fibrosis and CD31 immunostaining for cardiac angiogenesis were performed. A $p<0.05$ was considered significant. Values were mean ± SE. LP was associated with heart hypertrophy ($p<0.05$ vs. NP) that was reversed 7-days PP (heart weight: NP=120 ± 3, LP= 163 ± 2, PP1= 145 ± 2, PP7= 117 ± 1 mg). Conversely, heart weight/body weight (hw/bw) ratio was decreased in LP ($p<0.05$ vs. NP) that was reversed in PP1 (hw/bw: NP=5.9 ± 0.1, LP= 4.5 ± 0.1, PP1= 5.6 ± 0.1). LV ejection fraction was reduced in LP ($p<0.05$ vs. NP) and was also restored at PP1 (NP=74 ± 4, LP= 57 ± 1, PP1= 73 ± 1%). Cardiac angiogenesis was significantly increased in LP ($p<0.001$ vs. NP), and was fully restored in PP7 ($p<0.001$ vs. LP) (Capillary density: NP=0.95 ± 0.01, LP=1.25 ± 0.02, PP7=0.98 ± 0.01 capillaries/myocyte). Similarly, VEGF was upregulated in LP, and was restored in PP7 (NP=1 ± 0.1, LP=1.4 ± 0.1, PP7=0.83 ± 0.1). There was no increase in cardiac fibrosis in pregnancy-induced heart hypertrophy. Transcript levels of extracellular matrix (ECM) degrading enzyme MMP2 were downregulated in LP ($p<0.05$ vs. NP) and were restored at 7-days PP (NP=1 ± 0.01, LP=0.47 ± 0.03, PP7=0.70 ± 0.1). In conclusion, pregnancy-induced heart hypertrophy is associated with increased cardiac angiogenesis, lack of fibrosis, decreased MMP2 and decreased diastolic function of the heart that are reversed postpartum. We speculate that physiologic heart hypertrophy of pregnancy has minimal cardiac ECM remodeling.

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Apolipoprotein-A1 Mimetic Peptide 4F Rescues Severe Pulmonary Hypertension in Rats and Inhibits Human Pulmonary Artery Smooth Muscle Cell Proliferation In Vitro

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Pulmonary hypertension (PH) is a characterized by arterial obstruction resulting from proliferation of pulmonary artery smooth muscle and endothelial cells. Genetic deletion of apolipoprotein-A1 increases airway hyperresponsiveness, inflammation, and collagen deposition in the lung. Apolipoprotein-A1 mimetic peptide 4F protects endothelial function, causes vasodilation, decreases inflammation and oxidative stress in lungs, yet its role in treating PH and right ventricular (RV) dysfunction is not known. We hypothesized that 4F may rescue pre-existing severe PH. We also investigate the effects of 4F on human pulmonary artery smooth muscle cell (hPASMC) proliferation in vitro as a possible mechanism of rescue by 4F. Twenty three rats were randomly divided into 4-groups. PH was induced by monocrotaline (MCT, 60mg/kg, s.c.). Severe PH was well-established at day-21 (PH, n=6) that progressed to RV failure (RVF, n=6) by day-30. One MCT-group was treated with 4F (50mg/kg/day, s.c., n=6) from day-21 to 30. Saline-treated rats served as control (CTRL, n=5). Serial echocardiography was performed to monitor cardiopulmonary hemodynamics. Cardiac catheterization was performed terminally to record RV-pressure (RVP). hPASMCs proliferation was assessed by MTT-assay. $p<0.05$ was considered significant. Values were mean ± SE. Rats developed severe PH 21-days after MCT (RVP=67.12 ± 1 vs. 29.8 ± 1 mmHg in CTRL, RV/LV+IVS= 0.65 ± 0.05 vs. 0.23 ± 0.02, RV-ejection fraction (RVEF)= 40 ± 1 vs. 65 ± 1%, all $p<0.05$ vs. CTRL), which progressed to RVF by day-30 [RVP=74 ± 1; RV/(LV+IVS)=0.68 ± 0.05; RVEF=28.6 ± 1%, $p<0.05$ for all vs. CTRL]. 4F-therapy from day-21 to 30 resulted in rescue of PH (RVP=47 ± 3 mmHg, RV/LV+IVS= 0.38 ± 0.02, RVEF= 51.7 ± 3%, $p<0.05$ vs. PH and RVF). 4F also inhibited hPASMC proliferation (~60% inhibition at 100ng 4F/ml medium, $p<0.05$). In conclusion, 4F rescues pre-existing severe PH and RV-dysfunction. Inhibition of PASMC proliferation may be one of the key mechanisms in this rescue.

