## GW26-e0093

### Expression of 5-Lipoxygenase and 5-Lipoxygenase-Activating Protein in Immune Cells

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**OBJECTIVES** Arachidonic acid (AA), an omega-6 (n-6) fatty acid, can be converted to inflammatory leukotrienes (LTs) by 5-lipoxygenase (5-LO), which is activated by 5-lipoxygenase-activing protein (FLAP) and then lead to the expression of LTB4, which play a role in coronary heart disease (CHD).omega-3 (n-3) fatty acids are the metabolic counterparts to n-6 fatty acids and have anti-inflammatory effects. Thus, the tissue ratio of n-6/n-3 fatty acids may influence LT production. However, whether alteration of tissue n-6/n-3 fatty acid ratio has an effect on 5-LO and FLAP expression is not well known. The fat-1 transgenic mouse, expressing an n-3 fatty acid desaturase, is capable of endogenously converting n-6 fatty acids to n-3 fatty acids, and thereby has a tissue n-6/n-3 fatty acid ratio close to 1:1. The aim of the study was to compare 5-LO and FLAP expression status in immune cells between fat-1 transgenic mice and wild type (WT) littermates.

**METHODS** Immune cells from fat-1 transgenic mice (n=5) and WT mice (n=5) were harvested from the spleen and cultured for 24h. Mononuclear cells were also isolated from the blood. Cellular n-6/n-3 fatty acid profiles were analyzed using gas chromatography. The mRNA and protein expressions of 5-LO and FLAP in the cells were evaluated using real time RT-PCR and immunoblot (WB) assays, respectively. the expression of LTB4 level was assessed by Elisa assay.

**RESULTS** He fat-1 transgenic mice showed a lower ratio of n-6/n-3 fatty acids than WT mice in both splenocytes and blood monocytes (AA: 4.53±0.10 VS 2.16±0.09; EPA: 0.13±0.07 VS 0.36±0.15; DHA: 0.49±0.14 VS 2.17±0.76; Total ω-3: 0.68±0.29 VS 2.66±0.70; ω-6/ω-3: 9.49±4.70 VS 1.32±0.36). The mRNA expressions of 5-LO and FLAP were significantly lower in the cells of fat-1 mice than in those of WT mice. Accordingly, the protein levels of 5-LO and FLAP were also markedly lower in fat-1 mice than in WT mice. The LTB4 level was also lower in fat-1 mice than WT mice.

**CONCLUSIONS** Our findings demonstrate that a decreased tissue n-6/n-3 fatty acid ratio reduces 5-LO and FLAP expression. This study suggests a role for tissue n-6/n-3 fatty acid ratio in the 5-LO pathway of AA metabolism in immune cells, and a new mechanism for the anti-inflammatory effect of n-3 fatty acids, omega-3 fatty acid may play a role in the prevention of CHD.

## GW26-e1234

### A prescription of Jiashen reduces postinfarct left ventricular remodeling via inhibition of TGF-β/Smads signaling pathway

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**OBJECTIVES** To explore the mechanisms underlying A prescription of Jiashen(PJS)-mediated cardioprotection, we determined whether PJS reduces early left ventricular remodeling via inhibiting TGF-β/Smads signaling pathway in a rat model of myocardial infarction(MI).

**METHODS** Male Sprague-Dawley rats were subjected to sham-operation or MI by ligating the left anterior descending coronary artery. The rats with MI were treated with vehicle, PJS at the dose of 3 g/kg/day and 6 g/kg/day, or losartan at the dose of 10 mg/kg/day 24h after MI. The rats in the vehicle group and the sham-operation group were intragastrically given at the same dosage of normal saline. One weeks later, we determined the effects of PJS and losartan on cardiac function, and the distribution and level of collagen using echocardiography, Masson's trichrome stain and hydroxyproline assay, respectively. The myocardial expression of TGF-β/Smads signaling pathway was determined by the use of Western blot.

