(ΔpCa_{50}=0.08 ± 0.02), while no significant difference was present in sham hearts (ΔpCa_{50}=0.01 ± 0.02).
These measurements indicate that economy of myofilament contraction is reduced in post-MI remodeled myocardium.

3723-Pos
Myosin Heavy Chain Isoform Expression and Contractile Function in Mechanically Unloaded Left Ventricles Following Left Ventricular Assist Device (LVAD) Implantation
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The ventricles of human myocardium normally express low levels of α myosin heavy chain (MHC) on a predominately β MHC background. However, in heart failure the distribution changes to ~100% β MHC with virtually undetectable levels of α MHC, a change that has been associated with contractile dysfunction. In cases of severe failure, surgical implantation of a left ventricular assist device (LVAD) may be used as destination therapy and has been previously associated with improvements in contractile function in single myocytes. Here, we used post-LVAD myocardium in which the heart has been explanted for transplantation to test the hypothesis that mechanical unloading of ventricular myocardium increases contraction kinetics, possibly through the re-expression of α MHC. Measurements of the maximal rate of ATP utilization and isometric force in permeabilized multicellular preparations revealed no significant difference between failing myocardium prior to LVAD implantation (pre-LVAD) and post-LVAD myocardium. Tension cost, which is calculated as the rate of ATP utilization divided by the isometric force, was also similar between groups. For comparison, normal myocardium displayed maximal rates of ATP turnover that were approximately 2.5-fold greater than in pre- and post-LVAD myocardium. SDS-PAGE indicated virtually undetectable levels of α MHC in pre- and post-LVAD myocardium, while protein phosphorylation gels revealed significant differences in the basal level of phosphorylation of myosin binding protein-C, TnT, and TnI between both groups. These results suggest that while mechanical unloading of failing myocardium may not cause a re-expression of α MHC, improvements in contractile function following LVAD implantation may be associated in part with alterations in the phosphorylation status of key regulatory proteins. This work supported by NIH RO1-HL61635 (RLM).

3724-Pos
Myofibrillar Protein Expression and Contractility in Neonates and Infants with Congenital Right Ventricular Outflow Obstruction
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In this study we investigated the postnatal developmental changes in sarcomeric protein expression in parallel with contractile parameters in myofibrils isolated from small resections from the right ventricular (RV) outflow tract in 25 patients with Tetralogy of Fallot and related congenital heart diseases (CHD). These CHDs are associated with RV hypertrophy and outflow tract obstruction. The age of the patients ranged from 4 days to 38 months. The resections were procured during surgical correction of the cardiac malformation and would have been otherwise discarded. In the neonate (4 days old), the expression of slow skeletal TnI (ssTnI) and atrial light chain (ALC-1) was ~82% and ~50% respectively and declined to respectively < ~8% and ~3% at the age of 38 months. This down-regulation in ssTnI and ALC-1 expression correlated ($p<0.05$) with the decline in Ca^{2+}-sensitivity from $pCa_{50} = 5.95$ in the neonate to $pCa_{50} = 5.33$ in 38 months old infants. Neither contraction nor relaxation kinetics correlated with ssTnI expression. However, ALC-1 expression correlated positively with the activation kinetics, k_{ACT} and force redevelopment, k_{TR} ($r = 0.62$, $p<0.05$). The time course of relaxation is biphasic with an initial slow quasi-linear decay followed by a fast exponential decay. The rate constant of the fast exponential decay, k_{REL} correlated positively with ALC-1 expression ($r = 0.57$, $p<0.05$). In summary right ventricular hypertrophy associated with congenital heart disease does not prevent the developmental down-regulation of ssTnI and ALC-1 although we cannot exclude that this down-regulation occurs at a slower rate than in healthy infants. The change in ssTnI expression correlates with the expected decrease in Ca^{2+}-sensitivity while ALC-1 expression appears to modulate crossbridge turnover kinetics in agreement with studies in animals.