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Genistein Therapy Reverses Lung Inflammation and Fibrosis during Severe Pulmonary Hypertension through Estrogen Receptor Beta

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Pulmonary Hypertension (PH) is a disease of increasing pulmonary arterial pressure characterized by extensive lung inflammation and fibrosis. We have previously shown that Genistein, a soy isoflavone, can rescue severe PH. Since Genistein is a selective Estrogen Receptor Beta agonist, here we examined whether Genistein can reverse fibrosis and inflammation induced by PH via an Estrogen Receptor Beta dependent mechanism. PH was established by treating male rats with monocrotaline (MCT, 60mg/kg, s.c.). At day 21 when severe PH was established, rats were treated with 10 day Genistein therapy (Gen group), Genistein therapy in the presence of selective Estrogen Receptor β antagonist PHTPP (Gen+PHTPP group), or were left untreated to develop right ventricular failure (RVF group). RVF animals developed severe pulmonary hypertension (RVP=72.96 ± 1.39mmHg vs 31.15 ± 0.56 mmHg in CTRL; RVEF=28.76 ± 0.79% vs 66.22 ± 1.40% in CTRL, all $p<.05$). Genistein therapy restored these abnormalities (RVEF=65.67 ± 1.08%, RVP = 43.34 ± 4.08 mmHg, $p<0.05$ vs RVF). Interestingly, in the presence of Estrogen Receptor Beta antagonist PHTPP, Genistein failed to rescue these animals (RVP=61.22 ± 4.40, RVEF=42.27 ± 2.7%, p=n.s. vs RVF). Masson's Trichrome staining revealed extensive lung fibrosis in RVF group, which was also restored with Gen therapy. Again, Gen+PHTPP group showed no reversal of pulmonary fibrosis. Immunoperoxidase staining showed an increase in ED-1 positive cells in the lung of RVF-group indicating increased inflammation. This change was reversed entirely by Genistein therapy. RT PCR also revealed that pro-inflammatory molecules TNFα and IL-1β were both elevated more than 2-fold in the lung of RVF animals and reversed with Genistein therapy (all $p<0.05$). Interestingly, Gen+PHTPP animals still showed significantly elevated ED-1 positive cells in the lung as well as elevated TNFα and IL1β (all p=n.s. vs RVF). These results suggest that genistein induced reversal of lung inflammation and fibrosis during pulmonary hypertension is mediated through Estrogen Receptor β.

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Estrogen Treatment Reverses Heart Failure-Induced Cardiac Fibrosis and Inflammation

