C57BL/6 mice using a frailty index (FI) tool. Contractile function was evaluated in Langendorff-perfused hearts before and after 30 mins ischemia and 40 mins reperfusion. Troponin levels were assessed in coronary effluent during reperfusion.
Results: FI scores increased with age. Under normoxic conditions, left ventricular developed pressure (LVDP) plus rates of pressure development (+dP/dt) and decay (-dP/dt) declined with age and this was graded by frailty (r=-0.51, p=0.0007; r=-0.48, p=0.002; r=-0.56, p=0.0002 for LVDP, +dP/dt and -dP/dt). All hearts, regardless of age (adult: x=55.7±4.7 mmHg; Aged: x=48.8±3.3 mmHg; p=0.23) or FI (r=0.15, p=0.33) developed similar levels of peak contracture during ischemia and similar results were seen in reperfusion. Interestingly, troponin release was significantly higher in adult animals (x = 3.99 ± 0.59 ng/ml) compared to aged animals (x=2.09±0.47 ng/ml; p<0.05), which suggests that IR injury is greater in younger, less frail hearts. In support of this, mean LVDP, +dP/dt, and -dP/dt at the end of reperfusion were actually higher in aged animals compared to adult mice. When plotted as a function of frailty, LVDP and –dP/dt were graded by FI score (r=0.31, p=0.046; r=0.32, p=0.039). **Conclusions:** Paradoxically, hearts from older, frailer mice recovered better following exposure to IR. This suggests that, while frailty reduced basal contractile function, it did not impair recovery of function following IR.

P2-48

MuRF1-Related Metabolic Alterations in HL-1 Cardiomyocyte Induced by Cyclic Stretch

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Background. The giant myofibrillar protein titin acts as a stretch sensor with its spring-like confirmation and sterically inhibited titin kinase (TK) regions which are exposed with stretch to allow binding to proteins that act as scaffold proteins which then regulate nuclear transcription factors. We have recently identified that the TK-binding MuRF1 ubiquitin ligase inhibits PPARalpha activity in cardiomyocytes via ubquitination. A link between stretch and TK-associated MuRF1 has not previously been established. **Methods.** HL-1 atrial cardiomyocyte cells were plated on silastic membranes coated with gelatin/fibronectin and transduced with Ad.shRNA MuRF1 (or Scramble control) to knock-down MuRF1 protein to <25% of controls and subjected to 15%

biaxial stretch at 1 Hz using the Flexcell FX-5000™ Compression System. We first determined PPARalpha localization and activity in stretch and no-stretch controls cells. In parallel, we collected cell media and performed GC-MS non-targeted metabolomics to identify the role of MuRF1 in the dynamic metabolic changes in cardiomyocytes. **Results.** Transient MuRF1 knock-down itself did not alter nuclear PPARalpha localization and activity, whereas stretch of cells with MuRF1 knock-down resulted in an increase in PPARalpha nuclear localization and activity without affecting overall PPARalpha concentration. Non-targeted metabolomics analysis performed on 15, 30, and 60 minutes stretch (vs. non-stretch) HL-1 with MuRF1 knock-down media identified eight ANOVA significant metabolites involved in 3 metabolic pathways: 1) citric acid cycle (citric acid-isocitric acid, glutamic acid), 2) amino acid degradation pathway (2-ketovaline, 2-ketoleucine), and 3) uracil-arginine metabolism (uracil, putrescine) in addition to 6-aminohexanoic acid-delta-aminolevulinic acid and putrescine. Two these metabolites are altered by 60 minutes of stretch in cell media from cells with full MuRF1 expression. **Discussion.** These studies identify MuRF1's role in maintaining PPARalpha's nuclear localization and activity during biaxial cyclic stretch, while regulating two metabolites involved metabolic adaptations to stretch.

