TU-001

M22α Ubiquitination Promotes G6PD Activation and NADPH Production, Contributing to GSH Homeostasis and VSMC Survival

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Objective: To identify the relationship between dihydronicotinamide adenine dinucleotide phosphate (NADPH) production and SM22α activity in the development and progression of vascular diseases.

Methods: Vascular smooth muscle cells (VSMCs) were transduced for 24 hours with the respective adenovirus constructs, using a multiplicity of infection of 100. VSMC apoptosis was determined by TUNEL assay. For in vivo analyses using a carotid artery ligation model, 25% pluronic F-127 gel containing the adenovirus at a concentration of 10^{10} pfu/mL was spread evenly around the outside of the left carotid arteries of the subject mice. Carotid arteries were harvested 14 days after ligation.

Results: We showed that the expression and activity of glucose-6-phosphate dehydrogenase (G6PD) are promoted in platelet-derived growth factor (PDGF)-BB–induced proliferative VSMCs. Platelet-derived growth factor–BB induced G6PD membrane translocation and activation in an SM22α K21 ubiquitination–dependent manner. Specifically, the ubiquitinated SM22α interacted with G6PD and mediated G6PD membrane translocation. Furthermore, we found that tumor necrosis factor receptor–associated factor (TRAF) 6 mediated SM22α K21 ubiquitination in a K63-linked manner on PDGF-BB stimulation. Knockdown of TRAF6 decreased the membrane translocation and activity of G6PD, in parallel with reduced SM22α K21 ubiquitination. Elevated levels of activated G6PD consequent to PDGF-BB induction led to increased NADPH generation through stimulation of the pentose phosphate pathway, which enhanced VSMC viability and reduced apoptosis in vivo and in vitro via glutathione homeostasis.

Conclusions: We provide evidence that TRAF6-induced SM22α ubiquitination maintains VSMC survival through increased G6PD activity and NADPH production. The TRAF6–SM22α–G6PD pathway is a novel mechanism underlying the association between glucose metabolism and VSMC survival, which is beneficial for vascular repair after injury but facilitates atherosclerotic plaque stability.

TU-002

PGE_2 promotes biliary cholesterol excretion and attenuates diet-induced atherosclerosis by activation of EP3-mediated HNF4α/CYP7A1 pathway in liver

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Objective

Inflammation has been proposed to influence multiple aspects of cholesterol metabolism. Prostaglandin E_2 (PGE_2) is an important lipid mediator in inflammation. However, whether or how PGE_2 regulates hepatic cholesterol metabolism remains unknown.

Methods

Plasma, hepatic cholesterol and bile acid levels were assayed in western diet-fed mice. Bile acid composition in serum and liver were analyzed by LC-MS. Bile acid related genes were determined by RT-PCR and western blot.

Results

PGE_2 receptor subtype 3 (EP3) expression was upregulated in livers when exposed to a high-cholesterol diet. Deletion of EP3 receptor in liver resulted in hypercholesterolemia and augmented diet-induced atherosclerosis in mice by suppression of hepatic bile acid synthesis. CYP7A1, catalyzing the first and rate-limiting step in the bile acid synthetic pathway, was down-regulated in EP3-deficient livers. Forced expression of CYP7A1 in liver rescued the impaired biliary cholesterol excretion in EP3 deficient mice. Mechanistically, we found that EP3 regulates CYP7A1 expression via depressing PKA-dependent phosphorylation of nuclear receptor HNF4α, which reduced its transcriptional activity.

Conclusion

Our results demonstrated that EP3 receptor modulates biliary cholesterol excretion in

liver through PKA/HNF4α/CYP7A1 pathway, also provided new evidence for a direct link between inflammatory eicosanoid and cholesterol homeostasis.

TU-003

Protective Effect of Omega-3 Polyunsaturated Fatty Acid in Myocardial Infarction in Mice-- A Metabolomics Based Study

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Objective: ω-3 polyunsaturated fatty acids (PUFA) have beneficial effects on many pathological processes especially the cardiovascular disease. PUFA comprise hundreds of bioactive molecules derived from complex metabolism network. ω-3 PUFA may protective against coronary heart disease (CHD), in which their protective metabolites are thought to play an important role. However, the underlying mechanisms by which dietary PUFA protect against acute myocardial ischemia (AMI) are largely unknown. This study aimed to investigate whether ω-3 PUFA metabolites play a role in the prevention and treatment of myocardial infarction in a mouse model and explore the possible mechanism. **Method:** we established a mouse model of myocardial infarction to test our hypothesis. After feeding with chaw diet or high ω-3 PUFA diet for 3 weeks, the protective effects in Infarction size and heart function were evaluated and the plasma samples were analyzed by a metabolomic approach. **Result:** The results revealed that ω-3 PUFA diet could significantly reduce the size of infarction. A LC-MS/MS based eicosanoid metabolomic method was developed, which could measure 32 arachidonic acid (ARA) metabolites and 37 ω-3 PUFA derived products. Using this metabolomic method, we subsequently quantified eicosanoids in mouse plasma and heart with ω-3 PUFA supplementation and myocardial infarction model. Correlation network analysis on mouse plasma data indicated a obvious change of metabolic profiles amoung different groups was observed. Further, we utilized a fat-1 transgenic mice, an experimental model to endogenously synthesize ω-3 PUFA to confirm our finding with diet. In vitro, the effects of eicosapentaenoic acid (EPA) on the response of neonatal rat cardiomyocytes to simulated ischemia (SI) and hypoxia. Cardiomyocytes isolated from the hearts of new born Wistar rats were cultured with or without EPA and exposed to 12 h hypoxia followed by detection of apoptosis and cell death. In this work, a systematic eicosanoid metabolomic analysis was performed in order to study the impact of fed ω-3 PUFA diet and myocardial infarction model. **Conclusion:** we proved a metabolic evidence that the role of ω-3 PUFA in the myocardial infarction. The work also proved the highly-specific eicosanoid metabolomic method, which would be a powerful tools for systematically analyze eicosanoid metabolism in diseases.

TU-004

A Comparative Study on High-Fat Diet Induced Metabolic Abnormalities in Male and Female C57BL/6 Mice

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Background: With the rise in incidence of metabolic syndrome (MetS) among pre- and post-menopausal women, female C57BL/6 mice with perturbed metabolic state can play a role model for targeting MetS linked comorbidities. **Methods:** In this study we compared metabolic, cardiac, hepatic, pancreatic, and renal changes in male as well as female C57BL/6 mice fed with either high-fat diet (HFD) or low-fat diet (LFD) for 16 weeks. **Results:** Within both the sexes, mice fed with HFD showed a significant gain in body weight, body mass index (BMI), energy intake, and abdominal circumference. These changes were accompanied by compromised glucose and insulin tolerance, hyperinsulinemia, dyslipidemia, elevated plasma IL-6, and TNF-α concentration. Histologically, hepatocytes showed an elevated fat accumulation with mild focal swelling, suggesting the initiation of non-alcoholic steatohepatitis (NASH). This was also appended by an increase in plasma activities of liver enzymes. The pancreas showed upsurge in number of β-cells with subsequent increase in size of islet implying its compromised state. While the kidney showed mild tubulointerstitial fibrosis indicating initiation of kidney impairment, heart showed mild degenerative changes in cardiac fibres denoting absence of cardiac

remodelling due to HFD. **Conclusions:** Male and female C57BL/6 mice showed variations in physical development wherein, male mice had greater body weight, BMI, central adiposity, and energy intake as compared to female mice. Further, both male and female C57BL/6 mice fed with HFD developed features of NASH, hyperinsulinemia, dyslipidemia, impaired glucose and insulin tolerance but the magnitude of these abnormalities was found to be less in female mice.

TU-005

Role of hyperhomocysteinemia in Alzheimer's neurodegeneration and the protections

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Background: Hyperhomocysteinemia (HHcy), beta-amyloid (Abeta) overproduction and tau hyperphosphorylation are critical etiological and pathological factors in Alzheimer disease, however, whether and how HHcy affects Abeta production and tau phosphorylation are not fully understood. **Methods:** Intraperitoneal or the vena caudalis injection of homocysteine were used in rats to produce the model of HHcy; ELISA, immunohistochemistry and Western blotting with site-specific phosphor-tau antibodies were employed for measurement of Abeta, tau phosphorylation and the related protein kinases and protein phosphatases. **Results:** We found that Intraperitoneal or the vena caudalis injection of homocysteine for two weeks remarkably increased the plasma level of homocysteine. At mean time, the HHcy rats showed the increased levels of Abeta, phosphorylated tau at multiple Alzheimer-associated sites, the activity of glycogen synthase kinase-3 (GSK-3) with spatial learning and memory deficits, while the activity of protein phosphatase-2A (PP-2A) decreased. Simultaneous supplementation of folate and vitamin-B12, betaine, or a synthesized juxtaposition (named SCR1693) composed of an acetylcholinesterase inhibitor (AChEI) and a calcium channel blocker (CCB) attenuated the hyperhomocysteinemia-induced Abeta overproduction, tau hyperphosphorylation and restored the activity of GSK-3 and PP-2A with improvement of spatial learning and memory capacities. **Conclusions:** HHcy can induce Alzheimer-like histopathologies and behavioural deficits, and supplement of folate and vitamin-B12, betaine, SCR1693 can efficiently attenuate the toxic effects of HHcy in rats.

TU-006

Bilirubin mediates heme oxygenase-1-induced vascular benefits in diabetic mice

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Background. Heme oxygenase-1 (HO-1) exerts vasoprotective effects. Such benefit in diabetic vasculopathy is not clear. We have demonstrated that bilirubin mediates HO-1-induced vascular benefits in diabetes (Liu et al., 2015, Diabetes 64:1564-75). Methods. Diabetic db/db mice were treated with HO-1 inducer hemin for 14 days and aortas were used for functional and molecular studies. NO generation was measured in cultured endothelial cells. Results. Hemin treatment augmented endothelium-dependent relaxations and elevated Akt and eNOS phosphorylation in diabetic mouse aortas, which were reversed by HO-1 inhibitor SnMP or HO-1 silencing. Hemin administration increased serum bilirubin, and ex vivo bilirubin treatment improved relaxations in diabetic mouse aortas. Biliverdin reductase silencing reduced the effect of hemin. Chronic bilirubin treatment improved the relaxations in diabetic mouse aortas. Hemin and bilirubin reversed high glucose-induced reductions in Akt and eNOS phosphorylation and NO generation. Biliverdin reductase silencing inhibited the effect of hemin but not bilirubin. In addition, bilirubin augmented acetylcholine-induced relaxations in renal arteries from diabetic patients. Conclusion. HO-1-induced recovery of endothelial function in diabetic mice is mediated mainly by bilirubin, which preserves NO bioavailability through the Akt/eNOS/NO pathway, indicating that bilirubin is a potential therapeutic target for clinical intervention against diabetic vasculopathy (supported by CUHK2/CRF/12G and T12-402/13N).

TU-007

Inhibition of miR-92a Improves Endothelial Function in Diabetes

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Rational: Cardiovascular disease is a major complication of diabetes and the leading cause of morbidity and mortality. Endothelial dysfunction is the hallmark and also the trigger for the development of diabetic cardiovascular diseases. MicroRNAs (miRNAs), critical regulators of gene expression, possess a wide spectrum of biological functions including regulation of endothelial function. MiR-92a, abundant in endothelial cells, has been reported to control endothelial function through regulating several target genes in cell studies. However, the pathophysiological role of miR-92a in endothelial dysfunction in diabetic animals remains unclear, and whether inhibition of miR-92a improves endothelial function in diabetes deserves investigation.

Objective: To investigate the effect of miR-92a inhibition and subsequent signaling in the improvement of endothelial function in diabetes.

Results: The expression of miR-92a is higher in the aortas of *db/db* diabetic mice compared with those of non-diabetic *db/m*+ mice, accompanied with impaired acetylcholine-induced endothelium-dependent relaxations. Inhibition of miR-92a restores the impaired relaxations, and normalizes the decreased phosphorylation of eNOS at Ser1177 in *db/db* mouse aortae. Likewise, diabetic risk factor, advanced glycation end products (AGEs) increases the miR-92a expression in human umbilical vein endothelial cells (HUVECs), which is reversed by NF-κB inhibitor. In addition, inhibition of miR-92a recovers the diminished nitric oxide (NO) levels in AGEs-treated HUVECs.

Conclusion: The present study provides new evidence that miR-92a inhibition improves endothelial function in diabetes, likely through increasing the expression and activity of eNOS/NO signaling in endothelial cells.

TU-008

MicroRNA-18□ suppresses LXR□ expression in human neuroblastoma cells and hepatocytes

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The liver X receptor α (LXRα, NR1H3) and LXRβ (NR1H2) are members of the nuclear hormone receptor superfamily. They play a critical role in the transcriptional control of lipid metabolism. MicroRNAs (miRs) are regarded as important negative regulators of gene expression. It has been reported that miR-1/miR-206 suppress LXRα-induced lipogenesis in hepatocytes. However, the regulation of LXRβ by microRNAs hasn't been reported. In this study, we found that miR-18□ repressed LXRβ expression in both human neuroblastoma cells and hepatocytes at both mRNA and protein levels. In addition, bioinformatics analysis predicted a same putative target-site for miR-18□ located within the 3'-untranslated region (3'-UTR) of LXRβ mRNA. The luciferase reporter gene assay in HEK293 cells revealed that miR-18a directly targeted the 3'-UTR of LXRβ mRNA. Taken together, we for the first time demonstrated that miR-18□ repressed LXRβ expression by targeting the 3'-UTR of LXRβ mRNA.

TU-009

Phosphodiesterase-5 inhibition protects against the development of diabetic cardiomyopathy in type-2 diabetes mellitus

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Background: Diabetes mellitus (DM) is associated with a special heart disease, termed diabetic cardiomyopathy. The pathophysiological role of cGMP signaling has been intensively investigated in DM. The second messenger cGMP, broken down by the phosphodiesterase-5 enzyme (PDE5), has been shown to exert cytoprotective effects. We investigated the effect of chronic inhibition of PDE5 by vardenafil in type-2 DM related cardiomyopathy.

Methods: For type-2 DM Zucker Diabetic Fatty (ZDF) rats were used. ZDF Lean (ZDFL) rats served as controls. Animals received either vehicle (ZDFL, ZDF) or 10mg/kg BW vardenafil per os (ZDFLVard, ZDFVard) from 7 to 32 weeks of age. Cardiac morphology was followed by echocardiography. Left ventricular (LV) function was assessed using a pressure-volume (P-V) conductance microcatheter system. Gene expression analysis of atrial natriuretic factor (ANF; qRT-PCR),

cardiomyocyte diameter/tibia length (CD/TL) and Masson's staining (fibrosis score (FS)) were used to prove pathological myocardium hypertrophy.
Results: Cardiac hypertrophy (echocardiography: LV anterior wall thickness in systole (LVAWs): 2.81±0.1mm; relative wall thickness (RWT): 0.49±0.02; LVmass/TL: 0.30±0.01g/cm; CD/TL: 3.53±0.02µm/cm; ANF: 3.04±0.26 vs ZDFL (LVAWs: 2.53±0.04mm; RWT: 0.43±0.02; LVmass/TL: 0.23±0.004g/cm; CD/TL: 3.09±0.02µm/cm; ANF: 0.92±0.17); $p<0.05$) and fibrotic remodelling (FS: 1.05±0.09 vs ZDFL (0.57±0.13); $p<0.05$) have been observed in ZDF. Drug treatment significantly decreased myocardial hypertrophy and fibrosis (LVAWs: 2.47±0.05mm; CD/TL: 3.15±0.02; ANF: 1.39±0.21; FS: 0.59±0.08 vs ZDF; $p<0.05$) in DM. PV analysis showed impaired diastolic function and increased cardiac stiffness (time constant of LV pressure decay (τ): 9.17±0.25ms; slope of end-diastolic P-V relationship (EDPVR): 0.078±0.002mmHg/µl vs ZDFL (τ: 8.18±0.13ms; EDPVR: 0.045±0.003mmHg/µl); $p<0.05$) while contractility parameters and blood pressure remained unchanged in ZDF. Vardenafil improved diastolic parameters (τ: 8.62±0.34ms, EDPVR: 0.062±0.006mmHg/µl vs ZDF; $p<0.05$). Vardenafil did not have any effects in ZDFL.
Conclusions: We reported that chronic administration of vardenafil prevents DM associated myocardial complications. PDE5 inhibition might be an important target to improve cardiovascular outcome in diabetic patients in the future.

TU-010

Central Body Fat Distribution Attenuates Heart Rate Recovery after Maximal Exercise in Young Healthy Obese Women

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Background: Obesity causes negative changes in the hemodynamic and autonomic control, what have an adverse effect on the cardiovascular risk. The attenuation of the decline in heart rate recovery after maximal exercise test (ΔHRR) reflects a vagal dysfunction, what is an independent predictor of mortality. Thus, the aim of this study was to test the hypothesis that obese women with central body fat distribution had lower ΔHRR compared with obese women with peripheral body fat distribution. **Methods:** Fifty-one healthy young obese women with waist circumference (WC) >88 cm, were divided into two groups: With central fat distribution (CF), defined as waist-to-hip ratio (WHR) >0.85 (n=24, 33.5±1.4 y), and with peripheral fat distribution (PF) with WHR ≤0.85 (n =27, 32.3±1.3 y). All volunteers were submitted to maximal cardiopulmonary exercise test. **Results:** CF and PF showed similar body mass index (33.20 ± 0.51 vs. 33.56 ± 0.50 kg/m^2, respectively, p=0.612). As expected, CF had higher WHR (0.91±0.01 vs. 0.80±0.01, $p<0.001$) and WC (108.17±1.42 vs.102.69±1.42, p=0.009). Interestingly, despite similar BMI, CF had attenuated ΔHRR at first minute compared with PF (13.4±1.5 vs. 18.3±1.5 beats, p=0.026). In addition, ΔHRR was associated with WHR (r=-0.31, p=0.025) and WC (r=-0.38, p=0.006). **Conclusion:** Our data suggest that in young obese women, WHR is a better risk related marker of central body fat distribution that impairs the vagal autonomic function, characterized by attenuated of 1st min of heart rate recovery after maximal exercise test.

TU-011

Deficiency of Annexin-A1 Exaggerates Diabetic Cardiomyopathy in a Mouse Model of Type 1 Diabetes

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Background: Diabetes is a chronic metabolic disease associated with low-grade inflammation and increased risk of heart failure. We have recently shown that deficiency of anti-inflammatory protein

annexin-A1 (ANX-A1) exaggerates myocardial infarction; its impact on other cardiac pathologies has not been investigated. The aim of this study was to test the hypothesis that deficiency of ANX-A1 exaggerates diabetic cardiomyopathy in Type 1 diabetic (T1D) mice.

Methods: T1D was induced in 6-week-old *ANX-A1*$^{+/+}$ and *ANX-A1*$^{-/-}$ male mice via streptozotocin (55mg/kg/day i.p. for 5-days), and mice followed for 16wks. At study end, cardiomyocyte hypertrophy, cardiac inflammation, remodelling, and dysfunction were assessed. Blood glucose and body weight were monitored fortnightly.

Results: T1D significantly increased blood glucose levels, with cardiac inflammation and remodelling; cardiac function was also impaired (See Table). Interestingly, cardiac inflammation and remodelling (but not diastolic dysfunction) were further exaggerated in *ANX-A1*$^{-/-}$ T1D mice.

Conclusion: This study was the first to demonstrate the deficiency of *ANX-A1* exacerbates diabetes-induced cardiomyopathy in T1D. ANX-A1 may thus represent a therapeutic target for the management of diabetes-induced heart failure.

RESULTS:	***ANX-A1*$^{+/+}$**		***ANX-A1*$^{-/-}$**	
	non-diabetic	**diabetes**	**non-diabetic**	**diabetes**
n	10	9	8	6
Systemic characteristics				
Blood glucose (mM)	8.7±0.2	30.3±1.0*	9.4±0.4	30.6±1.4*
Final bodyweight (g)	33.4±0.6	28.7±1.0*	31.1±0.3	25.8±0.6*
Cardiac inflammation and remodelling				
LV macrophage content (AU)	64.8±2.0	64.1±2.2*	61.9±3.6	92.1±5.0*$^{\#}$
β-MHC:18s mRNA(fold)	1.0±0.3	5.1±1.4*	1.0±0.5	11.1±5.3*
CTGF:18s mRNA (fold)	1.00±0.23	2.02±0.16*	0.92±0.19	1.50±0.36*
Cardiac collagen content (AU)	4.1±0.4	8.8±0.4*	4.2±0.4	11.7±0.1*$^{\#}$
Cardiac function				
LV E:A ratio (AU)	1.95±0.08	1.50±0.08*	1.81±0.10	1.43±0.11*
LV-dP/dt (mmHg/s)	9340±560	8070±284	8710±514	7181±890
LV+dP/dt (mmHg/s)	12100±652	9940±553	9100±444	7860±1020

*p<0.05 genotype non-diabetic counterparts; $^{\#}$p<0.05 vs diabetic ANX-A1$^{+/+}$ (2-way ANOVA, followed by Tukey's post-hoc test). β-MHC, β-myosin heavy chain; CTGF, connective tissue growth factor.

TU-012

β-adrenergic and AMPK signaling regulates cardiomyocyte glycogen autophagy in metabolic stress settings.

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Autophagy disturbance and glycogen mishandling have been observed in the diabetic heart. We have recently demonstrated that an autophagy process specific for glycogen ('glycophagy') is modulated by metabolic stress and is an important regulator of glycogen content in the heart. The aim of this study was to investigate the upstream glycophagy signaling mechanisms.

Excised hearts from type 1 (STZ rat) and type 2 (db/db mouse) diabetic rodents were analyzed for glycogen content, and expression of glycogen regulatory enzymes. Fixed heart tissue was processed and imaged by electron microscopy. β-adrenergic signaling activation by 10-6M isoproterenol perfusion of isolated rat hearts was used to determine β-adrenergic involvement in glycophagy response in non-diabetic hearts. A role for AMPK signaling was investigated using 1mM AICAR treatment (AMPK activator) of neonatal rat ventricular myocytes (NRVMs) cultured in 5mM or 30mM glucose.

In vivo cardiac glycogen was elevated in type 1 and type 2 diabetic rodent models (3.9-fold and 1.9-fold respectively), and this was not associated with changes in glycogen synthase and phosphorylase activation. Glycophagy involvement was evidenced by accumulation of glycogen in phagosome double-membrane structures visualized by electron microscopy. Ex vivo isoproterenol-induced β adrenergic activation markedly increased expression of glycophagy markers, GABARAPL1 and acid α-glucosidase (3 fold and 1.5-fold respectively, $p<0.05$) coincident with depletion of glycogen content (71% lower after 5min, fully depleted after 60min, $p<0.05$). In vitro, activation of AMPK attenuated high glucose-induced glycogen accumulation in NRVMs with no change in phosphorylase activation, suggesting a role for AMPK stimulated glycophagy-breakdown of glycogen.

This is the first study to show that diabetes-induced cardiac glycogen accumulation is linked with induction of glycophagy. Furthermore, these findings suggest that β adrenergic and AMPK signaling positively regulate glycophagy. Glycophagy may be an important new target for rescue of diabetic cardiomyopathy and further mechanistic interrogation of these signaling pathways is warranted.

TU-013

Aromatase expression in the myocardium and pericardial adipose – a potential arrhythmogenic modulator?

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In obesity, increased pericardial adipose deposition is associated with elevated incidence of atrial fibrillation. Estrogen-only hormone supplementation therapy also increases risk of atrial fibrillation. Adipose is a major endocrine/paracrine tissue capable of sex steroid synthesis, converting testosterone to estrogen via aromatase action. Thus links between adipose, aromatase, estrogen and arrhythmia are postulated. The aim of this study was to identify a potential role for pericardial fat-derived aromatase in regulating local cardiac sex steroid balance and arrhythmia propensity.

Atrial appendage tissues were obtained from coronary artery bypass patients. In parallel studies, myocardium and pericardial adipose were excised from anaesthetized male/female Sprague Dawley rats (SpD), from Hypertrophic Heart Rats (HHR) and control Normal Heart Rats (NHR). Using Western immunoblotting, aromatase was detected in both human and rat myocardium and pericardial adipose. In SpD, aromatase expression was greater in female myocardium (female vs. male, arb.units; 1.30 vs. 0.97, $p<0.05$) and pericardial adipose (1.68 vs. 0.75, $p<0.05$). In both male and female SpD, aromatase levels were approximately 30-fold greater at 50wks (aged) vs 8wks young adult controls (50wk vs. 8wk, arb.units; female 2.139 vs. 0.044, $p<0.05$; male 1.022 vs. 0.033, $p<0.05$). Aromatase expression was increased in both male/female hearts with an underlying pathological hypertrophy (HHR vs. NHR, arb.units; female 1.158 vs. 2.139, $p<0.05$; male 1.218 vs. 1.022, p=ns) compared with NHR controls.

This is the first study to show aromatase expression in atrial and pericardial adipose tissue. Data indicate the pathophysiological importance of aromatase is modulated according to sex, age and hypertrophic status. These findings suggest that increased pericardial adipose deposition (ie in aging & obesity) provides capacity for augmented steroid conversion through elevated levels of aromatase. The paracrine actions of locally synthesized estrogens in the heart may exert important influence on myocyte contractility and viability, and on arrhythmia vulnerability.

TU-014

Effects of perindopril on cardiovascular function in middle- aged diet-induced rat models of the metabolic syndrome

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RAAS blockade remains a mainstay of cardiovascular pharmacology for the treatment of hypertension, heart failure, left ventricular hypertrophy, vascular dysfunction, diabetes and renal disease yet its role in modulating body mass and weight loss following the metabolic syndrome is yet to be fully established. This study aimed to assess the effect of perindopril (P) on preventing cardiovascular dysfunction in animal models of metabolic syndrome with diet induced obesity and hypertension. Sixteen week old male WKY and SHR rats were randomly assigned to one of eight treatment groups; WKY, WKY+P WKY-HFHC, WKY-HFHC+P, SHR, SHR+P, SHR-HFHC, and SHR-HFHC+P. Rats in HFHC groups were fed a high fat high carbohydrate diet for a period of 20 weeks, while control rats were fed standard chow. Treatment with perindopril (1mg/kg/day) was administered to rats for 12 weeks commencing at week 8 of the 20 week treatment period. Perindopril treatment had significant impacts on body weight and fat mass (WKY-HFHC - 31±1*mg/g bwt; WKY-HFHC+P – 19±3**mg/g bwt) in HFHC fed animals, preventing obesity-induced cardiovascular dysfunction in these animals. Perindopril treatment also prevented the development of hypertension in normotensive HFHC fed rats (WKY-HFHC - 162±3*mmHg; WKY-HFHC+P -

136±4**mmHg). Improvements in a number of metabolic parameters were also noted. Decreased oxidative stress, improved lipid profiles and vascular function, in addition to prevention of cardiac fibrosis and electrical dysfunction were observed in obese rats with and without genetic hypertension. It was also found that perindopril had very little anti-hyperglycaemic effect in these rats indicating that the beneficial effects observed in this study occurred independently of any blood glucose lowering activity. Perindopril's antihypertensive effects have been extensively studied in various hypertensive disease contexts; however this study has provided some insight into perindopril's effects in obesity and the metabolic syndrome, intervening at both primary and secondary end points.

TU-015

Protein content of serum exosomes are correlated to atherosclerosis

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Cell-derived exosomes have been demonstrated to be efficient carriers to transfer proteins and other cellular contents to surrounding or distant cells. An exosome can be beneficial or harmful, depending on the cell it comes from. Atherosclerosis is one of the main reasons of coronary heart disease. Since arteries expose to serum constantly, we believe the proteins in serum exosomes are closely related to atherosclerosis. Exosomes were isolated from sera of age-match healthy and atherosclerosis patients using a method combining commercial kit and ultracentrifuge. The will identify by electron microscope, Nanosize and biomarkers. The protein contents of the exosomes were analysed by protein mass spectrometry. We found that in exosomes isolated from the sera of atherosclerosis patients contained significantly more proteins that promote inflammation, immune reaction and proteinase activity. In the mean times, proteins responsible for metabolism and transportation of lipid and cholesterol as well as for proteinase inhibition were decreased. The changes of the proteins were proportional to the size of atherosclerosis plaque. Thus, increased bad exosomes in serum may be an etiological factor of atherosclerosis. Further identification of the source of bad exosomes may reveal new mechanisms and risk factors of atherosclerosis.

TU-016

Common Variation in WNK1 and Blood Pressure Responses to Dietary Sodium or Potassium Interventions: A Family–Based Association Study

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Objects: WNK1(With No-lysine Kinase 1) could regulate numerous sodium or potassium transport related ion channels involved in sodium or potassium transport in the kidney, and involve in blood pressure. Common variations in WNK1 were associated with hypertension and sodium or potassium homoeostasis. However, because of interference between gene and environment interactions, it is difficult to fully detect genetic contribution of WNK1 gene polymorphism to BP variability. Our aim was to detect the effect of common WNK1 variants on the shift of blood pressure under strict dietary intervention of salt or potassium intake.

Methods: 342 subjects from 126 families were selected from a rural community of Northern China. They were sequentially maintained on normal diet for 3 days at baseline, a low-salt diet for 7 days (3 g/day, NaCl), then a high-salt diet for 7 days (18 g/day), and high-salt diet with potassium supplementation for another 7 days (4.5 g/day, KCl). Five single nucleotide polymorphisms were selected from WNK1 gene. Single marker and haplotype analyses were conducted using the Family Based Association Test program.

Results The data shown that rs880054 and rs12828016 were associated with DBP response during low-sodium or high-sodium intervention, and rs2301880 was significantly associated with SBP, DBP and MAP responses to high-sodium intervention (all $P<0.05$). Regretful, no associations for WNK1 SNPs and the constructed haplotype blocks of WNK1 with blood pressure responses to high-salt-and-potassium supplement intervention reached nominal statistical significance.

Conclusions: Our data support the hypothesis that the WNK1 gene might be

mechanistically involved in the variation in blood pressure response to dietary sodium and potassium intake among individuals , and these genetic variants might contribute to the variation of this complex phenotype.

Key Words: blood pressure; gene polymorphism; potassium; sodium; WNK1

TU-017

High Salt Intake Fail to Enhance Plasma Adiponectin in Normotensive Salt-Sensitive Subjects

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Objects: Evidences show that salt could modulate adiponectin and inflammation level in normal individuals. Therefore, we hypothesized that abnormalities of adiponectin and inflammation may be the potential mechanism of salt sensitivity. Aims of the study were to investigate whether different alteration of adiponectin and inflammation level in response of high salt were exhibited between normotensive salt sensitive and salt resistant subjects.

Methods 30 normotensive subjects (aged 25 to 50 years) were selected from a rural community of Northern China. All of the people were sequentially maintained on 3 days baseline investigate, a low-salt diet for 7 days (3 g/day, NaCl), then a high-salt diet for 7 days (18 g/day). ***Results:*** Salt-sensitivity was diagnosed in 10 subjects who exhibited a response of the increase in mean BP by ≥10% from low-salt period to high-salt period. Plasma adiponectin higher significantly in high salt intake than low salt diet(6.1±1.3vs7.1±1.7μg/ml, *P*=0.047) in normotensive salt resistant subjects but not in normotensive salt sensitive subjects (6.4±2vs5.9±2.1μg/ml, *P*=0.481). High salt intake increased markedly plasma TNF-α(*P* <0.0001) and MCP-1(*P*<0.0001) in normotensive salt sensitive subjects as well as normotensive salt resistant subjects. No significant change of plasma hs-CRP was observed.

Conclusions: Our data indicates that the disturbance of adiponectin exists in normotensive salt sensitive subjects during high salt diet, which may be a novel underlying mechanism of salt sensitivity.

Keyword sodium-dependent, adiponectin, inflammation, normotensive

TU-018

Effects of renin-angiotensin system inhibitors on renal expression of renalase in Sprague-Dawley rats fed with high salt diet

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Objects: To investigate the effect of a high salt diet on renal expression of renalase and the potential role of local renin-angiotensin system (RAS) in this process.

Methods: Sprague-Dawley (SD) rats were divided into normal-salt (NS), high-salt diet (HS), high-salt intake with hydralazine group (HS+H), high-salt diet with enalapril group (HS+E) and high-salt diet with valsartan group (HS+V), for 4 weeks. Systolic blood pressure (SBP) was monitored. Blood and urine samples were collected at the end of intervention. Renin activity, angiotensin II (Ang II) and Ang II type 1 receptor (AT1R) were detected by real-time PCR. Renalase mRNA and protein were measured by real-time PCR, western blot and immunohistochemistry.

Results: After 4 weeks, SBP and proteinuria were significantly increased in HS versus NS group. Dietary salt intake caused a dramatic decrease in expression of renalase in kidney. Renal cortex renin, Ang II and AT1R increased significantly in HS and HS+H. Urinary protein was positively correlated with renal renin, Ang II and AT1R. In addition, in HS+E and HS+V, enalapril or valsartan failed to influence renal expression of renalase but abolished the increase of proteinuria, renal cortex renin, Ang II and AT1R when compared with HS.

Conclusion: The present study indicates that a high salt intake reduces the renal expression of renalase, and renal RAS may be not involved in the regulation of renalase in SD rats fed with high salt.

Keywords: renin-angiotensin system; renalase; salt; proteinuria

TU-019

Efficacy and safety of losartan/amlodipine single pill versus free combination at the same dose in hypertensive patients with metabolic syndrome

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Background: The blockage of the RAS through ARB aids in slowing down the processes of endothelial dysfunction and subsequent atherosclerosis. This results in reduced oxidative stress, improved vasodilation and improved endothelial function. The renin-angiotensin system (RAS) is a common link between hypertension and comorbidities of obesity and metabolic syndrome (MS). CCBs inhibit the flow of extracellular calcium through ion-specific channels that span the cell wall. This causes vascular smooth muscle cells to relax and thereby results in vasodilation, blood pressure lowering and reduced peripheral arterial resistance.

Aim of this work to estimate the effects of losartan 50 mg/amlodipine 5 mg in single pill versus free combination of losartan 50 mg + amlodipine 5 mg.

Material and method: 82 patients with MS who have second or third stage hypertension were enrolled in this study (aged 48-65 years old (mean: 53±8). Anthropometric and laboratory data obtained at baseline and at the 4rd, 8th, and 12th months of follow-up were compared in the two groups.

Results: After 1 month of the treatment BP was well controlled in both treatment groups, however, patients under single pill combination tended to show a better positive response to the treatment than patients under free combination (87.4% vs. 81.2%; P<0.05) and higher percentage of controlled patients (88.3% vs. 77.9%; P<0.05). At week 12, office SBP (22.4±12.9 vs. 21.1±13.8; P<0.002), and DBP (16.2±8.4 vs. 13.4±8.2; P<0.02) decreases were still in favor of the single pill leading to high levels of response to the treatment (88.4% vs. 86.2%; P<0.05) and BP control (82.2% vs. 81.2%; P<0.02). All treatments were well tolerated.

Conclusions: Losartan 50 mg /Amlodipine 5 mg in single pill tend to show better positive response and higher percentage of controlled patients already after one month of treatment compared to a free combination in patients not controlled by previous antihypertensive therapy in patients with metabolic syndrome.

TU-020

Cardiogenetics Mapping of Cardiovascular Diseases and Using Those Variants as a Biomarker

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Genetic variation is a rich source of knowledge for cardiovascular disease because many, if not all, cardiovascular disorders are highly heritable. Genetic risk scores are a useful tool for examining the cumulative predictive ability of genetic variation on cardiovascular diseases (CVDs). Important considerations for creating genetic risk scores include the choice of genetic variants, biochemical parameters, and ethnicities.

The questions still remain about the ultimate clinical utility of the genetic risk score, further investigation in high-risk populations and new ways to combine genetic risk scores with traditional risk factors may prove to be fruitful.

To investigate the CVD genetic risk score profile, we compared 144 subjects with a cardiac problem and 180 without; we based on HapMap, 1000 genome and dbSNP datas and picked previously identified 36 different SNPs on 24 different genes that are suggested to have association with CVDs for different populations. This study is the first analysis of the highest SNP coverage that shown the association of genetic variants with CVDs in North Cyprus. Our data is the first data shown the association of all 24 gene and 36 polymorphism to CVD and thus these data are demonstrating the cardio-genetic profile of North Cyprus. North Cyprus has a unique mixture of allele distribution for each SNP to the other close by country neighbors. Thus, SNP-SNP interactions and also their relation with biochemical pathways might play critical role for developing genetic related diseases like CVD, metabolic syndromes etc. To conclude, this study will

help for understanding the genetic profile of CVDs in the Island and also will be great source and useful tool for prevention of CVDs.

TU-021

Enhanced CD34 expression was an potential independent prognostic factor for breast cancer

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The aim of the present study was to investigate the immunohistochemical expression of cluster of differentiation (CD)34 and vascular endothelial growth factor (VEGF) in breast cancer tissue, and their prognostic significance. High CD34 expression levels (microvessel density, >15/HPV) were identified in 27.3% (12/44) of cases, exhibiting no significant correlation with the clinicopathological characteristics of the patients. However, Kaplan-Merer analysis demonstrated that the survival time of patients with high CD34 expression was significantly shorter than that of patientow CD34 expression (50.0% vs.90.6%; P=0.003) Samples with high VEGF expression levels (++or+++) accouunted for 63.6% (28/44) of the total number of cases. High VEGF expression was significantly prevalent in patients aged ≥50 years conmpared with patients aged <50 years (78.6% vs.37.5%;P=0.006).Furthermore, all patients with vascular invasion exhibited high VEGF expression levels; thus, patients with vascular invasion presented with significantly higher VEGF expression rates conmpared with patients with no vascular invasion(100% vs.55.6%;P=0.018). However, Kaplan-Merer analysis demonstrated that high VEGF expression was not correlated with the overal survival of the patients (P=0.366). By contrast, Cox multivariate analysis identified that clinical stage, triple-negative subtype and age were independent prognostic factors for patients with breast cancer (P=0.005, P=0.006 and P=0.032, respectively), and that CD34 expression was a potential independent prognostic factor (P=0.055).Therefore, the present study determined that for patients with breast cancer, a high level of CD34 expression may be a potential indicator of a poor prognosis.

TU-022

High fat diet increases the activity of cardiac ryanodine receptors in lipid bilayers

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Mice fed with high fat diet become obese in a few weeks and develop cardiac hypertrophy after 4 month. Intracellular calcium plays a key role in cardiac physiology and pathology but calcium handling proteins in the heart of obese animals has not been characterized. Activity of ryanodine receptors (RyR2), the calcium release channels of the sarcoplasmic reticulum (SR), is redox dependent. Since obesity induces oxidative stress, we hypothesized that a redox dependent change in RyR2 activity occurs in obese mice. Therefore we investigated single channel activity of RyR2 incorporated in planar bilayers.

Single RyR2 channels present in SR vesicles obtained from mice hearts can be classified, according to their response to cytoplasmic calcium, into low, moderate or high activity. Channels from hearts of animals fed with control diet exhibit moderate activity with higher frequency (15 out of 21 channels) and low or high activity with lower frequency (3 out of 21 channels in each case). In mice fed with high fat diet, 10 out of 19 RyR2 channels recorded, displayed high activity while 8 showed moderate and only 1 channel showed low activity. Therefore, high-fat diet induced a marked change in the distribution of RyR2 responses increasing the fraction of high activity channels from 14 % to 53 %, and reducing the fraction of moderate activity channels from 71 % to 42 % and that of low activity channels from 14 % to 5 %. Addition of apocynin to the diet had no effect on channel activity in control mice, but prevented the change induced by the high fat diet. Therefore, high fat diet increases the sensitivity of RyR2 channels to calcium, favoring calcium-induced calcium release, probably via a redox dependent mechanism.

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TU-023

Tetrahydroxystilbene glucoside inhibits excessive autophagy and improves microvascular endothelial dysfunction in prehypertensive spontaneously hypertensive rats

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Aims: Autophagy exists in vascular endothelial cells, but the relationship between autophagy and vascular dysfunction in hypertension remains elusive. This study aimed to investigate role of autophagy in vascular endothelial dysfunction in prehypertension and hypertension, and underlying mechanisms. Furthermore, we determined if and how tetrahydroxystilbene glucoside (TSG), the active ingredient of *Polygonum multiflorum Thunb* with cardiovascular protective properties in Chinese medicine, influences vascular endothelial function. **Methods:** Age-matched male spontaneously hypertensive rats (SHRs) and Wistar Kyoto rats (WKY) aged 4 weeks and 12 weeks were randomized into 4 groups and treated for fortnight by gavage with a) vehicle (normal saline), b) TSG (100 mg/kg/day), c) rapamycin (i.p., 1 mg/kg/day), or d) TSG + rapamycin, and the vascular function of their isolated aorta and mesenteric artery was assessed *in vitro*. HUVECs were incubated serum-starved to induce excessive autophagy, and then incubated with DMEM and treated with a) 10 nmol/L insulin-like growth factor 1 (IGF-1), b)100 μmol/L TSG, c) pre-treated with rapamycin for 1 h and further incubated with TSG. **Results:** Compared with WKY, young and adult SHRs showed endothelial dysfunction of the aorta and mesenteric artery, along with decreased pAkt, pmTOR, and autophagic marker protein p62 and increased LC3 II/I in microvascular but not aortic tissues. TSG administration for fortnight significantly improved mesenteric vascular endothelial function, increased levels of pAkt and pmTOR, and decreased autophagy. Pretreatment of young SHRs with the mTOR inhibitor rapamycin blocked the antiautophagic and vasodilative effects of TSG. Moreover, TSG significantly activated Akt-mTOR signaling in HUVECs and reduced the autophagic levels *in vitro*, which were almost completely blocked by rapamycin. **Conclusions:** Microvascular endothelial dysfunction in prehypertensive SHRs is attributable to excessive autophagy in vascular tissues. TSG partly restores microvascular endothelial dysfunction through activating Akt/mTOR pathway and consequently suppressing autophagy.