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Heart failure is generally characterized by increased fibrosis and inflammation, which lead to functional and contractile defects. Recently we discovered that estrogen (E2) therapy can rescue advanced heart failure (HF) induced by pressure overload in mice by restoring left ventricular (LV) pressure and function. Here, we investigate the effects of E2 on reversing the adverse remodeling of the LV occurring during HF. Trans-aortic constriction procedure was used to induce HF. Once the ejection fraction reached ~30%, one group of mice was sacrificed and the other group was treated with E2 (30 μg/kg/day) for 10 days. In vitro, co-cultured neonatal rat ventricular myocytes and fibroblasts were treated with angiotensin II (AngII) in the presence or absence of E2. Quantitative real-time PCR showed that the transcript levels of the profibrotic markers collagen I, TGFβ, fibrosin 1 (FBRS) and lysil oxidase (LOX) were significantly upregulated in HF (from 1.00 ± 0.16 to 1.83 ± 0.11for collagen 1, 1.00 ± 0.86 to 4.33 ± 0.59 for TGFβ, 1.00 ± 0.52 to 3.61 ± 0.22 for FBRS and 1.00 ± 0.33 to 2.88 ± 0.32 for LOX) and were reduced with E2 treatment to levels similar to CTRL. In vitro studies validated our in vivo findings, as E2 also restored AngII-induced upregulation of LOX and TGFβ from 6.87 ± 0.26 in AngII to 2.80 ± 1.5 in AngII+E2 and 3.30 ± 0.25 to 1.59 ± 0.21 in AngII+E2, respectively (values normalized to CTRL). Furthermore, the pro-inflammatory interleukins IL-1β and IL-6 were upregulated from 1.00 ± 0.19 to 1.90 ± 0.09 and 1.00 ± 0.30 to 5.29 ± 0.77 in HF, respectively, and reversed to CTRL levels with E2 therapy. The anti-inflammatory interleukin IL-10 was downregulated from 1.00 ± 0.17 to 0.49 ± 0.03 in HF and reversed to 0.67 ± 0.09 with E2 treatment. This data strongly suggests that one of the mechanisms for the beneficial action of estrogen on left ventricular heart failure is through reversal of inflammation and fibrosis.

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Estrogen Directly Reverses Cardiac Remodeling Associated with Pulmonary Hypertension Induced Right Ventricular Failure

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Pulmonary hypertension (PH) leads to right-ventricular hypertrophy and failure (RVF). RVF involves adverse remodeling of the ventricular extracellular matrix (ECM). Recently we found that estrogen (E2) rescues PH-induced RVF. Here we explore whether the rapid restoration of RV function by E2 therapy during PH-induced RVF is in part due to a direct effect of E2 on the adverse ECM remodeling of the RV. In vivo, rats were injected with monocrotaline. At day 21, when PH had established, rats either received E2 (E2-group) for 10 days or were left untreated to develop RVF (RVF-group). In vitro, co-cultured neonatal rat ventricular myocytes and fibroblasts were treated with Angiotensin II in the presence or absence of E2 (AngII and AngII+E2 resp.). In vivo, E2 reversed RV fibrosis (4.06 ± 0.52% in CTRL, 33 ± 3.2 in RVF, 5.66 ± 0.33% in E2 group, all $P<.05$). In vitro, E2 similarly inhibited AngII induced increase in Collagen I transcript in cultured myocytes and fibroblasts (1.49 ± 0.04 in AngII, 0.75 ± 0.04 in AngII+E2, normalized to CTRL, $p<.05$). In vivo, E2 reversed PH induced increases of ECM remodeling enzymes OPN, ADAM15 and ADAM17 in the RV (9.33 ± 2.07 in RVF, 0.45 ± 0.16 in E2 for OPN; 2.13 ± 0.19 in RVF, 0.47 ± 0.07 in E2 for

ADAM15; 1.85 ± 0.04 in RVF, 1.3 ± 0.14 in E2 for ADAM17. All normalized to resp. CTRL, all $p<0.05$). In vitro, E2 also reversed expression of these genes induced by Angiontensin II (2.74 ± 0.19 in AngII, 1.72 ± 0.01 in AngII+E2 for OPN; 1.31 ± 0.06 in AngII, 0.88 ± 0.02 in AngII+E2 for ADAM15; 1.55 ± 0.01 in AngII, 1.09 ± 0.002 in AngII+E2 for ADAM17. All normalized to resp. CTRL, all $p<0.05$). E2 therapy was associated with complete reversal of RV fibrosis and changes in OPN, ADAM15 and ADAM17 expression. This data indicates that E2 has a direct effect on mitigating the adverse remodeling of the RV during PH.