3725-Pos
Sex Dimorphic Myofilament Function and AMPK Expression in R403Q Hearts
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Male mice expressing an autosomal dominant mutation in alpha-myosin heavy chain (R403Q) develop hypertrophic cardiomyopathy characterized by progressive left-ventricular dilation and cardiac dysfunction whereas females do not. We wished to determine whether these sex dimorphisms were due to underlying differences in myofilament contractile function. Therefore, we determined the sensitivity of the myofilaments to Ca^{2+} in demembranated cardiac trabeculae (CT) from wild-type (WT) and R403Q male and female mice (10-12 months of age). We demonstrate that the R403Q mutation did not affect Ca^{2+}-sensitive tension development in CT from males. While Ca^{2+}-sensitivity was greater in both male WT and R403Q CT compared to WT females, they were less sensitive to Ca^{2+} than CT from female R403Q hearts. We also determined rates of tension redevelopment (k_{tr}) following a release-restretch protocol in CT from WT and R403Q male and female hearts at the same age. CT from R403Q male hearts exhibited an enhanced k_{tr} compared to WT males. The k_{tr} in WT female CT was similar to WT males. The k_{tr} in R403Q female CT measured between WT and R403Q males. We hypothesized that the sex dimorphisms in myofialment function reflect an increase in the energetic cost of contraction when expressing the R403Q mutation. Therefore, we measured levels of Adenosine monophosphate-activated kinase (AMPK), a central sensor of the cellular energy state. Total AMPK protein levels were significantly increased in 10-12 month male R403Q hearts compared to WT controls. Female R403Q hearts showed the opposite: total AMPKα expression was lower compared to WT controls. We conclude that (1) the increased Ca^{2+}-sensitivity may provide sufficient contractile support in female R403Q hearts maintaining a compensated state, and (2) the increased AMPK expression in male R403Q hearts is indicative of an increased energetic demand caused by the mutation.

3726-Pos
Intralipid Protects Cardiac Function of Late Pregnant Mice against Ischemia/Reperfusion Injury
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Female mouse hearts show better functional recovery after ischemia/reperfusion (I/R) injury compared with males. However, the vulnerability of isolated late pregnant (LP) hearts to I/R injury is unknown. Here we investigated the susceptibility of isolated mouse hearts in LP and postpartum (PP) to I/R injury. Isolated hearts (Langendorff) from female mice in diestrus stage (NP), LP, one day PP (PP1) and 7 day PP (PP7) were subjected to 20 minutes of global normothermic (37°C) ischemia followed by 40 minutes of reperfusion. The heart function was recorded throughout the experiments and infarct size was assessed by triphenyltetrazolium staining at the end of reperfusion. Although the function was similar in all 4 groups before ischemia, the functional recovery of LP hearts at the end of reperfusion was significantly lower compared to NP hearts; the rate pressure product (RPP) was reduced from 12926 ± 1479mmHg*beats/min in NP to 1614 ± 438mmHg*beats/min in LP mice. Interestingly, the RPP recovered partially in PP1 to 4716 ± 584mmHg*beats/min and almost fully back to NP levels one week PP. Consistent with the functional recovery findings, the infarct size was markedly larger in LP (59.7 ± 5.2%) compared with NP (15.2 ± 0.8%). The infarct size was restored partially in PP1 and fully back in PP7. Recently we have observed that Intralipid can protect the male mouse heart against I/R injury. To test whether Intralipid can improve the heart function in LP mice, 1% Intralipid was applied to isolated LP hearts at the onset of reperfusion. Intralipid treatment significantly improved the cardiac function of LP mice (RPP=11565 ± 1599mmHg*beats/min) and reduced the infarct size (17 ± 1.1%) to similar values as in NP. In conclusion, isolated LP hearts have high vulnerability to I/R injury and postischemic treatment with Intralipid can protect the heart against I/R injury.

3727-Pos
Intralipid Induces Cardioprotection against Ischemia-Reperfusion Injury by Inhibiting the Mitochondrial Permeability Transition Pore Opening Via the PI3K/AKT Pathway
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Acute myocardial infarction is a major cause of mortality, and the best hope of salvaging viable myocardium is by rapid cardiac reperfusion. A novel cardioprotective drug which could be applied at the time of reperfusion after acute infarction would be ideal. Here we tested the hypothesis that administration of Intralipid at the onset of reperfusion protects the heart against ischemia reperfusion (I/R) injury. Isolated hearts (Langendorff) from male mice were subjected to 20 minutes of global normothermic (37°C) ischemia followed by 40 minutes of reperfusion with Krebs Henseleit buffer (CTRL) or with additional 1% Intralipid (ILP). Postischemic treatment with Intralipid significantly improved the cardiac function; the rate pressure product (RPP) was increased from 3432 ± 334mmHg*beats/min in CTRL to 15405 ± 1011mmHg*beats/min in ILP. Consistent with the higher functional recovery in ILP, the infarct