P2-49

Restoring TFEB action attenuates cardiomyocyte dysfunction following nutrient overload

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Background: Our laboratory demonstrated that lipid (palmitate) overload within the cardiomyocyte decreases nuclear transcription factor EB (TFEB), the master regulator of lysosomal metabolism, signalling and autophagy. Since the lysosomal function is disrupted in the obese and diabetic heart, we hypothesized that restoring TFEB content in the cardiomyocyte attenuates dysfunction induced by nutrient overload.
Methods and results: To examine whether graded palmitate concentration inhibits TFEB, rat cardiomyofibroblasts (H9c2) were incubated with palmitate (0.2-1.2mM). Immunoblot analysis revealed that 0.6 mM palmitate and above, augmented cleaved caspase-3, whereas exceeding 0.8 mM palmitate declined TFEB protein. Compared to chow, feeding high fat-high sucrose (HFHS) in C57BL6J mice decreased nuclear TFEB and lysosomal cathepsin activity in the heart at 8, 12, and 16 weeks post diet but not at 2 and 4 weeks. In the absence of nutrient overload, myocyte contractile function was comparable between WT and mice with tamoxifen-inducible cardiomyocyte-specific TFEB deletion. Therefore, loss of TFEB precipitates cardiomyocyte stress only if myocyte threshold of loading lipids exceeds its metabolic capacity. To assess whether the toxic effect of palmitate is inhibited by augmenting TFEB, neonatal rat cardiomyocytes (NRCM) and H9c2 cells were transduced with adenoviruses overexpressing WT-TFEB or constitutively active phosphorylation-resistant TFEBS142A or their viral control before incubation with 1.2 mM palmitate for 16 h. Overexpression of WT and TFEBS142A blunted the effect of palmitate to cause loss of cellular viability by reducing cleaved caspase-3 protein content and augmenting LAMP-2A content and cathepsin proteolytic activity. Adenoviral transduction of NRCM and H9c2 cells with TFEBS142A enhanced insulin-stimulated AKT Serine473 phosphorylation when compared to control, signifying that increased TFEB content likely protects against lipid overload by modulating insulin control of metabolism and survival.
Conclusions: Restoring TFEB action during nutrient overload attenuates lysosomal dysfunction and loss of viability, likely by sensitizing the cardiomyocyte to insulin-signalling.

P2-50

GDF15 a novel circulating cardiokine is secreted from the atrial tissue of obese patients with established heart disease

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Background:

Obesity is an important risk factor for cardiovascular disease (CVD). Growth differentiation factor 15 (GDF15) is a hormone that can regulate body growth, increases during tissue injury and inflammatory states and is associated with cardiometabolic risk in children and adults. Indeed, our work has identified GDF15 as a novel cardiokine that could mechanistically link obesity to CVDs. Due to the heterogeneity of heart tissue composition, the source of GDF15 in the human heart remains unenxplored.

Methods and results:

We aimed to characterize GDF15 expression in patients with established heart disease at the New Brunswick Heart Centre (NBHC). Patients requiring elective cardiac surgery were enrolled (n=75) and classified as normal, pre-obese and obese Classes I-III based on BMI. Circulating GDF15 was quantitated from pre-surgery plasma using Luminex assay. Expression (mRNA, protein) was determined in cardiac tissues (right atrial appendage-AA and epicardial adipose tissue-EAT) from the same group of patients.

Baseline clinical characteristics were similar between groups except for functional class (NYHA III/IV in 90% of Class III vs 17% in normal; $p<0.01$) and longer hospitalization in Class III patients (median 2d longer). Plasma GDF15 showed significant dose dependent increase with obesity ($p=0.009$). Expression of GDF15 was significant in AA compared to EAT from the same patients, suggesting that diseased heart tissue may be a more important source for circulating GDF15 than organ adipose tissue. This increase in GDF15 in AA appears to correlate with our previous work demonstrating significant number of inflammatory mononuclear CD45+ cells in AA.

Conclusions:

Our data suggests that significant increase of plasma GDF15 correlates with obesity severity in patients with heart disease. High expression in AA might indicate it as an important source of circulating GDF15 and supports a role for inflammation in atrial disease. Our data proposes a mechanism by which nutrient stress can significantly augment cardiac GDF15 expression and provides a link between inflammation, GDF15 and obesity.