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G-Protein Coupled Estrogen Receptor 1, but not Estrogen Receptors Alpha and Beta, Mediates Rapid Estrogen-Induced Cardioprotection during Ischemia/Reperfusion Injury in Male Mice

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Mouse heart possesses three types of estrogen (E2) receptors, ERα, ERβ and the G-protein coupled estrogen receptor1 (GPER1). We previously reported that rapid E2-induced cardioprotection is abrogated in GPER1$^{-/-}$, and that this action is mediated by pERK1/2 and pGSK-3β stimulation in absence of pAkt increase.

Here, we investigated the potential role of ERα, and ERβ in mediating the rapid cardioprotective action of E2 in ischemia/reperfusion injury in male mice, and further corroborated the role ERK1/2 and GSK-3β without the participation PI3K/Akt pathway.

We first quantified mRNA absolute levels of the three receptors in heart, and found that ventricles express much higher levels of GPER1 than ERα and ERβ. E2 (40 nM) treatment in the Langendorff model of ischemia/reperfusion improved cardiac functional recovery, reduced infarct size, and increased calcium-retention-capacity (a measure of mitochondrial transition pore, mPTP, opening) to the same degree in WT, ERα$^{-/-}$ and ERβ$^{-/-}$, further confirming no role of ERα and ERβ in rapid E2-induced cardioprotection. As previously reported, these beneficial effects were completely abrogated in GPER1$^{-/-}$. The involvement of MAPK-ERK1/2 pathway is further supported by the loss of E2-induced increase of calcium-retention-capacity by treatment with U01261, an ERK1/2 pathway inhibitor. The rapid E2 action did not involve PI3K pathway as the E2-induced pGSK-3β high level was not affected by treatment with LY294002, a PI3K inhibitor.

In summary, in male mice, only GPER1 activation mediates the rapid E2-induced cardioprotection against ischemia/reperfusion injury via phosphorylation of ERK1/2 and GSK-3β leading to increased mitochondrial calcium-retention-capacity reflecting a reduction of mPTP opening. Supported by NIH and AHA.

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Palmitate Improves Basal and β-Stimulated Left Ventricle Function in Diabetic Mouse Hearts

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The heart from a diabetic animal exhibits dysfunction when exposed to challenging energetic and tissue redox conditions. We showed that when cardiomyocytes from type-2 diabetes (db/db) mice are exposed to high glucose (HG) and β-adrenergic stimulation (via isoproterenol, ISO), these cells show blunted β-contractile reserve and increased oxidative stress. Treating these cells with the fatty acid (FA) palmitate (Palm) offsets those changes, an effect likely due to its ability of generating more reducing equivalents. Yet whether Palm infusion may benefit contractile performance and vascular tone of db/db hearts subjected to metabolic/redox stress is unclear. Using a Langendorff approach, we perfused wild type (WT) and db/db mice with HG (30mM glucose) + ISO (10nM), in absence or presence of Palm. WT hearts were infused with 0.2mM Palm and db/db ones with 0.4mM Palm to mimic higher circulating FA content in diabetic animals. Under HG, coronary perfusion pressure (CPP) was higher in db/db hearts (92 ± 8 vs. 63 ± 7 mmHg, $p<0.05$). This increase in tone was obviated by Palm. WT had better myocardial performance than db/db mice. For instance, dP/dtmax was 4653 ± 460 in WT vs 3474 ± 185 mmHg/sec in db/db ($p<0.05$). Although markedly blunted, ISO response was still present in db/db hearts (2821 ± 83 vs 3474 ± 185 mmHg/sec, $p<0.05$). However, HG fully blocked ISO-response. Infusing Palm to db/db preserved ISO response under HG (2392 ± 505 vs 3106 ± 425 mmHg/sec, $p<0.05$). Our study reveals that preferential FA oxidation improves heart LV function in diabetic mice subjected to combined energetic and redox challenge. This beneficial effect of Palm may be due to glucose-to-FA substrate shift and also to improved redox balance as shown in isolated cardiomyocytes.