size was markedly smaller in ILP (19 ± 3%,n=8) compared to CTRL (55.6 ± 3.4%,n=10). The inhibition of mitochondria permeability transition pore (mPTP) opening during reperfusion has been shown to induce cardioprotection. To investigate whether intralipid-induced cardioprotection occurs by inhibition of the mPTP opening, we compared the viability of isolated mitochondria by calcium overload. Postischemic administration of Intralipid inhibited the opening of the mPTP as calcium retention capacity was higher in the ILP group compared to control (2.7 ± 0.06 *vs.* 1.5 ± 0.11 μM/mg-mitochondrial protein, $p<0.05$). To identifiy the key signaling molecules involved in regulating mPTP opening, Western Blot analyis of heart lysates was performed. The activity of AKT/ERK1/GSK were respectively 2.3, 5 and 2.7 fold higher in ILP compared to CTRL. The involvement of PI3K/AKT pathway was further investigated by LY294002, a specific inhibitor of PI3K. The Intralipid-induced cardioprotection was fully abolished in the presence of LY294002, indicating the cardioprotective action of Intralipid is mediated via PI3K/AKT pathway.

3728-Pos

Proteasome Activity is Reduced at the end of Pregnancy and Fully Restored to Non-Pregnant Levels One Week Postpartum in the Murine Hear

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The proteasome is the major protein degradation system in the heart, and its activity has been shown to be affected during pathological cardiac diseases. Proteasome dysfunction in the hypertrophic heart leads to accumulation of abnormal proteins and has been proposed to contribute to the transition to heart failure. Pregnancy places an increased demand on the healthy female's heart resulting in ventricular hypertrophy and diastolic dysfunction as a result of volume overload and increased stretch and force demand. Since the molecular signature of pregnancy-related heart hypertrophy differs significantly from that of pathological hypertrophy, we investigated if the proteasome proteolytic pathway is affected by pregnancy in the mouse heart. We measured the transcripts and protein levels of proteasome subunits as well as proteasome activity in four groups of female mouse hearts: i) non pregnant (NP) at diestrus stage, ii) late pregnant (LP), iii) one day post-partum (PP1) and iv) 7 days post-partum (PP7). Real Time PCR showed that the transcript levels of RPN2 and RPT4 (subunits of 19S) as well as β2 and α7 (subunits of 20S) did not change with pregnancy. Western blot analysis of heart lysates also revealed no significant differences in the expression levels of α7 (a subunit of 20S), RPN2 and RPT4 (subunits of 19S) subunits in the four groups mentioned above. The β1 (caspase-like) and β2 (trypsin-like) activities of the proteasome were significantly decreased in LP. The β5 (chymotrypsin-like) activity was significantly decreased 1 day post-partum. Interestingly, all three proteolytic activities of the proteasome were restored to normal levels 7 days post-partum. These results suggest that the proteasome proteolytic pathway is affected by pregnancy and is restored to NP levels soon after delivery.

3729-Pos

Sepsis Related C5a Peptide Causes Calcium Overload in Adult Cardiac Myocytes

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Septic cardiomyopathy is an acute cardiac syndrome that occurs after the onset of sepsis due to infectious agents such as bacteria, viruses and fungi. During septic cardiomyopathy cardiac output falls due to waning contractile function of the heart. However, very little is known about the precise cause of cardiac failure in cases of sepsis. One factor induced during sepsis is the complement activation product C5a. C5a is a peptide that acts through a G-protein coupled receptor (C5aR) and affects cardiac myocyte contractility by unknown mechanisms. Here we have tested the effect of C5a peptide on single adult cardiac myocyte calcium homeostasis. Cardiac myocytes were isolated from healthy rats and intracellular calcium transients were monitored (fluo-4AM) before and after C5a peptide treatment. Intracellular calcium was monitored by two different methods: 1) using a conventional photomultiplier tube and, 2) using a high speed digital CCD camera (200frames/s) to image whole cell calcium transients and waves. Recombinant C5a was applied to cardiac myocytes during electrical pacing (0.5Hz, 40V). After application of C5a (82ng/mL) intracellular calcium concentrations and calcium transient amplitudes initially rose (from F/Fo=1.43 ± 0.12 to 1.86 ± 0.4, n=4). Calcium transient duration was also prolonged after C5a addition (half width=260.2 ± 29.0 ms to 318.7 ± 47.1ms) and spontaneous calcium transients and waves were observed in the diastolic period between electrical stimuli. Consequently the amplitude of calcium transients and contractions varied from stimulated beat to beat after C5a addition. Paradoxically at higher pacing frequencies (3Hz) calcium transient amplitude was smaller after C5a application (F/Fo=1.46 ± 0.2 vs. 1.32 ± 0.04, n=4) and prolonged (half width=127.0 ± 1.15 vs. 167.0 ± 15.6 ms). Spontaneous calcium transients were also observed in the absence of electrical stimulation following C5a treatment. These data suggest that C5a peptide acts through its receptor C5aR to cause cardiac myocyte intracellular calcium overload.