P2-51

Adverse Cardiometabolic Outcomes in Obese Patients Correlates Strongly with Defective Branched-chain Amino Acid Catabolism

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Background:Dysregulated branched amino acid (BCAA) metabolism is an important predictor of impaired insulin sensitivity and cardiovascular dysfunction. BCAA uptake is facilitated by branch chain aminotransferase (BCAT) to yield branched-chain α-keto acids (BCKA). Mitochondrial oxidation of BCKA is catalyzed by branched chain ketoacid dehydrogenase (BCKDH), enzyme sensitive to inhibitory phosphorylation by BCKD kinase. However, the clinical association between dysregulated BCAA catabolism and cardiovascular dysfunction merits investigation. Furthermore, the tissue-specific role of BCAA in inducing insulin resistance (IR) is unexamined.
Methods and Results: Atrial appendage (AAT) and subcutaneous adipose (SAT) tissues were collected from 136 patients during cardiac surgery with informed consent. Immunoblot analysis revealed that phosphorylated BCKDH correlated positively with BMI, length of stay (LOS) and HOMA IR in SAT. DLD1, an important regulator of BCKDH-E3 component, correlated negatively with BMI in SAT. KLF15, a transcriptional regulator of BCAA oxidation, negatively correlated with BMI in both SAT as well as AAT; while BCAT2 positively correlated with BMI in AAT thus explaining IR associated BCAA catabolic defects. Total serum BCAA levels measured using UPLC-MassSpec correlated negatively with KLF15 protein expression. Collectively, these findings suggest that impaired BCAA catabolism triggered by the decreased KLF15 expression is strongly associated with obesity-induced IR. To characterize the tissue responsible for facilitating BCAA induced IR,C57BL/6J mice were fed either chow, HFHS, HFHS+150%BCAA, LF or LF +150%BCAA for 16 weeks. Following insulin stimulation, AKT Ser473 phosphorylation was comparable in mouse heart, liver, EDL, and soleus between the BCAA supplemented diet groups. Notably, BCAA supplementation in the LF group reduced insulin-stimulated AKT phosphorylation in the gastrocnemius muscle.
Conclusion:BCAA catabolizing enzymes and their transcriptional regulator, KLF15 are co-ordinately regulated in SAT and AAT tissues from obese patients. This contributes to incomplete BCAA oxidation in fat leading to systemic elevation of BCAA and its metabolites.

P2-52

Cyclophilin D-mediated regulation of the permeability transition pore is altered in mice lacking the mitochondrial calcium uniporter

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Knockout (KO) of the mitochondrial Ca^{2+} uniporter (MCU) abrogates mitochondrial Ca^{2+} uptake and permeability transition pore (PTP) opening. However, hearts from global MCU-KO mice are not protected from ischemia-reperfusion injury. We aimed to investigate the hypothesis that adaptive alterations occur in cell death

signaling pathways in the hearts of global MCU-KO mice. First, we examined whether cell death may occur via an upregulation of necroptosis in MCU-KO. However, our results show that neither RIP1 inhibition nor RIP3 knockout afford protection against ischemia-reperfusion injury in MCU-KO as in WT hearts, indicating that the lack of protection cannot be explained by upregulation of necroptosis. Further, overexpression of RIP3 increases cell death in WT but not in MCU-KO. Instead, we have identified alterations in CypD signaling in global MCU-KO hearts. In the presence of a calcium ionophore, MCU-KO mitochondria take up calcium and do undergo PTP opening. Furthermore, PTP opening in MCU-KO mitochondria has a lower calcium retention capacity, suggesting that the calcium sensitivity of PTP is higher. Phosphoproteomics identified an increase in phosphorylation of CypD-S42 in MCU-KO compared to WT, and immunoprecipitation experiments showed an increase in CypD phosphorylation in MCU-KO at the protein level. We investigated the interaction of CypD with the putative PTP component ATP synthase and identified an approximately 50% increase in this interaction in MCU-KO cardiac mitochondria. Mutation of the novel CypD phosphorylation site S42 to a phosphomimic reduced calcium retention capacity and increased CypD-ATP synthase interaction by ~50% in comparison to a phospho-resistant mutant. Taken together these data suggest that MCU-KO mitochondria exhibit an increase in phosphorylation of CypD-S42 which decreases the calcium sensitivity of the PTP thus allowing activation of PTP in the absence of an MCU-mediated increase in matrix calcium.