Keywords: Autophagy; Prehypertension; Vascular endothelial dysfunction; Mesenteric arteries.

TU-024

The effects of epicatechin on vascular smooth muscle cells in an animal model of obesity.

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Background

Metabolic syndrome (MetS) is a significant public-health challenge worldwide leading to CVD and cardiovascular dysfunction. Flavonoids, such as epicatechin have been shown to prevent the development and progression of cardiovascular disease associated with obesity, however the precise mechanisms remain unknown. Therefore, the aim of this study was to assess the vascular response of epicatechin in tissues from an animal model of obesity.

Methods

18 male Wistar rat were randomly divided in two groups (Control (n=10) or High-Fat High-Calorie (HFHC) (n=8)). HFHC animals were treated for a period of 20 weeks, after which assessment of biometrics, organ weight and vascular function were made.

Results

HFHC treated animals demonstrated a significant increase in body weight (C – 658.81±11.64; HFHC – 771.42±23.4*g), fat mass, serum glucose (C – 8.88±0.87; HFHC – 11.59*mmol/L), cholesterol and triglycerides and left ventricular organ mass and a significant decrease in serum nitric oxide levels. HFHC mesenteric arteries demonstrated no change to sodium nitroprusside or noradrenaline but exhibited a reduced relaxation to acetylcholine. Concentration-response curves revealed epicatechin alone did not alter vasoreactivity in either control or HFHC animals. In pre-contracted arteries, epicatechin induced a significant relaxation in control animals that was reduced in HFHC animals. In contrast, epicatechin alone induced a significant contraction in aortas from HFHC animals whilst no

change was observed in control tissues. In pre-contracted aortas, epicatechin caused a significant relaxation in control animals that was reduced in HFHC animals.

Conclusion

Results suggest a diet high in fat and carbohydrates may contribute to the development of metabolic syndrome and its associated cardiovascular complications. In healthy animals, epicatechin may improve cardiovascular function by inducing nitric oxide dependent vasorelaxation in conduit and resistant arteries, suggesting a diet rich in flavonoids may improve cardiovascular health. However, in endothelium compromised individual, consumption of epicatechin will achieve minimum cardioprotective effects.

TU-025

THE LACK OF TOLL LIKE RECEPTOR 4 DID NOT PREVENT THE DIABETES-INDUCED CARDIAC ELECTRICAL CHANGES

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Background and Aims: Different studies have shown the important role of inflammation in Diabetes Mellitus (DM). In the last decade the presence and function of cardiac Toll Like Receptors 4 (TLR4), which were typically associated tothe innate immune system, has been studied. Several groups demonstrated that the lack of this receptor can prevent distinct cardiac diseases, such as cardiac hypertrophy. In the present work we investigated whether the lack of TLR4 could prevent the DM-induced cardiac and renaldysfunction. **Method:** male wild type and $TLR4^{-/-}$ mice were used. In order to induce diabetes both groups were treated with streptozotocin (STZ: 50mg/kg/day/i.p for 5 days). ECG was recorded 8 weeks after DM induction, when all animals were euthanized. Intracellular microelectrodes were usedfor ventricular action potential recordings. Urea and creatinine in serum was measured by colorimetric tests. qRT-PCR was used to assess vimentine mRNA expression. **Results:** Cardiac electrical remodeling was observed in wild type diabetic mice. This remodeling resulted in longer QT and QTc interval and a corresponding delay in repolarization (phase 3) of the cardiac action potential. The lack of TLR4 did not prevent/improve these cardiac electrical changes. In contrast,while the DM-mice presented impaired renal function the $TLR4^{-/-}$ diabetic mice showed conserved kidney function. Similar urea and creatinine levels and comparable vimentin mRNA expression in the $TLR4^{-/-}$ diabetic mice when compared to either wild type or $TLR4^{-/-}$ non-diabetic mice were observed.**Conclusions:**even though TLR4has been reported as an important key molecule in cardiac diseases, such as cardiac hypertrophy, in DM model this receptor is not involved in cardiac electric remodeling. However, the presence of TLR4 appears important in the pathogenesis of DM-induced renal diseases.

TU-026

Carbonic anhydrase and ion transporters in diabetic cardiomyopathy

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Diabetic cardiomyopathy (DC) describes diabetes-associated changes in the structure and function of the myocardium which commonly leads to heart failure. Myocardial intracellular pH (pHi) in the heart is regulated by acid/base transporters (ABT) such as the NHE1 Na^+/H^+ exchanger, the NBC Na^+/HCO_3^- cotransporter, and the AE Cl^-/HCO_3^- exchanger, among others. pHi alterations lead to changes in heart function and changes in the activity/expression of NHE1, NBC, and AE have been associated with cardiac disorders. Conversely, carbonic anhydrases (CAs) enzymes are widely distributed in all organs and tissues, catalyzing the reversible conversion of CO_2/HCO_3^-. Functional/physical interaction between CA and the AE, NBC, and NHE ABT occurs in cardiac muscle cells, maximizing ion fluxes, creating a membrane transport metabolon (MTM). Herein, we study the role of the MTM in cardiac dysfunction linked to obesity and DC. We characterized the expression and function of CA, and NHE1 and NBC ABT, using an obese mice model

(C57BL6 $ob^{-/-}$ mice). So far our results showed DC features in the heart of $ob^{-/-}$ female mice starting as soon as 8 weeks old, characterized by an increased septum thickness and posterior wall thickness, and increased left ventricular diameter. Furthermore, $ob^{-/-}$ mice had increased left ventricular mass and left ventricular mass index, indicating cardiac hypertrophy. To study the role of NHE1 in the $ob^{-/-}$ and wild type ($ob^{+/+}$) mice, isolated cardiomyocytes were loaded with the BCECF-AM fluorescent dye and the NHE1-dependent pHi recovery measured in myocytes subjected to NH_4Cl-induce acid loading, monitored by epifluorescence. Preliminary results showed increased NHE1 activity in the hypertrophic myocardium of $ob^{-/-}$ compared to $ob^{+/+}$ mice, 0.30 ± 0.02 vs. 0.20 ± 0.02 pH units.min^{-1}.100, respectively (n= 5, $p<0.01$), measured in isolated cardiomyocytes. We conclude that activation of NHE1 is a component that may prompt and/or accentuate NHE1-induced myocardial pathology in the DC.

TU-027

High intensity exercise reduces fibrosis and hypertrophy but not oxidative stress in diabetic cardiomyopathy

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Diabetic cardiomyopathy refers to the cardiac manifestations observed in the heart as a result of altered glucose homeostasis that is reflected as fibrosis, cellular hypertrophy, increased sources of oxidative stress, such as the NADPH oxidases (NOX), apoptosis, and finally systolic and diastolic dysfunction. Exercise is known to exert salutary effects on cardiovascular function, mainly through the increase in the expression of nitric oxide synthase, particularly eNOS.

Aims: We tested the hypothesis that chronic exercise could reverse the cardiac maladaptations and oxidative stress that are produced by diabetes.

Methods. Diabetes was induced in Sprague-Dawley rats by a single dose of alloxan (200/mg kg, i.p). Diabetic rats were randomly assigned to a sedentary group (n=5) or submitted to a program of exercise on a motor-driven treadmill (80% of maximal aerobic capacity) 5 days/week, for 4 weeks (n=5). Another group of normoglycemic rats was used as control (n=5). Cardiac fibrosis was evaluated by Sirius red staining, hypertrophy was estimated measuring the perimeter and cross sectional area of cardiac myocytes in haematoxylin & eosin stained sections. The levels of NOX and NOS enzymes were evaluated by real-time PCR and Western Blotting. Cardiac levels of tetrahydrobiopterin were analyzed by HPLC.

Results. Chronic exercise reduced cardiac fibrosis: 4.43 ± 0.9 % control, 8.68 ± 0.7 % diabetic and 5.72 ± 0.7 % diabetic + exercise, $p<0.05$, ANOVA. Cellular hypertrophy was also reduced in diabetic rats by exercise: myocyte perimeter 297 ± 17 µm2 in control group, 446 ± 26 diabetic group and 363 ± 14 diabetic + exercise; myocyte perimeter: 73 ± 7 µm in control group, 89.5 ± 4.3 diabetic group, 78.7 ± 2.7 diabetic + exercise, $p<0.05$. Biochemically, exercise increased the levels of the NADPH oxidases NOX2 and NOX4 mRNA levels ($p<0.05$, ANOVA). Neither diabetes nor exercise induced changes in the levels of cardiac eNOS ($p=0.4139$). On the contrary, diabetes increased the level of uncoupled eNOS, evaluated as the ratio of eNOS dimer/monomer: 1.3 ± 0.36 in control group, 0.38 ± 0.04 diabetic group and 0.26 ± diabetic + exercise, $p<0.05$. Furthermore, exercise was unable to restore the intracardiac levels of tetrahydrobiopterin, an essential cofactor for NOS activity, that were reduced in diabetic rats: 2.69 ± 1.3 nmol/L in control group, 0.31 ± 0.04 diabetic group and 0, 36 ± 0.06 in diabetic + exercise, $p<0.05$.

Conclusions. These results suggest that chronic exercise is able to reverse cardiac remodelling in the diabetic heart, but is unable to restore the nitroso-redox imbalance imposed by oxidative stress. This later could by restored by pharmacological manipulations.

TU-028

Secondary (symptomatic) hypertension in patients with metabolic syndrome

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Aim: To identify secondary hypertension (SHT) among patients with metabolic syndrome (MS).

Materials and methods: In Bakulev SCCVS, from 2010-2015, 599 patients aged 48-79 years were diagnosed with MS and arterial hypertension (AH). The duration of hypertension was 9 + 15 years.
Results: For the examination of the patients were used medication samples, dynamic renal scintigraphy, ultrasound of the aorta, the great arteries, multislice computed tomography (MSCT) and magnetic resonance imaging (MRI) with the introduction of contrast agents into the abdominal cavity and retroperitoneal space. In a comprehensive study of 599 patients in 25% patients the diagnosis of MS could not be confirmed and were identified as various types of SHT. Of the 599 patients with MS, parenchymal (nephrogenic) hypertension was diagnosed in 17.4%, Reno vascular hypertension with lesions of arteries in 2.2%. occlusion of renal arteries with shrinking of kidneys in 0.4% patients. With the help of MSCT and MRI with contrast in 5.4% patients were identified changes in the adrenal glands. Adrenal pheochromocytoma was diagnosed in 2.2% patients, Conn's syndrome in 3.3% patients. Conservative therapy and surgical interventions for treating SVT showed good and satisfactory effects in 75-85% patients, helped in minimizing the dosages antihypertensive drugs, improved the quality of life.
Conclusion: Thus, a thorough examination of patients with highly informative diagnostic methods (ultrasound, MDCT, MRI) helped in an early diagnosis SHT, significantly reducing the proportion of MS.

TU-029

The impact of *diabetes mellitus* on miR expression of patients with or without heart failure

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Diabetes mellitus (DM2) is an important risk factor for coronary artery disease (CAD). However, the direct involvement of DM2 in the pathogenesis of heart failure (HF) is still under investigation. The objective of this work was to assess changes in miR expression in diabetic patients with or without HF and to look for possible targets of these miRs. Based on their clinical profiles, patients were divided into 4 groups: CAD (n=9), CAD+DM2 (n=11), both with normal cardiac function, HF (n=13) and HF+DM2 (n=7). Right atrium samples were obtained from these patients during CABG and the relative quantification of 20 miRs was analyzed by qRT-PCR. The groups analyzed showed no differences in gender, body mass index, number of patients with hypertension or dyslipidemia. Ejection fraction (EF) and cavity diameters were preserved in all patients of CAD and CAD+DM2 groups, while in HF and HF+DM2 groups, EF was 40.5 ± 7.2% and 37.6 ± 11.0% respectively. It was verified the DM2 factor significantly downregulated the expression of miR-15a, -29a and -499 in CAD+DM2 group when compared to the CAD group. DM2 also upregulated let-7b expression in HF+DM group compared to HF group. MiR-1, -7, -9, -15b, -16, -21, -34a, -126, -133a, -145, -185, -192, -200a, -208a, -208b and -210 were not altered by DM2. Then, we used the database TargetScan to select possible target mRNAs, such as ATP2A2, SCN5A, KCNJ2 and HCN4 transcripts, whose deregulation is associated with arrhythmias and atrial fibrillation. By qRT-PCR, we confirmed an increased expression of KCNIP2, a predicted target of miR-29a, in CAD+DM2 group. Moreover, the transfection of pluripotent stem cell derived-cardiomyocytes with miR-29a inhibitor induced an upregulation of KCNIP2, indicating a possible mechanism by which diabetes promotes electrical changes in the heart.

TU-030

Nitric oxide bioavailability in rats with metabolic syndrome: effect of (–)-epicatechin in the heart

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Fructose overload promotes functional and metabolic changes in humans and animal experimental models. Evidence suggests that dietary flavonoids can prevent or attenuate the development of metabolic diseases. In this study we investigated the effects of (–)-epicatechin on the modifications induced by fructose overload in rat´s heart in terms of nitric oxide and superoxide metabolism. Male Sprague

Dawley rats were divided in three groups that received for 8 weeks: i) water and rat chow diet (C group), ii) 10% (w/v) fructose in the drinking water (F group); iii) 10% (w/v) fructose in the drinking water with (–)-epicatechin (20 mg/kg body weight/day) in the rat chow diet (FEC group). These conditions of fructose overload did not lead to heart hypertrophy or tissue remodeling. However, biochemical and molecular changes were observed and could represent the onset of functional alterations. In this line, an increase in nitric oxide synthase (NOS) activity was observed in FEC with respect to C and F ($p<0.001$ vs. C and $p<0.05$ vs F. These results were correlated with a higher level of eNOS phosphorylation and changes in the pattern of expression of iNOS and nNOS in the three groups studied. Regarding superoxide anion metabolism, a higher production of this oxidant was found in F group with respect to C and FEC ($p< 0.05$), associated with a higher expression of p47 subunit and NOX4.

Superoxide dismutase and glutathione peroxidase activities were lower in F group compared to C and FEC ($p< 0.05$). The higher oxidized/reduced glutathione ratio observed in F, was prevented by (–)-epicatechin.

In summary, (–)-epicatechin was able to ameliorate fructose induced biochemical modifications in the heart through modulating the expression and/or activity of specific proteins. Thus, resulting in a controlled oxidant metabolism favoring NO bioavailability in rats heart.

TU-031

Characterization of the CYP2C19*2 allelic variant distribution in Chilean coronary disease patients.

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Background: Clopidogrel is a widely used antiplatelet drug by patients undergoing percutaneous coronary interventions (PCI), being metabolized by the Cytochrome P450 2C (CYP2C) subfamily of enzymes. It has been reported that single nucleotide variants of CYP2C19 gene, the hepatic enzyme involved in biotransformation of clopidogrel to its active metabolite, can affect the metabolism and anti-platelet response of this drug and the use of an alternative antiplatelet medication has been recommended.

Objectives: The aim of this study was to assess the prevalence of the loss-of function allele CYP2C19*2 in a group of Chilean coronary disease patients.

Methods: 147 patients with history of coronary artery disease who underwent PCI were included. Clinical and demographic variables were registered. Single nucleotide polymorphism CYP2C19*2 (rs4244285) was genotyped by real-time PCR using a TaqMan® Drug Metabolism Genotyping Assay.

Results: General characteristics of the analysed population included: male sex 75.5%, age 63.7 ± 10 years, Diabetes mellitus 31.3%, smokers 19%, body mass index 28 ± 4 kg/m2, systolic blood pressure 134.5 ± 25 mmHg, total cholesterol 179.8 ± 132 mg/dL, and glycaemia 122.2 ± 53 mg/dL. The CYP2C19*2 genotype frequency for GG, AG and AA was 83%, 16.3% and 0.7% respectively, and the A allele presented a frequency of 8.8%. We found no significant differences in genotype frequency between men and women ($p= 0.12$) nor between patients divided by age (under 65 years and equal or older than 65 years, $p= 0.28$).

Conclusion: Our findings indicate the existence of a lower frequency of the CYP2C19*2 variant in Chilean patients with coronary artery disease, when compared to what has been reported for other populations. These results bring more information about metabolic phenotypes regarding the use of this drug in Chilean population. Fondecyt 1141292.

TU-032

Inhibition of phosphoinositide 3-kinase γ promotes cardiac mitophagy and prevents anthracycline-related cardiomyopathy

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PURPOSE: Anthracycline-induced cardiomyopathy has become a leading cause of morbidity and mortality among cancer survivors, but little is known about the underlying mechanisms. We demonstrated previously that phosphoinositide 3-kinase γ (PI3Kγ) promotes maladaptive cardiac remodeling and its inhibition prevents pressure overload-induced heart failure. Here we intend to investigate whether PI3Kγ inhibition is beneficial in a preclinical model of anthracycline-induced cardiomyopathy.

METHODS and RESULTS: Mice expressing a kinase inactive PI3Kγ (PI3Kγ kinase-dead; KD) and wild-type controls (WT) were injected with a cumulative dose of 12 mg/kg doxorubicin (DOX) via 3 weekly injections. DOX-induced systolic dysfunction was completely prevented in KD animals as compared to WT controls (% Fractional shortening: WT DOX 20.5 vs KD DOX 36.6). Accordingly, cardiac atrophy, cardiomyocyte apoptosis and collagen deposition were significantly lower in KD than in WT hearts. Mechanistically, PI3Kγ was found to serve as a negative regulator of cardiac mitophagy via a P-Akt/mTOR/Ulk-1 signaling axis. DOX-induced mitophagy was more pronounced in KD hearts and cardiomyocytes than in WT counterparts, as evidenced by increased expression of LC3II in mitochondrial fractions as well as accumulation of GFP-LC3 puncta, both well-established markers of autophagosome formation. This was paralleled by ultrastructural preservation of KD cardiomyocytes, while WT hearts displayed marked mitochondrial damage and vacuolization after exposure to DOX. Intriguingly, pharmacological inhibition of PI3Kγ with AS-605240 promoted cardiac mitophagy, prevented DOX-mediated contractile impairment and delayed tumor growth in Her-2/NeuT transgenic mice, a model of spontaneous mammary tumor growth.

CONCLUSION: Overall, these data suggest that PI3Kγ inhibitors may concomitantly prevent anthracycline-induced cardiomyopathy and tumor progression, by favoring cardiac mitohormesis and likely limiting a tumor-supportive inflammatory response.

TU-033

Oxidative Activation of cAMP-dependent Protein Kinase by Nitroxyl modulates Myofilament Protein Phosphorylation

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Background: Heart failure is a severe disease, which is defined by the heart's inability to maintain sufficient blood flow, accompanied by reduced force development and β-adrenergic desensitisation. Nitroxyl (HNO), released by donors such as 1-Nitrosocyclohexylacetate (NCA), shows positive inotropic and lusitropic properties, which are maintained under failing conditions. However, how NCA exerts these beneficial actions remains elusive. In healthy hearts β-adrenergic stimulation and subsequent cAMP-dependent protein kinase (PKA) activation is the major pathway for adjustments concerning cardiac performance. PKA, a heterotetrameric enzyme consisting of two regulatory (PKA_{reg}) and two catalytic (PKA_{cat}) subunits, phosphorylates proteins involved in excitation-contraction coupling, leading to increased cardiac output. Notably, besides typical receptor-mediated activation, PKA can be activated directly by oxidants, causing dimerisation of PKA_{reg} subunits by interdisulfide formation.

Hypothesis: The HNO donor NCA leads to oxidative activation of PKA and subsequent phosphorylation of myofilament proteins.

Results: Western Blot analysis of NCA-treated adult rat ventricular myocytes (ARVMs) showed an increased phosphorylation of the PKA substrate cardiac myosin-binding protein C (cMyBP-C). Förster resonance energy transfer (FRET) measurements of cells expressing an A-kinase-activity reporter (AKAR3) confirmed NCA-mediated PKA activation. To observe oxidative modifications of PKA, ARVMs were harvested under non-reducing conditions after incubation with NCA. Interestingly, increased PKA_{reg} dimer formation in these samples was detected by Western Blotting. Furthermore, we could show an intradisulfide bond forming within the PKA_{cat}. *In vitro* kinase assays with PKA_{cat} again allowed detection of this intradisulfide formation within PKA_{cat} after exposure to NCA, which was accompanied by decreased kinase activity. Preincubation of PKA_{cat} with ATP prior to NCA treatment restrained this inhibitory effect.
Conclusion: HNO released by NCA leads to oxidation and thus activation of PKA which phosphorylates the sarcomeric protein cMyBP-C. Resultant protein phosphorylation is a net product of inhibitory intradisulfide formation within PKA_{cat} and activatory interdisulfide formation of PKA_{reg} subunits.

TU-034

Testosterone activates MEF2 through CaMKII and androgen receptor to induce cardiomyocyte hypertrophy

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Ca^{2+}/Calmodulin-dependent protein kinase (CaMKII) and androgen receptor are involved in cardiomyocyte hypertrophy. CaMKII regulates myocyte-enhancer factor 2 (MEF2) that plays a key role in controlling cardiomyocyte growth. However, whether CaMKII/MEF2C signaling pathway is involved in testosterone-induced cardiomyocyte hypertrophy remains unknown. The aim this work was to investigate the testosterone effects on the CaMKII-MEF2C pathway in hypertrophy. Our results showed that testosterone (100 nM) increased the phosphorylation of both CaMKII (Thr286) and phospholamban (Thr17) in neonatal rat cardiomyocytes. Moreover, testosterone stimulated the nuclear translocation of MEF2C and MEF2-luc activity. These effects were prevented in cardiomyocytes pretreated with AIP (a CaMKII inhibitor) or bicalutamide (an androgen receptor inhibitor) and also by use of siRNA to MEF2C and CaMKIIδ. Transfection of cardiomyocytes with a constitutively active isoform of CaMKII (CaMKII-T286D) results in an increased MEF2-luc activity. Testosterone enhances MEF2-luc activity in T286D cardiomyocytes and it was suppressed by bicalutamide suggesting that MEF2C activation involve both canonical androgen receptor as well as Ca^{2+}-mediated pathways. Cardiomyocyte hypertrophy was assessed by increases in β-myosin heavy chain and skeletal α-actin proteins, aminoacid incorporation and cell size. All these parameters were increased by testosterone and prevented by AIP, siRNA-CaMKIIδ and siRNA-MEF2C. Collectively, these evidences suggest that testosterone activate CaMKII/MEF2C signaling pathway to induce cardiomyocyte hypertrophy.

TU-035

Acetylation of SERCA2a inhibits its function and is modulated by SIRT1

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During the diastole of heart pumping, calcium ions in the cytosol are re-sequestered into the sarcoplasmic reticulum (SR) by the cardiac SR Ca^{2+}-ATPase pump (SERCA2a). Reduced levels and activity of SERCA2a are hallmarks of heart failure. Restoration of SERCA2a expression level via a gene transfer improves cardiac function, energetics, and survival in rodent and porcine models of heart failure. In addition, phase 1 and 2 human trials, in which the SERCA2a gene was delivered to the myocardium of patients with advanced heart failure, have confirmed SERCA2a as an effective therapeutic target. We showed recently that the activity of SERCA2a is enhanced by conjugation of small ubiquitin-related modifier 1 (SUMO1) at two specific lysine residues. However, the roles of other post-

translational modifications of SERCA2a are unknown. Here, we show that the activity of SERCA2a is impeded by acetylation at lysine 492 (K492), and that this inhibitory event can be reversed by SIRT1, a NAD^+-dependent class III histone deacetylase. SIRT1 interacted directly with and deacetylated SERCA2a *in vitro*, and downregulation of SIRT1 increased SERCA2a acetylation and decreased its enzymatic activity *in vitro* and *in vivo*. Concomitant with reductions in its enzymatic activity, an increase in SERCA2a acetylation was observed in failing hearts, and these defects were restored by β-lapachone (β-lap), a metabolic activator of SIRT1. Structural modeling analyses suggested that acetylation at K492 may prevent ATP accessing its binding pocket in SERCA2a. These results indicate that acetylation is a critical post-translational modification of SERCA2a that is implicated in reduced function of this calcium pump, and that SIRT1 can restore the contractile dysfunction of failing hearts via deacetylation of SERCA2a.

TU-036

Contribution of serotonergic 5-HT_{2B} receptors to the mobilization of bone marrow endothelial progenitors in cardiac valve degeneration

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Valvular heart disease (VHD) is one of the most frequent cardiovascular pathology in industrialized countries. Chronic use of anorexigens, amphetamine or ergot derivatives targeting the serotonin system has been associated with VHD.

The first aim of this study was to characterize the pattern of serotonergic expression in various human VHD. In a second part of the work, we investigated the contribution of serotonin (5-HT) effectors in a model of valve degeneration induced by nordexfenfluramine the main metabolite of the anorexigens dexfenfluramine and benfluorex.

Surprisingly, we found that valve lesions were made by numerous non-proliferative $CD34^+$ endothelial progenitors both in humans and mice VHD. Chronically activated 5-HT_{2B} receptors by nordexfenfluramine in mice mimicked early steps of mitral valve remodeling attested by increased valve thickness and cell density. Lesions were totally prevented by blocking 5-HT_{2B} receptors (SB206553 or $Htr_{2B}^{-/-}$ mice) and both 5-HT_{2A} and 5-HT_{2B} receptors (ritanserin or $Htr_{2A/2B}^{-/-}$) but not 5-HT_{2A} receptors alone ($Htr_{2A}^{-/-}$).

We observed that valve lesion associated endothelial progenitors originated from bone marrow that shared 5-HT_{2B} receptor expression and were mobilized by serotonergic 5-HT_{2B} receptor stimulation, revealing crucial contribution of bone marrow derived endothelial progenitor cells in valve tissue homeostasis and remodelling.

TU-037

Metformin attenuates angiotensin II induced transforming growth factor-β1 production through the inhibition of HNF4α by AMPK activation

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Background : Metformin appears to provide cardiovascular protection that cannot be attributed to its antihyperglycemic effects. Angiotensin II (AngII) induced transforming growth factor β1 (TGFβ1) plays an important role in cardiac fibrosis. Metformin might play its cardioprotective role through inhibition of cardiac fibrosis and TGFβ1 production. This study investigated the effect of metformin on cardiac fibrosis and TGFβ1 production induced by AngII and the underlying mechanisms. **Methods :** Wild-type and AMPKα2-/- C57/BL6 mice were subcutaneously injected with metformin or saline and were infused with angiotensin II (AngII) (3mg/kg/day) for 7 days using mini-pump. Adult mouse cardiac fibroblasts were isolated for in vitro experiments. **Results :** Metformin inhibited AngII induced cardiac fibrosis and TGFβ1 production in the wild-type mice but not in AMPKα2-/-

mice. In wild-type cardiac fibroblasts (CFs), metformin inhibited TGFβ1 expression and production induced by AngII. AMPK inhibitor, compound C, reversed the effects of metformin. In vivo, AMPKα2 deficiency further increased AngII-induced TGFβ1 production. Furthermore, bioinformatics predicted the presence of an HNF4α binding site in the promoter region of the *Tgfb1* gene. Using HNF4α adenovirus, overexpressing HNF4α led to a 1.5-fold increase in TGFβ1 mRNA expression. HNF4α siRNA blocked AngII induced TGFβ1 production. Luciferase assays confirmed that HNF4α acted on the TGFβ1 promoter region. In CFs, metformin inhibited AngII induced increase of HNF4a protein level and binding activity with TGFβ1 promoter using CHIP assay.
Conclusions : Metformin inhibited AngII induced cardiac fibrosis and TGFβ1 production. Metformin inhibited TGFβ1 production through AMPK activation. The underlying mechanism is that AMPK activation inhibited AngII induced HNF4α and then decreased TGFβ1 expression. These findings provided a new mechanism for the cardioprotective effects of metformin.

TU-038

Chronic inflammation inhibits myofibroblast activation through macrophage Ccl12 secretion

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Background: Chronic inflammation is a risk factor for adverse remodeling post-myocardial infarction (MI). Cross-talk between the inflammatory and fibrotic response is needed for inflammation resolution and stable scar formation, and the macrophage is a prime intermediary cell. Previously, we showed chronic lipopolysaccharide (LPS) accelerated macrophage infiltration at day 1, resulting in increased cardiac rupture post-MI. We hypothesized that chronic inflammation would exacerbate macrophage secretion of pro-inflammatory cytokines to subsequently decrease activation of the reparative fibroblast.
Methods: We infused C57BL/6J mice (5 months old; n≥6/sex/group) with subseptic levels of LPS (0.8 ug/g/day) for 28 days to simulate chronic inflammation. Coronary artery ligation was performed and macrophage phenotype, fibroblast activation and proliferation, and extracellular matrix (ECM) deposition were evaluated at day 7 post-MI. Stimulation of resident cardiac fibroblasts with macrophage conditioned media, with and without Ccl12 blocking antibody, was performed to dissect signaling mechanisms of action.
Results: Macrophage associated pro-inflammatory cytokine genes were elevated in the infarct tissue of the LPS mice at day 7 post-MI, with Ccl12 demonstrating the largest expression change ($p<0.05$). By immunofluorescence, markers of reparative fibroblast activation (□-smooth muscle actin and F-actin) were decreased in day 7 post-MI cardiac fibroblasts from LPS exposed mice compared to controls ($p<0.05$). By *in vivo* BrdU labeling, post-MI fibroblasts isolated from LPS exposed mice were 3-fold more proliferative than non-exposed fibroblasts ($p<0.05$). Collagen III, fibronectin, and lysyl oxidase were at least 2-fold lower in the infarcts of LPS mice at day 7 post-MI (all $p<0.05$). Stimulation of resident cardiac fibroblasts with macrophage conditioned media from LPS mice decreased ECM expression, differentiation, and increased proliferation compared to controls; selective Ccl12 inhibition reversed the secretome effect ($p<0.05$).
Conclusion: Our study revealed for the first time that chronic inflammation increases Ccl12 production in macrophages to stimulate fibroblast dysfunction and adverse cardiac wound healing.

TU-039

HnRNPA1 regulates neointima formation through modulating vascular smooth muscle cell functions

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Background Our previous study reported that hnRNPA1 regulates vascular smooth muscle cell (VSMC) differentiation from stem cells *in vitro* and *in vivo.* However, little is known about the functional involvements of hnRNPA1 in VSMC functions and neointima formation. In the current study, we aimed to investigate the functional roles of hnRNPA1 in the contexts of VSMC functions, injury-induced intimal hyperplasia, and human neointima lesions.

Methods and results Primary mouse aorta VSMCs were isolated and showed that hnRNPA1 expressions were consistently regulated in VSMCs upon various pathological stimuli. hnRNPA1 over-expression in VSMCs significantly reduced VSMC proliferation and migration, while decreased hnRNPA1 promoted VSMC proliferation and migration, respectively. Moreover, hnRNPA1 exerted its effects on VSMCs via regulating IQ motif containing GTPase activating protein 1 (IQGAP1), a well-reported VSMC function modulator. Our data suggested that IQGAP1 expression was negatively regulated by hnRNPA1 through two ways. Firstly, hnRNPA1 protein directly bound to IQGAP1 mRNA AU-rich elements and consequently decreased IQGAP1 mRNA stability. Secondly, hnRNPA1 was involved in miR-124 biogenesis and up-regulated miR-124 expression which then post-transcriptionally reduced IQGAP1 mRNA and protein expression levels. Furthermore, hnRNPA1 expression was dramatically down-regulated during wire-injury induced neointima formation. In accordance, peri-vascular ectopic over-expression of hnRNPA1 markedly inhibited VSMC proliferation and attenuated wire-injury induced neointimal hyperplasia. Importantly, decreased expression of hnRNPA1 was observed in human atherosclerotic neointima lesion.

Conclusions Our data have demonstrated that hnRNPA1 is a critical regulator in VSMC functions and neointima formation, suggesting its potential therapeutic application for vascular diseases.

TU-040

Bach1 represses Wnt/β-catenin signaling and angiogenesis

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Background: Wnt/β-catenin signaling has an important role in the angiogenic activity of endothelial cells (ECs). Bach1 is a transcription factor and is expressed in ECs, but whether Bach1 regulates angiogenesis is unknown. Objective: This study evaluated the role of Bach1 in angiogenesis and Wnt/β-catenin signaling.

Methods and Results: Hind-limb ischemia (HLI) was surgically induced in Bach1$^{-/-}$ mice and their WT littermates and in C57BL/6J mice treated with adenoviruses coding for Bach1 or GFP. Lack of Bach1 expression was associated with significant increases in perfusion and vascular density and in the expression of pro-angiogenic cytokines in the ischemic hindlimb of mice, with enhancement of the angiogenic activity of ECs (e.g., tube formation, migration, and proliferation). Bach1 overexpression impaired angiogenesis in mice with HLI, and inhibited Wnt3a-stimulated angiogenic response and the expression of Wnt/β-catenin target genes, such as interleukin 8 (IL-8) and VEGF, in human umbilical vein endothelial cells (HUVECs). IL-8 and VEGF were responsible for the anti-angiogenic response of Bach1. Immunoprecipitation and GST pull-down assessments indicated that Bach1 binds directly to TCF4 and reduces the interaction of β-catenin with TCF4. Bach1 overexpression reduces the interaction between p300/CBP and β-catenin, as well as β-catenin acetylation, and chromatin immunoprecipitation experiments confirmed that Bach1 occupies the TCF4-binding site of the IL-8 promoter and recruits histone deacetylase 1 (HDAC1) to the IL-8 promoter in HUVECs.

Conclusion: Bach1 suppresses angiogenesis after ischemic injury and impairs Wnt/β-catenin signaling by disrupting the interaction between β-catenin and TCF4 and by recruiting HDAC1 to the promoter of TCF4-targeted genes.

TU-041

Kruppel-like Factor 2 Mediates the Suppressive Effect of Statin on BMP4-Smad Signaling

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Rationale: Bone morphogenic protein 4 (BMP4) is a pro-inflammatory and oxidative protein in vascular endothelial cells (ECs). Statins, the HMG-CoA reductase inhibitors, exert anti-inflammatory and anti-oxidant effects by upregulation of Kruppel-like factor 2 (KLF2) in ECs. Whether and how statins modulate BMP4 signaling in ECs is largely unknown.

Objective: We aim to investigate the effects of statins on BMP4-triggered signaling and function in ECs. Moreover, we also study the role of KLF2 induced by statins in modulation of BMP4-Smad signaling.

Results: *Ex vivo* treatment of mouse aortic rings with statins restored BMP4-induced impairment of endothelium-dependent relaxations (EDR) and this beneficial effect was abolished by Ad-KLF2-shRNA transduction. Oscillatory shear stress (OSS) induced BMP4-Smad activation was also attenuated by statin treatment and is dependent on KLF2 levels. Statin treatment of human umbilical vein endothelial cells (HUVECs) for 24 hours suppressed the expression of BMP4 and Smad1 at both mRNA and protein levels, which was abolished in KLF2-silenced HUVECs. Statins inhibited BMP4-induced expression of pro-inflammtory genes such as ICAM-1 and COX-2, phosphorylation of Smad1/5 and Smad1/5-mediated gene transcription, which are also abrogated in HUVECs with KLF2 knockdown. KLF2 overexpression by Ad-KLF2 in HUVECs showed that KLF2 directly suppresses the expression of BMP4 and Smad1 and BMP4-initiated Smad phosphorylation, indicating the negative regulatory effect of BMP4-Smad signaling by KLF2. Moreover, luciferase assay showed that KLF2 inhibited the promoter activity of BMP4 and Smad1.

Conclusion: Statins have suppressive effects on BMP4-Smad signaling through upregulation of KLF2, which negatively regulates BMP4 and Smad1 expression at transcription levels. (This study is supported RGC CRF)

TU-042

A role for antioxidants in reversing Tiotropium induced cardiotoxicity

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Tiotropium bromide is a long-acting muscarinic receptor antagonist (LAMA) used in the treatment of chronic obstructive pulmonary disease (COPD); a progressive inflammatory condition of the airways. LAMAs target muscarinic receptors to result in dilation of airway smooth muscle. Recently, clinical studies have correlated the use of anti-muscarinics with cardiovascular events, including stroke and myocardial infarction. Cardiac damage is often associated with reactive oxygen species (ROS) production and calcium overload. However, ROS also function as second messengers and are known to result in the activation of Akt. Although Akt is associated with promoting cell survival, constitutive activation of Akt can itself result in cell death.

The aim of this study was to assess the cardiotoxicity and associated intracellular mechanisms of Tiotropium using a whole heart model. Langendorff hearts were subjected to a stabilisation period (20 minutes), followed by reperfusion (155 minutes) ± Tiotropium bromide (10nM-0.1nM) and the anti-oxidant, Resveratrol (10μM) alone or combined with Tiotropium (1nM). Following reperfusion, hearts underwent triphenyl-tetrazolium chloride staining to assess infarct/risk ratio (%) or were snap-frozen for western blot analysis of p-Akt (Ser473) expression.

Tiotropium (10nM-0.1nM) administration during reperfusion, significantly increased infarct/risk ratio (%) compared with normoxic controls in a concentration dependent manner. Administration of Resveratrol (10μM) showed no significant difference with respect to controls (12.28±1.5% vs. 10.27±1.94%), however co-administration of Resveratrol with Tiotropium (1nM) attenuated infarct development (11.99±1.71% vs. 18.69±1.79%, $p<0.0002$, n=3/4). Western blot analysis showed significant increase in p-Akt (Ser473) expression in Tiotropium treated groups compared to time-matched control (79.10±20.04% vs. 26.86±2.70%, $p<0.01$ at 1nM), which was abrogated by Resveratrol administration 79.10±20.04% vs. 32.05±1.62%, $p<0.05$).

This is the first pre-clinical study to suggest that Tiotropium increases infarct/risk ratio in a whole heart model, which may account

for adverse cardiac side-effects seen clinically. This also proposes a role for Resveratrol in reducing Tiotropium mediated cardiotoxicity.

TU-043

MicroRNA-26a Inhibits Vascular Smooth Muscle Cell Proliferation and Neointimal Hyperplasia by Targeting MAPK6

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Abstract

Rationale—Saphenous vein graft disease is a timely problem in coronary artery bypass grafting. Long term patency of vein grafts is limited due to neointimal formation caused by vascular smooth muscle cell (VSMC) migration and proliferation in the intima. Therefore, identifying novel strategies to prevent neointimal thickening is important. Understanding the role of microRNA provides a opportunity to identify both functional drivers of VSMC proliferation and possible therapies of vascular pathology.

Objective—Because microRNA-26a (miR-26a) is involved in regulation of functions of various cells, we investigated the effect of miR-26a on the proliferation and migration of VSMC and the development of neointimal hyperplasia after autogenous vein graft.

Methods and Results— Using quantitative reverse-transcription polymerase chain reaction, we identified that miR-26a was one of the most significantly down-regulated microRNAs in jugular veins which were interposed in the rat carotid artery. miR-26a was also markedly down-regulated in VSMCs from rat jugular vein stimulated with Platelet-derived growth factor-BB (PDGF-BB). Overexpression of miR-26a inhibited VSMC cell proliferation and migration. MAPK6 was predicted as one of the top targets of miR-26a by using several computational miRNA target prediction tools, and was negatively regulated by miR-26a in VSMCs. Luciferase assay showed miR-26a substantially repressed wild type MAPK6-3'-UTR-luciferase activity in VSMCs, but not mutant MAPK6-3'-UTR-luciferease reporter. Furthermore, Knocking-down of MAPK6 reduced cell proliferation and migration, whereas overexpression of MAPK6 enhanced VSMCs proliferation and migration, which consisted in the activation of Akt and Erk. Data from co-transfection experiments also revealed that miR-26a inhibited VSMC proliferation and migration through modulating MAPK6 gene expression levels.

Conclusions—.These results have demonstrated that miR-26a is an important regulator in VSMC functions and neointima hyperplasia, suggesting its potential therapeutic application for saphenous vein graft disease (This projector found by NSFC 11172176).

TU-044

New insights into adrenergic regulation of cardiac remodelling

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Background: Heart failure is characterized by enhanced sympathetic nervous activity and subsequent activation of adrenergic receptors (ARs) through release of stress hormones (catecholamine). Thus, these exist unwanted signalling via ARs activation leading to cardiac remodelling and dysfunction, constituting causal mechanism in heart failure initiation and progression. Whilst the responsible AR signal mechanisms remain undefined. The conventional view of GPCR signalling holds that the receptor couples with its G protein only at the cell membrane. Recent studies, including those from our group, have shown that several events are thought to terminate this signalling pathway. **Methods:** Western blot, real time PCR, single molecule image, mice models were used. **Results:** (1) Binding of the β-arrestins to the receptor are involved in β2-AR-mediated p38 MAPK earlier activation. (2) Internalization of the receptor through the process of endocytosis is associated in α1A-AR induced ERK activation, which is independent of Gq/PLC/PKC signalling. (3) α1-AR induces STAT3 activation mainly through transactivation of Epidermal Growth Factor Receptor (EGFR) in neonatal rat

cardiomyocytes, which plays an essential role in α1-AR-induced cardiac hypertrophy. **Conclusions:** The new insights of adrenergic receptor signalling is one receptor and four signalling pathways. The signalling pathways and core molecules can be clarified in the cardiac remodelling induced by adrenergic activation, providing clues and theory basis for develop more specific treating target for cardiac remodelling.

