CONCLUSIONS FF treatment restored autophagy in the WT diabetic mice but not in the FGF21-KO diabetic mice. Mechanistic study with H9C2 cells in vitro showed that autophagy, measured by cytoplasmic form microtubule-associated protein 1A/1B-light chain 3 (LC3) expression, was significantly inhibited by high glucose (HG, 30 mM) that also significantly increased inflammation, oxidative stress, and fibrosis. These HG effects were prevented by FF treatment. Inhibition of autophagy by 3-methyladenine (3MA) or inhibition of Sirt1 by sirtinol abolished FF protection against HG-induced effects. Together, these results suggested that FF could prevent DCM by inducing FGF21, which in turn enhances the Sirt1-mediated autophagy.

GW26-e1079

Comparative Proteomics Reveals Abnormal Binding of ATGL and Dysferlin on Dysfunctional Cardiac Lipid Droplets

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OBJECTIVES Excessive retention of neutral lipids in cardiac lipid droplets (LDs) is a common observation in cardiomyopathy. We anticipate that the systematic investigation of the cardiac LD proteome will help to dissect the underlying mechanisms linking cardiac steatosis and myocardial dysfunction.

METHODS LDs isolated from Sprague-Dawley rat hearts were analyzed using morphological and biochemical approaches and then subjected to iTRAQ quantitative proteomic analysis. The LD localization of the identified LD proteins was verified by immunofluorescence assays. The set of dysferlin truncation mutants were used to determine the LD-binding structure.

RESULTS 771 heart LD proteins were identified and categorized into 10 functional groups, including 467 proteins previously unreported on LDs. The LD localization of these proteins was verified by immunofluorescence assays. The most noteworthy finding was the identification of the membrane resealing protein, dysferlin. An analysis of dysferlin truncation mutants indicated that its C2 domain was responsible for its LD localization. Using the iTRAQ quantitative proteomic method we determined that the quantity of 30 proteins was increased and 16 proteins was decreased in LDs from pressure overload-induced dysfunctional heart, compared with normal hearts. Notably, adipose triacylglycerol lipase (ATGL) was dramatically decreased and dysferlin was substantially increased on dysfunctional cardiac LDs, a finding that was confirmed using immunoblotting.

CONCLUSIONS This study for the first time reveals the dataset of the heart LD proteome in healthy tissue and the variation of it under cardiac dysfunction. These findings highlight an association between the altered LD proteinlocalization of dysferlin and ATGL and myocardial dysfunction.

GW26-e1360

Effects of Sirt1 on Protection Against Atherosclerosis Plaque via Anti-angiogenesis in Mice

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OBJECTIVES Atherosclerosis (AS) is an age-related disease attributed to vascular endothelial cell injury Angiogenesis might contribute to the progression of the atherosclerotic plaque SIRT1, has been reported to have the function of anti-angiogenesis and prevent senescence In this report, the present study aims to investigate whether resveratrol, an activator of SIRT1, is capable of protecting against atherosclerosis progress via anti-angiogenesis.

METHODS 25 Apolipoprotein E-knockout mice were divided into three groups: control group (n =5) with normal diet, high-fat diet (HFD) group (n=10) for 16 weeks, high-fat diet(16 weeks)+ resveratrol (2 months) group (HFD+RES) (n=10) following with resveratrol intraperitoneal injection (100 mg/kg/day, i.p) for two months Body weight, serum total cholesterol(TC), triglyceride(TG), LDL-C, HDL-C level and oil red O stain were performed to determine the establishment of atherosclerosis model. Immunofluorescence staining of CD31 was used to detect angiogenesis in atherosclerotic plaque. In vitro HUVECs study included six groups: control group, H2O2 treated group (HO), H2O2+resveratrol group (HO+ RES), sh-SIRT1 group, sh-SIRT1+ H2O2 group (sh-SIRT1+HO) and sh-SIRT1+ H2O2+ resveratrol group (sh-SIRT1+ HO+ RES) Oxidative stress damage was induced by H2O2. HUVECs proliferation was evaluated with wound assay and transwell migration assay. HUVECs apoptosis was assessed with TUNEL. Intracellular production of reactive oxygen species (ROS) was detected by a fluorescence probe-2′,7′-dichlorofluorescin diacetate (DCFH-DA). Subsequently, Western Blot was performed to determine relative changes of proteins such as SIRT1, HIF-1α and VEGF. We also explored the dose-dependent effect of resveratrol on HUVECs subjected to oxidative stress.