**RESULTS** Compared to MI+vehicle rats, PJS at the dose of 6g/kg/day reduced the increases in left ventricular end-diastolic dimension (0.82±0.04 vs. 0.66± 0.04 cm, P<0.05) and left ventricular end-systolic dimension (0.61±0.03 vs. 0.40±0.04 cm, P<0.05), and the decreases in ejection fraction(55.26±4.60% vs. 70.75±5.61%, P<0.05) and fractional shortening (25.68±2.91% vs. 36.69± 3.94%, P<0.05) at week 1 after MI. In addition, treatment with PJS at the dose of 6 g/kg/day inhibited the increased levels of collagen (10.84±0.79vs. 16.75±1.53 pg/mg protein, P<0.05) and the expression of transforming growth factor-β1(TGF-β1), phosphorylated Smad 2(p-Smad 2), and phosphorylated Smad 3(p-Smad 3) compared to MI+vehicle rats at week 1 after MI (TGF-β1: 1.06±0.01 vs. 1.39 ±0.01%GAPDH arbitrary units; p-Smad 2: 1.52±0.00 vs. 2.06± 0.03% GAPDH arbitrary units; p-Smad 3: 0.50±0.00 vs. 1.05±0.01% GAPDH arbitrary units, P<0.05). Losartan treatment has the similar results with PJS at the dose of 6 g/kg/day.

**CONCLUSIONS** Our data demonstrated that PJS treatment improved cardiac function, and reduced myocardial fibrosis at the early stage of MI. The results were associated with the inhibition of the expression of TGF-β/Smads signaling expression. Our results suggest that PJS may improve left ventricular remodeling possibly via attenuating TGF-β/Smads signaling pathway in the early period of MI.

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## GW26-e1430

### Fufangxueshuantong ameliorates diabetic cardiomyopathy in rats by attenuating cardic and metabolic dysfunction

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**OBJECTIVES** Diabetic cardiomyopathy (DCM) can cause diastolic and systolic dysfunction damage, resulting in myocardial ischemia and heart failure. Fufangxueshuantong (FXST), which is composed of *Panax notoginseng*, *Salvia miltiorrhiza*, *Astragalus membranaceus* and *Scrophularia ningpoensis*, has been used for the treatment of angina pectoris and diabetic retinopathy for years. However, whether FXST has an effect on DCM is unknown. Metabolic dysfunction occurs in hearts and contributes to DCM. Adenosine monophosphate-activated protein kinase (AMPK), peroxisome proliferator activated receptorγ coactivator 1 (PGC1α) and silent information regulator 1(sirt1) signaling pathways sense the metabolic demands and impinge on expression of genes encoding for metabolic enzymes (citrate synthase(CS), etc). So the present study aims to demonstrate that whether FXST treatment can ameliorate cardiac function in diabetic rats and to illustrate its effect on energy metabolic mechanism.

**METHODS** SD rats were randomly divided into 3 groups: normal group, diabetic group, diabetic+FXST group. 20 weeks after streptozocin induction, FXST or water was administered for 16 weeks. Cardiac dimensions and function were determined by echocardiography. Left ventricular internal diameter during diastole (LVIDd) and systole (LVIDs) were assessed in M-mode, and ejection fraction (EF), fractional shortening (FS) and left ventricular mass (LV mass) were calculated with the M-mode measurements. Doppler echocardiography was used to measure IVRT (Isovolumic relaxation time) and E/A (Ratio of peak early to late transmitral blood flow velocities). After echocardiographic detection, the indicators of energy metabolism (CS, AMPK, PGC1α, sirt1) in hearts were detected through PCR.

**RESULTS** Echocardiography revealed that in the diabetic group, a decrease in E/A and an increase in IVRT were observed in the rats (vs. normal group). Following treatment with FXST in the diabetic rats, E/A was found to be upregulated, (vs. diabetic group). In the diabetic group, a decrease in EF, FS, LV mass and an increase in LVIDd, LVIDs were observed in the rats (vs. normal group). Following treatment with FXST in the diabetic rats, EF and FS were found to be upregulated, while LVIDd and LVIDs were markedly decreased (vs. diabetic group).

Real time PCR analysis revealed decreased content of PGC1α, sirt1 and increased levels of CS, AMPK in the diabetic group when compared with the normal group. However, increased levels of (CS, AMPK, PGC1α, sirt1) were observed in the FXST-treated group when compared with the diabetic group.