TU-045

ER Stress mediates cardiac ion channel changes in heart failure

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Introduction: Heart failure (HF) is associated with endoplasmic reticulum (ER) stress and activation of the unfolded protein response (UPR). UPR inhibits protein translation. Ion channel downregulation is associated with arrhythmic risk. We hypothesized that UPR could be contributing to electrical remodelling in HF.

Methods: Hypertensive HF was induced in C57BL/6 mice by unilateral nephrectomy, deoxycorticosterone acetate pellet implantation, and salt water substitution. Sham operated mice were used as controls. After 6-7 weeks, isolated ventricular myocytes were utilized for whole-cell patch clamp recording, and heart tissue was used for mRNA measurements. GSK2606414, a specific inhibitor of protein kinase R like ER kinase (PERK), was applied to myocytes at 4 nM for 20 h (30 min pretreatment when coapplied with tunicamycin, 10 μg/ml, 20 h).

Results: HF myocytes showed classical electrical remodelling with action potential duration prolongation (APD_{90}: 203±26 vs. 108±16 ms of sham, $P<0.05$) and DADs. PERK activation in HF myocytes was indicated by elevated mRNA and/or protein levels of Grp78, phospho-PERK, phospho-eIF2α, ATF4, and CHOP. Peak I_{Na} and three types of K^+ currents (I_{to}, I_{K1}, and I_{Kslow}) were decreased significantly in HF group, while L-type Ca^{2+} current and some other types of K^+ currents were not affected. These changes were similar to those observed in myocytes treated with the classic UPR inducer, tunicamycin. An inhibitor of the PERK branch of the UPR, GSK2606414, restored I_{Na} and $I_{K,slow}$, and shortened the APD of the DOCA myocytes.

Conclusions: UPR appears to be responsible for reductions of I_{Na}, I_{to}, I_{K1} and $I_{K,slow}$ in heart failure. $Na_v1.5$ and $K_v1.5$ were downregulated by the PERK branch of UPR. Inhibiting the UPR may be a novel antiarrhythmic strategy.

TU-046

The neuro-cardiac interaction defines an extracellular microdomain required for neurotrophic signaling

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Purpose: Cardiac activity is tuned by sympathetic neurons (SNs), whose survival depends on limiting amounts of neurotrophins released by the myocardium. This study aims i) to determine whether specific cellular structures are present at the SN/cardiomyocyte (CM) contact site, ii) to investigate the role of SN/CM interaction in NGF-mediated signaling.

Methods and results: Electron microscopy and immunofluorescence on mouse heart slices and rat SN/CM co-cultures showed close association between SNs and CMs and enrichment of the NGF receptor (TrkA) at the contact site. These data support that specialized and locally organized signaling domains exist (neuro-cardiac junction, NCJ).

We tested the functional role of the NCJ in sustaining neuronal survival. Silencing of NGF expression by CMs in co-cultures led to 66% decrease of neuronal density, supporting that SN viability depends on NGF released by CMs. SNs cultured on NGF-silenced CMs showed 20% decrease in the NCJ area when compared to those on wild type CMs of the same culture. Moreover, NGF uptake was observed only in processes contacting NGF-overexpressing CMs, supporting that the NCJ is central to neurotrophin-mediated signaling. Consistently, cultured SNs in contact with CMs survived NGF withdrawal, whereas neurons alone treated with CM-conditioned medium did not survive because of the very low NGF concentration (1.61 pg/mL). Conversely, NGF concentration at the contact site was estimated by using the TrkA inhibitor K252a and resulted about 1000-fold higher (1.75

ng/mL), supporting that the NCJ allows amplification of intercellular NGF signaling. Dystrophin accumulation on CM membrane contacted by SNs was observed in mouse cardiac slices. Consistently, hearts from mdx mice showed 74.36% decrease of innervation, with no significant changes of NGF expression, supporting that ablation of dystrophin impairs cardiac SNs.
Conclusions: Taken together, our results suggest that NGF-dependent signaling to the neurons requires a direct and specialized interaction with myocytes.

TU-047

Chronic lead exposure impairs vascular reactivity through oxidative stress dependent mechanism: MAPKs pathway activation

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Introduction: Chronic exposure to lead (Pb) alter cardiovascular parameters. Aim: To investigate chronic exposure to Pb low concentrations in aorta and VSMC function, assessing oxidative stress and MAPKs pathway. **Methods:** Rats were treated with lead for 30 days (1st dose 10mg/100g, subsequent doses 0.125mg/100g/day im) and controls, saline-im. Vascular reactivity to phenylephrine (Phe) was measured in the presence and absence of endothelium and after 30 min of incubation with L-NAME and apocynin (APO). Isolated aortas were processed to obtain primary cultures of VCMS. Pb (20 µg/dL) was used to stimulate the cells for 48h. **Statistical analysis:** mean ± SEM; One way ANOVA or Student *t*-test. $p < 0,05$. Ethics Committee (UFES-063/2011) and (UAM-CEI-22-488). **Results:** The treatment produced blood lead concentration of 21,7 µg/dL and increased vascular reactivity to Phe. Removal of the endothelium and incubation with L-NAME increased reactivity with lower proportion compared to the Pb group. APO reduced vascular reactivity to Phe in both groups, but with greater magnitude in Pb group. Pb increased gp91phox, Cu/Zn-SOD and Mn-SOD protein expression. In VSMC, NADPH oxidase activity and superoxide anion production were also enhanced by Pb and normalized by Celecoxib, Rofecoxib, ML171, Tempol and Mito-TEMPO. Pb augmented NOX1, NOX4, Mn-SOD and EC-SOD expression. Celecoxib reversed the upregulation of NOX1 and NOX4. Pb also induced the activation of ERK1/2 and p38MAPK signaling pathways that augmented NOX1 and NOX4 expression but did not induce proliferation or migration of VCMS. **Conclusion:** Our findings suggest that treatment with low doses of lead, below the reference values, increased BP, promoted vascular dysfunction and activate the MAPKs signaling pathways, which are associated with the NADPH oxidase activation. Since lead is reported to be involved in hypertension development, its exposure should be considered an environmental risk factor for cardiovascular disease.

TU-048

mTORC1 and mTORC2 preserve cardiac function by regulating metabolism and contractility

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The mammalian target of rapamycin (mTOR), an evolutionary conserved serine/threonine kinase of the phosphatidylinositol-3-kinase-related kinase family, integrates intracellular and environmental cues such as amino acid availability, growth factors, energy status and stress. In response to these stimuli, mTOR regulates metabolic mechanisms including protein turnover, nucleotide synthesis and lipid synthesis, to ultimately control cellular growth. To exert its best-characterized function of protein synthesis, mTOR must be assembled in the multiprotein complex mTORC1. We have previously shown that cardiomyocyte-specific deletion of raptor, an essential and specific component of mTORC1, leads to cardiac dysfunction and death under basal conditions and that functional deterioration is accelerated in pressure-overloaded hearts. The dysfunction was related not only to reduced protein synthesis and the consequent lack of adaptive hypertrophy, but also to reduced mitochondrial content and a change in energy substrate use.

In contrast, cardiac rictor-deficient mice (rictor encodes an essential and specific component of mTORC2) had no phenotype during growth or adulthood under basal laboratory conditions up to 54 wks of age. However, aortic constriction-induced pressure overload significantly increased rictor protein levels along with PKCβII and PKCδ phoshorylation in control mice, but not in the cardiac rictor knockout mice. Pressure overload resulted in hypertrophy with maintained ventricular function in controls, but led to systolic dysfunction in the rictor-deficient hearts, without having any effects on cardiac weight, hypertrophy markers, or fibrosis. Our data suggest that mTORC2 regulates metabolism and contractility of the heart via PKCβII and PKCδ. As several compounds inhibiting both mTOR complexes are in clinical trials for the treatment of cancer, special attention should be paid in these studies to patients with concurrent cardiovascular disease such as hypertension or valve disease. On the other hand, our novel insights into cardiac mTORC2 signaling may also open up new avenues for the treatment of cardiac disease.

TU-050

Uncovering novel signaling components for DCM development - a phospho-proteomics approach

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Background

The muscle-lim protein (MLP/CSRP3) knockout mouse has long been used as a model to investigate dilated cardiomyopathy (DCM) disease mechanisms. Known pathway components that are involved in the development of the disease include PKCalpha, phospholamban, and proteins associated with beta-adrenergic signaling.

In addition, we identified CARP1/Ankrd1 as essential for DCM development. Hearts of double knockout mice do not develop the disease, and present with normal cardiac morphology and physiology. As a molecular mechanism we showed that CARP1 is required for the pathological activity of PKCalpha in MLP knockout hearts.

Methods

We probed for novel pathway components implicated in the development of DCM by using cardiac extracts of MLP knockouts, and CARP1-MLP double-knockouts. Because of the role that posttranslational modifications by PKCalpha play in DCM pathology, we utilized stable isotope dimethyl-labeling in a differential phospho-proteomics approach to identify candidate proteins with disease associated changes to their phosphorylation pattern. Biochemical and cell-biological assays were used to further characterize their involvement in DCM development.

Results

We identified 13'000 differentially labeled phospho-peptides from approx. 600 proteins that are representative of every cell compartment. One of the proteins identified is AHNAK-1, which is known to take part in cardiac signaling pathways, including PKC, and in addition is thought to regulate cardiac calcium channel activity. Along the AHNAK-1 polypeptide chain, we identified several novel phosphorylation sites that are differentially phosphorylated between diseased MLP hearts and healthy CARP1-MLP double knockout hearts. Intriguingly, a C-terminal mutation in AHNAK-1 known to cause cardiomyopathy in humans affects one of these novel phosphorylation sites in in-vitro kinase assays.

Conclusions

Further analysis of AHNAK-1, and other identified candidate proteins will give better insights into DCM mechanisms, and may reveal novel targets for a treatment of this disease.

TU-051

β_1-Adrenergic stimulation induces HDAC5 nuclear accumulation by B55α-PP2A-mediated dephosphorylation

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When localized to the nucleus, histone deacetylase 5 (HDAC5) prevents cardiomyocyte hypertrophy by repressing MEF2 transcription factors. Stimulation of G_q protein-coupled receptors induces HDAC5 nuclear export via its phosphorylation at S259/S498. In contrast, stimulation of β-adrenergic receptors has been proposed to induce both phosphorylation-independent nuclear export and protein kinase A (PKA)-dependent nuclear accumulation through S279 phosphorylation. We aimed to: (1) definitively determine the impact of β-adrenergic signaling on the

phosphorylation, localization and function of HDAC5 in adult rat ventricular myocytes (ARVM); (2) delineate the relative importance of altered phosphorylation at S259/S498 and S279 in regulating HDAC5 localization in this cell type. Towards these aims, we established a new confocal microscopy method to objectively quantify the whole-cell nuclear/cytoplasmic distribution of GFP-tagged HDAC5 in living ARVM. Isoprenaline (ISO; 10 nM) induced HDAC5 dephosphorylation at all three sites and HDAC5 nuclear accumulation, which was blocked by PKA inhibition. Mutation of S259/S498 to non-phosphorylatable alanine promoted nuclear accumulation and MEF2 inhibition, whereas ablation of the S279 phosphorylation site had no effect on these parameters and did not block ISO-induced nuclear accumulation. HDAC5 dephosphorylation was sensitive to PP2A inhibition with okadaic acid. Furthermore, co-immunoprecipitation experiments revealed a specific interaction of HDAC5 with the PP2A regulatory/targeting subunit isoform B55α, as well as PP2A catalytic and scaffolding subunits, and these interactions increased >3-fold with ISO stimulation. We conclude that β-adrenergic stimulation induces HDAC5 nuclear accumulation by a mechanism that is PKA-dependent but requires B55α-PP2A-mediated dephosphorylation of S259/S498 rather than PKA-mediated phosphorylation of S279.

TU-052

Identification of a high affinity, high efficacy adenosine A_{2B} receptor agonist with potent anti-fibrotic activity

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Background. The adenosine A_{2B} receptor (A_{2B}AR) has been therapeutically implicated in the heart with key roles in ischemia-reperfusion injury, inflammation and fibrosis. However to date, effective modulation of A_{2B}AR signalling has been limited by a lack of potent agonists. Recent screening of an adenosine receptor bitopic agonist, VCP746, revealed significant and previously unappreciated A_{2B}AR activity. VCP746 is a hybrid ligand comprised of an orthosteric agonist moiety (VCP900) and an adenosine A_1 receptor (A_1AR) allosteric modulator moiety (VCP171). This study aimed to rigorously characterise the binding and function of VCP746 at the A_{2B}AR and examine its anti-fibrotic activity in cardiac fibroblasts.

Methods. The affinity and efficacy of VCP746 was examined in FlpInCHO cells stably expressing the human A_{2B}AR. Agonist concentration response curves were generated across multiple functional pathways and compared to conventional A_{2B}AR agonists NECA and BAY60-6583. The ability of VCP746 to inhibit TGFβ- or angiotensin II- mediated collagen synthesis was measured by [^{3}H]-proline incorporation in isolated neonatal rat cardiac fibroblasts (NCFs).

Results. VCP746 had a significantly higher affinity and potency than NECA or BAY60-6583 at the A_{2B}AR. Functional assays demonstrated VCP746 stimulated robust increases in cAMP accumulation, ERK1/2 phosphorylation, IP_1 accumulation and Ca^{2+}. In primary NCFs, VCP746 stimulated potent inhibition of both TGFβ- and angiotensin II- mediated collagen synthesis in NCFs (pIC_{50} 7.6 $\pm$ 0.4 and 7.8 $\pm$ 0.4, respectively; n=4-6). The influence of VCP746 on collagen synthesis was selectively reversed in the presence of an A_{2B}AR antagonist, demonstrating that these effects were mediated through A_{2B}ARs endogenously expressed in NCFs.

Discussion. VCP746 was found to be the highest affinity and highest efficacy A_{2B}AR agonist identified to date. Furthermore, VCP746 displayed potent anti-fibrotic effects in NCFs, thus we believe that VCP746 will provide a novel tool to further investigate the role of the A_{2B}AR in cardiac (patho)physiology.

TU-053

Catestatin modulates adrenergic signaling and reverses the hypertrophic effects of norepinephrine in H9c2 cardiac myoblasts

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Background: Upon treatment with 2 and 100μM Norepinephrine (NE), H9c2 cardiac myoblasts elicit hypertrophic and apoptotic responses respectively. The two respective pathways are distinguished by the induction of distinctive redox-kinase signaling pathways. In mammalian heart, NE is co-released with Catestatin (CST), a catecholamine release inhibitory peptide derived from chromogranin A. CST plays an important role in the regulation of cardiovascular functions and associated diseases including hypertension, cardiomyopathy, myocardial infarction and heart failure; but the mechanisms of its actions are not known. Here, we aim to explore its mechanism of action; its downstream signaling pathway and target genes involved in the induction of cardiac hypertrophy.

Results and conclusion: We demonstrate that CST reverses the induction of fetal genes in H9c2 cardiac myoblasts by norepinephrine (NE). CST attenuates the ROS generated by NE treatment as evidenced by redox sensitive fluorescent probes DCFH-DA, HPF, DHE and Amprex red. Luciferase and gel shift assay shows that it modulates the redox responsive transcription factors AP-1 and Nrf2, either alone or in presence of NE. Expression of *fosB* and AP-1 promoter reporter constructs is also modulated by CST alone or in association with NE, though it has preference for the β- rather than α-AR signaling. However, it does not prevent apoptosis induced by a higher dose of NE. Effects of CST on reporter gene expression suggest that it acts through multiple signaling pathways. Taken together, this study suggests that CST modulates the adrenergic signaling by suppressing RO/NS generation and differentially modulating activities of AP1, FosB, Fra1 and Nrf2.

TU-054

Protective effect of Aronia melanocarpa on cardiovascular system in L-NAME-induced hypertension

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Background: Polyphenols are a class of natural products exhibiting multiple health benefits beyond their antioxidant potential. Aronia melanocarpa (black chokeberry) has attracted scientific interest due to its dense contents of polyphenols, especially anthocyanins. The aim of the present study was to analyze effects of non-alcoholic concentrate from aronia melanocarpa (AM) on blood pressure (BP), total NOS activity and cytokine level in the left ventricle of L-NAME-induced hypertensive rats.

Methods: 12-week-old male WKY rats were assigned to control group, group treated with L-NAME (40mg/kg/day), group treated with AM concentrate (1ml/kg/day), and group treated with combination of L-NAME (40mg/kg/day) and AM concentrate (1ml/kg/day) in tap water. The experiment lasted 3 weeks. BP was measured by the tail-cuff-plethysmography. NOS activity was determined by conversion of 3[H] Arginine to 3[H] Citrulline in the left ventricle (LV). Cytokine levels were investigated using the Bio-Plex Pro Cytokine kit in the plasma.

Results: After 3 weeks of L-NAME treatment BP was increased by 28% than the control group. AM reduced BP by 21% in L-NAME + AM group in comparison to L-NAME group. Moreover, AM inhibited TNF-α and IL-6 production in the plasma in L-NAME + AM group in comparison to L-NAME group. NOS activity of LV in L-NAME group was decreased by 40%, on the other hand AM was able to increase NOS activity on 90% of control level.

Conclusion: The results of our study show that active substances from Aronia melanocarpa may have positive effect on blood pressure, cytokine level and NOS activity in L-NAME induced hypertension.

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TU-055

Role of NADPH Oxidase-2 under adrenergic stimulation in cardiomyocytes

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Background: Reactive oxygen species is involved in the pathogenesis of cardiovascular diseases, including atherosclerosis, hypertension, cardiac hypertrophy and heart failure. Recent studies have emphasized on the role of NADPH oxidases (NOXs) in cardiac hypertrophy induced by pressure overload,

angiotensin II and phenylephrine. However, the role of specific NOX isoforms, site of ROS generation and underlying mechanism under adrenergic stimulation induced cardiac hypertrophy has not been explored.
Aim: In this study, we aim to investigate the spatial localization of ROS generation and involvement of specific NOX isoform under adrenergic stress leading to downstream signalling events.
Methods and Results: H9c2 cardiac myoblasts were treated with 2μM norepinephrine (NE) inducing ROS generation that was inhibited by NOX2 specific peptide inhibitor gp91ds-tat. Organelle specific hydrogen peroxide-sensitive GFP was used for monitoring ROS generation in cytosol, mitochondria, and ER of which cytosolic Hyper-GFP was primarily positive. Induction of cardiac hypertrophy marker genes (β-MHC, ANP) with 2μM NE treatment was restored by the NOX2 inhibition as measured by real-time PCR. Enhanced promoter activity of some of the stress induced transcription factors (AP-1, FosB) was also attenuated by NOX2 inhibition as estimated by promoter reporter assay. We hypothesize that under pathological condition, perturbation of this AR-NOX2 cross-talk cause β-AR malfunction. To understand the role of NADPH oxidase *in vivo*, we intraperitoneally injected rats with apocyanin, an inhibitor of NOXs, for two weeks and concomitantly, subcutaneous injection of isoproterenol was given to induce cardiac hypertrophy. Our data suggested partial reversal of cardiac hypertrophy marker proteins and genes with the inhibition of NOX by apocyanin.

TU-056

Nitro-oleic acid, a component of the mediterranean diet, prevents MKK3-p38αMAPK; dimer formation by steric obstruction of redox-sensitive cysteines.

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Abstract:
Background: p38α-MAPK (p38α), a serine-threonine kinase plays a pivotal role in a variety of biological processes and is thus activated by diverse stimuli including oxidant stress. This activation is achieved by its archetypal upstream kinase, MKK3, phosphorylating two key residues within the activation segment. Our purpose was to determine if such activation is dependent on redox-sensing cysteines within p38α.
Methods and Results: Following the exposure of rat cardiomyocytes or whole hearts to H2O2 (50 μM) p38α was activated and formed a heterodimer with MKK3 that was sensitive to reduction by mercaptoethanol. The abundance of this heterodimer was enhanced by co-administration of Auranofin (2 μM) suggesting redox cycling occurs in vivo. We predicted that Cys119 and/or Cys162, both close to the known MKK3 docking domain, could act as electron donors and form a disulphide bridge with MKK3. Dimer formation was reduced with p38α Cys119Ser and increased with p38α Cys162Ser suggesting these residues act as vicinal thiols. p38αCys119Ser/Cys162Ser was incapable of sensing H2O2. Similarly, heterodimer formation was abolished in H9C2 cells (rat heart embryonic myoblast cell line) expressing MKK3 and p38αCys119Ser/Cys162Ser following simulated ischaemia and reperfusion. p38α. The anti-inflammatory agents, 15d PGJ2, a naturally occurring end product of prostaglandin D2 metabolism and 10-nitro-oleic acid, a component of the Mediterranean diet, reduced p38α activation and covalently modified Cys119/Cys162, likely obstructing MKK3 access.
Conclusion: Our novel findings suggest that cysteines within p38α act as redox sensors dynamically regulating p38α activation.

TU-061

Pluripotent stem cell microRNA-294 as a mediator of cardiac proliferative response in the heart after myocardial infarction

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Rationale: Embryonic heart is characteristic of rapidly dividing cardiomyocytes required to build a working myocardium. In contrast, cardiomyocytes retain some proliferative capacity in the neonates but lose most of it

in adulthood. Embryonic stem cell cycle (ESCC) miRs are a class of microRNAs regulating the unique cell cycle of ESCs and their characteristic pluripotency. Nevertheless, expression of miR-294, a member of the ESCC miRs is lost during developmental transitions from the ESCs to mature cells. Effect of miR-294 to induce cardiac proliferation and heart function has not been previously studied.

Objective: To determine whether miR-294 drives cardiac proliferative response leading to augmentation of cardiac function after myocardial infarction.

Methods and Results: miR expression analysis in the heart during development revealed elevated levels of miR-294 in the prenatal stages while the expression was lost in the neonates and adults as confirmed by qRT-PCR. Neonatal ventricular cardiomyocytes (NRVMs) treated with miR-294 mimic to showed elevated mRNA levels of cell cycle markers (*E2F family and cyclins*) concurrent with increased expression of p-histone 3, Ki67 and Aurora B kinase (G2/M) as confirmed by immunocytochemistry compared to control cells. Cardiac progenitor cells (CPCs) engineered with miR-294 lentivirus led to increased proliferation and metabolic activity. AAV-9 carrying miR-294 was administered in mice subjected to myocardial infarction augmented cardiac function 8 weeks after injury. Increase myocyte proliferation was observed in the heart after miR-294 treatment as analyzed by BrdU uptake, p-Histone 3 and Aurora B expression by immunostaining. Concurrently, a decrease in infarct size along with decreased apoptosis was observed in the miR-294 hearts compared to the control. Furthermore, increased c-kit+ CPCs activation and proliferation was observed in the miR-294 receiving hearts.

Conclusion: Ectopic expression of miR-294 recapitulates embryonic signaling and enhances cardiomyocyte ability to proliferate together with CPC activation and expansion leading to augmented cardiac function in mice after myocardial infarction.

TU-062

Proliferation of the cardiac precursor cells expressing the Stem Cell Antigen-1 is modulated by activation of the Natriuretic Peptide Receptors.

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Introduction: A part of the cardioprotective role of the Brain Natriuretic Peptide (BNP) in mouse hearts is due to its effect on the cardiac precursor cell (CPC) proliferation and differentiation. Thus, in this study we identified the CPC subset able to respond to BNP as well as the signaling pathway involved.

Methods and results: We demonstrated by immunohistochemistry and by flow cytometry analysis that the c-kit$^+$ and the Sca-1$^+$ cell subsets in neonatal and adult murine hearts express the NPR-A and NPR-B receptors and are thus able to be stimulated by BNP. *In vitro*, BNP only stimulated the proliferation of the Sca-1$^+$ cells and not of the c-kit$^+$ cells. Among Sca-1$^+$ cells, BNP treatment led to increased number of Sca-1$^+$ Nkx2.5$^+$ cells, which were able to differentiate into cardiomyocytes.

To determine by which receptor BNP acts on Sca-1$^+$ cells to stimulate their proliferation, cells were isolated from neonatal hearts of mice deficient for the NPR-A (NPRA-KO) or NPR-B receptor. BNP stimulated the proliferation of the Sca-1$^+$ NPR-A KO cells but not of the Sca-1$^+$ cells lacking the NPR-B receptor, demonstrating that Sca-1$^+$ cell proliferation is linked to NPR-B activation. This was confirmed by stimulating the Sca-1$^+$ cells by the C-Natriuretic Peptide able also to activate the NPR-B receptor.

BNP binding to NPR-B receptor led in Sca-1$^+$ cells to Protein Kinase G activation and increased phosphorylation of phospholamban and p38. Reducing PKG activation inhibited BNP-induced-Sca-1$^+$ cell proliferation, whereas reducing p38 phosphorylation increased Sca-1$^+$ cell proliferation after BNP treatment. Phosphorylation of p38 was not mediated by BNP binding to NPR-B receptor but by its binding to NPR-A.

Conclusion: In this work, we identified the Sca-1$^+$ cells as being the targets of BNP *in vitro* and *in vivo*. BNP via NPR-B binding and PKG activation clearly stimulated the proliferation of the CPCs expressing Sca-1. Interestingly, is the dual role of the NPR-A and NPR-B receptors which control Sca-1$^+$ cell proliferation.

TU-064

Enzymatic degradation of 7-Ketocholesterol (7-KC), a new strategy for the treatment of Atherosclerosis.

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Background

7-ketocholesterol (7KC), an oxidized derivative of cholesterol, has been implicated in a variety of chronic diseases including atherosclerosis, Alzheimer's disease, Parkinson's disease, cancer and age-related macular degeneration.

It is formed by the autooxidation of cholesterol and especially cholesterol-fatty acid esters found in lipoprotein deposits, its elevated concentrations are associated with disruption of cellular homeostasis, decreased cell viability, and increased cell death.

Enzymatic cleavage of 7-KC can serve as a key solution for the cure of a number of chronic diseases directly associated with its accumulation.

Methods

Isolation of potential 7KC degraders was done from a diverse environmental samples. Molecular identification was done and HPLC analysis was carried out.

Results

Alcanivorax jadensis IP4 (accession number KP309836), isolated from sea water and sediment sample, Streptomyces auratus IP2 (accession number KP309837),Serratiamarcescens IP3 (accession number KP309838) isolated from soil, and ThermobifidafuscaIP1 (accession number KM677184), isolated from manure piles was found to effectively degrade 7-KC. All the isolates were capable of utilizing 7KC as the sole organic substrate, resulting in its mineralisation.

Further characterization of microbial genes and ultimately the enzymes involved in 7KC catabolism can lead to the development of a single potential therapeutic enzyme preparation to target number of above mentioned chronic diseases.

TU-065

Restoration of prostaglandin E2 levels in the mesenchymal stem cells prevents their rejection in the ischemic heart and preserves ventricular function

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INTRODUCTION: Allogeneic mesenchymal stem cells (MSCs) from young healthy donors are immunoprivileged. The initial phase I and II clinical trials with allogeneic MSCs suggested that transplanted cells were safe and improvement in the heart function was observed. However, the long term fate of the transplanted cells in these trials was not determined. We recently reported that MSCs lost their immunoprivilege late after implantation in the ischemic heart and were rejected, resulting in progressive deterioration of heart function. The present study reveals the mechanisms responsible for this post-transplantation immune switch in MSCs. **METHODS/RESULTS:** MSC immunoprivilege was found to be mediated by prostaglandin E2 (PGE2), the levels of this soluble factor decreased in rat MSCs after exposure to hypoxia/ischemic conditions which was associated with loss of immunoprivilege. We observed increased cytotoxicity in hypoxic MSCs caused by allogeneic T cells in the *in vitro* co-culture. Furthermore, blocking PGE2 biosynthesis in normoxic MSCs increased the immunogenicity of MSCs. MSCs immunoprivilege is reported to be established by the absence of major histocompatibility complex class II (MHC-II) molecules. Our data suggests that MHC-II expression increased in MSCs after exposure to hypoxia. PGE2 treatment of hypoxic MSCs decreased MHC-II expression and preserved their immunoprivilege. In a rat myocardial infarction (MI) model, allogeneic MSCs (3×10^6 cells/rat), with or without a biodegradable hydrogel that slowly released PGE2, were injected into the infarct region. Five weeks later, MSCs were rejected in the control group (no PGE2), but in the PGE2-treated group, significant number of the transplanted cells survived and heart function were significantly improved. **CONCLUSIONS:** Immunoprivilege of allogeneic MSCs was maintained by PGE2 mediated regulation of MHC-II levels, exposure to hypoxia/ischemia decreased PGE2 and increased MHC-II levels that was associated with loss of immunoprivilege and rejection of MSCs. Maintaining PGE2

levels preserved immunoprivilege and restored cardiac function after an MI.

TU-066

Upconversion Nanoparticle-mediated Photodynamic Therapy Induces THP-1 Macrophage Apoptosis and THP-1 Macrophage-derived Foam Cell Autophagy via ROS Burst

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Background: AS is a chronic disease characterized by accumulation of lipid and infiltration of inflammatory cells, which is the major cause of acute cardiovascular events. Of several contributing cell types, macrophages play a vital role of atherosclerotic plaque progression. Photodynamic therapy (PDT) has emerged as a useful therapeutic naturopathy not only in the treatment of cancer but also in the treatment of AS. Here we investigated the molecular mechanisms based on PDT, using mesoporous-silica-coated upconversion fluorescent nanoparticles encapsulating chlorin e6 (UCNPs-Ce6) in the induction of apoptosis in THP-1 macrophages and autophagy in THP-1 macrophage-derived foam cells. **Results:** Firstly, the induction of reactive oxygen species (ROS) and regulation of mitochondrial permeability transition pore (MPTP) to depolarize mitochondrial membrane potential (MMP) were observed in in THP-1 macrophages via UCNPs-Ce6-mediated PDT. Both Bax translocation and the release of cytochrome C were examined using immunofluorescence and Western blotting. Our results indicated that the levels of ROS were significantly increased in the PDT group, resulting in both MPTP opening and MMP depolarization, which led to apoptosis. In addition, immunofluorescence and Western blotting revealed that PDT induced both Bax translocation and the release of cytochrome C, as well as upregulation of cleaved caspase-9, cleaved caspase-3, and cleaved poly(ADP-ribose) polymerase. Moreover, we found that UCNPs-Ce6-mediated PDT could induce autophagy in THP-1 macrophage-derived foam cells, which showed the LC3-II/LC3-I conversion, increased expression of Beclin 1 and decreased expression of P62, and the formation of acidic vesicular organelles (AVOs). Assuredly, autophagy was induced by ROS and could be blocked by pretreatment with ROS inhibitor N-acetyl cysteine (NAC). Furthermore, The UCNPs-Ce6-mediated PDT induced autophagy was activated through PI3K/AKT/mTOR pathway. **Conclusion:** In summary, we demonstrated that ROS as vital intracellular mediators, produce by UCNPs-Ce6-mediated PDT can induce apoptosis in THP-1 macrophages and autophagy in THP-1 macrophage-derived foam cells.

TU-067

Bone marrow mesenchymal stem cells reprogrammed into cardiac progenitor cells by nano-protein transfection bio-unit

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AIM: Bone marrow mesenchymal stem cells (BMSCs) are stem cells from mesoderm period with potential of self-renewing, multiple differentiation, and are popular in clinical application because of the low immunogenicity. However, most of recent study only focus on their paracrine function, the ability of directly differentiating into cardiomyocyte has always been controversial, hence there is no doubt that direct BMSCs transplantation will fail to develop its full potential. Cardiac progenitor cells (CPCs) is a kind of specific stem cells from heart tissue, besides the basic characteristics of normal stem cells, they can differentiate into three heart spectrum directly (including cardiomyocyte, endothelial cells and smooth muscle cells in the heart), acting as a good “stemness” source. Therefore, this study will focus on the differentiation efficiency of how protein induced BMSCs reprogramming to generate CPCs.

METHODS: The nanometer reagents are set up in advance, then begin to transfect different kinds of proteins (green fluorescent protein GFP, Tbx5, Hand2, Mef2c and Gata4 transcription factor) into BMSCs. Lipofectamine-2000 was used as positive control. After inducting for 1 d, 3 d, 8 d and 15 d, the cells’ morphological

changes were observed respectively, total RNA and protein were extracted for detecting the expression levels of myocardial progenitor markers. Protein-induced CPCs (piCPCs) transplanted into rat hearts after myocardial infarction to observe if the cardiac function was improved.

RESULTS: Four transcription factors, Gata4, Hand2, Mef2c and Tbx5 all can entirely be targeted and led into the hBMSCs nucleus when modified by nano-protein transfection bio-unit. Fluorescence microscope observation revealed a near 100% efficiency of transfection. The whole process of transfection took about more than 15 d to differentiate into myocardial line. With regard to the expression of protein, we found the expression of makers of all three cell lines of cardiac progenitor cell: endothelial cell line (such as CD31), cardiomyocyte line (such as Nkx2.5) and smooth muscle cell line (such as sm_MHC and α-SMA) all had expressions at the 15d after transfection. After reprogramming, H3K4me3 and H3K9ac increased on the -10kb enhancer region of Nkx2.5. In rats, the hearts undergoing piCPC transplantation showed decreased fibrosis compared with those treated with vehicle at 4 weeks after myocardial infarction.

CONCLUSION: Nano-protein transfection bio-unit can control the gene expression of the host cells, leading to complete transformation of the parental phenotype using a method that is virus free and does not introduce any foreign genetic material into the recipient's system. This protein reprogramming strategy lays the foundation for future refinements and might provide a good source of CPCs for regenerative approaches.

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TU-068

Beta 2 adrenegic receptor expression and activation of endogenous progenitor cells

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Background: Endogenous progenitor cells may participate in cardiac repair after a myocardial infarction. The beta adrenergic pathway has been proposed to induce proliferation and migration of progenitor cells. However, the mechanisms have not yet been clarified.

Methods and Results: The mechanism underlying beta adrenergic signaling on endogenous c-kit+/CD45- cardiac cells was investigated by inducing myocardial infarction in adult mice. Hearts were dissociated and flow cytometry analysis demonstrated that one week after ligation, the percentage of c-kit+/CD45- cells expressing beta 1 or beta 2 adrenergic receptor was significantly increased (88.1±3% and 106.8±36.5% increase compared to sham respectively). Flow cytometry studies on cultured cardiac c-kit+/CD45- cells confirmed increased beta 1 and 2 adrenergic receptor expression in response to stress conditions, specifically hypoxia (5%) or serum starvation. Interestingly, stress conditions altered localization of the beta 2 adrenergic receptor by increasing membrane expression. The beta 2 adrenergic receptor signaling pathway was stimulated in adult sham mice with the agonist fenoterol (0.25 mg/kg/day) administered in drinking water. Seven days after treatment the mice and non-treated controls were sacrificed and progenitor cells were measured by flow cytometry in the heart and blood. Fenoterol increased the proliferation and percentage of c-kit+/CD45- cells in the heart (123.3±86.2% and 70.9±44.6% increase compared to control respectively). Fenoterol treatment also elevated levels of circulating endothelial progenitor cells (158.5±87.9% compared to control) and c-kit+/CD45- cells (70.6±33.5% increase) in the peripheral blood.

Conclusion: Beta adrenergic receptor expression in cardiac c-kit+/CD45- cells is increased after coronary ligation *in vivo* and in stress conditions *in vitro*. A beta 2 adrenergic receptor agonist may be used to improve endogenous cardiac repair through the activation of progenitor cells.

TU-069

Adult ovine cardiomyocytes express the cell cycle-inhibiting gene *Meis1*. A potential target for cardiac regeneration based on cardiomyocyte division

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Introduction: *Meis1* is a transcription factor known to regulate adult cardiomyocyte cell cycle. In mice it keeps cell cycle arrest through interactions with cell cycle inhibiting proteins (p15, p16, p19, p21), and its decreased expression is a mitogenic stimulus for postnatal cardiomyocytes. Meis1 inhibition may therefore be a potential means to promote adult cardiomyocytes division and this, in turn, could represent a potential cardiac regenerative therapy. We thus aimed at searching for *Meis1* and other cell cycle regulatory genes in adult sheep cardiomyocytes

Methods: Primers were designed targeting *meis 1*, *cdkn1a*, *cdkn2aip*, *cdk2*, *cdk4*, *cdk6*, *ciclin E1*, *ciclin D2* and *gapdh* genes using the information of exon junctions to detect only mRNAs. End point RT-PCRs were performed from adult healthy sheep myocardium RNA. PCR products were recovered and molecularly cloned into a generic plasmid. The plasmids, each containing the target sequences of one gene, were sequenced to confirm that the insert corresponded to the desired fragment. These plasmids were used as calibrators of real-time-PCR with SyBrGreen to obtain efficiency and dynamic range parameters.

Results: The genes *meis1, cdkn1a, cdkn2aip, cdk2, cdk4, cdk6, ciclin E1* and *ciclin D2* were expressed in ovine myocardium. Real time PCR was optimized employing the calibrator plasmids, obtaining a dynamic range of 10^2–10^8 for all genes, and adequeate efficiencies for quantitative estimations. The ratio target gene/GAPDH for the analyzed genes was: *Meis1*: 2.7, *cdk4*: 2.5, *cdk6*: 2.8, *Ciclin D2*: 3.6.

Conclusion: *Meis 1* is expressed in adult sheep myocardium, thus being a potential target for silencing strategies aimed at fostering adult cardiomyocyte cell cycle reentry and divison.

TU-070

High doses of high mobility group box-1 (HMGB1) protein increase capillary and arteriolar densities and induce overexpression of genes involved in angiogenesis and cell proliferation in ovine infarct border zone

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Introduction: In mice with AMI, administration of the pro-inflammatory protein HMGB-1 improved heart function and induced angiogenesis due to VEGF overexpression. CKit+ cells were also detected, suggesting cardiomyogenesis. However, neither the effects nor the optimal dose of HMGB-1 in large mammalian models of AMI have been addressed, this being important to develop translational therapies for humans. We thus assessed the effect of two doses of HMGB-1 on microvascular neoformation and the expression of genes involved in angiogenesis and cell proliferation in the infarct border zone of sheep with AMI.

Methods: Twenty-one sheep with AMI received, 4 hours after coronary ligation, a total of 10 µg (high dose, n=7) or 1 µg (low dose, n=7) of HMGB1 in 10 intramyocardial injections in the peri-infarct zone. Placebo animals (n=7) received PBS. One week later, animals were sacrificed to quantify capillary and arteriolar densities (anti- lectin and smooth muscle actin immunohistochemistry, respectively) and expression of *vegf, ckit, gata 4 and nkx2.5* genes (RT-qPCR).

Results: Arteriolar density was higher than placebo in the high-dose group (39.4±11 vs. 23.2±4 arterioles/mm^2, $p<0.05$, X±SD, ANOVA-Bonferroni) and in low-dose group (39±14, p=NS) although not statistically significant. Capillary density was higher than placebo in high-dose group (2828±511 capillaries/mm^2 $p<0.05$) vs placebo (1711±194 cap/mm^2, $p<0.01$) but not in the low dose group (2341 ± 379 capillaries/mm^2, p=NS). *Vegf, ckit, and nkx2.5* expression was significantly higher than placebo only in the high-dose group. *Gata4* was significantly higher than placebo in both high and low-dose groups.

Conclusions: In a large mammalian model of AMI, high, but not low, dose of HMGB1 injected in the peri-infarct zone induced overexpression of angiogenic and cell proliferation genes and microvascular proliferation. Studies addressing whether high-dose HMGB1 is in fact cardioprotective in terms of infarct size limitation and cardiac function improvement in the long term are ongoing.

TU-071

$P2Y_2$ nucleotide receptor prompts human cardiac progenitor cell activation

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Heart failure is a leading cause of death in the US due to the limited capability of adult mammalian heart to regenerate following injury. Autologous stem cell therapy holds promise for regeneration of injured myocardium after myocardial infarction. However, stem cells derived from diseased organs exhibit impaired proliferative and migratory capabilities and increased susceptibility to cell death. Empowering stem cells from diverse origins, including cardiac progenitor cells (CPCs), with pro-survival genes has been attempted. Despite the well-established roles of purinergic signaling mediated by extracellular nucleotides in regulating diverse cellular responses in cardiovascular diseases, it has not been well-defined in CPCs. This study shows, for the first time, that the majority of P2 purinergic receptors are expressed and exhibit functional responses to ATP and UTP in mouse and human CPCs. The G protein-coupled $P2Y_2$ receptor ($P2Y_2R$) is a pivotal stress detector that senses ATP and UTP accumulated in extracellular space following injury/stress and responds with the proper regenerative responses. $P2Y_2R$ induces cardioprotective responses in animal models as well as human cardiomyocytes and regulates a wide range of signaling pathways that are crucial to tissue repair in various experimental models and in stem cells from diverse origins. Hence, we aimed to determine whether $P2Y_2R$ plays similar roles in CPCs. $P2Y_2R$ stimulation with UTP enhances human CPC (hCPC) proliferation and migration. UTP-induced proliferation in hCPCs involves activation of the canonical ERK1/2 pathway. UTP also induces YAP activation revealing a novel link between extracellular nucleotides released during cardiac ischemia and extracellular matrix sensing and Hippo signaling that have been recently implicated in cardiac regeneration. Interestingly, hCPCs that exhibit relatively slower growth kinetics and higher levels of senescence markers show dramatic decreases in $P2Y_2R$ expression compared to fast-growing hCPCs consistent with our hypothesis that overexpressing $P2Y_2R$ participates in rejuvenating hCPCs and improving their regenerative potential.