RESULTS As compared with control group mice, HFD mice showed increased body weight, serum TC, TG, LDL-C level and reduced HDL-C. The size of tissues positive for oil red O expression was higher in the HFD mice ($P<0.05$), indicating the model of AS was established successfully. Subsequently, immunofluorescence staining of CD31 showed that angiogenesis in HFD+RES group significantly decreased compared with the HFD only group, indicating that resveratrol was capable of inhibiting angiogenesis in AS plaque. In vitro results revealed that H2O2 induced oxidative stress damage on HUVECs, evidenced by increased ROS generation. Furthermore, HIF-1α and VEGF expression were increased whereas SIRT1 expression was reduced ($P<0.05$). Resveratrol reversed these changes in HO group, however this effect was diminished in sh-SIRT1+ HO group. Moreover, high-concentration resveratrol inhibited tube formation and cell migration in HUVECs by contributing to apoptosis ($P<0.05$).

CONCLUSIONS High-concentration resveratrol protects against atherosclerosis and improves vascular function in HFD mice through anti-angiogenesis in plaque. SIRT1 plays an important role in the resveratrol beneficial effects against atherosclerosis.

GW26-e1553

Long-Term Treatment With Ivabradine in Transgenic Atrial Fibrillation Mice Counteracts HCN Channel Overexpression and Reduces Atrial Fibrillation Incidence

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OBJECTIVES Current studies show that ivabradine, a specific heart rate lowering drug, it selective inhibitor of funny (I_f), performing anti-arrhythmic effect of some disease condition, such as heart failure, myocardial ischemia, and so on. But little is known regarding the long-term ivabradine treatment on I_f current and prevention of atrial fibrillation (AF).

METHODS We firstly investigated the I_f current and the HCN channels expression between the wild-type (WT) mice and the transgenic mice overexpressing heart-specific (pro) renin receptor (TG), a useful mouse model of AF, and then examined the effects of ivabradine on the I_f current and HCN channels expression, and the prevention of AF episode.

RESULTS Compared with WT mice, the enhanced I_f current density (at -170mV: TG, -39.6±4.6pA/pF; WT, -26.9±3.0pA/pF, $p<0.001$) and the faster activation kinetic ($V_{1/2}$: TG, -109.45±1.35 mV; WT, -128.20±1.65 mV), as well as the mRNA of HCN2 and HCN4, accompanying the HCN4 protein were significantly increased in atrial myocytes from TG mice. After treatment with ivabradine for 4 months (7mg/kg per day orally), it partially reverses the electrophysiological remodeling occurring in TG mice, and I_f density was reduced in the atrial myocytes (at -170mV: TG, -17.7±3.0pA/pF; WT, -18.0±3.1pA/pF, $p=0.89$). The effects of ivabradine on electrophysiological remodeling were accompanied by an inhibition of upregulation of HCN2 and HCN4 protein in atrium tissue. Furthermore, we found that ivabradine significantly reduce the incidence of AF among TG mice (41.2% in TG mice, 16.7% in TG+ivabradine mice, $p<0.01$), most likely by suppressing an increase in automaticity.

CONCLUSIONS Thus, our findings provide the first evidence that ivabradine reduced the incidence of AF in mice, and the anti-arrhythmic of ivabradine was beyond the hate rate reduction, it partially due to counteract the HCN overexpression and reverse electrophysiological cardiac remodeling by reducing I_f gain-of-function.