**CONCLUSIONS** Our study demonstrated that diabetes induced diabetic cardiomyopathy, characterized by both diastolic and systolic dysfunction and metabolic dysfunction in heart. And FXST protected DCM via attenuating cardiac function. In addition, therapeutic FXST administration can promote gene levels of energy metabolism. These findings provide evidence as to the cardiac protective efficacy of FXST to DCM.

## GW26-e1454

### Cardioprotective effect of propofol against oxygen glucose deprivation and reperfusion injury in H9c2 cells

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**OBJECTIVES** The intravenous anesthetic propofol is reported to be a cardioprotective agent against ischemic-reperfusion injury in the heart. However, the regulatory mechanism still remains unclear.

**METHODS** In the study, we used H9c2 cell line under condition of oxygen glucose deprivation (OGD) followed by reperfusion (OGD/R) to

induce in vitro cardiomyocytes ischemia-reperfusion injury. Propofol (5-20 μM) were added to the cell cultures before and during the OGD/R phases to investigate the underlying mechanism.

**RESULTS** Our data showed that OGD/R decreased cell viability, increased lactate dehydrogenase leakage, reactive oxygen species and malondialdehyde production in H9c2 cells, all of which were significantly reversed by propofol. Moreover, we found that propofol increased both the activities and protein expressions of superoxide dismutase and catalase. In addition, propofol increased FoxO1 expression in a dose-dependent manner and inhibited p-AMPK formation significantly.

**CONCLUSIONS** These results indicate that the propofol might exert its antioxidative effect through FoxO1 in H9c2 cells, and it has a potential therapeutic effect on cardiac disorders involved in oxidative stress.

## GW26-e2343

### Echocardiography-guided percutaneous laser ablation of canine ventricular septum

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**OBJECTIVES** Currently surgical myectomy and ethanol ablation are two established interventions for relieving the left ventricular outflow obstruction in hypertrophic cardiomyopathy (HCM) patients. The limitations in safety and efficacy in these interventions call for minimally invasive, potentially safer and more efficacious approach. The aim of this study is to validate the feasibility of echocardiography-guided percutaneous per-ventricular laser ablation of the canine ventricular septum.

**METHODS** Six domestic dogs were chosen for the study. Laser (Nd: YAG, 800-1064 nm-wavelength, 300μm-diameter fiber, Echo Laser X4, Elesta S.R.L., Italy) was used. The laser passed through a needle (21G, PTC, ECOCHIBA, Italy) that inserted from the right ventricle into the targeted septum under the guidance of the echocardiography via a percutaneous route. Laser ablation was performed as follows: 1 W laser for 3 and 5 min at 180 and 300 J, respectively. Echocardiography, serology examination and pathology were performed to assess the results of laser ablation.

**RESULTS** There was no death or major complication, i.e. tamponade, pericardial effusion or ventricular fibrillation. The real-time echocardiography monitor of M-mode, 2D (LVEF), PW Doppler and TDI presented no significant variation before and after the laser ablation. Contrast echocardiography confirmed the perfusion defects in the ablated septal regions. The laser ablated areas were well demarcated on pathology examination and the diameters of the ablated region were (mm) 4.42 ± 0.57 and 5.28 ± 0.83 for 3- and 5-minute ablation, respectively. Pre- and post-ablation cardiac enzymes (IU/L) were: AST: 39.17 ± 11.23 vs 183 ± 101.07 (p=0.02), LDH: 71 ± 33.89 vs 253.33 ± 179.63 (p=0.07), CK: 468.17 ±192.42 vs 2775.17 ± 1309.35 (p=0.007), and CK-MB: 174.33 ± 113.34 vs 897.17 ± 486.84 (p=0.03). Microscopically, the ablated myocardium showed contracted coagulative changes. Nuclei disappeared and a zone of vacuoles was formed with red blood cells infiltrating into the widened intercellular space.

**CONCLUSIONS** Our research showed that percutaneous laser ablation of the septum is feasible, potentially safe and efficacious and may become a viable alternative solution to septum ablation.