P2-53

Mitochondrial Ca^{2+} Uptake is Tightly Regulated by the C-Terminal End of EMRE

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The Essential Mitochondrial Ca^{2+} Uniporter (MCU) Regulator (EMRE) is a small protein with a single transmembrane domain in the inner mitochondrial membrane. EMRE is essential for mitochondrial Ca^{2+} uptake in cells. We have further characterized the *in vivo* role of EMRE by creating whole body EMRE knock out ($^{-/-}$) mice. Like $MCU^{-/-}$ mice, $EMRE^{-/-}$ mice are viable in a mixed CD1 background, yet are slightly smaller than wild-type mice. We confirmed that EMRE expression was absent in heart, liver, and brain. We then measured Ca^{2+} uptake in isolated mitochondria and showed that $EMRE^{-/-}$ mitochondria do not take up calcium. To investigate the hypothesis that the reconstitution of EMRE could restore Ca^{2+} uptake in $EMRE^{-/-}$ cells, we immortalized $EMRE^{-/-}$ mouse embryonic fibroblasts (MEFs). Full-length ($EMRE^{wt}$) and truncated EMRE ($EMRE^{\Delta}$) constructs were expressed in $EMRE^{-/-}$ MEFs via lentiviral infection. The $EMRE^{\Delta}$ construct lacks the highly-conserved final 17 amino acids at EMRE's C-terminus. $EMRE^{-/-}$ MEFs infected with $EMRE^{WT}$ expressed EMRE protein, but at lower levels than $EMRE^{+/+}$ parental MEFs. Mitochondrial Ca^{2+} uptake assays revealed a partial rescue of Ca^{2+} uptake in the $EMRE^{-/-}$ + $EMRE^{wt}$ cells, demonstrating that partial reconstitution of EMRE protein levels enables mitochondrial Ca^{2+} uptake. Notably, $EMRE^{-/-}$ + $EMRE^{\Delta}$ cells exhibited little or no Ca^{2+} uptake, similar to that of $EMRE^{-/-}$ cells, prompting us to explore the mechanism whereby the C-terminal region of EMRE facilitates mitochondrial Ca^{2+} uptake. Overall, these results show *in vivo* that EMRE is necessary for MCU function and that the C-terminal end is directly involved in this activity.

P2-54

Role of adrenergic receptor singnaling in embryonic ventricular cell proliferation and differentiation

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In adult hearts, cardiomyocytes (CM) that die in response to aging or pathological insults are replaced by scar tissue. Transplantation of embryonic cardiac progenitor cells (CPC) was shown to increase the contractile function of a failing heart. We recently demonstrated that a non-selective beta-adrenergic receptor (β-AR) agonist isoproterenol (ISO) can decrease proliferation of CPCs *in vitro* and reduce graft size after intracardiac cell transplantation. Whereas, a β_1-AR antagonist (Metoprolol) abrogated the anti-proliferative effects of ISO and increased graft size. Carvedilol, a commonly used heart failure medication is known to block both α- and β-AR subtypes. There is no information available on the expression profiles of different α-AR subtypes during cardiac ontogeny and whether these receptors play any role in proliferation and differentiation of embryonic ventricular cells. It is hypothesized that expression of alpha 1 AR subtypes are differentially regulated during embryonic heart development and alpha 1 AR signaling plays an important role in ventricular cell proliferation and differentiation. Total RNA samples isolated from different developmental stages of embryonic ventricles were processed for quantitative RT-PCR analysis using α1-AR subtype specific primers. These experiments revealed that α1B or α1D gene expression levels were significantly higher than that of α1A at several stages of cardiac development. Subcellular localization of alpha 1 AR subtypes in embryonic ventricular cells revealed the presence of alpha 1 A, B, and D subtypes in the nucleus as well as the cytoplasm. Embryonic ventricular cultures treated with Carvedilol in the presence or absence of ISO did not show any changes in cell size compared to control cultures. Additionally, cells treated with Carvedilol and Prazosin, resulted in no change in proliferation and

differentiation status of CPC and CM cells. Therefore, these results suggest that it may be safe to use non-selective adrenergic receptor blockers with cell transplantation studies.