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Mathematical Model of Oxygen Labeling to Study Heart Energy Transfer

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The mechanisms underlying the homeostasis of ATP, ADP, PCr and inorganic phosphate in heart under a wide range of workloads remain unclear. Fundamental to this search is an accurate understanding of the recycling fluxes of these metabolites between the mitochondrial inner membrane space and the ATPases on both the myofibrils and SERCA pumps. In addition to ^{31}P-NMR inversion and saturation transfer studies, dynamic ^{18}O labeling data has been used to analyze energy transfer. An integrative kinetic model that tracks the mass isotopomers of these four metabolites was constructed with the following compartments: mitochondrial matrix, mitochondrial intermembrane space, cytosol, and enzyme bound states of both ATPase and ATP synthase (since these reactions are not unidirectional with respect to oxygen exchange). A sensitivity analysis of this system was conducted with a jump to 30% $H_2{}^{18}O$ to find flux parameters that influenced the ^{18}O labeling state. Both the creatine kinase and adenylate kinase shuttle fluxes were modeled as being bidirectional to test assumptions made in previous studies that analyzed dynamic ^{18}O labeling data.

It was found that the sum of forward and reverse fluxes through each shuttle determined the labeling state such that if the sum is kept constant and the net flux is reduced, a very similar labeling state is predicted. Total creatine kinase and adenylate kinase fluxes that exceeded the ATP synthase rate were also found to give almost the same predictions of the labeling state. Model predictions of the ^{18}O labeling state of metabolites are very similar using energy fluxes reported in earlier studies of ^{18}O labeling in the heart and energy transfer analysis by ^{31}P-NMR inversion and saturation transfer.

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Oxygen Increases Heart Injury in Ischemia/Reperfusion

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Mice hearts were perfused using Langendorff apparatus with Krebs-Henseleit (KH) buffer oxygenated with 95% O_2 + 5% CO_2 at 37°C. Hearts were maintained suspended in the air in the humid chamber at 37°C for 20 min. Thereafter, hearts were either immersed in KH or kept in air in the same chamber at 37 °C, and subjected to 18 min of global ischemia by clamping the aorta. Afterwards, hearts were returned to air in the same humid chamber for 40, 60 or 90 min reperfusion. Heart function was monitored and myocardial infarction assessed by TTC staining at the end of the reperfusion. ROS generation was measured with Amplex Red in mitochondria isolated after 10 min of reperfusion.

When the hearts were immersed in KH, heart function and infarct size had reached an injured steady-state at 40 min of reperfusion. In contrast, hearts kept in air had a smaller reperfusion injury and infarct size at 40 and 60 min approaching steady-state at 90 min after reperfusion. Consistent with a lower injury in air, mitochondrial ROS production by stimulating complex I was much smaller in air-maintained than in immersed hearts. We tested whether increased oxygenation of the ventricle heart walls in the immersed heart might be the cause of higher damage. To reduce oxygen, the KH solution was bubbled with N_2 only. In this condition, the cardiac functional recovery was improved, and the infarct size measured at 60 min after reperfusion was reduced. In conclusion, 40 min reperfusion is sufficient to reach a steady-state infarct size when the hearts are immersed in KH during ischemia, while longer reperfusion time is required if the hearts kept in air. The injury installation during the reperfusion depends on the oxygen surrounding the heart during ischemia. Supported by NIH and AHA.

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The Deleterious or Protective Action of ROS in the Heart Depends on their Production Site in the Mitochondrial Electron Transfer Chain

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ROS generation has been implicated in cardiac damage during ischemia/reperfusion injury, and also in cardioprotection by preconditioning with a series of