## GW26-e2407

### Protective Effects of Dihydromyricetin and Myricetin against Myocardial Ischemia/Reperfusion Injury In Vivo and In Vitro

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**OBJECTIVES** The aims of this study were to determine whether Myr or DMY exert any cardioprotective effect against I/R injury and investigate the responsible underlying mechanisms.

**METHODS** The study utilized an in vitro rat cardiomyocyte H9C2 model of hypoxia/reoxygenation (H/R) injury and an in vivo rat model of MI/R injury. H/R injury was determined by Cell Counting Kit-8 (CCK-8) assay and lactate dehydrogenase (LDH) leakage assay. In the in vivo experiment, histopathology staining was examined. Additionally, cardiac injury markers maleic dialdehyde (MDA), superoxide dismutase (SOD), glutathione reductase (GSH), myeloperoxidase (MPO), nitric-oxide synthase (NOS), $Ca^{2+}$-$Mg^{2+}$-ATPase and $Na^{+}$-$K^{+}$-ATPase were detected. TUNEL assay and caspases activation assays were used to investigate apoptosis. To assess levels of apoptotic regulators, immunohistochemical staining and real-time PCR were employed.

**RESULTS** Both Myr and DMY have no cytotoxic effect at the concentrations of 0.5-80 μM Myr or 10-200 μM DMY for 24 hours in H9C2 cells. after being subject to H/R, cellular viability was significantly reduced in the H/R group ($P < 0.01$ compared to control), and LDH leakage was highly increased compared to control ($P < 0.01$). DMY or Myr post-conditioning significantly increased cell viability compared with the H/R group. DMY (25, 50 and 100 μM) or Myr (20 and 50 μM) markedly reduced H/R-induced cell death and decreased LDH leakage ($P < 0.05$ or $P < 0.01$). These results indicate that DMY and Myr significantly preserved cellular viability post-H/R injury in a dose-dependent manner. Both DMY and Myr protected myocardium against I/R (or H/R) injury by increasing NOS, SOD, GSH and ATPases activity, decreasing MDA content and MPO activity at different degrees and attenuating histopathology injury. Meanwhile, DMY and Myr inhibited the cardiomyocyte apoptosis. The level of Bcl-2 protein and mRNA were restored to the normal level by DMY or Myr pharmacological postconditioning. In contrast, the Bax protein level and mRNA level were markedly reduced by DMY and Myr pharmacological postconditioning.

**CONCLUSIONS** DMY and Myr pharmacological postconditioning could protect against myocardial I/R injury in both in vivo and in vitro models, which are related to apoptosis pathway and antioxidant activity.

## GW26-e2435

### Role of calreticulin-induced mitochondrial damage in high glucose induced apoptosis in myocardial cells

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**OBJECTIVES** To observe the effect of high glucose on the protein expression of calreticulin (CRT) and its association with cell apoptosis and mitochondrial dysfunction in the cardiomyocytes.

**METHODS** AC-16 cardiomyocytes were randomly divided into normal glucose group, high glucose group, high glucose+ CRT siRNA and isotonic control group. The cell apoptotic rate, reactive oxygen species (ROS), mitochondrial membrane potential level, respiratory enzyme activities, and protein expression of CRT were observed.

**RESULTS** Compared with the cardiomyocytes in normal glucose group, the apoptotic rate of cardiomyocytes and ROS production increased in high glucose groups, accompanying with the decreases in the mitochondrial membrane potential level and enzyme activities of the respiratory chain. The protein expression of CRT was significantly increased in high glucose group. However, compared with high glucose group, high glucose+ CRT siRNA decreased the expression of CRT and attenuated the damage of mitochondrion, but CRT siRNA did not reduce the ROS level in cardiomyocytes.

**CONCLUSIONS** High glucose brought about CRT over-expression to induce mitochondrial injury, which may be a reason of increasing myocardial apoptosis.

## GW26-e4818

### The L-carnitine Ameliorates Pulmonary Arterial Hypertension by Improving Energy Metabolism Dysfunction of Right Ventricular Failure

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**OBJECTIVES** L-carnitine is indispensable for energy metabolism and mitochondrial function in the myocardium. Although carnitine