3730-Pos

Short-Range Mechanical Properties of Myocardium from Diabetic Rats

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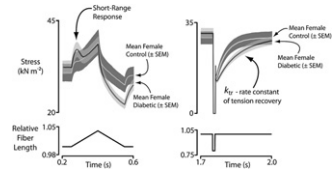
Diabetes mellitus is often associated with abnormalities in active relaxation and passive stiffness of the left ventricle but the molecular mechanisms responsible for the dysfunction are not yet clear. This study was designed to identify the molecular components that are responsible for the increased myocardial stiffness associated with diabetes. Multicelluar myocardial preparations were isolated from control Sprague-Dawley rats and an experimental group of rats injected 4 weeks previously with streptozotocin (model of Type I Diabetes). Preparations were subjected to paired ramp stretches/releases imposed under fiber length control in a series of calcium activations (pCa 4.5 - 9.0). The relative short-range force and elastic limits were substantially higher in the diabetic groups. The rate of tension recovery (k_{tr}) was considerably lower in the diabetic groups. Short range stiffness values did not differ in the control and diabetic animals. Gel electrophoresis showed that the relative content of slower beta Myosin heavy chain increased from 34 ± 15% in control hearts to 100% in the diabetic rat hearts. These results support the hypothesis that pathological changes in the mechanical properties of diabetic rat myocardium are mostly due to alterations in the active component (cycling cross-bridges) of ventricular stiffness.



3731-Pos

Increased Phosphorylation of Myofilament Proteins after Stretch in Rabbit Ventricular Myocardium

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After a change in muscle length, there is an immediate intrinsic response in the amount of developed force, followed by a slower response. Although it has been well documented that the slow force response is at least in part generated by modification of calcium handling, it is unclear whether regulation at the level of the myofilaments occurs during the slow force response. We set out to investigate myofilament calcium sensitivity and phosphorylation status of myofilament proteins after a step-wise change in cardiac muscle length. Ultra-thin right ventricular intact trabeculae were isolated from New Zealand White rabbit hearts and iontophoretically loaded with the calcium indicator bis-fura-2. Twitch force-calcium relationships and steady state force-$[Ca^{2+}]_i$ relationships were measured at slack and optimal muscle lengths at 37°C using potassium induced contractures. The EC_{50} significantly decreased with increase in muscle length, from 1467 ± 271 nM at the shortest muscle length to 653 ± 121 nM at the longest muscle length. Maximal active force development significantly increased from 19.7 ± 2.7 mN/mm^2 at the shortest muscle length to 51.8 ± 5.0 mN/mm^2 at the longest muscle length. No significant change in the myofilament cooperativity coefficient was found. Phosphoprotein analysis using ratiometric analysis of Pro-Q diamond staining and Sypro-Ruby staining of the same gel, revealed increased phosphorylation of tropomyosin, troponin I, and myosin light chain-2 at longer muscle lengths. Since the immediate response is seen virtually instantaneously, and post-translational modifications cannot occur within that timeframe, we hypothesize that these increases in phosphorylation occur during the slow response. Future studies will aim to elucidate the individual effects of the immediate response verses post-translational modifications during the slow response, and also determine to what extent increased phosphorylation of tropomyosin, troponin I, and myosin light chain-2 each play a role.

3732-Pos

A Systems Biology Approach to Restrictive Cardiomyopathy in Drosophila

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