TU-072

Polylactic acid sheets seeded with genetically modified ovine diaphragmatic myoblasts for myocardial regeneration

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Our aim was to isolate, culture and characterize ovine diaphragmatic myoblasts (DMs), transduce them with connexin 43 gene (Cx43) to induce connection between cells, and finally grow them on scaffolds made from different materials to generate DMs-carrying sheets for later application on infarcted areas of sheep hearts with coronary artery ligation. Sheep diaphragm biopsies were digested with collagenase. The extracted DMs were cultured on a feeder layer of autologous activated macrophages (MFD) and characterized with antibodies against desmin, sarcomeric α-actin, SERCA-2 ATPase and Ki-67. To promote inter- cell connections, DMs were transduced with a lentivirus encoding connexin-43 after testing transduction efficiency of diverse multiplicities of infection (MOI) using lentivirus-GFP. DMs were satisfactorily grown on MFD, were positive for all antibodies and were able to differentiate into myotubes expressing myo-D and myosin heavy chain. With MOI=100, transduction efficiency was 70.8% and Cx43 was extensively expressed. Finally, in order to select the most adequate material to build up DMs-carrying sheets, we seeded DMs on scaffolds made from ovine pericardium (OP, n=8), pig bladder extracellular matrix (ECM, n=8) and polylactic acid (PLA, n=8), and tested DMs confluence (C) at 4 days using a score in which 0=0% C, 1=1-20% C, 2=21-40% C, 3=41-60% C, 4=61-80% C, and 5=81-100% C. C was 0 with PB, 2.6±1.1 with ECM and 4.75±0.5 with PLA ($p<0.0001$ vs. PB and ECM; X±SD, ANOVA-Bonferroni). Conclusion: MDs were successfully isolated, cultured and transduced. PLLA was the most appropriate

material to generate DMs-carrying sheets. These results are the first step towards testing therapeutic efficacy of DMs in sheep models of myocardial infarction.

TU-073

Rapid Stabilisation of Atherosclerotic Plaque with 5-Aminolevulinic Acid-Mediated Sonodynamic Therapy

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Background: 5-Aminolevulinic acid-mediated sonodynamic therapy (ALA-SDT) effectively induces the apoptosis of atherogenic macrophages, but whether it can stabilise atherosclerotic plaque in vivo is unclear. Here, we used an animal model to evaluate the effects of ALA-SDT on plaque stabilisation.

Methods: Sixty rabbits were induced atherosclerotic plaques in the femoral artery with a combination of silastic tube placement with atherogenic diet, and randomly assigned into control (n = 12) and SDT (n = 48) groups. In the SDT group, after intravenous injected with ALA (60 mg/kg) animals underwent the treatment of ultrasound with intensities of 0.75, 1.00, 1.50 and 2.00 W/cm2 (n = 12 for each intensity). Seven days after the treatment, the plaque disruption assay was performed to test plaque stability.

Results: We found that ALA-SDT with ultrasound intensity of 1.5 W/cm2 showed the strongest efficacy to stabilise plaques. Under this condition, the frequency of plaque disruption decreased by 88 % ($p < 0.01$), positive area of macrophages reduced by 94 % ($p < 0.001$) and percentage content of lipids dropped by 60 % ($p < 0.001$), while percentage content of collagens increased by 127 % ($p < 0.001$). We also found that the plaque stabilisation by ALA-SDT was associated with increased macrophage apoptosis and apoptotic cell clearance. Moreover, ALA-SDT decreased the contents and activities of matrix metalloproteinase-2,9 and increased the levels of tissue inhibitors of matrix metalloproteinase-1,2 in plaques.

Conclusion: Our studies demonstrate that ALA-SDT promotes plaque stabilisation by inducing macrophage elimination and inhibiting matrix degradation. This method might be a promising regimen for atherosclerosis therapy.

TU-074

Cellular mechanisms of osteogenic differentiation in the development of aortic valve calcification

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Background: Interstitial cells of the aortic valve (VICs) may transform into osteoblast-like cells causing calcification and valve stenosis. The mechanism of this process is unclear. The process occurs exclusively on the aortic side of the valve leaflets, and not on the ventricular side. We sought to investigate the side-specific role of inflammation and mechanical stretch and to study if valvular endothelial cells (VECs) may have a role in osteogenic differentiation of VICs.

Methods: VICs were isolated from human aortic valves with or without calcification harvested during surgery. 1. VICs were cultured with or without osteogenic medium for 21 days. 2. VICs were cultured on collagen or elastin pre-coated plates (to simulate the aortic and ventricular side of the valve leaflets respectively) and subjected to 10% stretch at 1 Hz (FlexCell bioreactor), or lipopolysaccharide (LPS) 0,1µg/ml, or both. Calcification was assessed by Alizarin Red staining with quantification. 3. VECs were seeded over a 3D culture of VICs embedded in 2 mg/ml collagen gel and cultured for 21 days in osteogenic medium. The expression of mRNA of osteogenic markers (bone morphogenetic protein 2 (*BMP2*), beta-catenin (*BCAT*) and runt-related protein-2 (*RUNX2*) was evaluated by RQ-PCR.

Results: VICs from calcified valves cultured with osteogenic medium showed higher calcium accumulation and expression of osteogenic markers then from non-calcified. LPS triggered calcification in a culture of VICs on collagen, but not on elastin (Alizarin red staining and mRNA expression of osteogenic markers).

Mechanical stretch of fibroblasts cultured on collagen augmented the effect of LPS. VECs obtained from calcified valves stimulated osteogenic differentiation of non-calcified VICs.

Conclusion: Cell culture models of osteogenic differentiation and valve calcification were established. LPS-induced inflammation and stretch contribute to calcification of VICs on the collagen-coated surface in contrast to the elastin-coated. VECs may stimulate valve calcification by cross-talk with VICs.

TU-075

Cortical bone stem cells derived exosomes can promote cardiac repair mechanisms after myocardial injury

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Rationale: Adoptive transfer of stem cells into failing human hearts has been shown to be safe, but leads to modest improvements due to low cell retention and diminished viability of cells after transplantation in the ischemic environment. Recently we have shown in a mouse model that cortical bone derived stem cells (CBSCs) possess enhanced ability to improve cardiac function after MI mainly via secretion of paracrine factors. Since exosomes represent the active component of released factors whether CBSC derived exosomes have the potential to repair heart after injury in a cell autonomous manner is presently unknown.

Objective: Determine the therapeutic value of CBSC exosomes and their contents for myocardial repair.

Methods and Results: Exosomes were isolated from murine CBSCs by ultracentrifugation. The purified fraction of exosomes was analyzed for size by dynamic light scattering measurement and transmission electron microscopy and showed typical size range of exosomes from 30-100nm. CBSCs derived exosomes showed increased cardiac protection in vitro in NRVMs after hypoxic challenge as measured by TUNEL staining. To determine myocardial repair ability, CBSC exosomes (60μg) were injected in mice after myocardial infarction. Improved cardiac function was observed in CBSC exosomes injected mice compared to saline controls 6 weeks after MI. Importantly, CBSC exosomes treated animals showed increased myocyte survival and angiogenesis. The underlying mechanism for beneficial effects was tied to increased packaging of cardioprotective miRNAs in the exosomes compared to the parent cells as confirmed by MiRNA array analysis.

Conclusion: Exosomes derived from CBSCs provides a cell free system that uses the reparative power of CBSC to augment cardiac function after myocardial injury recapitulating our earlier findings with CBSCs. Increased packaging of cardioprotective miRs compared to the parent's cells highlighting a potential new insight into the salutary effects of exosome therapy.

TU-076

Detection of serum vascular endothelial growth factor and its clinical significance in lymphoma patients

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Abstract :

Objective: To explore the correlations of serum vascular endothelial growth factor (VEGF) levels with clinical tumor size in lymphoma patients and to evaluate the value of VEGF in the diagnosis of lymphoma.

Methods: Serum VEGF levels were detected by ELISA in 53 patients with lymphoma and 42 healthy controls.

Results: The serum VEGF levels in patients with lymphoma were (263.11±23.13) pg/ml, and the serum VEGF levels in healthy controls were (93.45±13.23) pg/ml. So, the serum VEGF levels in lymphoma patients compared to that in healthy controls was almost three-folds. It is significantly higher than their healthy controls (t=-3.810 , P ‹0.05). Serum VEGF level in lymphoma patients was significantly related to tumor size (t=-2.520 , P ‹0.05). The sensitivity of VEGF for the diagnosis of lymphoma were 90.0%, and was significantly higher than other common serum tumor markers and the specificity was 80.6%..

Conclusion: The level of serum VEGF significantly increased in lymphoma patients and was also associated with tumor size. which may have great clinical

significance for the screening and diagnosis of lymphoma.

Key words: vascular endothelial growth factor ; tumor markers ; lymphoma

TU-077

Genome-wide siRNA screening identifies cellular genes regulating AAV transduction in the cardiovascular system

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Recombinant adeno-associated viral vectors (AAVs) are currently considered as the vectors of choice for in vivo gene transfer for cardiovascular applications. Genetic simplicity, possibility of generating high-titer vector preparations, lack of inflammatory response and, most notably, capacity to deliver genes into postmitotic cells are among their most notable characteristics. Over the last few years, using AAV9, the most cardiotropic serotype, we have obtained extensive and persistent transduction of the myocardium in both small (rodent) and large (dogs, pigs) animals and taken advantage of this property to assess function of both protein coding genes and small RNA molecules.

Notwithstanding their favorable characteristics, several unknowns still hamper a broader and more effective use of these vectors. These limitations are mostly related to our still limited understanding of their interaction with the host cell proteins. To tackle this issue in a systematic manner, we performed an unbiased high throughput RNAi screening (18,120 human target genes) and identified 1,483 genes affecting vector efficiency more than 4-fold and up to 50-fold, either negatively or positively. Most of the identified factors have never previously been associated to AAV infection. The most effective siRNAs we identified were independent from the virus serotype or the cell type used and were equally evident for single-stranded and self-complementary AAV vectors. A common characteristic of the most effective siRNAs was the induction of cellular DNA damage and the activation of a cell cycle checkpoint. These characteristics appear important to explain the specific tropism of these vectors for post-mitotic cells, including cardiomyocytes, in which DNA damage recognition occurs through molecular pathways differing from those active in replicating cells.

TU-078

Synthetic patches "BASEX" for management of post-infarction aneurysm of heart

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Objective: To show the antimicrobial, thromboresistant, low porosity properties of the "BASEX" patches which can be used for geometric reconstruction(GR) of left ventricle(LV) following post-infarction left ventricular aneurysms of the heart.

Materials: "BASEX" (Bokeria-Abdulgasanov-Spyridonov explants) patches is being manufactured and used in our center since 1997. Domestic textiles were subjected to various modifications for producing "BASEX" patches. Medical gelatin was used as a base for modifying its coating. To maintain the antimicrobial and thromboresistant properties of the coatings were introduced, antimicrobials (ciprofloxacin, metronidazole) anticoagulants(heparin), antiaggregants(acetylsalicylic acid, dipyridamole). GR LV using "BASEX" patches were done on 742 patients. In 25% of patients were additionally done mitral valve interventions.

Results: Postoperative complications were observed in 18% patients. Major postoperative complications were CHF (21.6%), arrhythmias (22.9%) and neurological complications (8.9%). Hospital mortality was 6.4%. The main causes of deaths were heart failure, multiple organ failure and ventricular fibrillation.

Mural thrombosis around the patch was observed in 4 (0.54%) patients. Thromboembolic complications were not observed. Infection of patch was observed in 3 (0.4%) patients. First patient suffered from sepsis due to post-injection abscess 2 years after surgery. She was re-admitted to hospital in a terminal condition. Autopsy revealed an abscess above the patch with penetration into pericardial cavity. In second patient, 2 months after surgery complained of episodes of fevers, on

examination vegetations were noticed around the patch, patient refused to proposal for re-surgery, his further fate is unknown. In the third case infections were managed using conservative interventions.

Conclusion: Thus the synthetic patches "BASEX" which showcased antimicrobial, thromboresistant, low-porosity properties can widely be used in reconstructive corrections of the LV of heart.

TU-079

Role of miRNA-33a in Dilated Cardiomyopathy

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Background: Dilated cardiomyopathy (DCM) accounts for approximately 1/3rd of total cases of heart failure (HF) and is a leading indication for cardiac transplantation. Myocardin (MYOCD), a potent transcriptional co-activator of smooth muscle (SM) and cardiac genes, is upregulated in failing myocardium in animal models and human end-stage heart failure (HF). microRNAs (miRNAs) are 20-22 nucleotide long non-coding RNAs regulate gene expression. However, the role of miRNAs regulating MYOCD expression in heart failure remains unknown. The goal of this study was to identify the miRNAs regulating the cardiac MYOCD and to study the molecular and functional consequences of cardiac modulation of MYOCD specific miRNA in an animal model of HF/DCM.

Method and Results: Our study design included identification and validation of miRNA targeting MYOCD using *in silico* approach and 3'-UTR luciferase reporter assay and to study its cardiac expression in idiopathic DCM (IDCM) endomyocardial biopsies, renal artery ligation (RAL) rat model of HF/DCM. We identified and validated miRNA-33a as a putative regulator of MYOCD expression in cardiomyocytes. Cardiac miRNA-33a expression was significantly decreased in IDCM and in RAL. We studied the role of miRNA-33a in two important processes of cardiac remodelling that is cardiac hypertrophy and fibrosis. We also investigated if cardiac specific augmentation of miRNA-33a expression using a homing peptide conjugated siRNA could potentially modulate the cardiac remodelling and outcome in RAL. We observed that targeted modulation of miRNA-33a attenuated cardiac hypertrophy and fibrosis, decreased expression of hypertrophy and fibrotic genes and ameliorated the impaired diastolic dysfunction in RAL model of cardiomyopathy.

Conclusion/Significance: This data provide the first evidence that miRNA-33a is involved in regulating cardiac MYOCD expression as well as regulation of cardiac remodelling process and cardiac specific augmentation of miRNA-33a offers a putative therapeutic target in DCM.

TU-080

Symptomatic arterial hypertension: modern methods of diagnosis and treatment (results based on examination of 2050 patients)

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Aim: To diagnose secondary or symptomatic arterial hypertension (SHT) in patients with primary or essential hypertension (EHT)

Materials and methods: During 1986-2015 were examined 2050 patients aged 5-75 years with a diagnosis of EHT. With comprehensive examinations in 71.0% patients, EHT could not be confirmed.

Results: During comprehensive examinations, nephrogenic (parenchymal) hypertension (chronic pyelonephritis, nephrolithiasis, hypernephroma etc.) was diagnosed in 42.0%, coarctation of the aorta in 2.5%, Reno vascular hypertension in 5.3%, aneurysm in 9.7%, non-specific aortoarteritis and congenital hypoplasia in 1.0% patients. Endocrine hypertension was

diagnosed in 15.8% patients of which adrenal pheochromocytoma was the cause of hypertension in 1.8%, primary hyperaldosteronism (Conn's syndrome) in 9.8%, Cushing's syndrome in 0.8%, lesions of cerebral arteries in 1.8% patients. Medicinal hypertension was the cause of hypertension in 0,8%, alcoholic hypertension in 0.3%, cocaine hypertension in 0.3% and use of oral contraceptives in 0.5% patients.
Conclusion: Thus with comprehensive examinations (ultrasound, CT, MRI) of patients with EHT, the cause of hypertension could be confirmed in 70% patients. The widespread use of highly informative diagnostic techniques can significantly reduce the proportion of EAH. Upto 80% of small-sized tumors could not be detected by traditional methods. Surgical interventions in 80-85% patients helped minimize dosage of antihypertensive drugs, reduce cerebral and cardiac complications, improved quality of life. Lifetime antihypertensive therapy in SHT is indicated only to patients who are contraindicated to surgical, endovascular, endoscopic corrections or in its ineffectiveness.

TU-082

Increased Expression of Calreticulin in the Heart: Cardiac Fibrosis and Heart Failure

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One detrimental aspect of cardiac failure is an increase in fibrosis with surplus deposition of extracellular matrix proteins. This can reduce cardiac function but the underlying mechanism of why this happens is still unclear. Increased abundance of calreticulin in adult heart has been associated with dilated cardiomyopathy and heart failure. Here, we discovered that increased expression of calreticulin in the adult mouse heart leads to severe cardiac fibrosis. To investigate the mechanism behind calreticulin-dependent increase in cardiac fibrosis, we utilized microarray hybridization and monitored global gene expression in calreticulin transgenic hearts with impaired ER homeostasis. We observed significantly enhanced expression of TGF-ß1, a pleiotropic cytokine, as well as fibrillar collagens when compared with control hearts. Validation of protein expression showed that TGF-ß1 expression and secretion into the circulatory system was significantly increased as well as receptor-regulated Smad2/3 expression, also activated in calreticulin transgenic hearts. Several pro-inflammatory factors and markers of fibrosis, including NF□B p65, and pro-inflammatory cytokines, TNF□, IL-1ß, and IL-6, were noticeably up-regulated. The expression and localization of periostin, a ligand for integrins that supports cellular adhesion and migration, was increased in calreticulin transgenic hearts. Furthermore, ER stress was increased as measured by XBP1 splicing analysis (IRE1 activity), due to the overexpression of calreticulin in the heart. However, cardiac fibrosis triggered by calreticulin overexpression was effectively reduced by administration of tauroursodeoxycholic acid (TUDCA), possibly due to TUDCA's inhibitory effects on ER stress. We concluded that the mechanism leading to cardiac fibrosis in adult hearts overexpressing calreticulin may involve impaired ER homeostasis triggering activation of ER stress coping responses, activation the TGF-ß1/Smad2/3 signaling pathway which may lead to cardiac fibrosis with this pathogenesis suppressed by TUDCA treatment.
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TU-083

TRPV2 regulates the development of myocyte hypertrophy

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Background: Transient Receptor Potential Vanilloid (TRPV2) channels function as stretch mediated regulators of calcium homeostasis in various cell types. We have demonstrated that TRPV2 channels are fundamental in contractility and calcium handling in the cardiomyocyte. Herein, we tested the hypothesis that TRPV2 channels mediate the hypertrophic response of cardiomyocytes in vitro as well as with clinically relevant mouse models of hypertrophy via genetic ablation of the channel and with a TRPV2 blocker (tranilast).
Methods: Isolated ventricular myocytes were obtained from wild type (WT) mice, while WT and TRPV2-/- mice were used for in vivo experiments. Isolated myocytes

were exposed to phenylephrine (PE) as a hypertrophic stimulus and their calcium transients were measured via FURO-4. In-vivo mice were exposed to various hypertrophic stimuli including transverse aortic constriction (TAC), isoproterenol infusion and angiotensin II (Ang-II) infusion. Cardiac function was measured in vivo via echocardiography weekly and via invasive catherization at the terminal endpoint. Post mortem molecular markers of hypertrophy and failure as well as fibrosis and myocyte size were measured.

Results: We report that TRPV2 is upregulated in response to increased hypertrophic stimuli such as PE, TAC and Ang-II but not directly via adrenergic stimulation in vivo. The genetic deletion and pharmacologic blockade of TRPV2 inhibited the hypertrophic response as noted via echocardiography, histology and molecular markers of hypertrophy, but did not result in cardiovascular collapse as noted via echocardiography, invasive catherization or markers of failure. Interestingly, both TRPV2 deletion and blockade resulted in significantly reduced myocardial fibrosis.

Conclusions: We conclude that TRPV2, as a stretch mediated channel, modulates the development of cardiomyocyte hypertrophy and may be a target for the prevention of left ventricular hypertrophy in patients at risk for heart failure with preserved ejection fraction.

TU-084

Normalization of Cardiac Energy Metabolism and Left Ventricular Hypertrophy Precede Functional Recovery in the Regression of Heart Failure

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AIMS: Impaired cardiac substrate metabolism plays a key role in heart failure (HF) pathogenesis. Since many of these metabolic changes occur at the transcriptional level of metabolic enzymes, it is possible that this loss of metabolic flexibility is permanent and thus contributes to worsening cardiac function and/or prevents full regression of HF upon treatment. However, despite the importance of cardiac energetics in HF, it remains unclear whether these metabolic changes can be normalized. In the current study, we investigated whether reversal of an elevated aortic afterload in mice with severe HF would result in recovery of cardiac function, substrate metabolism and transcriptional reprogramming, as well as determine the temporal relationship of these changes.

METHODS AND RESULTS: Male C57Bl/6 mice were subjected to either sham or transverse aortic constriction (TAC) surgery to induce HF. After HF development, mice with severe HF (% ejection fraction <30) underwent a second surgery to remove the aortic constriction (debanding). Three weeks following debanding, there was a near complete recovery of systolic and diastolic function, and gene expression of several markers for hypertrophy/HF were returned to values observed in healthy controls. Interestingly, pressure overload-induced left ventricular hypertrophy (LVH) and cardiac substrate metabolism were restored at 1 week post-debanding, which preceded functional recovery.

CONCLUSIONS: Regression of severe HF is associated with early and dramatic improvements in cardiac energy metabolism and LVH normalization that precede restored cardiac function, suggesting that metabolic and structural improvements may be critical determinants for functional recovery.

TU-085

Genetic background does not affect progression to heart failure in a mouse model with genetic ablation of RyR2-S2808 phosphorylation

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Background. The pathophysiological relevance of cardiac ryanodine receptor (RyR2) phosphorylation has been of great interest for over 20 years. This field was boosted when Marks et al. proposed that S2808 hyperphosphorylation is a critical mediator of heart failure (HF) progression. Many laboratories, however, have been unable to reproduce key elements of this hypothesis, including a better outcome after myocardial infarction (MI) in a RyR2-S2808A mouse model. Our aim was to determine whether part of this discrepancy is due to the genetic background of the mice, since Marks' model was C57Bl/6 and that created by the Valdivia laboratory was Sv129. **Methods.** RyR2-S2808A Sv129

mice were backcrossed for seven generations with C57Bl/6J mice to obtain the congenic mouse line with >99% C57Bl/6 genetic background. Congenic RyR2-S2808A mice and C57Bl/6J wild type (WT) controls were then: 1) evaluated with a basal echocardiogram one week before MI; 2) subjected to MI by LAD coronary ligation; 3) followed-up with an echocardiogram one- and four-weeks post-MI. **Results.** The basal echocardiogram of S2808A and WT mice did not show statistical difference. MIs performed in both groups were significant, as determined by a decrease in ejection fraction (EF) and fractional shortening (FS), as well as dilation of the left ventricle (LV). S2808A mice did not show better outcome than WT mice up to four weeks post-MI. All parameters measured were comparable between groups, including EF (30.85±4.14% WT vs. 35.05±3.83% S2808A, p=0.47), FS (14.87±2.17% vs. 17.06±2.06% p=0.47), LV diameter (5.21±0.20 mm vs. 5.21±0.29 mm, p=0.99) and heart weight as percentage of body weight (0.72±0.03% vs. 0.70±0.05%, p=0.78). **Conclusions.** The genetic background of the mice is unlikely the source of discrepancy between results obtained by the Marks and Valdivia/Houser groups using different RyR2-S2808A mice. These data support the notion that RyR2-S2808 phosphorylation is not critically involved in HF progression.

TU-086

CDK6 mediates the effect of attenuation of miR-1 on provoking cardiomyocyte hypertrophy

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MicroRNA-1 (miR-1) is approved involved in cardiac hypertrophy, but the underlying molecular mechanisms of miR-1 in cardiac hypertrophy are not well elucidated. The present study aimed to investigate the potential role of miR-1 in modulating CDKs-Rb pathway during cardiomyocyte hypertrophy. A rat model of hypertrophy was established with abdominal aortic constriction (AAC), and a cell model of hypertrophy was also achieved based on PE-promoted neonatal rat ventricular cardiomyocytes (NRVCs). We demonstrated that miR-1 expression was markedly decreased in hypertrophic myocardium and hypertrophic cardiomyocytes. Dual luciferase reporter assays revealed that miR-1 interacted with the 3'UTR of CDK6, and miR-1 was verified to inhibit CDK6 expression at the posttranscriptional level. CDK6 protein expression was observed increased in hypertrophic myocardium and hypertrophic cardiomyocytes. Morover, miR-1 mimic, in parallel to CDK6 siRNA, could inhibit PE-induced hypertrophy of NRVCs, with decreases in cell size, newly transcribed RNA, expressions of ANF and β-MHC, and the phosphorylated pRb. Taken together, our results reveal that derepression of CDK6 and activation of Rb pathway contribute to the effect of attenuation of miR-1 on provoking cardiomyocyte hypertrophy.

TU-087

The interplay between genetic background and sexual dimorphism of doxorubicin-induced cardiotoxicity

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Background: Doxorubicin (DOX) is a very effective anticancer medication that is commonly used to treat both hematological malignancies and solid tumors. Nevertheless, DOX is known to have cardiotoxic effects that may lead to cardiac dysfunction and heart failure. In experimental studies, female animals have been shown to be protected against DOX-induced cardiotoxicity; however, the evidence of this sexual dimorphism is inconclusive in clinical studies. Therefore, we sought to investigate whether the genetic background could influence the sexual dimorphism of DOX-induced cardiotoxicity. **Methods:** Male and female Wistar Kyoto (WKY) and Spontaneous Hypertensive Heart Failure (SHHF) rats were used in this study. DOX was administered in 8 doses of 2 mg/kg/week; thereafter, the rats were followed for an additional 12 weeks. Cardiac function was assessed by trans-thoracic echocardiography, systolic blood pressure was measured by the tail cuff method, and

heart and kidney tissues were collected for histopathology. **Results:** Female sex protected against DOX-induced weight loss and increase in blood pressure in the WKY rats, whereas it protected against DOX-induced cardiac dysfunction and the elevation of cardiac troponin in SHHF rats. In both strains, female sex was protective against DOX-induced nephrotoxicity. There was a strong correlation between DOX-induced renal pathology and DOX-induced cardiac dysfunction. **Conclusions:** This study highlights the importance of studying the interaction between sex and genetic background to determine the risk of DOX-induced cardiotoxicity. In addition, our findings suggest that DOX-induced nephrotoxicity plays a role in DOX-induced cardiac dysfunction.

TU-088

Rnd3/RhoE is a Pro-Angiogenic Factor Regulating Responsive Cardiac Angiogenesis

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Background—The insufficiency of compensatory angiogenesis in response to pathological stimuli contributes to the transition to heart failure. HIF1α-VEGF signaling cascade controls angiogenesis in the heart in response to stress. One of the challenges in reprograming the insufficient angiogenesis is to achieve a sustainable tissue exposure to the pro-angiogenic factors such as by stabilizing HIF1α.

Methods and Results—In this study, we identified Rnd3, a small Rho GTPase, as a new pro-angiogenic factor participating in the regulation of HIF1α-VEGF signaling cascade. Rnd3 physically interacts with and stabilizes HIF1α, consequently promoting VEGFA expression and endothelial cell tube formation. To demonstrate this pro-angiogenic role of Rnd3 *in vivo*, we generate Rnd3 knockout mice. Rnd3 haploinsufficient ($Rnd3^{+/-}$) mice are viable, yet develop dilated cardiomyopathy with heart failure after transverse aortic constriction (TAC). The post-TAC $Rnd3^{+/-}$ hearts show significantly impaired angiogenesis and decreased HIF1α and VEGFA expression. The angiogenesis defect and heart failure phenotype are partially rescued by cobalt chloride treatment, a HIF1α stabilizer, confirming a critical role of Rnd3 in stress-responsive angiogenesis. Furthermore, we generate Rnd3 transgenic mice (MHC-Rnd3) and demonstrate that Rnd3 overexpression has a cardio-protective effect through reserved cardiac function and preserved responsive angiogenesis after TAC. Finally, we assess the expression level of Rnd3 in the human heart and detect significant downregulation of Rnd3 in patients with end-stage heart failure.

Conclusions—Rnd3 acts as a novel pro-angiogenic factor involved in cardiac responsive angiogenesis through HIF1α-VEGFA signaling promotion. Rnd3 downregulation observed in heart failure patients may explain the insufficient compensatory angiogenesis contributing to the transition to heart failure. The assessment of Rnd3 expression levels in patients could be a new reference biomarker for human heart failure.

TU-089

Telomeres in cardiac hypertrophy: tails of a broken heart

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Background: Cardiac hypertrophy, an abnormal increase in cardiac mass, is the most potent risk factor for heart failure after age. Association studies have shown that aberrations in telomere dynamics correlate with cardiac mass but possible causal mechanisms remain unknown. The aim of the study was to determine whether changes in cardiomyocyte telomere dynamics result in hypertrophy.

Methods: Telomere dynamics were altered in human primary cardiomyocytes (HPCs) with siRNA knockdowns of TERT, the catalytic component of the telomere-lengthening enzyme telomerase, and TRF2, an integral telomeric protein. Telomere elongation in HPCs was inhibited pharmacologically by suppressing telomerase activity (BIBR) and preventing its binding to telomeres (TMPyP). Cell size was measured by confocal microscopy and telomerase activity with the TRAP assay. Telomeric and hypertrophic gene expression as well as telomere length was determined using qPCR.

Results: Reduced expression of TERT ($p=<0.001$), TRF2 ($p=<0.001$), and

decreased telomerase activity (p=0.001 and p=0.004 for BIBR and TMPyP, respectively) significantly increased cardiac cell size. The hypertrophic gene NPPA was significantly upregulated following TERT-siRNA (p=0.04) and TRF2-siRNA (p=<0.001) treatments. However there was no significant change in telomere length in any of the groups (all p=<0.05).

Discussion: These findings demonstrate that disturbances in telomere dynamics are sufficient to induce cardiomyocyte hypertrophy, independently of telomere length. The involvement of TRF2 in maintaining telomere loops independently of length and the absence of telomerase activity at telomeres suggests an important role for shelterin structure in cardiomyocyte size and hypertrophic gene expression.

Conclusions: These results highlight the importance of telomere dynamics influencing hypertrophic gene expression and maintaining cardiomyocyte size in the absence of changes in telomere length.

TU-090

Improved metabolic function and contractility in *mdx* mice following treatment with morpholino oligomers.

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Duchenne Muscular Dystrophy (DMD) is an X-linked muscular disease that involves a fatal cardiac phenotype. DMD-associated cardiomyopathy is underpinned by disrupted cytoskeletal architecture and mitochondrial dysfunction, and current treatment strategies to date are limited to minimising symptoms of the disease. Here we report a recovery of metabolic and contractile function in *mdx* mice (a murine model of DMD) following treatment with antisense morpholino oligomers to induce skipping of dystrophin exon 23 (M23D). Optimal treatment regimen was first established by varying dosage and route of administration using a three week treatment trial in neonates. Activation of the L-type Ca^{2+} channel (LTCC) facilitates Ca^{2+} influx required for contraction, but also causes an increase in mitochondrial membrane potential (Ψ_m) in a Ca^{2+}-independent manner. This is dependent on the cytoskeleton and is disrupted in *mdx* mice. Recovery of metabolic function was assessed by monitoring LTCC-dependent increases in Ψ_m (JC-1 fluorescence) and mitochondrial oxygen consumption (flavoprotein autofluorescence) in isolated cardiomyocytes. A total weekly dose of 120mg/kg M23D administered s.c. was optimal in neonatal *mdx* cardiomyocytes, and restored the BayK(-)-mediated increase in Ψ_m (treated=29.0±2.0%, n=51; untreated=1.0±0.4%, n=22) and flavoprotein oxidation (treated=16.0±2.0%, n=25; untreated=2.0±0.5%, n=21). Using this treatment regimen, 24 week old adult mice with established cardiomyopathy were treated for 16 weeks. We report a post-treatment restoration of BayK(-)-mediated increases in Ψ_m (treated=32.0±3.1%, n=6; untreated age-matched=1.2±1.2%, n=4) and flavoprotein oxidation (treated=55.4±15.4%, n=17; untreated age-matched=4.1±1.6%, n=8). In addition, echocardiographic measurements revealed a decrease in end diastolic diameter in systole (treated=2.5±0.mm, n=4; untreated age-matched=2.8±0.0mm, n=3) and increase in fractional shortening (treated=37.0±1.6%, n=4; untreated age-matched=31.3±0.5%, n=3) in adult mice upon completion of 16 weeks treatment. These results indicate that treatment with M23D results in restoration of metabolic function and improvement in contractility in adult mice with established cardiomyopathy.

TU-091

Identification of miR-34 regulatory networks in settings of disease and antimiR-therapy: Implications for treating cardiac pathology and other diseases

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Expression of the miR-34 family (miR-34a, -34b, -34c) is elevated in settings of heart disease, and inhibition with antimiR-34a/antimiR-34 has emerged as a promising therapeutic strategy. Under chronic cardiac disease settings, targeting the entire miR-34 family is more effective than targeting miR-34a alone. The identification of transcription factor (TF)-miRNA regulatory networks has added complexity to understanding the therapeutic potential of miRNA-based therapies. Here, we sought to determine whether antimiR-34 targets secondary miRNAs via TFs which could contribute to antimiR-34-mediated protection. Using miRNA-Seq we identified differentially regulated miRNAs in hearts from mice with cardiac pathology due to transverse aortic constriction (TAC), and these miRNAs were also regulated by antimiR-34. Two clusters of stress-responsive miRNAs were classified as "pathological" and "cardioprotective". Using ChIPBase we identified 45 TF binding sites on the promoters of "pathological" and "cardioprotective" miRNAs, and 5 represented direct targets of miR-34, with the capacity to regulate other miRNAs. The expression of two "pathological" miRNAs (let-7e and miR-31) was independently experimentally validated in hearts from antimiR-34 treated TAC mice, and may explain why targeting the entire miR-34 family is more effective than targeting miR-34a alone. AntimiR-34 regulates the expression of other miRNAs and this has significant implications for drug development.

TU-092

Cardiac Thyrotropin Releasing Hormone (TRH) Inhibition attenuates the post-ischemic damage and improves ventricular function after myocardial infarction in rats

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Heart injury induces ventricular remodeling. Particularly acute myocardial infarction causes myocytes damage, reactive hypertrophy and interstitial fibrosis in the infarcted area.

We described TRH system hyperactivity in left ventricle (LV) hypertrophied SHR´s hearts. Indeed, TRH inhibition prevents cardiac hypertrophy despite the severe hypertension suggesting its involvement (Schuman et al, 2011). We observed that LV TRH overexpression in normal rats induces features of the hypertrophic phenotype (Schuman et al 2014).

Microarray studies revealed LV TRH increase after myocardial infarction (Jin H. et al 2004), and added to our reports, we hypothesized that LV TRH inhibition previous to infarct maneuver could attenuate cardiac remodeling damage.

Adults Wistar males were infarcted by permanent anterior descending coronary artery ligation simultaneously to 40ug LV SiRNA injection against TRH or scrambled siRNA (control). At day 6 ventricular function evaluation was performed (echocardiography) and 24h later animals were sacrificed for heart gene expression quantitation (RT-PCR).

Infarcted rats showed an expected significant decrease in ejection fraction and increases in heart rate and end diastolic volume compared to sham group and according to our hypothesis, the animals in which LV TRH system was blocked all these changes were not observed pointing out that LV TRH inhibition prior to injury improves ventricular function and decreases contractility and heart dilatation.

As expected, we found a LV TRH overexpression in infarcted rats injected with siRNA-Control accompanied by significant increases in BNP, ANP, β-MHC and Collagen III and decreases in SERCA2 and α-actin expressions in harmony to heart tissue damage profile including the contractility system.

LV TRH inhibition which reduced significantly TRH gene expression, blunted BNP, ANP, Collagen III and β-MHC increase and normalized the expression of SERCA2 and α-actin.

These novels results demonstrate the participation of TRH in post-ischemic remodeling and reveal that its inhibition attenuates the damage and improves ventricular function.

TU-093

Functional role of G9a-induced histone methylation in cardiac hypertrophy

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Cardiac hypertrophy is an initially adaptive response of the myocardium to increased work overload and can progress to heart failure (HF). At the molecular level, it's associated with a specific gene expression program. The role of histone methylation in regulating this program is poorly understood. Our group has recently shown that an epigenetic signature defined by methylation and acetylation of histone H3 regulates the gene expression changes accompanying cardiac hypertrophy. However, the molecular pathways that define this signature are not elucidated yet. Here, we show that histone methyl-transferase G9a is differentially regulated in cardiomyocytes of mice subjected to transverse aortic constriction (TAC) — a procedure that through pressure overload induces first compensated hypertrophy then HF — and in stressed human hearts.

G9a is a histone methyl-transferase that specifically mono and di-methylates Lys-9 of histone H3 and tri-methylates Lys-27 of histone H3, leading to transcriptional repression and these histone modifications contribute to cellular memory by establishing gene expression programs during development and subsequently stabilizing the differentiated state.

We first assessed whether G9a had a role in regulating cardiac function at baseline conditions in vivo. To this end, C57BI/6 mice were treated with a selective inhibitor (BIX-01294) up to four weeks via subcutaneous mini-osmotic pumps. Mice treated with the drug showed a significant decrease in cardiac function, as assessed by echocardiographic analysis, compared to control groups (untreated mice and mice treated with vehicle). Thus, baseline G9a inhibition seemed to cause progressively heart failure. To confirm that this effect was due to G9a in cardiomyocytes, we generated conditional cardiac G9a ko (KO) mice and we analyzed the effects of down-regulated G9a in the heart of these mice. After 4 weeks of the induction of the myocardial deletion of G9a, by echo analysis, biochemical and histological studies, we observed a HF phenotype similar to that of mice treated with G9a inhibitor, in KO mice compared to controls.

Data in vitro and in vivo will be presented in support of our hypothesis showing that G9a is important in defining the correct transcription program of cardiomyocytes and in regulating gene expression re-programming during cardiac hypertrophy. Our work may lead to the development of new therapeutic strategies for HF based on the modulation of this epigenetic enzyme.

TU-094

Guanylyl Cyclase-A Signaling Attenuates Deleterious Salt Effect on Aldosterone-Induced Cardiac Remodeling

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Background: Sodium causes the development of cardiovascular disease such as hypertension, cardiac hypertrophy and heart failure in conjunction with enhanced renin-angiotensin-aldosterone system (RAAS). Natriuretic peptide (NP), which is an important sodium regulator, prevents pathological cardiac alternations by counteracting RAAS. However, it is not elucidated whether NP inhibits sodium-effect on adverse cardiac alternations. We investigated whether salt excess exacerbates cardiac remodeling in mice with impaired NP signaling.

Methods and Results: Mice lacking the gene encoding the NP receptor (guanylyl cyclase (GC)-A) and wild type (WT) mice were assigned to vehicle or subpressor dose of aldosterone (100 ng/kg/min) administration group under low salt (0.001% NaCl), normal salt (0.6% NaCl) and high salt diet (6.0% NaCl) for 4 weeks. Salt load did not induce cardiac change in both vehicle and aldosterone groups in WT mice. On the other hand, cardiac hypertrophy and interstitial fibrosis were significantly exacerbated in a salt dependent manner in aldosterone groups of GC-A KO mice, associated with enhanced gene expression relevant to hypertrophy, fibrosis and oxidative stress (BNP, collagen1 and Nox4, respectively). Of note, salt excess increased the expression of Sgk1, an important downstream of mineralocorticoid receptor (MR), in aldosterone groups of GC-A KO mice.

These molecular changes were not observed in WT mice.

Conclusion: The present study demonstrates that salt excess induces cardiac remodeling in conjunction with aldosterone in GC-A KO, but not in WT mice. These data indicate that the GC-A signaling attenuates the deleterious salt effect on aldosterone-induced cardiac remodeling.

TU-095

The role of fibroblast and endothelial cell NADPH oxidase-2 in the development of cardiac fibrosis

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Background

NADPH oxidase-2 (NOX2) is elevated in myocardium of heart failure patients. Global NOX2 knockout (KO) mice have reduced cardiac fibrosis in models of elevated angiotensin II (Ang II) or chronic pressure overload. NOX2 is expressed in cardiomyocytes, fibroblasts, endothelial cells and inflammatory cells, but the NOX2-expressing cell type responsible for these anti-fibrotic effects is unknown.

Aim

To investigate the role of fibroblast and endothelial NOX2 in the development of cardiac fibrosis.

Methods

We generated inducible fibroblast-specific or endothelial-specific NOX2 KO mice by crossing *Col1a2*-Cre or *Cdh5*-Cre mice with a novel floxed-NOX2 mouse model. Cre recombinase expression was induced with tamoxifen and the fidelity of cell-specific targeting was evaluated by using a STOP-floxed tdTomato reporter strain.

Results

Both models were successfully generated. Flow cytometry of cardiac cellular digests of STOP-floxed reporter mice indicated that >96% of fibroblasts or endothelial cells underwent recombination. In a model of Ang II infusion (1.1 mg/kg/day), fibroblast-specific NOX2 KO mice developed less cardiac fibrosis than wild-type (WT) littermates (1.59% vs. 2.58%; *p<0.05*, n=4-6). However, transverse aortic constriction (TAC) caused a similar extent of cardiac fibrosis (7.62% vs. 7.21%; *p=0.97*) in fibroblast-specific NOX2 KO and WT controls. Endothelial-specific NOX2 KO subjected to TAC also developed similar fibrosis to WT littermates (11.8% vs. 9.61%; *p=0.69*). There were no significant differences in the extent of cardiac hypertrophy or contractile dysfunction between fibroblast-specific or endothelial-specific NOX2 KO and their respective WT littermate controls.

Conclusion

Although fibroblast NOX2 contributes to the development of Ang II-induced cardiac fibrosis it has no effect on TAC-induced fibrosis. Endothelial NOX2 is also dispensable for TAC-induced fibrosis. These results suggest that other NOX2-expressing cell types are required for the development of cardiac fibrosis in response to chronic pressure overload.

TU-096

NADPH oxidase-4 mediates cardiac adaptation to volume overload

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Background: Chronic pressure and volume overload induce concentric versus eccentric remodelling respectively. Distinct signalling pathways are likely involved in these responses but the underlying pathways are incompletely defined. NADPH oxidase-4 (Nox4), a reactive oxygen species (ROS)-generating enzyme, reduces detrimental cardiac remodelling during chronic pressure overload but its role in volume overload-induced remodelling is unknown.