P2-55

Epigenetic regulation of cardiac fibroblast senescence by class I histone deacetylases and ING2

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Fibrosis is estimated to contribute to 45% of deaths in the western world. A key cellular mediator of fibrosis is the activated myofibroblast, which is characterized by the secretion of extracellular matrix (ECM) proteins. Myofibroblasts are thought to initially have a positive mechanical influence following an injury. However, long-term activation of these cells can lead to excessive ECM accumulation, culminating in fibrosis and organ failure. Cellular senescence is an irreversible form of cell cycle arrest, associated with a senescence-associated secretory phenotype (SASP), which is characterized by production of ECM-degrading enzymes such as matrix metalloproteases. Thus, it has been proposed that induction of the SASP might be a promising strategy for the resolution of organ fibrosis. In this context, histone deacetylase (HDAC) inhibitors might be candidate therapies for senescence modulation, as this class of enzymes is intensely involved in the control of cellular proliferation. Here, adult rat ventricular fibroblasts stimulated with TGF-β were used as a cell-based model of myofibroblast activation, and a panel of isoform-selective HDAC inhibitors was tested. Inhibitors of class I HDACs (especially HDAC1 and HDAC2), but not class IIa or IIb HDACs, potently stimulated cardiac fibroblast senescence, as assessed by upregulation of p16 and p21 gene expression, enhanced b-galactosidase activity, and induction of the classical SASP pathway. The mechanism of stimulation of senescence by class I HDAC inhibitors appears to involve ING2, a methyl-histone reader protein and phosphoinositide receptor. These findings define a chromatin signaling program for cardiac fibroblast senescence that could be could targeted with 'epigenetic therapies' such as small molecule HDAC inhibitors to treat fibrosis of the heart.

P2-56

Branched chain α-ketoacids: Novel Regulator of Insulin and mTOR Signalling in Skeletal and Cardiac Muscle

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Background: Branched chain α-keto acids (BCKA) are intracellular catabolic product of branched-chain amino acids (BCAAs) catabolism. Mitochondrial oxidation of BCKA is catalyzed by branched chain ketoacid dehydrogenase (BCKDH), enzyme sensitive to inhibitory phosphorylation by branched chain ketoacid dehydrogenase kinase (BCKDK). Skeletal and cardiac muscle generate significant BCKA. Defective BCAA catabolism and elevated BCKA is central to the pathogenesis of obesity, insulin resistance and heart disease. However, the effect of BCKA on muscle insulin signalling is unexplored.

Methods and Results: To examine the direct effect of BCKAs on muscle insulin signaling, adult and neonatal rat cardiomyocytes (ARMCs & NRCM), mouse (C2C12) and rat (L6) skeletal myocyte were incubated with 0.4mM palmitate for 16 h followed by 30 mins 5mM BCKA (ketoleucine or ketovaline or ketoisoleucine) and 15 min 100 nM insulin stimulation. Immunoblot analysis revealed that in the absence and presence of palmitate, ketoleucine, ketovaline and to a lesser extent ketoisoleucine significantly reduced insulin-stimulated AKT Serine 473 and 308 phosphorylation with concomitant upregulation of mTOR signaling pathway. Acute exposure of ARCM to ketoleucine alone augmented electron transport chain capacity given elevated complex III/IV respiratory rate for TMPD/ascorbate after treatment with FCCP suggesting that BCKA directly impairs insulin signaling and remodels mitochondrial respiration. To simulate altered intramyocellular BCKA flux, BCKDH and BCKDK were adenovirally modified. Genetic (adenoviral) and pharmacological activation of BCKDH using BCKDK inhibitor BT-2 in C2C12 cells potentiated insulin signaling with a corresponding decline in the phosphorylation of ribosomal p70S6K indicating reduction in mTOR signaling. Overexpression of BCKDK diminished insulin signaling with concomitant hyperactivation of the mTOR signaling.

Conclusion: BCKDH and BCKDK exerts control of intramyocellular BCKA flux into mitochondrial oxidation thereby dictating BCKA availability for regenerating BCAA and altering mTOR regulation of insulin function.