Methods: Aortocaval fistula (ACF) was performed to induce volume overload in male, global Nox4-null mice (KO) and wildtype (WT) littermates. Animals were followed up for 2 weeks.

Results: 2 weeks of ACF in WT mice caused a significant increase in cardiac Nox4 mRNA (1.6 fold, $p<0.05$) and protein expression (2.0 fold, $p<0.01$) compared to sham controls but no change in Nox2 levels. KO mice developed significantly less LV hypertrophy (+25% vs +43% increase in LV/tibia length ratio, $p<0.01$) and less LV dilatation (echocardiographic LVEDD: 4.6

mm vs 5.1 mm, p<0.01) than WT animals after ACF. LV ejection fraction was similar in both genotypes following ACF, as were levels of ANP, BNP and SERCA-2α mRNA. Phospho-Akt levels increased in WT mice after ACF whereas levels decreased in KO mice (+29% vs -21%, p<0.05). The levels of phospho-Erk1/2 decreased to similar levels after ACF in both genotypes (-37% vs -29% in WT and KO respectively, p=n.s.).

Conclusion: Nox4 appears to be required for the development of eccentric cardiac remodelling and hypertrophy during chronic volume overload. Nox4-dependent activation of Akt may be involved since Akt is implicated in the development of adaptive cardiac dilatation during volume overload. Ongoing studies are assessing the impact of Nox4 deletion during more prolonged volume overload.

TU-097

Structural and functional changes in the murine heart during sustained β-adrenergic stimulation in vivo

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Purpose: To determine the structural and functional responses of the murine heart to sustained β-adrenoceptor stimulation in vivo.

Methods: C57/BL6J mice aged 8 weeks were randomly assigned to receive a subcutaneous infusion of saline or isoprenaline (30 µg/g/day) for 3 days (n=9/group) or 14-days (n=8/group). At the end of the 14-day infusion period, the mice in each group were randomly assigned to receive a single bolus intraperitoneal injection of saline or dobutamine (0.75 µg/mg) (n=4/group). Cardiac phenotype was assessed by high-resolution echocardiographic imaging and standard gravimetric and histological assays.

Results: β-adrenergic stimulation for only 3 days induced cardiac hypertrophy (significant increases in left ventricular (LV) wall thicknesses and heart weight, heart weight/body weight ratio and heart weight/tibia length ratio). Mice subjected to more prolonged β-adrenergic stimulation for 14 days exhibited comparable differences in cardiac structure relative to corresponding saline-infused mice, but at this stage such differences were accompanied also by enhanced LV function (significantly greater LV fractional shortening and ejection fraction) and increased heart rate. Interestingly, relative to mice that had received saline for an identical period, mice that had received isoprenaline infusion for 14 days exhibited significantly lower LV fractional shortening and ejection fraction following acute β-adrenergic stimulation with dobutamine, in the presence of a similarly elevated heart rate.

Conclusion: These observations indicate that, during sustained β-adrenergic stimulation by isoprenaline infusion at 30 µg/g/day, structural hypertrophic remodelling occurs predominantly within the initial 3 days and precedes persistent positive inotropic and chronotropic responses. Sustained β-adrenergic stimulation for 14 days induces a loss of contractile reserve, which is revealed only when an acute β-adrenergic stress is superimposed on the hypertrophied heart. Thus, acute β-adrenoceptor stimulation with dobutamine may be a useful method to unmask early signs of LV dysfunction in the remodelled heart, even when basal function appears enhanced.

TU-098

Distinct Roles of Intracellular Calcium Release Channels in Cardiac and Vascular Remodelling

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Background - Calcium release from intracellular stores controls countless cellular processes. Ryanodine receptors (RyRs) and inositol 1,4,5-trisphosphate receptors (IP3Rs) are the major calcium release channels on the endo/sarcoplasmic reticulum (ER/SR). RyRs and IP3Rs comprise macromolecular signalling complexes that include modulatory proteins which regulate channel activity in response to extracellular signals eventually resulting in intracellular calcium release.

Methods and Results - We investigated the respective functional roles of different RyR and IP3R isoforms in the pathophysiology of vascular and cardiac dysfunction. To this aim, we generated tissue specific knockout murine models via a *flox/cre* recombinant technique, targeting endothelial cells (EC), vascular smooth muscle cells (VSMC), and ventricular cardiomyocytes. Combining *in vivo*, *ex vivo*, and *in vitro* techniques we demonstrated for

the first time that: 1) IP3R1 is directly involved in nitric oxide (NO) production in EC via a calcineurin/nuclear factor of activated T-cells (NFAT) pathway, and its deletion in EC causes a hypertensive phenotype; 2) IP3R1 in VSMC is a key player in the vasomotor responses both in basal conditions and during neurohormonal overdrive – mediated by both adrenergic and renin-angiotensin systems – following myocardial infarction obtained via ligation of the left anterior descending coronary artery; 3) in ventricular cardiomyocytes, RyR2, but not IP3R2, has a crucial role in determining mitochondrial dysfunction in heart failure.

Conclusions - Taken together, our results provide robust evidence towards a tissue-specific functional predominance within intracellular calcium release channels: IP3Rs are crucial in modulating vascular tone whereas RyRs are the main players in the regulation of myocardial contractility.

TU-099

Inhibition of Rho Kinase (ROCK) Restores Ionic Currents and Prevents Electrical Remodelling of Heart in Pressure Overload Induced Hypertrophy Model

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Background: Various cardiovascular diseases like myocardial infarction, heart failure and cardiac hypertrophy are associated with the RhoA/Rho kinase (ROCK) signalling pathway. Although electrical remodelling of left ventricle has been studied in pressure overload (PO) induced cardiac hypertrophy, effect of ROCK inhibition with selective ROCK inhibitor fasudil on action potential (AP) prolongation and relevant currents have not been studied yet. This study examined the impact of ROCK inhibition on AP duration and repolarizing potassium currents.

Methods and results: PO model is created by transverse aortic constriction (TAC) of rats. SHAM animals underwent surgery without banding. All data taken from three groups; SHAM, TAC and fasudil-treated (5 mg/kg and 10 weeks) TAC (Tac+Fas) group. In TAC group, increased heart weight (HW), HW/body weight ratio and HW/tibia ratio were observed and fasudil treatment attenuated these ratios. There was a significant prolongation in TAC myocytes AP duration which was similar to control values in Tac+Fas group. Inward rectifier (I_{K1}) and transient outward (I_{to}) potassium currents were recorded in whole-cell configuration of patch-clamp by step pulse protocol. Both currents decreased significantly in TAC myocytes, despite inhibition of ROCK reversed these currents to control values.

Expression level of relevant proteins RhoA, ROCK1, ROCK2, Kir2.1 and Kv4.2 were also examined. According to western blot analysis, Kv4.2 didn't change significantly while RhoA increased and Kir2.1 decreased in TAC myocardium. ROCK1&2 expressions decreased significantly after 10 weeks in TAC hearts. Fasudil administration brought these proteins changed in TAC heart to control levels.

Conclusion: These findings suggest that fasudil improves AP duration due to restoration of potassium currents and underscore the role of RhoA/ROCK pathway in development of pathological cardiac hypertrophy. Therefore inhibition of this pathway may be a potential target for therapeutic purposes in future.

TU-100

Restrictive cardiomyopathy mutation TnI-R145W blocks PKA-PKC cross-modulation of human myofilament length dependent activation and relaxation kinetics

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Background: The Troponin-I (TnI) R145W mutation is associated with the presentation of restrictive cardiomyopathy (RCM), high diastolic filling pressures, and a severe adverse clinical outcome. The molecular mechanisms underlying RCM in patients carrying this mutation are uncertain. It has been suggested that increased myofilament calcium sensitivity plays an important role in the disease. Myofilament calcium sensitivity and myofilament relaxation kinetics determine diastolic stiffness of the heart. These parameters are influenced by factors that include both PKA and PKC mediated contractile protein phosphorylation, as well as sarcomere length (SL) mediated regulation of contractile function via the process of myofilament length dependent activation (LDA).

Methods: Permeabilized multicellular myocardial muscle preparations or single myofibrils were isolated from frozen human donor septum, followed by overnight exchange for recombinant troponin composed of hTnC, hTnT-*myc*, and WT or mutated/phosphomimetic hTnI: S23D,S24D (PKA), T144E (PKC), R145W (RCM), S23D,S24D,T144E (PKA+PKC), and S23D,S24D,R145W (PKA+RCM). Force was measured in skinned muscles over a wide range of free [Ca^{2+}] at short and long SL to derive myofilament Ca^{2+} sensitivity and LDA; activation/relaxation kinetics were measured in single myofibrils at saturated [Ca^{2+}].

Results: PKA phosphomimetic induced a large reduction in myofilament Ca^{2+} sensitivity and a strong increase in LDA, while PKC phosphomimetic induced a slight increase in myofilament Ca^{2+} sensitivity, but no change in LDA. RCM induced a large increase in myofilament Ca^{2+} sensitivity and a decrease in LDA. Finally, PKA phosphomimetic accelerated the kinetics of myofibril relaxation while PKC phosphomimetic was without affect on this parameter. In contrast, RCM induced a strong slowing of myofibril relaxation rate. Both PKC phosphomimetic (PKA+PKC) and RCM mutation (PKA+RCM) virtually eliminated the impact of PKA phosphomimetic on: myofilament Ca^{2+} sensitivity, LDA, and myofibril relaxation kinetics. Finally, the hcTnI-R145W mutation caused the threonine phosphorylation target on hcTnI-144 to be inaccessible to a panel of PKC kinases, thus, rendering this PKC target effectively phospho-null in RCM. **Conclusions:** Phosphorylation of cTnI at PKA target S23/S24 reduces myofilament activation, increased length dependency (LDA), and accelerates relaxation kinetics, events that are expected to lower diastolic stiffness of the heart. Phosphorylation of PKC target T144 induces opposite properties, expected to enhance diastolic stiffness. The R145W RCM associated mutation induces a phenotype that is similar to PKC phosphomimetic, but constitutively. Moreover, the RCM mutation interrupts the normal phenotypical cross-modulation property between PKA and PKC contractile phosphorylation, such that the relaxing impact of PKA mediated phosphorylation caused by decreased myofilament Ca^{2+} sensitivity, enhanced LDA, and accelerated relaxation kinetics is no longer possible. We propose that these myofilament based properties contribute to elevated diastolic stiffness of the heart in RCM patients, especially during episodes of elevated beta-adrenergic stimulation, such as in exercise.

TU-101

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Rationale:

Heart failure is one of the most severe burden of cardiovascular diseases due to its striking prevalence, mortality and morbidity. Besides the clinical classification of distinct etiologies the underlying molecular mechanisms still remain unclear. Recent findings suggest that inflammatory pathways play critical roles remodeling and progression of heart failure.

Myocyte enhancer factor 2 (MEF2) is a crucial inductor of pathologic cardiac remodeling due to its effects on fetal gene programs. In a lately in-vitro study, we could show that Prostaglandin E2 (PGE2) leads to a strong MEF2-activation. Furthermore the investigation provides data that PGE2 stimulates via EP3-receptor an intracellular signal transmission, which drives a protein kinase D (PKD) dependent hyperphosphorylation of histone deacetylase 5 (HDAC5) and the resulting nucleo-plasmatic shuttling of HDAC5 leads to the increased MEF2 activity.

The aim of the present work is a translation of these findings in different in-vivo models to figure out its relevance in inflammatory cardiomyopathies.

Results:

In an experimental model of cardiac myosin-induced myocarditis, the resulting setting of inflammation comes along with increasing levels of the PGE2-forming Cyclooxygenase 2 and Prostaglandin-E-synthase 1. Furthermore we see an increase of catalytic PKD-activity and consecutively an HDAC5-hyperphosphorylation. Additionally raised mRNA-levels of MEF2-target genes like Myomaxin and CCL3/CCL6 represent the induction of cardiac remodeling genes. We validated the mechanism in other models of inflammatory cardiomyopathies and see confirming results in a model of LPS-

induced septic cardiomyopathy and a model of coxsackie B3-induced viral myocarditis as well.

Conclusion:

Inflammation and autoimmune response seem to play crucial roles in the induction of ventricular remodeling and progression of heart failure. With these in-vivo data, we provide evidence that PGE2 mediates an epigenetic pathway containing EP3-receptor transmitted phosphorylation of PKD and HDAC5 leading to a nucleo-cytoplasmatic shuttling of HDAC5 with the consequence of a MEF2 deregulation and induction of pathologic remodeling. The described pathway provides a new link between cardiac inflammation and the initiation and progression of remodeling and heart failure. Further investigations on the distinct pathway and its relevance in the different etiologies of remodeling is urgently needed and could provide new therapeutic targets to alleviate the burden of heart failure.

TU-102

Effects of a Selective Class I HDAC 1/2 Inhibitor on Cardiac Remodeling in Mouse TAC

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Pan Class I HDAC inhibitors (HDAC 1/2/3) have shown benefits in preclinical heart failure models, however, given the severe cardiac toxicity phenotype of *Hdac3* mutant mice, it remains unclear whether a selective Class I HDAC 1/2 inhibitor would have enhanced efficacy. Therefore, the objective of this study was to evaluate the therapeutic potential of a potent and selective small molecule HDAC 1/2 inhibitor (MRL-001; IC50: HDAC1=2.9nM; HDAC2=27nM; HDAC3=2553nM) to improve cardiac structure and function in a mouse model of chronic moderate thoracic aortic constriction (TAC). The study was performed blinded in c57/b6 mice (n=15/group), with in-feed doses of MRL-001 (3, 10 and 30 mg/kg/day) with treatment beginning 3-weeks post-TAC for a duration of 10-weeks. Dose selection was based on the highest tolerated dose in a 28-day pharmacokinetic /safety study. MRL-001 did not significantly alter TAC-mediated increases in anterior and posterior wall thickness or left ventricular (LV) weight at any dose. Likewise, MRL-001 had no effect on the TAC-induced reduction in ejection fraction, stroke volume, and LV filling, or the prolonged LV relaxation as measured by tau. Low doses of MRL-001, however, significantly attenuated LV dilatation as measured by end diastolic dimensions, with highest dose showing no effect. In summary, while MRL-001 did not demonstrate benefit on cardiac hypertrophy or function, MRL-001 did demonstrate cardiac structural improvements at low doses. The highest dose of MRL-001 was not effective and trended to worsen LV function and structure. In conclusion, MRL-001 preserved normal LV dimensions after TAC, but at the lower doses. The lack of effect on cardiac function differed from the literature with pan Class I inhibitors. It is possible that Class I HDAC 1/2/3 is needed for enhanced benefit, and the cardiac toxicity observed in *Hdac3* mutant mice will not be observed in pharmacological studies. Future studies will address this hypothesis.

TU-103

Direct and Selective AMPK Activation Fails to Improve Cardiac Structure and Function in Mouse Pressure-Overload

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Because chronic treatment with indirect AMPK activators AICAR and metformin improves cardiac structure and function in multiple preclinical heart failure settings, AMPK activation has been proposed as a promising therapeutic target for heart failure. AMPK mediated cardiac benefits hypothesized to drive efficacy include increased glucose uptake and metabolism, improved insulin sensitivity, enhanced endothelial function, anti-inflammatory and anti-fibrotic effects. Here, we evaluated the efficacy of a potent, direct, and selective small molecule AMPK activator (MRL-002) in mouse TAC. The study was performed blinded and included 5 groups, sham-vehicle, sham-10 mg/kg/day, TAC-vehicle, TAC-1 mg/kg/day and TAC-10 mg/kg/day. Treatment was administered in feed beginning 4 weeks prior to TAC surgery. Echocardiography and invasive

hemodynamics were performed to assess dose response effects of AMPK activation on cardiac structure and function over 12 weeks post-TAC. Exposures for the 10 mg/kg/day dose were consistent with those required for glucose lowering in lean C57Bl6 mice. Echocardiography revealed expected increases in wall thickness at early time points in TAC-vehicle mice as well as progressive decreases in fractional shortening and increases in chamber dimensions at later time points. 1 mg/kg/day MRL-002 blunted the increase in interventricular septal thickness at diastole at 4, 6, and 8 weeks post-TAC. Posterior wall thickness at diastole trended lower in treatment groups at early time points. Fractional shortening decreased and chamber dimensions increased similarly in both vehicle and MRL-002 treatment groups. Heart weights were also similarly increased. Thus, in the long-term, MRL-002 failed to improve cardiac function or alter progressive remodeling in this model. In addition and unexpectedly, invasive hemodynamics revealed dose dependent increases in Tau indicating prolonged relaxation following long term treatment. Data from this study fails to replicate findings observed with indirect AMPK activators and refutes the hypothesis that chronic direct activation of AMPK as a therapeutic approach for heart failure.

WE-001

The transactivation activity of glucocorticoid receptor plays a key role in protecting heart against stress and that is suppressed under pressure-overload

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[Objective]

We previously reported glucocorticoids protect heart against ischemia-reperfusion injury (J Clin Invest. 2009, Hypertension 2014) and acute viral myocarditis (J Cardiol. 2013). In the present study, we investigated the role of cardiomyocyte glucocorticoid receptor (GR) in pressure-overload induced cardiac remodeling.

[Methods and results]

We made cardiac-specific conditional knockout of GR (GRCKO) mice and mineralocorticoid receptor (MR) (MRCKO) mice. GRCKO and MRCKO mice had no phenotype in the steady-state condition. GRCKO mice showed exaggerated cardiac hypertrophy and worse systolic function in comparison with their wild-type (WT) littermates after 4 weeks of transverse aortic constriction (TAC). MRCKO showed a similar degree of hypertrophy and systolic function in comparison with their WT littermates after 4 weeks of TAC. MR and GR, while functionally redundant in some contexts, cardiomyocyte GR played a distinct functional specificities, since neither genetic ablation of MR nor pharmacological blockade of MR by eplerenone rescued the phenotypes observed for GRCKO mice under pressure overload. DNA microarray analysis of GRCKO and MRCKO in the steady-state condition revealed that cardiomyocyte GR has distinct transcriptional specificities in comparison with MR. Interestingly, GR transcriptional activities were suppressed under the pressure overloaded. GR-selective agonist dexamethasone ameliorated TAC-induced hypertrophy and preserved LV systolic function in WT mice.

[Conclusions]

Heart is the most stressful organ in the body. Cardiomyocyte GR transcriptional activities protects heart against pressure-overload. Homeostatic role of glucocorticoids-GR signaling in cardiomyocytes has been underestimated because of systemic adverse effects induced by glucocorticoids.

WE-002

Gentianella acuta Improves Cardiac Function in a Model of Coronary Ligation Induced Heart Failure via a Mechanism of Against Endoplasmic Reticulum Stress-Associated Autophagy

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Background Increased endoplasmic reticulum(ER) stress is known to be one of the causes of cardiovascular damage. Gentianella acuta (Michx.) Hulten can treat hepatitis, jaundice, headache and fever in Mongojia native medicine. However, the cardioprotective effect of Gentianella acuta has yet to be examined. The aim of the current study is to investigate the cardioprotective effect of Gentianella acuta

on ER stress-induced heart failure (HF) rats and its possible mechanisms.

Methods HF was induced using coronary artery ligation in adult male Sprague-Dawley rats and Gentianella acuta was used. Thirty minutes after surgery, rats were randomly assigned to 3 groups: HF (n =12) alone, HF with high-dose Gentianella acuta, or HF with low-dose Gentianella acuta treatments. Rats in medicine-treated groups were given 0.06 mL/10 g (once a day) of Gentianella acuta by gavage based on different doses (1.2 or 0.3- g/Kg). Sham surgery was performed in another group of rats (n =12) without coronary artery ligation. Cardiac function was assessed by echocardiography and cardic index 4 weeks after HF. After treated with Gentianella acuta for 4 weeks, cellular levels of ER stress marker and autophagy marker were evaluated by western blot analysis, immunohistochemistry and real time RT-PCR respectively.

Results Gentianella acuta effectively inhibited ischemia-induced heart failure, as evaluated by biometric, echocardiography, and histological examinations. Consistently, western blot analysis and immunohistochemistry showed that the protein level of ER Stress markers and autophagy marker in cardiac tissue were significantly lower after treatment with Gentianella acuta than HF group. Meanwhile, Gentianella acuta significantly increased p-AKT and p-mTOR expression in cardic tissue. In addition, Gentianella acuta was also found to inhibit GRP78, ATF4 and LC3 mRNA expression induced by HF.

Conclusions Taken toghter, our results suggest that ER stress-associated autophagy is essential for HF, which can be effectively improved by Gentianella acuta.

WE-003

Gentianella acuta prevents isoprenaline-induced myocardial fibrosis in rat by reduction of myocardial TGF-β1/ CTGF expression

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Gentianella acuta (Michx.) was used as folk medicine to treat hepatitis, jaundice, headache and fever in Mongolia native medicine. It has been used as a health tea to treat heart diseases for many years in Hulunbeier districts of inner Mongolia. So we thought that Gentianella acuta could inhibit myocardial fibrotic formation. In this study, we investigated the effect and potential mechanisms of the extract of Gentianella acuta on myocardial fibrosis. A rat myocardial fibrosis model was established by hypodermic injection of isoproterenol (5 mg/kg bw/day) for 7 days, when these rat were simultaneously treated with extract of Gentianella acuta（1.2 g/kg, 0.6 g/kg, 0.6 g/kg）or saline by gavage for 21 days. After 21 days, the rats underwent electrocardiograph detection and were sacrificed. Myocardial fibrosis was observed by Masson staining. NF-κB, TGF-β1 and CTGF protein expression were detected by immunohistochemistry and western blotting, TGF-β1 and CTGF mRNA expression were detected Real-Time PCR.Treatment with Gentianella acuta could significantly improve myocardial fibrosis and decrease the collagen accumulation, hydroxyproline content in myocardial tissue. Gentianella acuta could attenuated the cardiac dysfunction and decreased the ST-segment-elevation in isoproterenol rats. Real-Time PCR results indicated that the mRNA expression of TGF-β1 and CTGF in myocardial tissue was decreased. Importantly, Gentianella acuta could significantly decrease the protein expressions of TGF-β1, CTGF and NF-κB in myocardial tissue. The results of this research indicated that Gentianella acutal extract improved the cardiac function and anti-fibrotic activity by reducted TGF-β1 and CTGF expression via inhibition of NF-κB in myocardial tissues.

WE-004

Phosphodiesterase 3A1 Prevents Cardiac Remodeling from Neurohormonal Activation

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Background: β-adrenergic receptor (βAR) signaling and renin-angiotensin-aldosterone system (RAAS) are pivotal mechanisms to induce cardiac remodeling, and recent studies have revealed that there is direct interaction between βAR and RAAS. Phosphodiesterase 3A (PDE3A) inhibits βAR/protein kinase A axis by metabolizing cAMP. Therefore, we hypothesized that

overexpressed PDE3A has cardioprotective effects against neurohormonal activation. **Methods and Results**: Isoproterenol (ISO, 30 mg/kg/day for 7 days) or angiotensin II (AngII, 800 ng/min/kg for 10 days) was continuously infused using osmotic mini-pump in wild-type (WT) mice and transgenic (TG) mice with cardiac-specific expression of exogenous PDE3A1. Both ISO and AngII infusion increased heart weight/body weight ratio in WT mice compared with WT mice given vehicle, but not increased in TG mice. The 8-OHdG, a marker of oxidative DNA damage, positive area was increased by ISO stimulation in WT hearts compared with vehicle hearts (14.9±3.7% vs. 7.4±1.1%, P<0.05), but not in TG hearts (13.9±1.9% vs. 12.0±2.8%, ns). Protein expression levels of Sirt1, which provides anti-oxidative effects, were upregulated in TG hearts compared to WT hearts in both basal (1.9±0.2 AU vs. 1.0±0.1 AU, P<0.01) and after ISO infusion (2.8±0.2 AU vs. 1.3±0.2 AU, P<0.01), suggesting that PDE3A has anti-oxidative effects by upregulating Sirt1-related signaling. AngII induced cardiac fibrosis in both WT and TG mice, but the extent of fibrosis was less in TG mice compared to WT mice (4.2±1.1% vs. 6.9±2.6%, P<0.05). Moreover, basal expression levels of transforming growth factor-β were lower in TG hearts compared to WT hearts (0.31±0.05 AU vs. 1.00±0.10 AU, P<0.01), and it remained lower levels after AngII stimulation in TG hearts compared to WT hearts (0.52±0.09 AU vs. 1.72±0.29 AU, P<0.01). **Conclusion**: We conclude that PDE3A prevents cardiac remodeling by neurohormonal activation.

WE-005

Angiotension-converting enzyme inhibitor-induced cough prevalence in resistant hypertension patients

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Background: Resistant Arterial Hypertension (RAH) is characterized by persistently high blood pressure values. Angiotensin Converting Enzyme (ACE) inhibitors in combination with other antihypertensive drugs are effective for RAH. According to the literature, the adverse effect of cough in patients using ACE inhibitors occurs in 5-20% of patients. However, in practice, the incidence appears to be higher, making it difficult the therapeutic adherence. **Objective:** To estimate the prevalence of cough induced by ACE inhibitors in patients with RAH. **Methods:** Cross-sectional study in a referral hospital in severe hypertensive cardiovascular disease. To assess the adverse effect cough in the use of ACE inhibitors, patients answered to a questionnaire and the blood pressure (BP) was measured on the day of the interview. **Statistical Analysis:** Data were analysed using IBM SPSS Statistics Program for Mac version 21. Frequency and percentage were used for qualitative variables and mean ± standard deviation for quantitative variables. **Results:** 120 patients were analysed and 70% were female (84). The average age was 62,1 ± 12,4 years. 100% (120) of the patients use or had used ACE inhibitors. The prevalence of cough was 64,2% (77). 71,7% (86) of the patients started using an angiotensin II receptor blocker as an ACE inhibitor substitute. 13,9% (12) of patients reported that the cough continued even after the discontinuation of ACE inhibitor. Patients used an average of 4,7 ± 1,2 antihypertensive medications. The average systolic pressure was 151,8 ± 27,6 mmHg and the average diastolic pressure was 88,6 ± 16,3 mmHg. **Conclusion:** We observed a high prevalence of cough associated with the use of ACE inhibitor in this population. Despite the large number of antihypertensive drugs in use, the BP was not controlled in most of the patients. It is possible that the non-use of ACE inhibitors may contribute to the low hypertensive control.

WE-006

Inhibition of Class I Histone Deacetylases Blunts Cardiac Hypertrophy via TSC2-dependent mTOR Repression

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Introduction: Histone deacetylases (HDACs) participate in the pathogenesis of pathological cardiac growth, and small molecular inhibitors of HDACs reduce and regress pathological hypertrophy. The mammalian target of rapamycin complex 1 (mTORC1) is an important regulator of cell growth. It has been shown that mTORC1 is active during cardiac hypertrophy, leading to increased protein synthesis. Inhibiting mTORC1 can repress pathological remodelling. Therefore, we hypothesized that class I HDACs regulate cardiac hypertrophy in an mTOR-dependent manner.

Results: To test this hypothesis, neonatal rat ventricular myocytes (NRVMs) were exposed to a variety of growth stimuli, and class I HDACs were inhibited by either pharmacological means or by knockdown of individual HDAC isoforms. We found that HDAC1, HDAC2 and HDAC3 act together to facilitate pathological and physiological cardiomyocyte hypertrophy. In addition, inhibition of class I HDACs decreases mTOR activation by hypertrophic growth stimuli. HDAC inhibition also decreased mTOR activity in the setting of pressure overload using an *in vivo* surgical model of transverse aortic constriction (TAC). Adult mice with conditional cardiomyocyte-specific knockout of both HDAC1 and HDAC2 together had improved function following TAC surgery as well as decreased mTOR activity. Tuberin (TSC2) is a component of the tuberin-hamartin complex, which inhibits mTOR. We found that inhibition of class I HDACs increased expression of TSC2 in NRVMs, in mice and in embryonic stem cell-derived cardiomyocytes. Using siRNA we observed that TSC2 is required for HDAC-dependent inhibition of mTOR in NRVMs. Furthermore, we showed that overexpression of TSC2 is sufficient to reduce NRVM hypertrophy.

Conclusion: These findings point to TSC2-dependent control of mTOR as a critical component of the mechanism through which HDAC inhibitors blunt pathological growth. Together, these results enhance our understanding of the function of HDACs in cardiac pathology and facilitate the ultimate translational application of HDAC inhibitors in the treatment of heart disease.

WE-007

Myosin Activator improves Actin Assembly and Sarcomere Function of Human Induced Pluripotent Stem Cell-Derived Cardiomyocytes with a Troponin T Point Mutation

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Background. We have investigated cardiac myocytes derived from human induced pluripotent stem cells (iPSC-CMs) from two normal control and two family members expressing a mutant cardiac troponin T (cTnT-R173W) linked to dilated cardiomyopathy (DCM). cTnT is a scaffolding protein of the sarcomeric thin filament. The loss of this basic charge, which is strategically located to control tension, has consequences leading to progressive DCM. iPSC-CMs serve as a valuable platform for understanding clinically-relevant mutations in sarcomeric proteins; however, there are important questions to be addressed with regard to stress on myocytes and adaptation over time.

Methods and Results. We model stress by plating iPSC-CMs on physiologically stiff substrates (100 kPa). During the first week of culture of the iPSC-CMs, we have determined structural and functional characteristics as well as actin assembly dynamics. Shortening, actin content and actin assembly dynamics were depressed in CMs from the severely affected mutant at one week of culture, but by two weeks differences were less apparent. Potential changes due to sarcomeric troponin and myosin isoform composition were also assessed. Furthermore, the troponin complex, reconstituted with wild-type cTnT or recombinant cTnT-R173W, depressed the entry of cross-bridges into the force generating state, which can be reversed by the myosin activator Omecamtiv Mecarbil. Therapeutic doses of this drug increased both contractility and the content of F-actin in the mutant iPSC-CMs.

Conclusions. Collectively, our data suggest the use of a myosin activation reagent to restore function within patient specific iPSC-CMs may aid in understanding and treating this familial DCM.

WE-008

Identification of calpastatin as a novel substrate of p38gamma mitogen activated protein kinase.

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Despite the high and preferential expression of p38γ mitogen activated protein kinase in the myocardium, little is known regarding its role in the heart. The aim of this study is to elucidate p38γ signalling in the heart, with a particular focus on its role in the progression of pathological hypertrophy following abdominal aortic banding. Comparisons of cardiac function and structure of wild type (WT) and p38γ knock out (KO) mice in response to abdominal aortic banding found that KO mice develop less ventricular hypertrophy than their corresponding WT controls, and have preserved cardiac function. Basally, p38γ myocardial staining is primarily localised at the membranes and throughout the cytoplasm. Following aortic constriction nuclear staining of p38γ increases but no nuclear accumulation of the other dominant isoform, p38α, occurs. This suggests differential roles of the two isoforms in the heart.

To elucidate its signalling pathway and identify endogenous substrates of p38γ we have generated an analogue sensitive p38γ, which is mutated at a gatekeeper residue, to specifically track endogenous substrates in the myocardium. The mutation allows only the mutant kinase, but not WT kinases, to utilise analogues of ATP that are expanded at the N-6 position and contain a visible tag on the γ-phosphate. Transfer of this tag to substrates allows subsequent isolation and identification by mass spectrometry.

Using this technique we have been able to identify, amongst others, calpastatin as a novel target of p38γ. Calpastatin is the natural and endogenous inhibitor of calpain proteases. Calpain proteases are activated by increased calcium signalling during cardiac hypertrophy and inhibition of calpain shows favourable improvements in cardiac function. We observed that phosphorylation of calpastatin by p38γ reduces the efficiency of calpastatin to inhibit calpain and we propose that this may be a mechanism by which p38γ mediates its pro-hypertrophic role in the heart.

WE-009

Increased activity of AMP deaminase by decreased interaction with PGM1 and depletion of F1,6P: a novel mechanism of diabetic cardiomyopathy

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Background: AMP deaminase (AMPD) critically regulates adenine nucleotide pool and thereby the amount of ATP production by catalysing conversion of AMP to IMP. We have recently demonstrated that afterload-induced diastolic dysfunction in a rat model of type 2 diabetes (T2DM), OLETF, is mediated by excessive activity of AMPD and consequent ATP depletion. Thus, AMPD is a promising therapeutic target for diabetic cardiomyopathy. Here, we examined the mechanism by which AMPD activity is increased in T2DM, focusing on its interacting proteins and its regulation by metabolic alterations in T2DM.

Methods and Results: OLETF showed 60% higher AMPD activity in the left ventricular myocardium than in the non-diabetic control, LETO. Western blot analyses revealed that protein levels of AMPD3, a cardiac isoform of AMPD, were comparable in OLETF and LETO, indicating that regulation of AMPD3 activity is modified in OLETF. However, reported regulatory mechanisms of AMPD activity, including PKC-mediated phosphorylation of AMPD, its interaction with calmodulin and tissue inorganic phosphate levels, were similar in OLETF and LETO. Metabolomic analysis of left ventricular myocardium revealed that fructose 1,6-diphosphate (F1,6P) level was substantially lower in OLETF than in LETO (131±12 vs. 289±56 nmol/g, $p<0.05$), indicating reduced activity

of phosphofructokinase 1 (PFK1), a kinase catalysing conversion from fructose 6 phosphate (F6P) to F1,6P, in OLETF. In vitro addition of F1,6P (20 mmol/L) to left ventricular tissue lysates reduced AMPD activity by 69% in OLETF, confirming that AMPD activity depends on the level of F1,6P. We next performed two-dimensional gel electrophoresis using anti-AMPD3 immunoprecipitates obtained from left ventricular tissues of OLETF and LETO. Among 15 protein spots observed, intensities of 2 spots were much lower in OLETF than in LETO. MALDI-TOF/MS analysis revealed that one of the spots contained phosphoglucomutase-1 (PGM1), a component of the glycogenolytic sarcoplasmic reticulum complex (GSRC) that regulates local ATP level in the immediate vicinity of the sarcoplasmic reticulum.

Conclusion: Reduction of F1,6P level by reduced PFK1 activity contributes to T2DM-induced upregulation of myocardial AMPD activity. The change in PFK1 activity may be attributable to reduction of AMPD3-PGM1 interaction in GSRC, which potentially modifies Ca^{2+}-calmodulin-mediated regulation of PFK1.

WE-010

Angiogenesis in patients with angiographically significant coronary artery diseases and chronic heart failure: endothelial progenitor cells, growth factors and cytokines

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Backround: Angiogenesis is a complex multifactorial process with involving different cellular , molecular proangiogenic and antiangiogenic factors and is a zone of intensive researches at the present time. Mature endothelial cells possess limited regenerative capacity. There is therefore much interest in circulating endothelial progenitor cells (EPCs). EPCs were first described in 1997 and have since been the subject of numerous investigative studies exploring the potential of these cells in the process of cardiovascular damage, repair and angiogenesis. Circulating EPCs are capable of differentiating into mature endothelial cells to assist in angiogenesis and vasculogenesis. Previous studies have suggested an inverse relationship between levels of circulating EPCs and the presence of coronary artery disease (CAD) or cardiovascular risk factors, whereas other studies have observed increased numbers of EPCs in the setting of acute ischemia.
Objectives: To investigate whether the number of EPCs in patients with CHF was associated with severity CAD in patients undergoing coronary angiography, their correlations with the severity of stenosis, cytokines activation, growth factors, other clinic indicators.
Methods: Peripheral blood EPCs assessed both as CD133+ cells and CD133+ cells coexpressing CD34 and vascular endothelial growth factor (VEGF) receptor-2 cells, plasma tumor necrosis factor-α (TNF-α), C-reactive protein, VEGF, granulocyte-colony stimulating factor (G-CSF), NT-probrain natriuretic peptide (NT-proBNP) were studied in 82 men with ischemic heart disease and CHF I-IV class (NYHA), undergoing coronary angiography. Patients with acute coronary syndroms were excluded.
Results: There was an decrease CD133+, CD34+/CD133+/VEGFR2+ cells in men with CHF and 3-vessel, 4-vessel CAD compared with 1-vessel CAD ($p<0.05$). Patients with occlusion of coronary artery had lower CD133+, CD34+/CD133+/VEGFR2+ cells ($p<0.05$). A significant decrease blood levels of VEGF were detected with 3-vessel, 4-vessel compared 1-vessel CAD ($p<0.05$). CD34+/CD133+/VEGFR2+ cells negative correlated to age, smoking, NYHA CHF class, left ventricular ejection fraction, number of miocardis infarction, NT-proBNP, and positive - to VEGF, CD34+, CD133+ cells. VEGF positive correlated circulating endothelial progenitor cells CD34+/CD133+/VEGFR2+ cells, CD133+ cells.
Conclusion: There were lower number of circulating EPCs was associated with the presence of significant angiographically CAD and the number of vessel CAD, and the EPCs number correlated with maximum angiographic stenosis in patients with CHF. There were VEGF decrease and cytokine activation in patients with ischemic heart disease with CHF especially with more severe NYHA class. VEGF level was

interdependent with important angiogenesis cells - circulating endothelial progenitor cells in CHF.

WE-011

Effects of phosphodiesterase-5 A (PDE5A) inhibition on the hypertrophied myocardium of spontaneously hypertensive rats (SHR).

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In a previous study we showed that an increased protein Kinase G (PKG) activity after PDE5A inhibition (sildenafil, "SIL") inhibits the myocardial Na^+/H^+ exchanger (NHE1). Since NHE1 hyperactivity is linked to the development of cardiac hypertrophy, our study was aimed to study the potential antihypertrophic effects of SIL on the hypertrophic myocardium of SHR. We initially tested the inhibitory capability of SIL (1μM) on NHE1 in isolated cardiomyocytes of SHR by comparing H^+ efflux (J_{H^+}) in the absence or presence of SIL at a common pH_i of ~6.8, during the pH_i recovery from an acidic load (ammonium prepulse in the absence of bicarbonate where the NHE1 is the only active pH_i regulatory mechanism). SIL significantly decreased J_{H^+}: (mmol /L/ min) 12.93±3.80, n= 5vs. 2.09±0.87, n=4 ($P < 0.05$), confirming its inhibitory effect on the NHE1. Then 8 months old SHR were chronically treated (3 months) with SIL (100mg/kg/day, orally through drinking water, n=4) and compared to age-matched untreated controls (n=6). SIL treatment decreased left ventricular weight to body weight ratio (hypertrophy index) from 3.2±0.1 (control) to 2.7±0.1 mg/g (SHR +SIL). Accordingly, cardiomyocytes cross-sectional area (CSA) from treated rats was significantly reduced (688 ±39 vs. 496±23μm^2, $P < 0.05$). SIL treatment also reduced myocardial interstitial fibrosis: (in percentage of total interstitial collagen) 7.01±0.018 vs. 1.36±0.003%, $P < 0.05$), which was in accordance to the lower myocardial stiffness detected in treated hearts by comparing length-tension curves in isolated papillary muscles ($P<0.05$, 2-wayANOVA). Finally, we measured kinases upstream NHE1. Not significant changes in ERK1/2-p90RSK MAP kinases phosphorylation, or in NHE1 protein expression were detected between groups. In summary, the results show that PDE5A acute inhibition by SIL inhibits NHE1 activity in SHR, suggesting that this effect would be responsible for the decreased cardiac hypertrophy and the lower stiffness observed in hearts from SIL treated SHR.

WE-012

Cardiomyocyte high Ca^{2+} operational levels linked with arrhythmogenic vulnerability in a rat model of hypertrophic heart failure with preserved ejection fraction

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The pathophysiology of heart failure with preserved ejection fraction (HFpEF) is characterised by near normal systolic function coincident with diastolic dysfunction and inadequate ventricular filling at normal pressures. While the extent of hypertrophy is a key diagnostic indicator in HFpEF, the underlying cellular aetiology of this disease is poorly understood, due partly to a lack of appropriate models.

The aim of this study was to characterize *in vivo* cardiac and isolated cardiomyocyte functional status in the Hypertrophic Heart Rat (HHR), a newly derived model of HFpEF. Echocardiography (GE Vivid 9) was performed in adult male HHR and NHR (Normal Heart Rat). Ventricles were used for fibrosis (picrosirius red staining) and protein quantification (immunoblotting). Single cardiomyocyte (fura 2-AM loaded) contractility and $[Ca^{2+}]_i$ measurements by edge-detection and microfluorimetry (3Hz, 2.0mM Ca^{2+}, 37°C) were performed.

Premature death in HHR was preceded by cardiac hypertrophy (HHR vs NHR: cardiac weight index, 4.6±0.2 vs 3.2±0.1mg/g; cardiomyocyte length, 163±2 vs 133±1μm) and *in vivo* diastolic dysfunction (E/E', 31.0±3.4 vs 21.7±2.5) with maintained systolic parameters (ejection fraction, 73.8±1.5 vs 80.1±0.9%). Diffuse interstitial fibrosis was not prominent in HHR but dispersed fibrotic foci were observed. Surprisingly, hypertrophic cardiomyocytes exhibited hypercontractile status (94%

shortening increase) and high Ca^{2+} operational levels (91% increase in transient amplitude) linked with arrhythmogenic vulnerability. This was associated with hyperphosphorylation of sarcoplasmic reticulum Ca^{2+} regulatory proteins.

In the HHR model of HFpEF, a distinctive cardiomyocyte Ca^{2+} dysregulation during progression to overt decompensated heart failure is revealed. This strongly supports the contention that progression to HFpEF has a cellular phenotype which is different to that observed in failure linked with reduced systolic dysfunction and ejection fraction (HFrEF). These findings demonstrate that therapies directed to increasing cardiomyocyte Ca^{2+} operational levels as appropriate for HFrEF phenotype may not be effective, and may be detrimental, in the HFpEF context.

WE-013

Alda-1 improves cardiac function in the heart failure mice carrying human aldehyde dehydrogenase 2 E487K variant

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The aldehyde dehydrogenase 2 (ALDH2) located in the mitochondrial matrix is crucial for the maintenance of cellular aldehydic balance. It plays a important role in metabolizing reactive aldehydes produced during oxidative stress. Currently, it is estimated that 8% of the world population have a point mutation in the ALDH2 gene (E487K) which reduces its enzymatic activity by 95%. We assess the impact of the E487K variant of ALDH2 on cardiac function in myocardial infarction-induced heart failure. For that, we used heterozygous and homozygous ALDH2 E487K knock-in and WT mice. We observed that sham mice carrying the ALDH2 variant have a reduced ALDH2 activity and protein levels compared to WT mice. We have also seen that animals with ALDH2 mutation develop cardiac dysfunction and ventricular remodeling equivalent to WT animals after myocardial infarction. However, animals with the mutation have a significant reduction in the basal and maximum oxygen consumption (estimated by respirometry and maximal running test). Isolated heart mitochondria from mutant mice validated the in vivo findings of reduced oxygen consumption. Finally, the sustained Alda-1 treatment (an ALDH2 allosteric activator) improved cardiac function of infarcted animals, regardless of genotype. Taken together our data suggest that mice carrying ALDH2 E487K (which is probably the most common human enzyme deficient worldwide) are responsive to Alda-1 treatment, even though the have lower ALDH2 protein levels and activity.

WE-014

Arterial hypertension due to adrenal pheochromocytoma: modern methods of diagnosis and treatment

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Aim: To identify pheochromocytoma of adrenal glands in patients with essential hypertension.

Materials and methods: From 1986 to 2015, 2050 patients with arterial hypertension were examined of which adrenal pheochromocytoma was the cause of hypertension in 1.8% patients. Extraadrenal forms of pheochromocytoma of heart, para-aortic space with a malignant course of hypertension was diagnosed in 3.0% patients.

Results: In 97.9% patients, there was a good and satisfactory hypotensive effect post-surgery. One patient had a relapse after 5 years. After radical surgery was normalized. Another patient with pheochromocytoma with a large heart was inoperable. Malignant pheochromocytoma with distant metastasis was diagnosed in 4 patients only with CT and MRI. Unfortunately, due to late diagnostic methods only metastases of the malignant growth could be determined. Small sized pheochromocytoma could not be diagnosed by ultrasound, angiography, the same was diagnosed in 32% patients exclusively using MRI and CT. 10 patients were identified extra adrenal single or multiple pheochromocytoma of diameter 1 to 4mm in para-aortic tissue around the renal arteries that were not diagnosed by

ultrasound and preoperative examination of hormones. After surgical intervention in patients with unilateral lesions of adrenal glands, 97.9% patients showed good hypotensive effects. Prolonged hypotensive effect has been observed in patients in whom tumor removal was performed with splanchnicganglionectomy, extended sympathectomy.
Conclusion: Thus, the widespread use of CT, MRI allows the diagnosis of pheochromocytoma in a timely manner and significantly reduces its complications.

WE-015

Arterial hypertension due to primary hyperaldosteronism: modern methods of diagnosis and treatment

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Aim: To identify primary hyperaldosteronism (Conn's syndrome) in patients with essential hypertension (EHT)
Materials and methods: From 1986 to 2015, 2050 patients aged 5 to 75 years with diagnosis arterial hypertension were examined, in 71.0% patients the diagnosis EAH could not be confirmed and was identified as various forms of secondary hypertension.
Results: With comprehensive examinations Conn's syndrome (primary hyperaldosteronism) with adrenal adenoma was diagnosed in 9.8% of patients. Small adenoma and macro- and micro nodular adrenal hyperplasia could not be diagnosed using the traditional ultrasound, angiography, so MRI and CT were used to diagnose them in 32.2% of patients.
During revision of retroperitoneal cavity in 22 patients was observed macro- or micro nodular adrenal hyperplasia. In 10 patients, small formations of diameter 1 to 4 mm were not diagnosed by ultrasound and hormone study before surgery. After the surgical interventions, good hypotensive effects were observed in 98% patients with unilateral lesions of adrenal glands and normotensive effects were observed in 65% patients with bilateral lesions of adrenal glands.
Prolonged hypotensive effects were observed in patients who underwent removal of the tumor of adrenal glands by epi- subphrenic splanchnicganglionectomy, extended sympathectomy. The surgical corrections of adrenal hypertension in 65-85% of patients showed good and satisfactory effects.
Conclusion: Thus, the widespread use of informative diagnostic methods (CT, MRI with contrast) helps in an early diagnosis and significantly reduces the complications.

WE-017

Arterial hypertension due to renal parenchymal lesions (diagnosis and treatment)

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Aim: To identify the hypertension (RHT) with renal parenchymal lesions in patients with essential hypertension.
Materials and methods: From 1986-2015, were examined 2050 patients aged 5-84 years with persistent hypertension and diagnosis essential hypertension.
Results: Following comprehensive examinations, RHT was diagnosed in 42.0% patients. After surgery, 87% patients showed good and satisfactory effects. Nephrectomy, decapsulation of the kidneys, splanchnicganglionectomy(SGE) of 62% patients led to normotension, 25% patients showed significant reduction in blood pressure, reduction in doses of antihypertensive drugs. In 13% patients the operation led to a decrease in blood pressure by 15-20 mm Hg. Renal cysts were found in 3.0%, polycystic in 0.7% patients. Removal of cysts, omental revascularization, expanded SGE in 65% of patients have led to a decrease in blood pressure. In 88% patients expanded SGE, kidney decapsulation with satisfactory effects were performed. Nephrolithiasis with chronic pyelonephritis was diagnosed in 4.2% patients, nephroptosis in 2.0% patients. Nephropexy, renal artery angioplasty, SGE led to normotension in 91.7% patients. In 2 patients due to the pronounced hypertensive nephrosclerosis, surgery did not lead to normotension. Ormond's disease (retroperitoneal fibrosis) with compression of the ureter was the cause of hypertension in 0.3% patients. Hypernephroma with hypertension was diagnosed in 0.3% patients and after surgery blood pressure returned to normal in all patients.

Conclusion.: Thus, diagnosis EHT should be considered only after excluding other forms of hypertension. Surgical interventions must be indicated following ineffective conservative treatment for patients with persistent RHT.

WE-018

Cardiac anti-fibrotic effects of direct AT_2 and Mas receptor stimulation in stroke-prone spontaneously hypertensive rats

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Background: Angiotensin II type II receptor (AT2R) and Mas receptor (MasR) belong to the 'protective arm' of the RAS, with AT2R or MasR stimulation known to evoke a number of cardiovascular effects, including acute vasodilatation and chronic anti-fibrotic effects. Compound 21 (C21) is the prototypical AT2R agonist, while Ang (1-7) has mainly been used in chronic studies to stimulate MasR, although being relatively nonselective. Therefore, we examined if selective AT2R (using C21) or MasR (using AVE0991) stimulation evokes similar anti-fibrotic phenotypes to that of combination treatment, which may implicate similar signalling mechanisms.

Methods: To investigate if AT2R and MasR pharmacological co-stimulation provide additional protection against end-organ damage in stroke-prone spontaneously hypertensive rats (SP-SHR) than either treatment alone. Adult male SP-SHR, aged 20-22 weeks, were treated for 4 weeks with either saline (n=7), AT2R agonist C21 (0.03 mg/kg/day, n=6), MasR agonist AVE0991 (24 µg/kg/h, n=3), or a combination of both (n=4), subcutaneously via osmotic mini-pump. Blood pressure (tail-cuff) was measured at days 0, 14 and 28 of the protocol. At the end of treatment, indices of aberrant cardiac remodelling (cardiac hypertrophy and interstitial fibrosis) were quantified.

Results: None of the treatments influenced elevated blood pressure or cardiac hypertrophy in SP-SHR. However, cardiac interstitial fibrosis as collagen volume fraction (assessed by picrosirius red staining) was strikingly attenuated from control levels (5.1%) to approximately half those levels by each treatment (2.5%, 2.4% for C21 and AVE0991, respectively, both $P<0.01$ versus untreated) while there was no additive anti-fibrotic effect of combination treatment (2.3%). Similar significant reductions were noted for vimentin and α-smooth muscle actin immunoreactivity suggesting that treatments reduced fibroblast number and differentiation to synthetic myofibroblasts.

Conclusion: Pharmacological stimulation of AT2R and/or MasR exhibited marked cardiac anti-fibrotic effects without influencing blood pressure. Ongoing studies will address whether similar mechanisms contribute to altered extracellular matrix.

WE-019

A simplified, Langendorff-free method for concomitant isolation of viable cardiac myocytes and fibroblasts from the adult mouse heart

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Objective

Recent advances in mouse genomics, epigenomics and transgenics offer huge potential for research in murine models of heart disease. However, the isolation of viable cardiac myocytes from the adult mouse heart is particularly challenging to most labs worldwide. Every established protocol to date relies on Langendorff apparatus or equivalent to allow retrograde aortic perfusion and digestion of the myocardium. This 45-year-old technique presents significant logistical, technical and financial barriers, and requires considerable training investment. We therefore sought to "re-invent" an alternative.

Method

We have developed a simplified method to introduce optimised digestion buffers to the intact mouse heart by intraventricular injection. Deep myocardial perfusion via the coronary vasculature was induced by clamping of the aorta. Myocyte fractions were subsequently separated from non-myocytes by gravity settling, and cells were analysed by quantitative real-time PCR, immunocytochemistry, calcium imaging and an xCELLigence RTCA CardioECR system to confirm healthy, viable properties, in addition to characteristic responses to hypoxia, neurohormonal and electrical stimulation.

Results

The technique elicits viable myocyte yields of up to 80%, which meets and even exceeds those reported in previous Langendorff-based protocols. Myocytes

could be maintained in culture for a week and displayed a full range of morphological and functional contractile characteristics, while cardiac fibroblasts could be concurrently cultured from the non-myocyte cellular fraction.

Conclusion

We propose a robust, convenient protocol for the isolation of adult mouse cardiac myocytes. The procedure is simple, flexible, does not require heparin pre-injection and uses only common surgical and laboratory equipment. We further demonstrate concurrent isolation and culture of myocyte and fibroblast populations, from the same adult mouse heart.

WE-020

Study of a possible paracrine communication between cardiac fibroblasts and myocytes induced by Galectin-3

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Introduction. Galectin-3 is a β-galactoside-binding protein that is being evaluated as a biomarker for the development of heart failure (HF). By now it is unknown if Gal-3 has a direct role in cardiac disease progression. On the other side, TGF-β1 is a cytokine related largely to cardiac remodelling and cardiac disease progression. Here, we show that Gal-3 has no obvious effect over cardiomyocytes biology. Instead, it activates cell signalling cascades and tgfb1 gene expression in fibroblasts. Our results suggest that a paracrine communication between fibroblasts and cardiomyocytes by means of TGF-β1 is established in response to Gal-3, explaining to some extent the deleterious actions of Gal-3 over cardiac tissue.

Methodology. Primary cultures of cardiac myocytes and fibroblasts were stimulated with Gal3 10 ug/ml. Cell death was evaluated by Flow cytometry through PI incorporation and MTT assay. The activation of signaling pathways was evaluated by western blot, while mRNA expression was analysed by RT-qPCR.

Results. The results obtained showed that Gal-3 has no effect over cardiomyocytes, at least at the times and concentrations used here. When fibroblasts were stimulated with Gal-3 the phosphorylation of ERK1/2 and AKT as well as the expression of tgfb1 was increased. The supernatant obtained from Gal-3-stimulated fibroblasts provokes a hypertrophic effect onto cardiomyocytes and the increase of anp mRNA expression. TGF-β1 released into the culture media of fibroblasts could mediate this effect.

Conclusions. Gal-3 is a biomarker for the development of HF, showing a straightforward relationship between plasma levels of Gal-3 and the impairment of cardiac function. Our results show for the first time the activation of pro-survival signaling pathways and TGF-β1 expression in fibroblasts in response to Gal-3.

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WE-021

Identification of emerging micro-rna markers for heart failure development

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Purpose: Heart failure is the final stage of several cardiovascular diseases. Despite the health burden associated with this pandemic, biomarkers aimed to assess the individual risk of developing HF are still lacking. Advances in bioinformatics have accelerated the pre-analytic phase of biomarker research using data mining strategies; we aim to identify potential microRNA (miR) biomarkers in plasma of non-ischemic HF patients combining in silico and in vivo approaches.

Methods: We systematically reviewed the literature for miR profiling and HF. Four

studies fulfilled quality criteria for analysis. Raw data was obtained from public databases. The largest dataset was normalized and analyzed by unsupervised hierarchical clustering to identify differentially expressed miRs using a fold change of 2 as cut-off with a false discovery rate < 1%. Results from the discovery dataset were contrasted with previously reported miRs using robust rank aggregation. We validate our predicted miRs in plasma samples from 12 HF patients and 5 healthy controls by RT-qPCR. miR-39 was used to standardize for extraction procedures.

Results: We identified nine differentially expressed miRs (let-7b, miR-100, miR-103, miR-199a and miR-23a), including three previously unreported (miR-125b, miR-140 and miR-15b). Relative expression of miR-23a was markedly up-regulated in plasma of HF patients (p=0.05). On the other hand, miR-140 was significantly down-regulated HF patients (p=0.03). Interestingly, miR-140 participates in the regulation of several genes of the Wnt and Akt/mTOR pathways, critical in the transition from compensate hypertrophy to overt HF (p=1.21e-08 for interaction).

Conclusion: Bioinformatics analysis allows to identify previously unreported miRs involved in HF development. This is a novel approach using public access data for identifying new potential biomarkers such as miR-140; its biological role in HF development or progression remains to be elucidated.

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WE-022

Role of carbonic anhydrase ix and hypoxia-inducible factor 1 in infarcted rat heart.

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Background. Acute myocardial infarction (MI) remains a leading cause of morbidity and mortality worldwide. MI refers to an oxygen blood perfusion reduction, hypoxia, severely altering cardiac function and myocardial energy metabolism. Studies made on hypoxic tissues of solid tumors revealed an increase expression of Hypoxia-Inducible Factor 1 (HIF-1). This transcription factor translocates to the nucleus, binds to DNA elements and stimulates the transcription of several genes including Carbonic Anhydrase IX (CAIX). During MI the increase expression of HIF-1 reduces infarct size, improving cardiac function. We have previously shown that heart CAIX plays a critical role in regulating myocardial intracellular pH, interacting with bicarbonate transporters (BT). However, the role of CAIX in MI is unknown.

Objetive. Our goal is to evaluate CAIX expression in ischemic myocardium and its relation to HIF-1 and BT.

Methods. To analyze the role of HIF-1, CAIX and BT in MI we used adult male Wistar rats. MI was produced by permanent ligation of the left anterior descending coronary artery “in vivo” and analyzed by histology. Heart samples were obtained from infarct, peri-infarct and remote heart regions. Expression of HIF-1, CAIX and BT was analyzed by western blot. Also, interaction of CAIX-BT was assessed by co-immunoprecipitation and colocalization.

Results and Conclusions. Infarcted Wistar rats showed a significant increased expression of HIF-1 and CAIX in the peri-infarct regions, compare to the remote heart areas. Peri-infarct regions show a marked physical interaction between CAIX and sodium bicarbonate co-transporter (NBC1). These results suggests that HIF-1 and its downstream target, such us CAIX, interacting with BT may improve cellular pH surroundings and survival mechanisms possibly attenuating progression of cardiac dysfunction after MI.

WE-023

The cMyBP-C E258K HCM-causing mutation does not affect mRNA splicing

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Hypertrophic cardiomyopathy (HCM) is the most commonly inherited cardiovascular disease, affecting approximately 0.2% of the general population. Mutations in *MYBPC3*, encoding cardiac myosin binding protein-C (cMyBP-C), are common causes of HCM. Many *MYBPC3* mutations cause aberrant mRNA splicing, leading to cMyBP-

C truncation and cause disease through a mechanism of haploinsufficiency. The E258K mutation in *MYBPC3,* a prevalent cause of HCM, has been postulated to alter splicing due to its location in the exon 6 splice donor site. Our previous data, however, indicated that it may act in a dominant negative manner by altering interactions with myosin-S2 and actin.

Here we investigate whether the E258K mutation alters RNA splicing and act through a mechanism of cMyBP-C haploinsufficiency, or as a true dominant negative missense mutation by assessing mRNA and protein levels in an E258K knock-in mouse model.

Applying an array of RT-PCR primers designed to detect all potential miss-spliced transcripts arising from this mutation no aberrantly spliced *Mybpc3* transcripts were found in mice heterozygous for E258K. Additionally, Myocardium expression of cMyBP-C protein in either heterozygous or homozygous E258K mice was similar to that of wild type control littermates and lacked evidence of truncated cMyBP-C. Interestingly, the E258K mutation results in reduced phosphorylation levels of cMyBP-C at S273 and S302, without affecting phosphorylation S282.

In this murine model, the E258K mutation does not affect mRNA splicing and does not appear to act through a mechanism of cMyBP-C haploinsufficiency. We previously showed that E258K cMyBP-C reduces its affinity for myosin S2 while increasing its affinity for actin, resulting in reduced twitch force amplitude and accelerated contractile kinetics. Taken together, these results suggest that this mutation acts in a dominant negative fashion.

WE-024

Neuregulin-1 Modulates Doxorubicin Cardiotoxicity In Mouse

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Neuregulin-1 (NRG1) signaling through tyrosine kinase receptors erbB2 and erbB4 is required for cardiac morphogenesis, and plays an essential role in maintaining the myocardial architecture during adulthood. Targeted immunotherapies blocking the survival of erbB2+ cancer cells revealed that an impaired NRG1 signal under anthracycline chemotherapy may lead to dilated cardiomyopathy in a subpopulation of treated patients. The ventricular-specific deletion of ErbB4 (erbB4-KO) manifested dilated cardiomyopathy, aggravated by the administration of anthracyclines (doxorubicin) (KOD). The exacerbated toxicity in KOD induced genes of the ubiquitin-proteasome system and autophagy. Myofibril proteins were largely ubiquitinated with the commonality of a subgroup of proteins in the erbB4-KO and the doxorubicin mice WTD. We aimed to investigate the activities underlying cardiomyocyte damage and moreover, to evaluate the therapeutic effect of recombinant NRG1β peptides. We first examined biomarkers of apoptosis and autophagy (e.g. active caspase3, LC3II/I), then characterized the ubiquitination profile of myofibrils in 2D gels towards the monitoring of the rNRG1β effect through the reversion of the molecular modifications observed in cardiotoxic conditions. We have identified new consistent biomarkers of pathology and suggest that rNRG1β protects from cardiotoxic injury

WE-025

2-deoxy-ATP enhances multiple kinetic parameters to improve cardiac function

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Hypertrophic cardiomyopathy (HCM) is a form of genetic heart disease often caused by point mutations in sarcomeric proteins. As the underlying mechanisms of genetic HCM continue to be unraveled, developing novel ways to modulate the actomyosin contractile apparatus is of growing interest and importance. The nucleotide analog 2-deoxyadenosine triphosphate (dATP) has recently garnered interest as potentially having therapeutic benefit for treatment of systolic and/or diastolic heart failure. dATP has been previously reported to enhance cardiac contractility, increase +dP/dt, and improve diastolic relaxation parameters in transgenic mice with elevated levels of dATP in the heart (Korte, 2011). To better understand potential therapeutic benefits of dATP on actomyosin, we characterize the mechanism of action for dATP using bovine cardiac myosin subfragments S1 and HMM in a variety of steady-state, transient, and single-molecule experiments. We report a 40% increase in unloaded *in vitro* motility sliding velocities, as well as increased

ATPase activity, ADP- and phosphate-release rates, and actin-binding affinities with dATP compared to ATP. The combination of transient kinetic rates and equilibrium constants of the actomyosin ATPase cycle, as well as basal myosin parameters, implicate ADP release as the primary contributor to the differences observed between the two nucleotides. We propose a model by which enhancing both cardiac contraction and relaxation kinetics can improve cardiac function and potentially serve as a therapeutic for genetic heart disease.

WE-026

Frailty, not age, predicts age-dependent cardiac contractile dysfunction under basal and ischemic conditions in Langendorff-perfused hearts from C57BL/6J mice

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Frail patients with cardiovascular disease (CVD) experience worse outcomes and higher mortality than non-frail patients, but the links between frailty and myocardial function are unclear. Here we investigated the impact of age and frailty on cardiac hemodynamic function under control conditions and after ischemia/reperfusion. Frailty was measured in male C57BL/6J mice (755-882 days; n = 18) using a novel frailty index (FI) developed in our laboratory based on the clinical assessment of health deficits. Hypertrophy was assessed by measuring heart weight to tibia length (HW:TL) ratios. The HW:TL ratio increased with frailty (r=0.42, P=0.01) but not with chronological age (r=0.24, P=0.15). Langendorff-perfused hearts were used to measure left ventricular developed pressure (LVDP), rate of pressure development (+dP/dt), rate of pressure decay (-dP/dt), and incidence of arrhythmias under normoxic conditions. Under these conditions, LVDP (r=0.64; P=0.004), +dP/dt, (r= 0.61; P=0.01), and -dP/dt (r=0.58; P=0.01) declined dramatically as FI scores increased. However, chronological age did not affect LVDP, +dP/dt, or -dP/dt (r= 0.11, 0.10, and 0.11 respectively; P =0.66, 0.70, and 0.65 respectively). Furthermore, frailty increased the incidence of arrhythmias (r=0.55; P=0.02) while age did not (r= 0.11; P=0.66). Hearts were then exposed to 30 min of ischemia followed by 40 min of reperfusion. Interestingly, contracture, a marker of ischemic damage, increased with frailty (r=0.52; P=0.03) but not with increasing age (r=0.03; P=0.92). By contrast, recovery of contractile function was poor after reperfusion in all aged hearts regardless of FI score or age. These results suggest that age-dependent hypertrophy, cardiac contractile dysfunction, and arrhythmias are more closely linked to frailty than chronological age. Thus, frailty disrupts cardiac structure, function, and may increase susceptibility to ischemic damage in the aging heart.

WE-027

RIP3 Mediates Ischemia- and Oxidative Stress-induced Myocardial Necroptosis via CaMKII/mPTP Signalling Pathway

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Background: Regulated necrosis (necroptosis) and apoptosis are crucially involved in multiple severe cardiac pathological conditions, including myocardial infarction, ischemia/reperfusion (I/R) injury, and heart failure. Whereas apoptotic signaling is well defined, the mechanisms that underlie cardiomyocyte necroptosis remain elusive.

Methods and Results: Here we show that both I/R injury and doxorubicin (Dox) stimulation increase both mRNA and protein levels of RIP3 in the hearts. In mice, receptor-interacting protein 3 (RIP3) deficiency ameliorates myocardial necroptosis and heart failure induced by I/R (30 min ischemia followed by 4 h or 8 wk reperfusion) or Dox treatment (20 mg/kg or 5 mg/kg×4, i.p.). Overexpression of RIP3 induces cardiomyocyte necroptosis evidenced by decreased intracellular ATP level and increased lactate dehydrogenase concentration in cell culture medium. RIP3 triggers myocardial necroptosis through activation of Ca^{2+}/calmodulin-dependent protein kinase (CaMKII), rather than the well-established RIP3 partners, RIP1 and MLKL (mixed lineage kinase domain-like

protein). Specifically, our *in vivo* and *in vitro* data indicate that I/R and Dox markedly increase myocardial CaMKII activation in wild type but not RIP3-deficient mice, and that inhibition of CaMKII protects the heart from I/R- and Dox-induced cardiomyocyte necropotosis, cardiac remodeling and heart failure. Mechanistically, RIP3 activates CaMKII via both direct phosphorylation and indirect reactive oxidative species-dependent oxidation, and subsequently triggers opening of the mitochondrial permeability transition pore (mPTP) and myocardial necroptosis.

Conclusion: These findings identify CaMKII as a new RIP3 substrate and delineate a RIP3-CaMKII-mPTP myocardial necroptosis pathway, a promising target for the treatment of ischemia– and oxidative stress-induced myocardial damage and heart failure.

WE-028

Cardiac-protection of acetylcholine on ischemia/reperfusion injury via regulation of TNF-α/TNFR signal pathway

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Background:

Recent studies reported ischemic heart disease is accompanied by substantial withdrawal of vagal activity, and over-production of tumor necrosis factor-alpha (TNF-α) worsen cardiac injury. However, it is not fully clear that the replacement of ACh for myocardial ischemia/reperfusion (I/R) modulated the production of TNF-α and TNF-α receptor1/2 (TNFR1/2) signal pathway.

Methods:

Langendorff- perfused rat hearts and H9c2 cells were subjected to global ischemia and reperfusion, or hypoxia/reoxygenation, respectively. Real-time PCR, western blot, TUNEL and Si RNA were used.

Results:

1) ACh abolished hypoxia-induced up-regulation of TNF-α mRNA and protein, caspase-3 activation, and reactive oxygen species (ROS) in cardiomyocytes. ACh treatment prevented the hypoxia-induced increase in p38 MAPK and JNK phosphorylation, and increased ERK phosphorylation in H9c2 cells. Co-treatment with atropine, a non-selective muscarinic acetylcholine receptor antagonist, or methoctramine, a selective type-2 muscarinic acetylcholine (M_2) receptor antagonist, abrogated the above effects of ACh.

2) Following Langendorff- perfused rat myocardial I/R injury, the cardiac dysfunction and myocardial infarction significantly increased and the expression of TNFR1, apoptosis signal regulating kinase 1 (ASK1) and activated caspase-8 were increased in left ventricle. Instead of TNFR1, TNFR2, Akt and ERK were not affect by I/R. Treated with ACh not only improved the cardiac function, decreased infarction area and apoptosis by TUNEL and Bcl-2/Bax, but also down-regulated the expression of TNF-α and TNFR1, and reduced the activity of ASK1 and caspase-8, finally inhibiting the cardiomyocyte apoptosis. Meantime, ACh up-regulated TNFR2 expression, Akt and ERK phosphorylation, which involved in survival pathway to protect myocardium against I/R injury.

3) Si RNA TNFR1 in H9c2 cells reduced HR-induced phosphorylation of ASK1 and caspase-3 activation. In addition, Si RNA TNFR2 eliminated ACh-increased phosphorylation of Akt and ERK after HR in H9c2 cells.

Conclusion:

ACh protected myocardium against I/R injury via inhibition TNF-α production and regulation of TNFR1/2 pathway.

WE-029

Acute hyperglycemia abolishes cardioprotection by remote ischemic perconditioning

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Background: Remote ischemic perconditioning (RIPerC) has a promising therapeutic insight to improve the prognosis of acute myocardial infarction. Chronic comorbidities such as diabetes are known to interfere with conditioning interventions by modulating cardioprotective signaling pathways, such as e.g., mTOR pathway and autophagy. However, the effect of acute hyperglycemia on RIPerC has not

been studied so far. Therefore, here we investigated the effect of acute hyperglycemia on cardioprotection by RIPerC.

Methods: Wistar rats were divided into normoglycemic (NG) and acute hyperglycemic (AHG) groups. Acute hyperglycemia was induced by glucose infusion to maintain a serum glucose concentration of 15–20 mM throughout the experimental protocol. NG rats received mannitol infusion of an equal osmolarity. Both groups were subdivided into an ischemic (Isch) and a RIPerC group. Each group underwent reversible occlusion of the left anterior descending coronary artery (LAD) for 40 min in the presence or absence of acute hyperglycemia. After the 10-min LAD occlusion, RIPerC was induced by 3 cycles of 5-min unilateral femoral artery and vein occlusion and 5-min reperfusion. After 120 min of reperfusion, infarct size was measured by triphenyltetrazolium chloride staining. To study underlying signaling mechanisms, hearts were harvested for immunoblotting after 35 min in both the NG and AHG groups.

Results: Infarct size was significantly reduced by RIPerC in NG, but not in the AHG group (NG + Isch: 46.27 ± 5.31 % vs. NG + RIPerC: 24.65 ± 7.45 %, $p < 0.05$; AHG + Isch: 54.19 ± 4.07 % vs. 52.76 ± 3.80 %). Acute hyperglycemia per se did not influence infarct size, but significantly increased the incidence and duration of arrhythmias. Acute hyperglycemia activated mechanistic target of rapamycine (mTOR) pathway, as it significantly increased the phosphorylation of mTOR and S6 proteins and the phosphorylation of AKT. In spite of a decreased LC3II/LC3I ratio, other markers of autophagy, such as ATG7, ULK1 phopsphorylation, Beclin 1 and SQSTM1/p62, were not modulated by acute hyperglycemia. Furthermore, acute hyperglycemia significantly elevated nitrative stress in the heart (0.87 ± 0.01 vs. 0.50 ± 0.04 μg 3-nitrotyrosine/mg protein, $p < 0.05$).

Conclusions:

This is the first demonstration that acute hypreglycemia deteriorates cardioprotection by RIPerC. The mechanism of this phenomenon may involve an acute hyperglycemia-induced increase in nitrative stress and activation of the mTOR pathway.

WE-030

LAPTM4b protects hearts from ischemia/reperfusion injury by promoting autophagy flux

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Myocardial injury following ischemia/reperfusion (I/R) is a common clinical scenario in patients suffering from ischemic heart disease. During myocardial I/R over-activated autophagy and accumulated autophagosome contribute to cardiomyocytes death. Thus, understanding how to accelerate autophagosome clearance and promote autophagy flux is important for the development of new cardioprotective approaches to alleviate I/R injury. Lysosome is responsible for the eventual degradation of autophagosome, however, how to promote lysosome flux via the regulation of lysosome function remains poorly understood. In the present study, we found a lysosomal membrane protein lysosomal-associated transmembrane protein 4b (LAPTM4b) was down regulated during myocardial I/R. Overexpression of LAPTM4b in neonatal rat cardiomyocytes preserved cell viability from hypoxia/reoxygenation (H/R) injury. Moreover, overexpression of LAPTM4b activated lysosomal function and promoted autophagy flux characterized by a decrease in the autophagosome of H/R myocytes, while knockdown of LAPTM4b blocked autophagy flux and aggravated cell death. We then constructed *LAPTM4b* knockout (*LAPTM4b*$^{-/-}$) mice by using Crispr/Cas9 system. The size of myocardial infarction/area at risk after I/R was significantly larger in *LAPTM4b*$^{-/-}$ mice than that in wide-type mice. Our results firstly report the involvement of a lysosomal protein LAPTM4b in myocardial I/R injury through the regulation of autophagy flux and acceleration of autophagosome clearance.

Key words: LAPTM4b; autophagy flux; ischemia/reperfusion injury;

WE-031

The Role of Calcium-sensing Receptors and Spermine in Hypoxia-induced Pulmonary Vascular Remodeling and the Mechanism

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Background Pulmonary vascular remodeling(PVR) is an importent pathological feature of hypoxia-induced pulmonary hypertension (HPH), which exact mechanism is unknown. Calcium-sensing receptor (CaSR) is an G-protein coupling receptor, and spermine is a polyamine. **Methods** We estabolished rat hypoxia models in vivo and in vitro by nitrogen or cobalt chloride (CoCl2), and observed CaSR expression, polyamine metabolism, PVR related parameters and signal pathways by RT-PCR, Western blotting, immunofluorescence, immunohistochemistry, confocal laser scanning microscopy, flow cytometric assay etc. **Results** Under hypoxic conditions, the expressions of CaSR, SSAT(a key enzyme of polyamine degradation), PCNA, OPN (osteopontin) and p-ERK, the intracellular concentration of calcium, the survival rate of cells and cell proliferation index (PI) were markedly increased, while the expressions of ODC(a key enzyme of polyamine biosynthesis), SMα-actin (SMAα) and calponin were significantly reduced. The agonists of CaSR ($GdCl_3$, Neomycin) enhanced but antagonist of CaSR (NPS2390) weakened the hypoxic effect. PD98059 (a MEK1 inhibitor) or LY294002 (a PI3K inhibitors) reversed the upregulation of PCNA expression and the increase of cell proliferation index induced by hypoxiain PASMCs. Exogenous spermine at low concentrations significantly inhibited hypoxia induced PASMC proliferation, leading to cell cycle arrest at the G1/G0 phase, decreased cyclin D1 expression, increased p27 expression, and suppressed the phosphorylation of ERK1/2, PI3K and AKT. **Conclusions** CaSR activation and polyamine disbalance are involved in the proliferation, phenotypic modulation of PASMCs and pulmonary vascular remodeling induced by hypoxia through MEK1/ERK1.2 and PI3K/AKT pathway. Exogenous spermineat inhibits the proliferation of PASMCs. Our study thus offer new insight into the prevention and treatment of hypoxia-induced pulmonary hypertension (HPH).

WE-032

Exogenous H_2S Contributes to Recovery of Ischemic Post-Conditioning-Induced Cardioprotection in the Aging Rat and Cardiomyocytes and the Related Mechanism

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Background Ischemic post-conditioning (PC) plays an important role in cardioprotection from ischemia/reperfusion (I/R) injury in young heart but not in aging. The physiological and pathological roles of hydrogen sulfide (H_2S) in the regulation of cardiovascular functions have been recognized. Whether H_2S is involved in the recovery of PC-induced cardioprotection in aging cardiomyocytes is unclear. **Methods** The aging rats (24-months-old) and the aging cardiomyocytes induced by D-galactose suffer from I/R (H/R) and PC. Western blotting, real time-PCR, TUNEL staining, confocal laser scanning microscopy, flow cytometric assay were used to detect apoptosis, oxidative stress and related signal pathways. **Results** Both I/R (H/R) and PC decreased cystathionine-γ-lyase (CSE) expression and the production rate of H_2S in aging heart. Supplementation of NaHS protected against I/R (H/R)-induced apoptosis, production increase of reactive oxygen species (ROS), the expression of cleaved caspase-3 and cleaved caspase-9, the release of cytochrome *c* (Cyt *c*), and mPTP opening. The addition of NaHS also counteracted the reduction of cell viability caused by I/R (H/R) and increased the phosphorylation of ERK1/2, PI3K, Akt, GSK-3β and mitochondrial membrane potential. Additionally, NaHS increased Bcl-2 expression, promoted PKC-ε translocation to the cell membrane, and activated mitochondrial ATP-sensitive K channels ($mitoK_{ATP}$). PC alone did not provide cardioprotection in I/R (H/R)-treated aging cardiomyocytes, which was significantly restored by the supplementation of NaHS. **Conclusion** The exogenous H_2S restores PC-induced cardioprotection in aging rat and cardiomyocytes via inhibition of oxidative stress and the inhibition of mPTP opening by the activation of the ERK1/2-GSK-3β, PI3K-Akt-GSK-3β and PKC-ε-$mitoK_{ATP}$ pathways. These findings provide a novel potential target for the treatment of aging ischemic cardiomyopathy.

WE-033

Cardiomyocyte-specific Runx1 deficiency protects the heart from ischemia-reperfusion injury in vivo.

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Coronary artery blockage leading to prolonged myocardial ischemia and cardiomyocyte cell death (myocardial infarction; MI) is a leading cause of death worldwide. Current treatment options include reperfusion of the myocardium to salvage reversible damage and limit further irreversible cell death. Paradoxically, the efficiency of this treatment is limited by ischemia-reperfusion injury. Identification of novel targets that have the potential to limit cardiac dysfunction caused by ischemia-reperfusion injury are urgently required. The transcription factor, Runx1, is activated in human and mouse cardiomyocytes post-MI. The functional role of Runx1 during ischemia-reperfusion injury remains unknown. Furthermore, whether Runx1 is also increased in an intermediate sized species, which could be utilised for future translational studies, has not been characterised. Here we show the importance of Runx1 during ischaemia-reperfusion injury using an in vivo mouse model of Runx1 deficiency and in separate experiments demonstrate that Runx1 is increased in rabbit myocardium post-MI. Echocardiography of a cardiomyocyte-specific Runx1-deficient mouse with ischemia reperfusion injury demonstrated preserved left ventricular function as measured by fractional shortening compared to control mice (45% versus 29% at 5 wk post-ischemia reperfusion; $P<0.05$). Western blot analysis revealed increased expression of Runx1 protein levels in the border zone and left ventricular region of post-MI rabbit hearts to 175% and 250% of sham levels respectively ($P<0.05$). These results demonstrate that Runx1 is a novel therapeutic target post-MI; in particular deficiency of Runx1 within cardiomyocytes is cardio-protective against ischemia-reperfusion injury. Increased expression of Runx1 within the rabbit reveals that this larger species could be used in future translational studies.

WE-034

Simultaneous Ultrasound Diagnosis and Treatment of Thrombosis using Activated Platelet Targeted Theranostic Microbubbles

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Molecular ultrasound imaging is an attractive non-invasive technology widely available for rapid clinical diagnosis. We hypothesized that thrombolytic drugs loaded microbubbles (MBs), which are selectively targeted to activated platelets, will allow high-resolution, real-time imaging of thrombosis, and at the same time offer potent thrombolytic efficacy without bleeding complications, and enable the immediate monitoring of success or failure of thrombolysis.

Our therapeutic agents/imaging particles, targeted theranostic microbubbles (TT-MB), consist of a fusion construct that combines the fibrinolytic drug urokinase, echo-enhancing microbubbles for visualization by ultrasonography, and an activated-platelet-specific single-chain antibody for targeting specifically to thrombi. In the ferric-chloride induced carotid artery thrombosis mouse model, treatment with TT-MB significantly reduced thrombus size after 45 min, while no significant difference was observed in the MB that were targeted but without urokinase (37.09 ± 5.6 vs. 97.16 ± 4.3, mean % change ± SEM, normalized to baseline thrombus size, $p<0.001$). The same degree of efficient thrombolysis was only achievable using a high dose of urokinase (NS). We also show that the targeting and thus clot-enrichment effect of TT-MBs results in a highly potent fibrinolysis that could only be matched using high doses of non-targeted urokinase. However, the latter is associated with a highly prolonged bleeding time (79.25 ± 6.5 vs. 1079.25 ± 260.7, seconds ± SEM, $p<0.001$). In contrast, TT-MB does not prolong bleeding time (NS).

In conclusion, activated platelet targeted microbubbles conjugated with recombinant

urokinase represent a novel and unique theranostic approach to simultaneously diagnose and treat thrombosis as well as to immediately monitor success or failure of thrombolysis. This unique technology holds promise for major progress towards rapid diagnosis and bleeding-free, potent therapy of the vast number of patients suffering from thrombotic diseases.

WE-035

Acetylcholine to Improve Calcium Dyshomeostasis in Cardiovascular Disease: Attenuated ER-PM contacts

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Background: The endoplasmic reticulum (ER) is an important organelle for the protein homeostasis and calcium (Ca^{2+}) storage in cells. It forms discrete junctions with the plasma membrane (PM) and membranes of organelles (such as mitochondria) that play critical roles in Ca^{2+} signaling during cellular bioenergetics, apoptosis and autophagy. We have confirmed that acetylcholine (ACh), the neurotransmitter of vagal nerve, could inhibit ER stress and protected cells in inflammatory injury, as well as inhibit the formation of ER-mitochondria junctions to attenuate $[Ca^{2+}]_{mito}$ overload in hypoxia/reoxygenation HUVEC. However, limited researches focus on the formation or dissociation of ER-PM complex in cardiovascular disease.

Objectives: In this work, we studied the structure and function of supramolecular complex involved in regulating Ca^{2+} homeostasis in cardiovascular disease.

Methods: The nanometers apart of ER-PM and ultrastructure of cell were measured by transmission electron microscope. Protein-protein interactions were measured by immunoprecipitation. Ca^{2+} concentration was measured by confocal microscope. The siRNA was employed to silence specific proteins.

Results: 1. Our results first demonstrated that the peripheral ER translocation into PM-junction sites, while ER dilation and $[Ca^{2+}]_{ER}$ depletion was induced by TNF-alpha. There was new NCX1-TRPC3-IP3R1 complex formed in the PM-junction sites. 2. The abdominal aortic coarctation promotes STIM1 junctional accumulation in rat heart, and then formed the STIM1-Orai1 complex. 3. Above two ER-PM complex involve in the $[Ca^{2+}]_{cyt}$ overload and apoptosis. 4. Interestingly, the activated M_3AChR by ACh could uncouple the NCX1-TRPC3-IP3R1 complex, then inhibit $[Ca^{2+}]_{cyt}$ overload and apoptosis. In fact, the protective effect of ACh was depended on the M_3/AMPK pathway.

Conclusion: ER mediated Ca^{2+} transport by connection of PM or mitochondria. ACh ameliorated Ca^{2+} dyshomeostasis by inhibition of ER-PM and ER-mitochondria connection simultaneously. It is suggested that the inhibition of ER-PM junction may be play an important role in cardiovascular protection.

Keywords: acetylcholine; endoplasmic reticulum; ER-PM contacts; $[Ca^{2+}]_{cyt}$ overload; cardiovascular disease

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WE-036

Dopamine D2 Receptors Is Iinvolved in the Cardioprotection of Ischemic Post-Conditioning in Rat by Activiting Autophagy

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Background: The physiological and pathological roles of dopamine D2 receptors (DR2) in the cardiovascular system have been recognized. Autophagy is associated with cardioprotection of ischemic post-conditioning (PC). However, the role of DR2 during this process is unclear. **Methods**: In this study, PC model in isolated rat hearts was established. To investigate the contribution of DR2 to autophagy activation, the rats were treated with 3-methyl-adenine (3-MA, the autophagy inhibitor), Bromocriptine (Bro,

DR2 agonist) and Haloperidol (Hal, DR2 antagonist). We observed cardiomyocytes damage, apoptosis and signal pathways by TUNEL and TTC staining, Transmission electron microscopy, Real-Time PCR, Western blotting etc. **Results**: We found that PC reduced I/R-induced cardiomyocytes damage, apoptosis and myocardial infarct size, and improved cardiac function. In addition, PC increased DR2 expression, the formation of autophagic vacuoles, the autophagic related protein levels of LC3-II and Beclin1, and decreased mTOR phosphorylation. Compared with PC, Bro further enhanced the cardioprotective roles of PC, but Hal and 3-MA canceled the protection of Bro. **Conclusion**: Taken together, these results suggest that the involvement of DR2 activation in PC-induced cardioprotection are associated with up-regulation of autophagy through inhibiting mTOR phosphorylation in isolated rat hearts.