P2-57

Patient specific iPSC-derived cardiomyocytes reveal abnormal signaling pathways underlying hypertrophic cardiomyopathy in Noonan Syndrome

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Noonan Syndrome (NS) is caused by mutations that affect the RAS/ERK1/2-MAPK pathway. Strikingly, >90% of NS patients with a mutation in the CR2 domain of *RAF1* exhibit hypertrophic cardiomyopathy (HCM). However, the molecular mechanisms that elicit HCM in these patients remain unknown.
Here, we modeled NS-associated HCM by differentiating *RAF1* S257L/+ and its isogenic corrected control (generated with CRISPR) iPSCs into cardiomyocytes (iCMs). We found that mutant iCMs phenocopy the pathology seen in NS by exhibiting hypertrophy and structural defects. At the molecular level, while S257L/+ iCMs had elevated RAF1 and MEK1/2 activities, ERK1/2 itself was modestly enhanced. To test whether ERK1/2 activation was responsible for increased size of S257L/+ iCMs, we inhibited MEK1/2 activity with three different inhibitors (U0126, PD or Tram) and found that, while neither PD nor Tram reduced mutant iCMs area, both rescued myofibrillar disarray, indicating that the MEK1/2-ERK1/2 pathway regulates myofilament organization but not cell size. Strikingly, U0126 was able to reduce iCMs surface area, suggesting that an ERK1/2-independent pathway was responsible for hypertrophy. Since U0126 also targets MEK5, we treated S257L/+ iCMs with a specific MEK5/ERK5 inhibitor and found that hypertrophy was reduced, indicating that the ERK5 pathway triggered hypertrophy in S257L/+ iCMs. Finally, using RNA-Seq, we uncovered that mutant iCMs displayed reduced expression of several genes necessary for cardiac development and proposed that at the transcriptional level deregulation of several extracellular matrix, cytosqueletal and actin dynamic regulator genes downstream of ERK5 or MEK1/2-ERK1/2 might underlie the hypertrophic phenotype of NS iCMs.

Overall, our NS iPSC-derived cardiomyocyte model deciphered the abnormal molecular mechanisms deployed in the heart of NS *RAF1* patients and defined the concomitant targeting of the ERK5 and MEK1/2-ERK1/2 pathways as a promising therapeutic for HCM in NS *RAF1* patients.

P2-58

Proximity-Labelling by BioID Reveals Pleiotropic Ski Interactome

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Introduction: Despite the improvements made in cardiac patient treatment and outcomes, current therapies only serve to alleviate the symptoms of cardiac fibrosis, rather than treat the disease itself. Our lab has previously established Ski as a potent inhibitor of TGF-β signalling in cardiac myofibroblasts, the primary effectors of fibrosis; however our recent investigations suggest that Ski may inhibit a multitude of pro-fibrotic pathways, including Hippo. To further expand the current knowledge on Ski's anti-fibrotic properties, and provide insight into potential mechanisms of action, we mapped its interactome using enzyme-catalyzed biotin proximity labelling (BioID2).

Methods: Using the BioID2 vector, E.coli BirA* biotin ligase was fused to the N-terminus of human Ski; the fusion protein was then expressed in HEK 293 cells in the presence of an excess of free biotin. Whole cell lysates were then subject to streptavidin-mediated protein capture, and potential c-Ski binding partners were identified by tandem time-of-flight mass spectrometry. The resulting 62 candidates included several known Ski interactors (eg. Smad2/4), but also revealed novel interactors which potentially link Ski's anti-fibrotic capacity and interaction with the Hippo signalling pathway. Candidate interactors were verified using dynamic cytoskeletal assays and immunoblotting.

Conclusion: Our data suggest that Ski serves not only as a Smad-dependent TGF-β inhibitor, but also interacts with the Hippo, Wnt/β-catenin, and FoxO signalling pathways--all of which are suspected contributors to fibrotic disease. In addition, Ski's interaction with various points of cytoskeletal dynamics further indicates that its functions in the cell go beyond simple inhibitory protein-protein interactions.