WE-037

Extracellular RNA induces ischemia/reperfusion injury by Tumor Necrosis Factor (TNF-α) – Shedding: The role of TNF-Receptor 1

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Background - During acute myocardial infarction, cardiomyocyte death has a great impact on the quality of life and survival of patients. Despite reopening/reperfusion of stenosed vessels, major organ damage remains. The initial mechanistic triggers of this myocardial “ischemia/reperfusion (I/R) injury” remain greatly unexplained. Hypothesis - We hypothesized that extracellular-RNA (eRNA), derived from damaged tissue, and tumor-necrosis-factor-a (TNF-α), may dictate cardiac I/R injury. **Methods and Results** - Following myocardial I/R in mice or I/R induced in the isolated Langendorff rat heart, increased eRNA levels were found together with cardiac injury markers such as troponin-I, creatine-kinase and LDH. Likewise, eRNA was released from cardiomyocytes under hypoxia and subsequently induced TNF-α liberation by triggering TNF-α-converting-enzyme (TACE) to provoke cardiomyocyte death. Conversely, TNF-α promoted eRNA release especially under hypoxia, feeding a vicious cell damaging cycle during I/R. Administration of RNase1 or TAPI (a TACE-inhibitor) prevented cell death and myocardial infarction. Likewise, RNase1 significantly reduced I/R-mediated energy exhaustion, opening of mitochondrial-permeability-transition-pores as well as oxidative damage in cardiomyocytes. Furthermore, as compared to isolated wild-type cardiomyocytes, in TNF-receptor-1 and TNF-α knockout cells, upon exposure to hypoxia cell viability decreased in a similar manner, but was not further reduced in the presence of eRNA. In contrast, in TNF-receptor-2 knockout cells eRNA significantly induced cell death, indicating that the lack of TNF-α and TNF-receptor-1 prevented eRNA-induced cell death. These findings were corroborated by the observation that TAPI-treatment of the isolated rat heart during an interval of 30min prior to the ischemic phase significantly decreased LDH release in comparison to the untreated I/R group. **Conclusions** - RNase1 and TAPI provide novel therapeutic regimen to interfere with the adverse eRNA-TNF-α interplay and significantly reduce or prevent the pathological outcome of ischemic heart disease. This as yet unrecognized fundamental pathomechanism is likely to operate in other organs and tissues as well.

WE-038

Endogenous annexin-A1 is cardioprotective against myocardial infarction in mice *in vivo*

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Background: Annexin-A1 (ANX-A1) is an endogenous anti-inflammatory protein that preserves left ventricular (LV) viability and function after an ischemic insult *in vitro*. However, its cardioprotective actions *in vivo* are largely unknown. The aim of this study was to test the hypothesis that ANX-A1 deficient (*ANX-A1*$^{-/-}$) mice have an exaggerated detrimental response to myocardial infarction (MI) *in vivo* compare to their wild type counterparts.

Methods: Adult male *ANX-A1*$^{+/+}$ and *ANX-A1*$^{-/-}$ mice were subjected to left anterior descending (LAD) coronary artery occlusion (1h) followed by reperfusion (24h or 48h), permanent LAD occlusion (8 days) or sham operation.

Results: Compared to *ANX-A1*$^{+/+}$ mice, *ANX-A1*$^{-/-}$ mice exhibited increased infarct size (24h; 34.8±1.7 vs. 49.3±5.4% $p<0.05$, n=8-9) and increased LV macrophage content (48h; 546±71 vs. 873±86 macrophages/mm^2; $p<0.05$, n=5-6). Eight days post-MI, there was a significant 2-fold up-regulation of hypertrophic *ANP* expression in *ANX-A1*$^{+/+}$ mice compared to sham animals ($p<0.05$), which tended to be further increased in *ANX-A1*$^{-/-}$ mice ($p=0.08$). This corresponded with increased heart weight in *ANX-A1*$^{+/+}$ compare to *ANX-A1*$^{-/-}$ mice (5.6±0.2 vs. 7.3±0.4mg/g; $p<0.001$) and LV weight (4.2±1.2 vs. 4.9±0.2mg/g; $p<0.05$) relative to body weight. In addition, pro-inflammatory *TNF-α* and pro-fibrotic *CTGF* gene expression were increased 2-fold in *ANX-A1*$^{+/+}$ mice, compared to a 7-fold elevation in *ANX-A1*$^{-/-}$ mice ($p<0.05$ vs. *ANX-A1*$^{+/+}$), and this was associated with increased LV collagen deposition after MI (19±2 vs. 41±7%, $p<0.01$, n=5-7). Moreover, *ANX-A1*$^{-/-}$ mice exhibited greater expansion of the hematopoietic stem cell population and altered pattern of mobilization relative to *ANX-A1*$^{+/+}$ mice after MI. Further, circulating neutrophil and platelet (but not monocyte) numbers were significantly increased in *ANX-A1*$^{-/-}$ mice after MI compared to *ANX-A1*$^{+/+}$, possibly as result of increased monocyte/macrophage infiltration into the injured *ANX-A1*$^{-/-}$ myocardium after MI.

Conclusion: In summary, ANX-A1-deficiency increased cardiac necrosis, inflammation, hypertrophy and fibrosis following MI. These findings suggest endogenous ANX-A1 limits LV damage *in vivo* and supports further development of novel ANX-A1 based therapies to improve cardiac outcomes after MI.

WE-039

HAX-1 regulates contractile recovery after ischemia/reperfusion injury by preventing SERCA2a degradation

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Cardiac SR calcium handling is critical for control of contractility, bioenergenetics, and cell death. We have previously shown that a mitochondrial protein, HAX-1, is an interacting partner of phospholamban (PLN) and can modulate SERCA2a activity. HAX-1 overexpression increases the inhibitory effects of PLN on the Ca-affinity of SERCA2a, resulting in depressed Ca handling and contractility. To examine the functional role of endogenous HAX-1 in the heart, we generated an inducible cardiac specific HAX-1 knockout model (HAXcKO). Full ablation of HAX-1 in adult hearts significantly enhanced SERCA2a activity, cardiomyocyte contractile parameters and Ca-kinetics without altering levels of Ca handling proteins (SERCA2a, PLN, RyR). The increased activity was half of that observed with PLN ablation or isoproterenol stimulation, suggesting that 50% of the physiological inhibition of PLN is mediated by HAX-1. Additionally, no alterations in apoptotic and ER stress markers (caspase 3/12, GRP94, and IRE-1) were associated with ablation of the anti-apoptotic HAX-1 protein. However, HAX-1 deficient hearts exhibited significantly reduced functional recovery upon ex vivo ischemia/reperfusion injury (I/R; 40 min no flow ischemia/60 min reperfusion). The rates of contraction/relaxation and left ventricular developed pressure recovered to only 25% of pre-I/R levels in HAXcKO hearts, compared to the 50% recovery in WTs. This diminished recovery was partially attributed to 40% reduction in SERCA2a protein in HAXcKO hearts, compared to a 20% decrease in WT. Accordingly, HAX-1 overexpression prevented loss of SERCA2a protein after I/R and enhanced contractile recovery. The alterations in SERCA2a degradation did not reflect changes in calpain 1 and 2 protein levels, while calpain activity was equally increased in the HAX-models due to loss of the endogenous calpain inhibitor, calpastatin. Thus, HAX-1 depresses SR Ca-cycling but

enhances functional recovery after ischemia/reperfusion, in part by preventing the degradation of SERCA2a protein.

WE-040

Adenosine A_1 receptor biased agonism in cardiac ischemia-reperfusion injury

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Background. Stimulation of the adenosine A_1 G protein-coupled receptor (A_1AR) is a powerful protective mechanism in cardiac ischemia-reperfusion injury (IRI). Despite this, therapeutic targeting of the A_1AR has been largely unsuccessful due to on-target adverse effects, including pronounced bradycardia, atrioventricular block and hypotension. Biased agonism has the potential to overcome these limitations by enabling the separation of therapeutic from adverse effects.

Aims. To compare the *in vitro*, *ex vivo* and *in vivo* signaling profile of the A_1AR biased agonist VCP746 to A_1AR prototypical agonists in cardiac IRI.

Methods. In the isolated heart model, perfused rat hearts were subjected to ischemia (30 min) and reperfusion (60 min). In the acute myocardial infarction model, the left anterior descending coronary artery was temporarily occluded for 30 min, followed by 120 min reperfusion. In both models, compounds were added at reperfusion and infarct size, heart rate and blood pressure (*in vivo* only) assessed. Signalling profiles were determined in isolated rat neonatal ventricular cardiomyocytes.

Results. VCP746 and prototypical agonists stimulated an A_1AR-dependent reduction in infarct size and an improvement in cardiac function post-IRI. However, in contrast to prototypical agonists, VCP746 had no significant haemodynamic effects. In isolated rat cardiomyocytes, A_1AR agonists stimulated ERK1/2 phosphorylation, inhibited cAMP accumulation, promoted cardiomyocyte cell survival and decreased glycolysis and oxidative metabolism after a period of simulated ischemia. Prototypical agonists stimulated a potent reduction in cardiomyocyte beat rate frequency via G protein-coupled inwardly-rectifying potassium (GIRK) channels. In contrast, VCP746 stimulated only a weak decrease in cardiomyocyte beat rate, suggesting signal divergence at the level of GIRK channel activation.

Discussion. Collectively, these studies demonstrate that VCP746 can promote cardioprotection in the absence of bradycardia, a profile suggestive of ligand bias. Insights into the signaling profile of VCP746 in cardiomyocytes suggest this signal divergence may involve GIRK channel activation.

WE-041

Deletion of the NADPH Oxidase Organizing Protein NoxO1 promotes angiogenesis

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Reactive oxygen species contribute to angiogenesis and vascular repair. NADPH oxidases are the main source of ROS in the vasculature. NoxO1 is a cytosolic protein facilitating assembly on the constitutively active NADPH oxidase of epithelial cells. Being constitutively active, we speculate that NoxO1 contributes to basal ROS formation in the vascular system and modulates angiogenic responses. This hypothesis was tested in NoxO1 knockout mice and cells obtained from these animals. Blood flow recovery after femoral artery occlusion was better in NoxO1-/- as compared to WT animals. Similar, ex vivo spheroid outgrowth assays revealed increased tube formation capacity in lung endothelial cells obtained from NoxO1-/- mice as compared to WT animals. In a spheroid confrontation assay, in which color labeled cells from WT and NoxO1-/- animals are directly studied within the same spheroid, the number of NoxO1-/- cells at the tips was higher than that of wildtype cells. These results suggest that deletion of NoxO1 favors the expression of a tip cell like phenotype.

The NOTCH pathway is one of the main switches for an endothelial cell from a tip cell into a stalk cell phenotype and activation of the NOTCH pathway results in expression of a stalk cell phenotype. Physiologically, NOTCH mediated signalling requires proteases, among them the alpha-secretase ADAM17, to eventually result in the formation of the active NOTCH intracellular signalling domain. Importantly,

ADAM17 activity was indeed reduced in NoxO1-/- cells when compared to wildtype as measured by the degradation of an artificial substrate.
We conclude that NoxO1 controls alpha-secretase activity. Deletion of NoxO1 therefore promotes a tip cell phenotype which results in increased angiogenesis.

WE-042

Cardioprotective reperfusion strategies improve cardiac recovery after global, warm ischemia in an isolated working rat heart model of donation after circulatory death

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Background: Donation after circulatory death (DCD) could improve cardiac graft availability, which is currently insufficient to meet transplant demand. However, in DCD heart transplantation, organs undergo an inevitable period of warm ischemia and most cardioprotective approaches can only be applied at reperfusion (procurement) for ethical reasons. Therefore, we investigated whether strategies applied at the onset of reperfusion may improve heart recovery after warm ischemia.
Methods: Isolated hearts of male Wistar rats were perfused in working-mode for 20 min (baseline), subjected to 27 min global ischemia (37°C), and 60 min reperfusion (n=43). Mild hypothermia (MH; 30°C, 10 min), mechanical postconditioning (MPC; 2x30 sec), hypoxia (HY; no O_2, 2 min) and low pH (pH 6.8-7.4, 3 min) were applied at the onset of reperfusion and compared with controls (i.e. no strategy applied). Data (mean±SD) were compared using t-tests; p-values were corrected for multiple comparisons.
Results: Post-ischemic recovery was higher in MPC, MH and HY treated hearts compared to controls. No difference was measured for low pH (see Table below).
Conclusions: MPC, MH and HY, but not pH, seem to improve hemodynamic recovery vs controls. Reduced necrosis (MH), increased oxidative metabolism (MPC) and decreased mitochondrial damage (HY) may contribute to improved functional recovery. Cardioprotective strategies applied at graft procurement, could improve DCD graft recovery and limit further injury; however, optimal reperfusion strategies remain to be identified.

	LV Work [%]	Cardiac Output [%]	dPdt max [%]	O_2 cons. [%]	Coronary Flow [mL/min]	LDH release [U*min^{-1}*g wet^{-1}]	Cyt c release [ng*min^{-1}*g wet^{-1}]
Control	44±7	5±8	57±10	41±15	13±3	396±276	45±20
MH	62±7*	20±18	74±12*	55±13	14±2	112±128*	31±23
MPC	65±8*	27±19*	74±7*	61±14*	17±3*	303±357	44±17
HY	61±11*	8±16	85±20*	50±12	16±3	265±318	28±10
Low pH	45±13	12±11	60±14	44±10	15±3	449±628	38±18

All parameters are reported as 60 min reperfusion values expressed as percentage recovery of baseline, except for coronary flow, cytochrome c (Cyt c) and lactate dehydrogenase (LDH) release, expressed as the absolute value at 10 min reperfusion.
*p<0.05 vs control; left ventricular (LV) work (developed pressure*heart rate) / dPdt max (maximum contraction rate) / O_2 cons (O_2 consumption)

WE-043

High circulating fatty acids prior to warm ischemia decrease cardiac recovery in an isolated rat heart model of donation after circulatory death

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Background: Insufficient cardiac graft availability could potentially be improved with donation after circulatory death (DCD). Preclinical studies suggest that high pre-ischemic levels of circulating fatty acids, as may expected with DCD, affect post-ischemic cardiac recovery. Therefore, we investigated whether acute cardiac exposure to high levels of fatty acids prior to global warm ischemia alters subsequent recovery.
Methods: Isolated hearts of male Wistar rats underwent 20 min baseline working-mode perfusion with glucose (11 mM) and either high fat (1.2 mM palmitate; HF) or no fat (NF), followed by 27 min global ischemia (37°C), and 60 min glucose only reperfusion (n=16). Additional hearts underwent 10 min reperfusion with radiolabelled glucose for measurement of glucose oxidation (GOX) and glycolysis (GLY; n=2-4). Release of lactate, cytochrome c and tissue glycogen content were also monitored. Data (mean±SD) were compared using t-tests; p-values were corrected for multiple comparisons.
Results: After 60 min reperfusion, percent recovery of rate-pressure product (peak systolic pressure*heart rate) was two-fold lower in HF vs NF hearts (31±17% vs 69±17% baseline; p<0.01). Trends toward

lower GLY and GOX rates, with a greater imbalance between GLY and GOX was measured in HF vs NF hearts during early reperfusion. Furthermore, lactate (10±2 vs 6±2 μmol*g tissue^{-1}; $p<0.05$) and cytochrome c release (18±9 vs 5±2 ng*min^{-1}*g wet^{-1}; $p<0.01$) were greater in HF vs NF hearts at 10 min reperfusion. Glycogen consumption during ischemia was not different between groups.

Conclusion: Acute pre-ischemic exposure of hearts to high fat significantly decreases hemodynamic and metabolic recovery upon reperfusion compared to no fat. Thus, pre-ischemic circulating fatty acid levels should be taken into consideration in pre-clinical models and clinical situations involving cardiac ischemia-reperfusion. In the context of DCD, pre-ischemic interventions are limited, but optimizing energy substrate metabolism at the time of procurement may facilitate use of these hearts.

WE-044

T185- Study and characterization of p38MAPK's key residue involved in Ischaemic Heart Disease

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p38 has been studied over the years and over hundred studies have shown it to be implicated in Ischaemic heart disease. p38 belongs to a family of MAPK and gets activated via classical 3 tiers of MAPKKK cascade. However during ischaemic condition, p38 gets activated via atypical activation mechanism involving a scaffolding protein Transforming growth factor-β-activated protein kinase 1 – binding protein 1 (TAB1), where TAB1 binds to p38α in a bipartite manner to induce structural changes within p38 that leads to its autoactivation. Both in-vivo and in-vitro model have shown this specific pathway of p38α activation to be the root of harmful outcomes seen during and after Myocardial Infarction. This specific pathway of p38 activation makes it a very attractive therapeutic target, as adverse effect from small molecules has been the major Achilles heel in drug discovery of p38 inhibitors. We recently published the crystal structure of p38α with TAB1 peptide in NSMB, and in this structure we made an observation that led us to hypothesise that Thr185 residue of p38 could play a pivotal role in the autoactivation process. Following our investigation, we present evidence to support our hypothesis that T185 plays a critical role in the structural changes during TAB1 induced auto-activation of p38 and without it the process is significantly compromised. Furthermore, with our on-going investigations we've collected some preliminary results to indicate that this residue may have additional functional role to play upon TAB1 induced autoactivation of p38 which could shed light on p38's mechanism of action under ischaemic stimuli, however further experiments are required.

WE-045

The inhibition of proteasomes prevents Mitofusin 2 and Miro 1 degradation in cardiomyocytes during ischemia-reperfusion

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During cardiac ischemia reperfusion (I/R) diverse mitochondrial proteins are degraded altering mitochondrial dynamics and inducing mitochondrial fragmentation (fission). Extensive mitochondrial fission impairs mitochondrial function and causes cardiomyocyte death. One strategy to reduce heart damage during I/R is the use of proteasome inhibitors, however the mechanism by which these inhibitors induce protection during I/R is still unknown. Mitofusin 2 (Mfn2) and Miro 1 are proteins implicated in transport and dynamics of the mitochondria. The consequences of I/R on Mfn 2 and Miro1 content in cardiomyocytes have not been studied. The aim of this work was to evaluate the content of these proteins and whether inhibition of the proteasome is able protect the mitochondria from I/R injury. Cultured neonatal rat cardiomyocytes were subjected to simulated I/R (sI/R) in the absence or the presence of the proteasome inhibitor MG132. Cell death was evaluated by lactate dehydrogenase release (LDH) and the relative content of mitochondria was determined by qPCR. Mitochondrial fusion and fission were evaluated by confocal microscopy using mitotracker green and the protein levels of Mfn2 and

Miro1 were determined by inmunowesternblot (WB).
In the absence of proteasome inhibitor, sI/R decreased the relative content of mitochondria, increased mitochondrial fission and produced cardiomyocytes death. Also, sI/R decreased the protein content of Mfn2 and Miro1. The inhibition of proteasomes by MG132 preserved the content of Mfn2 and Miro1. Mitochondrial fission was also prevented resulting in an increased number of cells that survived sI/R. Taken together, these data suggest that inhibition of the proteasome preserves the mitochondria explaining at least in part the protective effect of proteasome inhibition after I/R.
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WE-046

The new St Thomas' Hospital polarized cardioplegia shows non-inferiority and improved efficacy of myocardial protection in pigs undergoing cardiopulmonary bypass compared to St. Thomas' 2 cardioplegia

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Objectives: Increasingly, patients undergoing cardiac surgery are more elderly, sicker and hence require improved protection. We compared cardioprotective efficacy of a new St Thomas' Hospital Polarizing cardioplegia (STH-Pol: esmolol, adenosine, magnesium) to conventional St Thomas' Hospital cardioplegia (STH2: potassium, magnesium) in a pig model of cardiopulmonary bypass (CPB). Our hypothesis was the non-inferiority of depolarized versus polarized arrest.
Methods: Pigs (47±4kg) were anesthetized and monitored for baseline hemodynamic function. After sternotomy, CPB and aortic cross-clamping, hearts were arrested via antegrade warm (37°C) STH-Pol (n=7) or STH2 (n=6) for 60min ischemia followed by 60min on-pump reperfusion. After weaning from CPB, hearts were monitored for further 120min off-pump reperfusion before sacrifice and tissue sampling (for high-energy phosphates and electron microscopy). Recovery was measured as % of baseline (mean±SEM).
Results: Baseline hemodynamics were comparable. After 180min reperfusion, recovery of mean arterial pressure and heart rate were similar; however, in STH-Pol hearts had improved recovery of left ventricular systolic pressure (133±8 vs. 97±5 %, p<.01) and external heart work (145±16 vs. 88±10%, p<.05) than STH2 hearts. Coronary flow/heart weight was also higher during early (430±59 vs. 211±59%, p<.05) and late reperfusion (269±43 vs. 90±16 %, p<.01) in STH-Pol. Total creatine kinase release was lower in STH-Pol hearts during reperfusion (2016±262 vs. 1232±199 U/L, p<.05). Creatine phosphate levels in ST-POL hearts were higher (133±31 vs. 63±2 nmol/mg, p<.05). There was no difference in ultrastructure between groups.
Conclusion: Polarized cardiac arrest improves myocardial protection and reduces ischemic damage in a model of CPB in pig hearts. We therefore think this new concept of polarized cardioplegia should have clinical relevance.

WE-047

Quantitative assay of microvascular hyper-permeability following cardiac ischemia-reperfusion

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Background: Microvascular hyper-permeability is a common event following ischemia-reperfusion (IR) and impacts the efficacy of therapeutic interventions. Currently there has been lack of quantitative assay of microvascular hyperpermeability. We attempted to establish a quantitative method to determine microvascular permeability at both organ level and cellular level.
Methods: Male C57Bl/6 mice were subjected to left coronary artery occlusion

(0.25, 0.5, 1 and 4h) followed by reperfusion for 24 h (IR). Evans blue (EB, i.v.) was given 3h prior to termination. Heart tissues were homogenized and EB was extracted in trichloroacetic acid solution and quantified using spectrophotometer. Experiments were conducted to determine an optimal dose of EB (3.3, 10, 20 and 33 mg/kg, respectively). For in vitro permeability assay, mouse cardiac endothelial cells (H5V) were cultured in transwells for 4 days to confluency and maturation. Hypoxia-reoxygenation was induced. The permeability across endothelial cell monolayer was measured by adding FITC-dextran (mw=40KDa, 1μM) as fluorescent tracer into the upper chamber of transwells. Concentration of FITC-dextran in the lower chamber was determined.

Results: Compared to other doses, EB at 20 mg/kg yielded a high level of EB content in the infarct zone while EB content in non-infarct zone was minimal. So this optimal dose was used. And the results showed that IR induced a 15-fold increase in EB content in the infarct zone. The extent of microvascular leakage was dependent on the duration of ischemia of 0.25, 0.5, 1 and 4h, $P<0.001$ by ANOVA, n=6 each).

FITC-dextran leaking experimental results showed that the permeability of monolayer H5V cells increased over 50% at both hypoxia 16h and reoxygenation at 2h and 6h.

Conclusion: The microvascular permeability induced by myocardial IR can be quantified in vivo by using EB extraction method described and ex vivo by using FITC-dextran as indicator.

Key words: microvascular damage, ischemia-reperfusion, Evans blue

WE-048

Effects of hydrogen sulphide (H_2S) on oxidative stress in acute myocardial ischemia injury in isolated hearts in rats

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Objective: To observe the effects of H_2S on oxidative stress in myocardial ischemia injury in isolated heart in rats. **Methods:** The myocardial ischemia injury model was established by the ligation of coronary artery. Forty male SD rats, weighing 270±20g, were randomly divided into five groups: sham, model, and low, middle, high dose groups of NaHS. The left anterior descending coronary artery was ligated in rats of the model group, but the rats in the sham group were only threaded without ligation. The normal perfusate was replaced with NaHS perfusate (5μmol/L,10μmol/L,20μmol/L) accordingly in low dose, middle dose and high dose group of NaHS at 2h after ischemia. The content of MDA, the activities of LDH, SOD and GSH-P_X were respectively measured by spectrophotometry. The ultrastructural alterations of myocardium were observed by electric microscope. **Results:** Compared with those of the sham group, the activity of LDH in perfusate was significantly increased in the model group. Compared with those of the model group, the activity of LDH in perfusate was significantly decreased in low, middle and high dose groups of NaHS. The content of MDA in cardiac tissue was significantly increased, and the activities of SOD and GSH-P_X in cardiac tissue were significantly decreased in model group compared with those of sham group .The content of MDA was significantly decreased and the activities of SOD and GSH-P_X in cardiac tissue were significantly increased in the low, middle and high dose groups of NaHS compared with those of model group. The ultrastructure of the myocardial cells exhibited the myocardial cells were characterized by mitochondrial swelling, disappearance or deformation of mitochondrial cristae, disruption of nuclear membrane, and nuclear condensation in the model group. Compared with those of the model group, The myocardial ischemia injury was significantly decreased in NaHS treatment groups. **Conclusion:** It could be concluded that H_2S has certain protective effect against acute myocardial ischemic injury and the mechanism may be related to anti oxidation.

WE-049

Effects of Simvastatin on the Expression of P47phox in Renal Ischemia Reperfusion Injury

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Objective: To investigate the effects of Simvastatin (SIM) on the expression of P47phox in renal ischemia-reperfusion injury (RI/RI). **Methods:** Sixty male

Sprague-Dawley rats were divided into five groups randomly: (1) Sham group; (2) ischemia-reperfusion group (I/R); (3) low-dose SIM group (Sim-L, 5mg/kg/d); (4) middle-dose SIM group (Sim-M, 20mg/kg/d); (5) high-dose SIM group (Sim-H , 40mg/kg/d). Sim-L, M and H group rats were given oral SIM 5, 20 and 40 mg/kg/d treatment respectively for 2 weeks. The model of RI/RI was induced by bilateral clamping the renal artery and vein for 45 minutes followed by reperfusion. After 6 and 24 hours of reperfusion, the blood samples were taken for detecting contents of serum creatinine (Scr), urea nitrogen (BUN). After blood was taken, both side of kidney were excised for observing renal histological examination, content of Nitric Oxide (NO), activity of superoxide dismutase (SOD), the content of malondialdehyde (MDA) and the protein expression of P47phox were measured respectively . **Results:** After RI/RI, the renal tubule epithelial cells showed signs of damage in I/R group rats. the contents of Scr, BUN and MDA were significantly increased in I/R group than that of sham group ($P < 0.01$); Compared with the I/R group rats, contents of Scr, BUN and MDA were significantly lower in Sim-L , M and H groups ($P < 0.05$). Contents of NO and activity of SOD were significantly increased ($P<0.01$) in Sim-M and Sim-H groups; The expression of positive immunoreactive particles and protein of P47 phox were increased in I/R group rats than that of in Sham group rats. Compared with I/R group rats, both of positive immunoreactive particles and protein expression of P47phox were decreased in Sim-M and Sim-H group rats, but not in Sim-L group rats. **Conclusions:** These results suggest that SIM could reduce renal tissue injury and down-regulated the expression of P47phox of renal tissue in RI/RI rats. It is indicated that the protective effects of SIM to the RI/RI may be related to block the NAD (P) H oxidate pathway and anti-free radical damage.

WE-061

A pathogenic *MYBPC3* 25-bp polymorphic variant causes hypertrophic cardiomyopathy in South Asian descendants

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South Asians account for 25% of the world's population, but they hold a disproportionate 60% of the world's cardiovascular disease burden. Hypertrophic cardiomyopathy (HCM) is predominantly caused by mutations in sarcomeric genes, including *MYBPC3,* the most common HCM-associated gene. Previously, we identified a *MYBPC3* 25-bp polymorphic variant (*MYBPC3*$^{\Delta Int32}$), which is inherited in 4% of South Asian descendants. *MYBPC3*$^{\Delta Int32}$, an intronic 25-bp deletion in *MYBPC3* at the 3′ region, is characterized by incomplete penetrance and expressivity. While those carrying this variant are at high risk for developing HCM and heart failure, its functional and molecular effects remain unknown. Using cultured adult rat cardiomyocytes *in vitro*, we showed that *MYBPC3*$^{\Delta Int32}$ was unable to incorporate into the sarcomere, which resulted in contractile dysfunction. In the current study, a genetically engineered mouse model expressing a moderate amount of *MYBPC3*$^{\Delta Int32}$ was established with HCM phenotype, including diastolic dysfunction. Furthermore, to determine the prevalence of this variant among South Asians in the United States, we screened 1162 subjects and determined a variant frequency of 6.80% and an allele frequency of 3.57%, a higher prevalence than was initially expected in this cohort study. Four homozygous subjects were identified. Following prevalence studies, clinical studies, including echocardiogram and electrocardiogram analyses, were performed on 15 positive subjects, compared to 15 non-carriers, to determine the presence of any sign of HCM. Our data again confirmed incomplete penetrance. Overall, therefore, we determined that *MYBPC3*$^{\Delta Int32}$ alone is sufficient to promote the development HCM, implicating the translational importance of these studies in the context of the development of heart disease among South Asian populations.

WE-062

Lack of essential myosin light chain phosphorylation impairs cardiac ability to adapt to augmented physical demand.

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Cardiac ability to adapt its function to the body's demand is pivotal for normal heart function. Modulatory proteins adjunctive to actin and myosin largely accounts for this ability. Among others, the regulatory (RLC) and the essential myosin light chain (ELC) are part of myosin molecules and contribute to modulation of cardiac contraction. Mutations in RLCs and ELCs cause cardiomyopathy in humans. While the role of RLCs in cardiac physiology and pathophysiology is well established, the precise function of ELCs in the heart and its contribution to human cardiomyopathy remains unclear. Similar to RLCs, ELCs are subject to phosphorylation. However, the exact role of ELC phosphorylation for normal heart function and in disease is unknown. To model human haploinsufficiency, we used the adult heterozygous zebrafish mutant *lazy susan* (*laz*^{m647}) carrying a nonsense mutation in ELC, resulting in the removal of the highly conserved phosphorylation site S195. By echocardiography we found that these zebrafish display signs of systolic dysfunction. When subjected to forced swimming, heart function severely deteriorated, causing heart failure and sudden death. We used native heart tissue to show that ELC becomes phosphorylated after physical stress. Additionally, *in vitro* motility analysis of zebrafish actin sliding on ventricular myosin derived from wildtype (wt) and *laz* mutant zebrafish after rest or physical stress reveals that C-terminal phosphorylation critically modulates cross-bridge activity, cycling kinetics and filament velocity, specifically after stress. Our model enabled us to analyze acto-myosin interaction in native composition of wt and mutant protein. Further, calcium-dependent force measurements and calcium transient recordings demonstrates impaired calcium handling in *laz* mutant cardiomyocytes, again specifically after physical stress. Our study employed for the first time adult heterozygous zebrafish to provide novel mechanistic insights into ELC-mediated adaptation of cardiac function after physical stress and might contribute to a better understanding of pathomechanisms in ELC-linked cardiomyopathy.

WE-063

CYP2C19 and PON1 genetic variants as potential predictors for the risk of bleeding in antiplatelet-treated patients

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Bleeding has emerged as an important outcome in antiplatelet treatment after percutaneous coronary intervention (PCI), but the study on the relationship between genetic variations with inter-patient variability of bleeding is lack, compared to major adverse cardiac events (MACE). The present study was aimed to evaluate the contribution of 13 genetic variants to the risk of MACE and the occurrence of bleeding events in Han Chinese patients after PCI. Five hundred and twenty Han Chinese patients undergoing PCI and received dual-antiplatelet therapy were sequentially recruited and followed up to 1 year. Thirteen variants in ABCB1, CYP2C19, PON1, P2RY12, P2RY1 and ITGB3 were genotyped. The effect of genetic variants on MACE in 1 year and bleeding in 6 months was assessed. CYP2C19*2 allele was significantly associated with a higher risk of the efficacy endpoint of MACE (HR per allele, 2.00; 95%CI: 1.02-3.92), while a low risk of safety endpoint of bleeding events (OR, 0.42; 95%CI, 0.25-0.70). Univariate analysis indicated PON1 g.*2435 A allele and p.Gln192Arg G allele were associated with a lower risk of bleeding (OR, 0.54; 95%CI, 0.30-0.99, and OR, 0.47; 95%CI, 0.25-0.86), while p.Leu55Met T allele was associated with a higher risk of bleeding (OR, 2.83; 95%CI, 1.12-7.15). PON1 g.*2435G>A and p.Leu55Met were still significantly associated with the risk of bleeding in 6 months when adjusted for other variables. This study confirmed CYP2C19*2 and identified for the first time PON1 genetic variants as potential predictors for the risk of bleeding events in clopidogrel-treated patients after PCI.

WE-064

ADP-stimulated contraction: a predictor of thin-filament activation in cardiac disease

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Background Diastolic dysfunction is general to all idiopathic dilated (IDCM) and hypertrophic cardiomyopathy (HCM) patients. Relaxation deficits may result from increased actin-myosin formation during diastole due to altered tropomyosin position, which block myosin-binding to actin in the absence of Ca^{2+}. We investigated if ADP-stimulated force development (without Ca^{2+}) can be used to reveal changes in actin-myosin blockade in human cardiomyopathy cardiomyocytes.

Methods Force measurements were performed in single membrane-permeabilized cardiomyocytes at sarcomere length of 2.2 µm in the absence of Ca^{2+}, but in the presence of mM levels of ADP. Exogenous protein kinase A (PKA)-treatment was performed to determine whether myofilaments are sensitive to kinase treatment. Cardiac samples from HCM patients, harboring thick- (*MYH7*$_{mut}$, *MYBPC3*$_{mut}$) and thin-filament (*TNNT2*$_{mut}$, *TNNI3*$_{mut}$) mutations, and IDCM, were compared with sarcomere mutation-negative HCM (HCM_{smn}) and non-failing donors.

Results Myofilament ADP-sensitivity was higher in IDCM and HCM compared with donors, while it was lower for *MYBPC3*. Increased ADP-sensitivity in IDCM, HCM_{smn} and *MYH7*$_{mut}$ was caused by low phosphorylation of myofilament proteins, as it was normalized to donors by PKA treatment. Troponin exchange experiments in a *TNNT2*$_{mut}$ sample corrected the abnormal actin-myosin blockade. In *MYBPC3*$_{trunc}$ samples, ADP-sensitivity highly correlated with cardiac myosin-binding protein-C (cMyBP-C) protein level. Incubation of cardiomyocytes with cMyBP-C antibody against the actin-binding N-terminal region reduced ADP-sensitivity, indicative of cMyBP-C's role in actin-myosin regulation.

Conclusions In conclusion, ADP-stimulated contraction can be used as a tool to study how protein phosphorylation and mutant proteins alter accessibility of myosin-binding on actin. Our data provides a mechanism of how phosphorylation alterations and/or expression of mutant proteins increase actin-myosin interactions, that precede Ca^{2+} rise, and could contribute to the impaired myocardial relaxation observed in human cardiomyopathy.

WE-065

Cross-bridge dynamics is determined by two velocity dependent kinetics; implications on the adaptive and synchronous cardiac function

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Introduction: The cardiac muscle has a remarkable ability to adjust function to changes in demands, as described by the Frank-Starling Law, the Fenn effect, and the high contractile efficiency. The loading conditions determine the force per cross-bridge, cross-bridge recruitment and the sarcomeric energy consumption. The length, stress, and velocity of shortening were suggested as possible modulators of cross-bridge dynamics.

Methods: The study tested the effects of the initial length or isometric stress (n=9), and the sarcomere shortening velocity (n=9) on cross-bridge dynamics in the intact rat trabeculae, under constant activation. Sarcomere length was measured by laser diffraction and ramp shortenings at various velocities were imposed with a fast servomotor.

Results: Both stress decline and redevelopment responses revealed two distinct kinetics: a fast and a slower phase. The fast (3 msec) and slow phases depicted linear dependencies of the rate of stress changes on the instantaneous stress levels. The rate coefficients of the two phases were independent of the initial length or stress level. However, they were tightly dependent on the shortening velocity (V_{SL}). An increase in the V_{SL} expedited the rates of both phases in a linear mode; the rate coefficients for the first and second phases were 286±28 + 39±2 V_{SL} [s^{-1}] and 35.7±4.8 + 9.5±1.4 V_{SL} [s^{-1}], respectively.

The fast kinetics is more than 5 time faster than the slow kinetics, at all velocities. The fast kinetics determines the force per cross-bridge and the second is ascribed to cross-bridge cycling and determines the number of strong cross-bridge.

Conclusion: Cross-bridge dynamics is modulated by the velocity and not by the length or stress. These features shed light on theories of muscle contraction, and are essential for the regulation and synchronization of all the sarcomeres and myocytes in the myocardium and for adapting function to match the demands.

WE-066

The functional association between the Sodium/Bicarbonate Cotransporter and the Soluble Adenylate Cyclase (sAC) modulates basal cardiac contractility

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In addition to the adenylate cyclase (AC) embedded in the plasma membrane, another source of cyclic AMP (cAMP) was identified in the heart, the soluble AC (sAC). However, the cardiac physiological function of sAC is unknown. On the other hand, the cardiac Na^+/HCO_3^- cotransporter (NBC) promotes the cellular co-influx of HCO_3^- and Na^+. Since sAC activity is mainly regulated by HCO_3^-, our purpose was to investigate the potential impact on cAMP-dependent cardiac contractility of the relationship between the activity of NBC and sAC. Rat ventricular myocytes were loaded with Fura-2 or Fluo-3 in order to measure Ca^{2+} transient amplitude (CaT) by epifluorescence or Ca^{2+} sparks frequency (CaSF) by confocal microscopy, respectively. Sarcomere shortening as contractility index was measured simultaneously with epifluorescence. The NBC blocker S0859 (10 µM) induced a negative inotropic effect (NIE) in the presence of HCO_3^- (Control: 19.1±3.2% vs. S0859: 14.6±2.6%; n=9, $P<0.05$) which was associated with a decrease of 18.5±2.6% in CaT. S0859 did not induce a NIE in the absence of HCO_3^-. The selective inhibitor of sAC, KH7 (1µM) decreased contractility (Control: 15.7±0.7% vs. KH7: 11.3±0.9%, n=5, $P<0.05$) and CaT (15.7±4.9%) only in HCO_3^-. Moreover, S0859 did not add more NIE in the presence of KH7 (KH7+S0859: 11.1±0.9%, n=5). Since cAMP activates the kinase PKA, which in turn increases Ca^{2+} release through sarcoplasmic reticulum RyR channels, CaSF was measured as an index of RyR open probability. The increase in CaSF observed when field stimulation frequency was increased from 0.5 to 3 Hz (Control variation ratio: 1.23±0.1) was reversed in the presence of S0859 (0.62±0.2, n=5, $P<0.05$) only when HCO_3^- was present in the extracellular medium. In summary, the results demonstrated that the complex NBC-sAC plays a relevant role in Ca^{2+} handling and basal cardiac contractility.

WE-067

Proteomic analysis of excitation-contraction coupling abnormalities in a rat model of heart failure with preserved ejection fraction

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Background:

Heart failure with preserved ejection fraction (HFPEF) is a chronic heart disease with high morbidity and mortality. HFPEF is characterized by diastolic dysfunction, which leads to elevated cardiac filling pressures. Currently, there is no effective treatment, perhaps because the underlying cellular mechanisms of HFPEF remain to be elucidated. In this study we focus on investigating of HFPEF-associated maladaptive calcium responsive changes induced with in a rat model of salt-sensitive hypertension.

Methods:

Dahl salt-sensitive rats received either a high salt (hypertension-induced HFPEF) or a low salt diet (control) for 6 weeks. Following treatment, rat hearts were either harvested for 1) hemodynamic characterization followed by cell isolation for analysis of intracellular calcium (Ca^{2+}) excitation-contraction (EC) coupling or 2) liquid chromatography-tandem mass spectrometry analysis quantifying protein amounts.

Results:

Using the patch clamp technique in isolated myocytes obtained from control and HFPEF hearts, we found defective EC coupling with reduced EC coupling gain, slowed Ca^{2+}

uptake, and slowed relaxation consistent with myofilament phosphorylation. Consistent with these findings, proteomic analysis revealed HFPEF-induced changes ($p<0.05$) in the protein quantity of Ca^{2+} binding and/or Ca^{2+} handling proteins (e.g., for HFPEF vs control, the RyR2-inhibiting protein chloride intracellular channel protein 2 increased by 108%; Sodium/potassium-transporting ATPase subunit beta-1 decreased by 20%), as well as associated kinases and their activators (e.g., Transforming protein RhoA increased by 69%, Serine/threonine-protein kinase PAK 2 increased by 325%; cAMP-dependent protein kinase (PKA) type I-alpha regulatory subunit decreased by 15%).

Conclusion:

Maladaptive changes in key Ca^{2+}-signaling proteins lead to disruption of EC coupling and slowed cardiac relaxation. Pharmacologic targeting of these proteins may be of benefit for treating HFPEF.

WE-068

Insulin Treatment Did Not Prevent Cardiac and Baroreflex Dysfunctions in a Model of Type 1 Diabetes

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Background: The mechanisms underlying the increased risk in Type 1 Diabetes *mellitus* (T1DM) patients even on insulin treatment are not well understood. **Objective:** Evaluate the effects of insulin replacement therapy on cardiac, autonomic and oxidative stress (OS) parameters in a model of T1DM. **Methods:** Male Wistar rats (230-260g) were divided into 3 groups (n=7/group): control (C), diabetic (D, streptozotocin 50mg/kg) and diabetic treated daily with insulin subcutaneously (DTI). At 30 days, cardiac function was assessed by echocardiogram. Baroreflex sensitivity and cardiac autonomic tonus were evaluated. OS analysis was performed in heart tissue. **Results:** The diabetic groups showed hyperglycemia (>350mg/dL) at the beginning of the protocol. Insulin therapy normalized the glycemia (DTI:126±10, C:128±7 vs. D:439±21mg/dL). There was a reduction in the left ventricle mass (LVM) in D group and these changes were not observed in DTI group (LVM- C:1.04±0.04, D:0.82±0.03 and DTI:1.04±0.03g). It was observed impairment in systolic function (shortening fraction) of diabetic group that was reversed with insulin treatment. Regarding diastolic function, the isovolumetric relaxation time (IVRT) was increased and E/A wave ratio (EA) was decreased in D group, which was not reversed in DTI group (IVRT-C:1.29±0.11, D:1.68±0.11 and DTI:1.60±0.08ms/bpm; EA-C:2.45±0.38, D:1.53±0.10 and DTI:1.69±0.17). The baroreflex sensitivity was impaired in D group in relation to C in both bradycardic and tachycardic responses (C:-1.36±0.11, D:-1.06±0.05, DTI:-1.32±0.07 and C:3.18±0.17, D:2.59±0.18, DTI:2.58±0.15bpm/mmHg, respectively). The tachycardic responses dysfunction was not normalized by the insulin treatment. The insulin treatment normalized mean arterial pressure, heart rate, intrinsic heart rate, as well as the vagal and sympathetic tonus which were impaired in D group. These benefits of insulin treatment were reflected on the analysis of OS, where the diabetic group had higher oxidized glutathione (D:0.0242±0.0008 vs. C:0.0169±0.0012, DTI:0.0183±0.0009nmol/g tissue) and increased lipid peroxidation (D:2.54±0.21 vs. C: 1.95±0.10, DTI: 1.59±0.18μmoles/mg protein). **Conclusion:** Despite the insulin treatment normalized blood glucose, cardiac morphometry and systolic function, cardiac autonomic control and oxidative stress, it was not able to attenuate diastolic dysfunction and the tachycardic response of baroreflex, suggesting a remaining cardiovascular risk even after insulin replacement in this model of experimental T1DM.

WE-069

Nitric oxide and CaMKII: critical steps in the inotropic response to IGF-1

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Cardiac adaptation to aerobic exercise training includes improved cardiomyocyte contractility, by a non-yet clarified mechanism in which nitric oxide (NO) and CaMKII have been implicated. At the cellular level, IGF-1 is the main mediator of the adaptive response to exercise. Our purpose was to explore the effect of IGF-1

on mice cardiomyocyte contractility and the underlying signaling pathway.
IGF-1 (10nmol/L) increased cardiomyocyte shortening (128.12±4.62%, n=8 vs basal; p<0.05), effect abrogated by inhibition of NO production with the non-selective nitric oxide synthase inhibitor L-NAME (2.5 mmol/L; 103.2±3.02%, n=5) or nitroguanidine (NG, 240 nmol/L), specific inhibitor for the neuronal isoform (nNOS, 97.4±1.21%, n=5) and by CaMKII inhibition with KN93 (101.50±2.04%, n=6). In agreement, a significant increase in NO production in response to IGF-1 (133.75±2.17%, n=16) was detected by epifluorescence with DAF-FM. Again, this was prevented by L-NAME (110.36±3.20%, n=11) and NG (114.44±1.83%, n=9), confirming the involvement of nNOS but not altered by KN93 (135.22±1.36%, n=9) suggesting that CaMKII activation was downstream NO production. We explored the pathway involved in nNOS activation by measuring AKT phosphorylation. As expected, IGF-1 increased P-AKT (185.90±10.18%, n=3; p<0.05). Since NO-dependent CaMKII activation has been proposed, we next determined CaMKII activity (P-CaMKII) and the phosphorylation of its downstream target Thr17-phospholamban, detecting a significant increase in both in the presence of IGF-1 (227.19±29.43% and 143.34±5.44%, n=3 respectively) but not when NO production was prevented by NG (126.61±5.48 and 65.76±15.04, n=3 respectively). Interestingly, similar results showing nNOS and CaMKII activation were obtained in the hypertrophied myocardium of mice subjected to swimming training.
In conclusion, our results support a critical role of CaMKII in the positive inotropic effect of IGF-1. Our findings suggest that IGF-1 through the IGF-1R triggers the phosphorylation of AKT which in turn activates nNOS and increases NO production which would be responsible for CaMKII activation.

WE-070
oxiCaMKII-dependent RyR2 phosphorylation mediates contractile dysfunction associated with sepsis.

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In sepsis, there is a recognized association between cardiac dysfunction and mortality. Contractile dysfunction associated with sepsis has been attributed to a decrease in the amplitude of the intracellular Ca^{2+} transient and recent studies have proposed that altered ryanodine receptor (RyR2) function is responsible for sarcoplasmic reticulum (SR) Ca^{2+} loss and reduced Ca^{2+} transients.
We examined the subcellular mechanisms involved in SR Ca^{2+} loss and contractile dysfunction associated with sepsis.
Using a colon ascendens stent peritonitis mouse model of sepsis (CASP) and Sham controls, we observed that after 24hs CASP mice had significantly elevated proinflammatory cytokine levels, reduced ejection fraction and fractional shortening (EF%54,76±0,67; FS%27,53±0,5) compared to sham (EF%73,57±0,2; FS%46,75±0,38). At the cardiac myocyte level, CASP cells showed reduced cell shortening, Ca^{2+} transient amplitude and SR Ca^{2+} content compared to Sham cardiomyocytes. CASP hearts showed a significant increase in oxidation-dependent calcium and calmodulin-dependent protein kinase II (CaMKII) activity (CASP 0.92 ± 0.1 AU, Sham 0.56 ± 0.05 AU) which could be prevented by pretreating animals with the antioxidant Tempol (1mM for 7 days in drinking water).
Pharmacological inhibition of CaMKII with 2.5µM KN93 prevented the decrease in cell shortening, Ca^{2+} transient amplitude and SR Ca^{2+} content in CASP myocytes. Contractile function was also preserved in CASP myocytes isolated from transgenic mice expressing a CaMKII inhibitory peptide (AC3-I) and in CASP myocytes isolated from mutant mice that have the RyR2 CaMKII-dependent phosphorylation site (Ser 2814) mutated to alanine (2814A). Furthermore, CASP 2814A mice showed preserved EF and FS (EF%59,54±3,42; FS%35,88±5,58) compared to sham 2814A mice EF%65,89±6,95; FS%33,33±2,38) .
Results indicate that oxidation and subsequent activation of CaMKII has a causal role in the contractile dysfunction associated with sepsis. CaMKII, through phosphorylation of RyR2 would lead to Ca^{2+} leak from the SR, reducing SR Ca^{2+} content, Ca^{2+} transient and contractility.

WE-071

Silencing of the epidermal growth factor receptor (EGFR) blunts the slow force response to myocardial stretch

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Myocardial stretch induces a biphasic force increase: A first phase due to the Frank-Starling mechanism, followed by a slower one called slow force response (SFR). The SFR is due to a complex autocrine mechanism that appears to involve Angiotensin II (AII)-triggered EGFR transactivation and the consequent generation/release of reactive oxygen species (ROS) leading to Na^+/H^+ exchanger (NHE1) activation. In order to conclusively prove the role of the EGFR in the SFR, we developed a lentivirus carrying a siRNA against EGFR (siEGFR), and injected it into the rat cardiac left ventricular wall (n=8). A scramble (siSCR) sequence was used as control (n=9). After 4 weeks, EGFR protein expression showed a 48±15% reduction in siEGFR-injected hearts compared to siSCR (100±6%, $p<0.05$). Isolated rat papillary muscles from both groups were then stretched from 92 to 98% of L_{max}. The SFR was 131±2% of initial rapid phase in siSCR ($p<0.05$ vs. rapid phase) and was blunted in siEGFR-expressing muscles (102±1%, $p<0.05$ vs. siSCR). Basal myocardial oxidative stress estimated by T-BARS was not affected by the reduction in EGFR expression: (in nmol/gr tissue) 1.29±0.09 siEGFR vs. 1.38±0.06 siSCR. However, AII or EGF-mediated ROS production (assessed by lucigenin method in cardiac tissue slices) was significantly reduced in siEGFR-injected hearts: AII (1nM) from 226±27 siSCR to 113±9 siEGFR ($p<0.05$); EGF (0,1ug/ml) from 175±19 to 102±7 ($p<0.05$) respectively. Finally, we studied the EGFR silencing effect over the reported AII-dependent NHE1 activity by measuring pH_i (BCECF, papillary muscles) in bicarbonate-free medium. 1nM AII significantly increased pH_i by 0.18±0.06 units in the siSCR group ($p<0.05$), effect that was completely blunted in the siEGFR one (-0.12±0.03). Taken together, we can conclude that EGFR activation after stretch is crucial for the development of the SFR, effect that would result from preventing ROS-mediated NHE1 activation.

WE-072

Thioredoxin 1 (TRX1) overexpression cancels the slow force response (SFR) development

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The stretch of cardiac muscle increases developed force in two phases. The first phase occurs immediately after stretch and is the expression of the Frank-Starling mechanism, while the second one or slow force response (SFR) occurs gradually and is due to an increase in the calcium transient amplitude. An important step in the chain of events leading to the SFR generation is the increased production of reactive oxygen species (ROS) leading to redox sensitive ERK1/2, p90RSK and NHE1 phosphorylation/activation. Conversely, suppression of ROS production blunts the SFR. The purpose of this study was to verify whether overexpression of the ubiquitously expressed antioxidant molecule TRX1 affects the SFR development and NHE1 phosphorylation. We did not detect any change in basal phopho-ERK1/2, phopho-p90RSK and NHE1 expression in mice with TRX1 overexpression compared to wild-type (pERK1/2:105.4±9.9%, n=4; p-p90RSK: 111±15%, n=3; NHE1:100±13%, n=4, ns) Isolated mouse papillary muscles (wild-type, WT, or with TRX1 overexpression) were stretched from 92 to 98 % of Lmax. The SFR was 137±1% of the initial rapid phase in wild-type mice (n=8, $P<0.05$ vs. rapid phase) while it was completely canceled in TRX1 overexpressing animals (100±3%, n=7, $P<0.05$ vs. control SFR). The increase in NHE1 phosphorylation induced by stretch was significantly higher in WT mice (156±6% of control, n =3, $P<0.05$) compared toTRX1 overexpressing mice (126.1±8.4% of control, n=3, ns). These results, although preliminary, suggest that mitigation of ROS formation after stretch by increasing the myocardial antioxidant defense precludes the NHE1 phosphorylation induced by stretch and consequently the SFR development.

WE-073

Dynamic resistance exercise training induces skeletal muscle and cardiac hypertrophy and improves baroreflex sensitivity in female hypertensive rats

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Background: Guidelines recommend the association of aerobic and resistance exercise training (RT) in the management of hypertension. However, few studies have focused in the evaluation of the effects of dynamic RT in hypertensive subjects, mainly in females. **Objective:** Evaluate the effect of the RT on blood pressure (BP), baroreflex sensitivity (BRS), physical capacity, muscular and cardiac mass in female spontaneously hypertensive rats (SHR). **Methods:** 16 adult female SHR were divided into 2 groups (n=8): sedentary (SF) and trained (TF). The maximum load tests (MLT) were performed before and after the RT. The RT was performed in ladder for 8 weeks (40-60 % MLT). BP was directly recorded in awake rats and BRS was tested using vasoactive drugs. After euthanasia, heart and skeletal muscles were weighed. **Results:** The maximal load was increased in TF group (>140%). No differences between groups were observed for BP (mean BP, TF: 165±12 vs. SF: 171±17 mmHg), heart rate (TF: 378±25 vs. SF: 373±41 bpm) or metabolic parameters (glicemia: 103±6 vs.107±9 mg/dl; triglycerides: 112±10 vs. 135±30 mg/dl, TF vs. SF, respectively). The BRS was improved for bradycardic response in TF group (TF: -1.35±0.39 vs. SF: -0.86±0.16 bpm/mmHg); however, there was no difference between groups for tachycardia response. The soleus, gastrocnemius and right ventricle weights were similar between groups. However, the plantar muscle (TF: 0.187±0.02 vs. SF: 0.144±0.03 g) and the left ventricle weights (TF: 0.737±0.06 vs. SF: 0.580±0.06 g) were increased in TF group. **Conclusion**: The RT in female SHR did not induce adverse effect or reduce BP, however it induced an increase in strength, skeletal muscle and cardiac hypertrophy, as well as, improved BRS. These data reinforces the importance of the association of moderate intensity dynamic RT to aerobic exercise training in the management of hypertension in females.

WE-074

Effect of aging on heart function and calcium handling: impact of NOX inhibition

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Cardiac aging is characterized by alterations in contractility and calcium handling. It has been suggested that oxidative stress may be involved in this process. The superoxide generating system NADPH oxidase (NOX) is expressed in the heart (NOX2 and 4). We and others have reported that in cardiac failure, the NOX-derived superoxide is increased, with a negative impact on calcium and contractility. We tested the hypothesis that calcium transients and contractility in aged rat cardiomyocytes are disturbed by NOX.

Hearts and cardiomyocytes were obtained from adult (5 months-old) and aged (20 months-old) Sprague-Dawley rats, and were treated with apocynin (50 µmol/L), a NOX inhibitor. Cells were field-stimulated from 0.5 to 4 Hz and $[Ca^{2+}]i$ was monitored with fura-2.Contractility was evaluated as dP/dtmax in isolated hearts, challenged with isoproterenol.

Cardiac response to isoproterenol was depressed in aged hearts compared to adults ($p<0.005$), but was restored by apocynin treatment.

$[Ca^{2+}]_i$ transients amplitude was increased in aged cardiomyocytes ($p<0.005$) and was further increased by apocynin treatment. Time-50 to peak $[Ca^{2+}]i$ was increased in aged myocytes ($p<0.05$), suggesting impairment in RyR2, and was improved by apocynin treatment. Time-50 to maximal relaxation was increased in aged myocytes ($p<0.05$) and reduced towards normal by NOX inhibition.

Using thapsigargin to block SERCA2 function, we submitted myocytes to tetanic stimulation to evaluate the myofilaments Ca^{2+} sensitivity. By comparing the amplitude of the tetanic contraction achieved, with the level of $[Ca^{2+}]_i$ evoked, we found that myofilaments Ca^{2+} sensitivity was reduced in aged myoctes ($p<0.05$).

At the protein level, SERCA expression was reduced in aged hearts while

phospholamban was not different between both groups.
In conclusion, contractility in response to isoproterenol was depressed in aged hearts, and in aged myocytes $[Ca^{2+}]_i$ level was higher, as a result of diminished myofilaments Ca^{2+} sensitivity. NOX inhibition increased $[Ca^{2+}]i$ transients amplitude and improved Ca^{2+} kinetics, and improved contractility. These results suggest a NOX dependent effect in aged myocytes at the level of RyR2 and SERCA2 and myofilaments.

WE-075

Mechanisms of sex-difference in serotonergic and α_1-adrenergic vasoconstriction in the internal mammary artery of patients going through coronary artery bypass graft

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Background: Females have poorer outcomes following coronary bypass surgery (CABG) than males and sex-differences in the internal mammary artery (IMA) vasoconstrictor properties have been proposed to contribute to this differential outcome. The objective of this study was to determine the role of: (1) endothelial integrity, (2) nitric oxide (NO) and (3) prostaglandins (PG) in mediating sex-differences in IMA vasoconstriction to serotonin (5HT) and α_1-adrenergic agonist phenylephrine (PE).

Methods: Contractile responses of male (n=60) and female (n=50) IMA to 5HT or PE were generated in the presence or absence of an intact endothelium. Nitric oxide synthase (NOS) and cyclooxygenase (COX) inhibitors were used to evaluate the role of NO and PG in mediating the sex-dependent vasoconstriction in the presence of 5HT or PE. Electron paramagnetic resonance (EPR) was used to quantify NO release in response to the endothelium-dependent vasodilator (A23187).

Results: Female IMA's had increased sensitivity to 5HT and PE than males. (1) Endothelial denudation abolished this sex-difference for both 5HT and PE, implicating the involvement of an endothelial factor. (2) NO did not contribute to the sex-difference for either agonist since EPR-assessed NO production did not differ or NOS inhibition have no impact. (3) However, COX inhibition abolished female IMA hypersensitivity to 5HT and PE.

Conclusions: These data indicate that the female IMA hypersensitivity to the 5HT and PE are mediated via an endothelium-dependent COX pathway. Ongoing studies are investigating the potential autocoids involved. Therapies targeting this pathway may negate the sex-difference and improve outcomes amongst women undergoing CABG.

WE-076

The Effects of Sildenafil, phosphodiesterase 5 inhibitor, on the Expression of α and β Myosin Heavy Chains in Hypoxia induced Right Ventricular Hypertrophy in Mice

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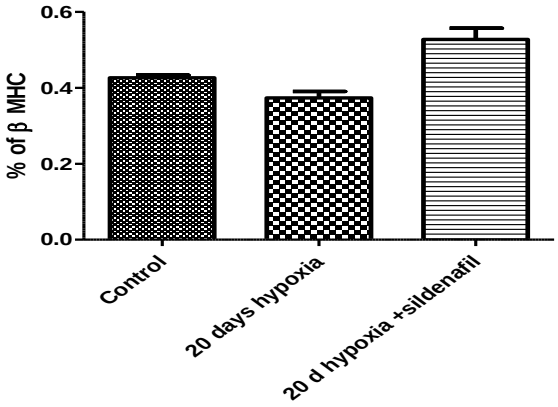
Introduction: Myosin heavy chains are known to be the main contractile protein in muscles. It is present in the cardiac muscle in two forms alpha (α), and beta (β). The contractile properties of the cardiac muscle are determined by the types of myosin heavy chain (MHC) present in the muscle . The expression of these MHCs can be altered by many physiological and pathological conditions. In this study we investigated the effect of sildenafil on MCHs isoforms in hypoxia induced right ventricular hypertrophy in mice.

Method and results:

Right ventricular hypertrophy was induced by exposing the animals to low oxygen tension (11%) in normobaric chamber for 20 days. 32 mice were were distributed randomly into: 10 as control (C). 10 were exposed to hypoxia for 20 days without sildenafil treatment (I) and 12 were given sildenafil orally at dose of 30 mg.Kg-1.day-1 while they were exposed to hypoxia for 20 days (II). MCHs isoforms were detected using two ELISA kits containing antibodies against α and β MHCs. Compared to control group C, mice exposed to hypoxia (group I) showed a significant increase in right ventricle weight to body weight mg/g ratio, (0.89±0.13 in group C and 1.3± 0.3 in group I, P> 0.001) but significant changes

in ratio of group II, 0.91+ 0.15). Expression of β MHC isoform was significantly decreased in mice group I (P>0.001), while mice exposed to hypoxia and treated with sildenafil showed significant shift of MHC towards β isoform (P>0.000). Hypertrophied right ventricle expresses more α myosin heavy chain and this is beneficial to the heart since hearts with more α MHC have more ATPase activity and powerful and fast contraction. Conclusion: sildenafil reduced hypoxia induced right ventricular hypertrophy and caused a shift in MCH towards β form whichs makes the heart contraction more economical (i.e using less ATPase).



WE-077

Bisphenol S depresses myocardial function through an estrogen receptor-□-dependent cascade

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Bisphenol A (BPA) is an estrogenic endocrine disrupting chemical that has been linked to a variety disorders including diabetes, cancer, and cardiovascular disease. The link between BPA exposure and widespread health concerns led to its reduced use in consumer products such as food packages and baby bottles. Bisphenol S (BPS) is a common substitute for BPA even though it has similar estrogenic potential. Like BPA, BPS leaches from products and accumulates in people who use BPS containing products. Despite the similar potential to cause health problems, investigations into the cardiovascular effects of BPS exposure are limited. We treated Langendorff perfused mouse hearts with BPS (10^{-9}M), BPA (10^{-9}M), or DPN (□-estrogen receptor agonist, 10^{-9}M) for 15 min and measured the impact on myocardial contractility. In females BPS depressed left ventricular developed pressure by 15% through a reduction in systolic pressure. The effects of BPS were greater than the depressant effects of BPA (10%), but they were similar to those seen with □-estrogen receptor activation. The □-estrogen receptor antagonist PHTPP blocked the effects of BPS. Although the myocardial impact of □-estrogen receptor activation was similar in males and females (~15%), the effects of BPA and BPS were attenuated in males. BPS had no impact on cardiac myofilament activation in either males or females suggesting that its mechanism of action is through another molecular pathway. This is the first study showing that BPS rapidly and significantly depresses heart function through □-estrogen receptors. These data call into question the safety of BPS in consumer products.

WE-078

Sarcoplasmic reticulum (SR) calcium transport in atrial myocytes isolated from healthy human hearts

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Background: The current information on Ca^{2+} handling in human myocardium comes from studies using tissue from explanted hearts or patients undergoing cardiac surgery. As Ca^{2+} handling may be altered in these diseased hearts, it is possible that information from these experiments carry an inherent bias in the case of control preparations. Because data from healthy human hearts are scarce, our aim was to investigate SR-cytosol Ca^{2+} transport during a twitch in atrial cardiomyocytes isolated from healthy human.

Methods: A segment of the left atrium of donor hearts, resected during orthotopic transplantation, was used for cardiomyocyte isolation with collagenase. Cytosolic free Ca^{2+} concentration ($[Ca^{2+}]_i$) was measured with indo-1 AM, in cells paced at 0.5 Hz at room temperature. The SR content (SR[Ca^{2+}]), the SR fractional release during a twitch (FR), and the integrated Ca^{2+} fluxes carried by SR Ca^{2+}-ATPase (SERCA) and Na^+/Ca^{2+} exchanger (NCX) were estimated from the $[Ca^{2+}]_i$ decline phase of different types of Ca^{2+} transients (Gen Physiol Biophys 31:401-8, 2012). The protocols were approved by the institutional Committee for Ethics in Research (CAAE/FCM/UNICAMP, number 32931014.0.0000.5404).

Results: In 10 cells (3 hearts), SR[Ca^{2+}] was 104.4±8.8 µmoles/liter of non-mitochondrial cell water, and FR was 0.41±0.08. The fluxes mediated by SERCA and NCX (and their respective percent contribution to cytosolic Ca^{2+} removal) were 43.06±9.62 µmol/L (86%) and 3.80±0.82 µmol/L (9%), respectively, while the remaining 5% were carried by slower transporters.

Conclusions: According to our knowledge, we here describe for the first time relevant quantitative data on Ca^{2+} handling in atrial myocytes from non-diseased human hearts, which may contribute to the establishment of reference values for comparison with those from cells isolated from diseased patients, and be helpful in the choice of the best animal models.

Acknowledgments: R&D-CEB staff, CAPES, CNPq, Brazilian Ministry of Health, FINEP, Dr. Lars S. Maier.

WE-079

Endothelial ATP-binding Cassette G1 in Mouse Endothelium Protects against Hemodynamic-induced Atherosclerosis

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Abstract

Aims—Activated vascular endothelium inflammation under persistent hyperlipidemia is the initial step of atherogenesis. ATP-binding cassette G1 (ABCG1) is a crucial factor maintaining sterol and lipid homeostasis by transporting cholesterol efflux to high-density lipoprotein. In this study, we investigated the protective effects of ABCG1 in endothelial inflammation activation during early-stage atherogenesis in mice and the underlying mechanisms.

Methods and results--Endothelial cell-specific ABCG1 transgenic (EC-ABCG1-Tg) mice were generated and cross-bred with low-density lipoprotein receptor–deficient (*Ldlr*$^{-/-}$) mice. After a 4-week Western-type diet, compared with *Ldlr*$^{-/-}$ mouse aortas, EC-ABCG1-Tg/*Ldlr*$^{-/-}$ aortas showed decreased early-stage lesions, as evidenced by decreased lesion area, lipid content, collagen deposition and macrophage infiltration. In addition, the expression of EC activation markers and inflammatory factors was decreased in EC-ABCG1-Tg/*Ldlr*$^{-/-}$ aortas. Adenoviral overexpression of ABCG1 blunted cholesterol- and TNFα-activated ECs *in vitro*. Furthermore, the lesion area in the EC-ABCG1-Tg/*Ldlr*$^{-/-}$ mouse aortic arch but not thoracic aorta was significantly reduced, which suggests a protective role of ABCG1 under atheroprone flow. *In vitro*, adenoviral overexpression of ABCG1 attenuated EC activation caused by oscillatory shear stress. In exploring the mechanisms of ABCG1 attenuating endothelial inflammatory activation, we found that ABCG1 inhibited oscillatory flow-activated nuclear factor kappa B and NLRP3 inflammasome in ECs.

Conclusions-- ABCG1 may play a protective role in early-stage atherosclerosis by reducing endothelial activation induced by oscillatory shear stress via suppressing the inflammatory response.

WE-080

High-throughput screens to discover inhibitors of leaky ryanodine receptor calcium channels

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Using fluorescence lifetime (FLT) detection of fluorescence resonance energy transfer (FRET), we have developed and validated high-throughput screening (HTS) methods to discover compounds that modulate the ryanodine receptor (RyR) Ca^{2+} (Ca) channel for therapeutic applications. Regulation of cellular Ca homeostasis is critical for skeletal and cardiac muscle

function, and RyR is a central player. In cardiomyocytes, high Ca "leak" via the cardiac isoform of RyR (RyR2), and reduced SR Ca uptake, conspire to reduce the SR Ca content and elevate diastolic $[Ca]_i$, both of which are hallmarks of heart failure (HF). RyR2s that open inappropriately during diastole contribute to both systolic and diastolic dysfunction and arrhythmias in HF. Therefore, inhibitors of the RyR2 leaky state could become highly effective drugs. Our HTS methods specifically detect binding of key RyR modulatory proteins (CaM and FKBP12.6) that have been implicated in controlling the pathological RyR2 leak. Thus, under oxidizing conditions that mimic a pathological state, a drug that restores normal CaM and/or FKBP binding may correct the leaky RyR2 state. Integration of fluorescently labeled FKBP12.6 and CaM and FRET enables translation of these tools into ultrasensitive HTS assays to assess the RyR leaky conformation. We have carried out a pilot screen of the 727-compound NIH Clinical Collections, which yielded several compounds that changed FRET by >3SD (a typical threshold used to select hits in primary HTS). Ongoing studies will show how the HTS structural readout correlates with effects on RyR function.

WE-081

Functional crosstalk of RyR2 and $InsP_3R2$ mediated SR-Ca^{2+} release in atrial cardiomyocytes

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Inositol 1,4,5-trisphosphate ($InsP_3$)-induced intracellular Ca^{2+} release (IP3ICR) has been implicated in modulatory functions of excitation-contraction coupling (ECC) in cardiac myocytes. Recently augmented inositol 1,4,5-trisphosphate receptor ($InsP_3R2$) expression and function has been linked to a variety of cardiac pathologies including cardiac arrhythmogenicity although its role in ECC in atrial and ventricular myocytes is not conclusively characterized. We aimed to elucidate local crosstalk mechanisms between $InsP_3R2$ and cardiac ryanodine receptors (RyR2s) in an $InsP_32$ TG mouse model that exhibits increased cardiac specific $InsP_3R2$ activity.

Using rapid two-dimensional Ca^{2+} spark analysis (x,y confocal images, 150 Hz), we report in this study that in cardiac cells, local Ca^{2+} release by $InsP_3R$ (Ca^{2+} puffs) directly activates RyRs to trigger elementary Ca^{2+} release events (Ca^{2+} sparks) with a 266 ms delay of onset and vice versa, but with a delay of 47 ms. Endothelin-1 (ET-1), which activates phospholipase C (PLC) and subsequent $InsP_3$ production, triggered an increase in Ca^{2+} spark frequency from 2.3 to 9.2 Ca^{2+} sparks 1000 μm^{-2} s^{-1}. Inhibition of the intracellular $InsP_3$ pathway in the presence of phenylephrine by application of the PLC inhibitor U-73122 abolished the Ca^{2+} puff occurrence and lead to a decrease of Ca^{2+} spark frequency from 5.1 to 1.8 Ca^{2+} sparks 1000 μm^{-2} s^{-1}. IP3ICR is under local control of Ca^{2+} release by RyRs open probability. In our study, this was mimicked by UV-flash photolysis of caged Ca^{2+}, promoting Ca^{2+} puffs in the presence of intracellular $InsP_3$.

These results strongly support the concept that IP3ICR can effectively modulate RyRs openings and Ca^{2+} spark probability in order to shape global Ca^{2+} transients and contractility in cardiac myocytes. We conclude that IP3ICR and highly efficient $InsP_3$ dependent SR-Ca^{2+} flux is the main mechanism of functional crosstalk between InsP3Rs and RyRs leading to increased ECC sensitivity. By using this TG mouse model which exhibits cardiac specific functional overexpression of $InsP_3Rs$ in a similar fashion to human cardiac disorders, this work provides novel perspectives for local control of Ca^{2+} signaling mechanisms in cardiac myocytes under physiological and pathophysiological conditions.

WE-082

Influence of ACE inhibitors on frailty and cardiac function in middle-aged female C57BL/6 mice

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Objective: ACE inhibitors improve exercise capacity in functionally impaired older adults without cardiovascular disease and improve physical performance in aged rodents. This suggests these drugs might attenuate frailty. We determined whether chronic treatment with ACE inhibitors attenuates frailty and whether this is accompanied by changes in cardiac function in middle-aged mice.

Methods: One-year old female C57BL/6 mice were treated with either an ACE inhibitor (enalapril; 40 mg/kg/day; n=5) or

placebo (n=5) for ≈3 months. Frailty was quantified with a frailty index (FI) as accumulation of clinically apparent health deficits. Blood pressure (BP) was measured with a tail-cuff; *in vivo* cardiac function was measured using echocardiography. Contractile function and calcium homeostasis (fura-2) were measured simultaneously in field-stimulated cardiomyocytes (2 Hz).

Results: Results showed that FI scores were higher in placebo mice when compared to enalapril-treated mice after ≈3 months of treatment (0.21 ± 0.03 vs. 0.14 ± 0.01, $p<0.05$). BP was not significantly different between the drug and placebo groups. Echocardiography showed no changes in *in vivo* heart structure or systolic and diastolic contractile function with enalapril treatment. Heart rate was unaffected by drug. Cardiomyocytes obtained from enalapril-treated mice showed increased cell shortening (1.59 ± 0.22 vs 3.01 ± 0.47 % resting cell length, $p<0.001$), increased velocity to peak contraction (0.068 ± 0.005 vs 0.133 ± 0.016 µm/ms, $p<0.001$) and increased velocity to ½ relaxation (0.044 ± 0.005 vs 0.100 ± 0.016 µm/ms). No significant changes occurred in underlying calcium transients.

Conclusion: These results suggest that ACE inhibitor treatment may enhance cellular contractile function independent of effects on calcium homeostasis. Furthermore, ACE inhibitors attenuate frailty in middle-aged animals, even in the absence of cardiovascular disease.

WE-083

Chronic testosterone withdrawal modifies cardiac contraction and calcium homeostasis in ventricular myocytes isolated from gonadectomised C57BL/6 male mice

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Objective: The influence of testosterone on cardiac function is not well understood. This study determined the impact of chronic testosterone withdrawal on cardiac contractile function and calcium homeostasis.

Methods: Male C57BL/6 mice had either a bilateral gonadectomy (GDX) or a sham operation at 4 weeks of age. Ventricular myocytes were isolated (age 7-11 mos) by enzymatic digestion and cells were used for field-stimulation, current clamp, and voltage clamp studies (2 Hz; 37°C). Western blot experiments used protein from ventricular homogenates. Contractions and calcium transients (fura-2) were measured simultaneously.

Results: Peak calcium transients and contractions were similar in myocytes from GDX and sham-operated controls, although calcium transients (44±2.3 vs 54±2.7 ms, $P<0.05$) and contractions (28±1.5 vs 39±3.1 ms, $P<0.05$) were prolonged by GDX. Action potential duration also was prolonged in GDX myocytes compared to sham controls (56±3.0 vs 74±4.6 ms, $P<0.05$) although resting membrane potentials were not different. When the duration of depolarization was controlled with voltage clamp, GDX suppressed peak contractions and calcium transients, with no difference in E-C coupling gain. Calcium currents from GDX myocytes had a smaller peak (5.9±0.4 vs 4.5±0.4 pA/pF, $P<0.05$), prolonged decay (13±0.8 vs 17±1.6 ms, $P<0.05$), with no difference in current density compared to sham. Sarcoplasmic calcium content (10 mM caffeine) was attenuated by GDX, while fractional release was unaffected. Western blots of key calcium handling proteins (Cav1.2, NCX, SERCA, RYR) showed no change in expression in sham vs GDX hearts. By contrast, calcium sparks in fluo-4 loaded myocytes were smaller (0.381 vs 0.373 F/F_0, $P<0.05$), less frequent (7.9±0.9 vs 5.8±0.9 /100µm/sec, $P<0.05$), and decayed more slowly in GDX myocytes (20±0.6 vs 22±1.6 ms, $P<0.05$) when compared to sham.

Conclusion: Low testosterone levels disrupt calcium homeostasis and prolong cardiac relaxation. This may promote diastolic dysfunction in older men with very low testosterone levels.

WE-084

Force-frequency relationship in rat ventricular myocytes; elucidating the intracellular mechanisms.

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The force–frequency relationship (FFR) is an important intrinsic regulatory mechanism of cardiac contractility. While an increase in contractile force after elevation of the stimulation frequency (positive FFR) is

elicited in ventricular myocytes of most mammalian species, a decrease (negative FFR) or no effect (flat FFR) in contractile force in response to an elevation of the stimulation frequency is also present in some species or pathological situations, including rat and in human heart failure. It is known that reactive oxygen species (ROS) can act as intracellular signaling molecules activating diverse kinases as CaMKII and p38 MAPK. In addition, it was demonstrated that p38 MAPK activation induces a negative inotropic effect in ventricular myocytes mediated by a decrease in myofilament response to Ca^{2+}. The involvement of ROS and p38 MAPK activation during the FFR, however, has not been studied yet. Therefore, our aim was to evaluate the FFR in rat ventricular myocytes and elucidate the intracellular molecules implicated in such process. Cell shortening was recorded with an edge detector in isolated cardiac ventricular myocytes of Wistar rats. The stimulation frequency was set to 0.5, 1 or 2 Hz. In parallel experiments, Ca^{2+} transient and pH_i were also recorded by epifluorescence. Data are shown as percentage change at 2 Hz vs 0.5 Hz. * indicates $p<0.05$ vs Control. Increasing frequency from 0.5 to 2 Hz decreased Control cell shortening (-15.41±4,02 %; n=20). This negative FFR was changed to positive FFR when the myocytes were pre-incubated with the ROS scavenger MPG 2 mM (27,87±4,60 %; n=11*), the NADPH oxidase blocker, Apocynin 300 µM (16,48±3,20 %; n=10*) or inhibiting mitochondrial ROS production with 5-hydroxidecanoate (5-HD) 500 µM (30,31±5,81 %; n=6*). Similar results were obtained when the cells were pre-incubated with the CaMKII blocker, KN93 2.5 µM or the p38 MAPK inhibitor, SB-203580 10µM (23,01±6,28 %; n=7*, 37,13±7,62 %; n=7*; respectively). Ca^{2+} transients or pH_i did not significantly change in Control or after ROS production inhibition. In conclusion, our results indicate that the activation of the intracellular pathway involving ROS-CaMKII-p38 MAPK is responsible for the negative FFR of rat cardiomyocytes, likely by desensitizing the response of contractile myofilaments to Ca^{2+}.

WE-085

RyR2 haploinsufficiency in a rabbit model is compensated by fine-tuning channel activity

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Several reports suggest that RyR2 expression is decreased in Heart Failure and Hypertrophic Cardiomyopathy, but the contribution of RyR2 downregulation to the pathology of the disease is unknown. Using CRISPR/Cas9 technology, we generated a RyR2 knockout rabbit model to determine the cardiac effects of RyR2 deficiency. Mating of heterozygous knock-out rabbits does not yield homozygotes, highlighting RyR2 relevance for development (105 kits, $p<0.001$). Heterozygous hearts show haploinsufficiency, with 33.8±6.1% of RyR2 expression in the left ventricle (LV) and 54.2±19.5% in the atria (n=5 per genotype, $p<0.05$). Remarkably, heterozygous animals subjected to echocardiography (n=7 per genotype) and electrocardiography (n=5-9 per genotype) are not different to wild-type littermates. Additionally, haematoxylin/eosin and Masson's trichrome staining of LV and atrial biopsies showed no difference in the general microscopic structure between genotypes (n=3 per genotype). To determine the mechanism that prevents an abnormal phenotype in heterozygous knock-out hearts, we looked at the expression of excitation-contraction proteins. The expression of SERCA2a, NCX, Cav1.2 and phospholamban (n=3-5 per genotype) in heterozygous rabbits was comparable to that observed in wild-type animals. Using [^{3}H]ryanodine binding assays, we tested the sensitivity of RyR2 to increasing [Ca], between 10 nM and 100 µM. Wild type and heterozygous LV samples showed the same Ca-dependent activation (EC_{50} 1.15±0.09 and 1.03±0.05 µM, respectively; n=5 per genotype). However, the maximum [^{3}H]ryanodine binding normalized to RyR2 density was 1.91-fold higher in heterozygous samples, suggesting that remaining RyR2 channels are more active. Finally, the phosphorylation of RyR2-S2808 and RyR2-S2814 was not different between genotypes, but RyR2-S2031 was significantly more dephosphorylated in the heterozygous LV (n=3 per genotype, $p<0.05$). These data suggest that a large RyR2 protein reserve sustains normal cardiac function, at least under basal (non-stimulated) conditions. Moreover, RyR2 activity can be fine-tuned through phosphorylation to compensate for protein deficiency.

WE-086

Thyroid Stimulating Hormone can directly modulates the cardiac electrical activity

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Background: The electrocardiogram of hypothyroid patients shows a series of abnormalities of cardiac repolarization due to a reduction of some repolarizing K+ currents and an increase of the L-type calcium current. Experimental and clinical works called into question the unique role of T3 and T4 in these mechanisms and correlated serum TSH levels with the repolarization abnormalities in patients with subclinical and overt hypothyroidism. The aim of the present study was to investigate the direct effects of TSH upon cardiac electrical properties.

Methods: We studied the direct acute (30 min) and long term (24 h) effects of the activation of TSH receptor by the TSH (30mUI/ml) on adult rat cardiac preparations. We first studied acute effects in electrocardiograms records of Langendorff perfused hearts and also, using patch clamp technique, we recorded Ito current in ventricular myocyte. The long term effects were studied in action potentials of epicardial strips and the transient outward K+ current, Ito, and the L-type Ca2+ current, ICa-L, in ventricular myocytes are recorded too. Finally, Kv and Cav channels subunits mRNA expression was determined by qRTPCR.

Results: TSH has no acute effects on cardiac electrical activity. However, prolonged exposition to TSH increased the action potential duration through a reduction of the amplitude of Ito current in adult ventricular myocytes due to a reduction in Kv4.2, Kv4.3 and KChIP2 mRNA expression. Interestingly, TSH had no effect on either ICa-L current or Cav1.2 mRNA expression.

Conclusion: These results support the idea that some of the electrical abnormalities seen in hypothyroid hearts, such as increase in ICa-L, are due to the reduction of T3 levels, and introduce the possibility that others, such as TSH elevation, could also be involved in this cardiac electrical disturbances.

WE-087

miR-19b deficiency impairs cardiac repolarization in zebrafish

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The most fatal complication of heart failure (HF) is sudden cardiac death which results mostly from impaired electrical activity of the heart and arrhythmias. During HF electrical remodeling includes the prolongation of the ventricular action potential duration (APD) that may be interpreted as an acquired long-QT syndrome. Molecular mechanisms contributing to the action potential (AP) perturbation are still inadequately understood. microRNAs are small noncoding RNAs that post-transcriptionally fine-tune gene expression by translational repression or transcript destabilization. By now, several microRNAs are known to be dysregulated during HF, suggesting a potential involvement in the development and progression of the disease. Here, we identified miR-19 to be an important regulator of heart function. Zebrafish lacking miR-19 developed severe bradycardia and reduced cardiac contractility. While the mammalian genome encodes for two isoforms of miR-19, zebrafish express four members (19a-d). We found that the reduction of miR-19b specifically deploying morpholino mediated knockdown and CRISPR/Cas9 induced knockout is sufficient to cause bradycardia and reduced cardiac contractility. Moreover, miR-19b deficiency results in increased sensitivity to an AV-Block, which is a characteristic feature of long QT-Syndrome in zebrafish. Recordings of ventricular APs from paced hearts demonstrated that APD is significantly prolonged and repolarization is impaired in miR-19b deficient zebrafish. Strikingly, by reduction of miR-19b we were able to normalize the arrhythmogenic phenotype of a short QT-mutant zebrafish.

Mechanistically, miR-19b regulates the expression and thereby modulates the function of several cardiac ion channels crucially involved in shaping the AP. In conclusion, we identified miR-19b as a novel and essential modulator of the electrical activity of the heart and establish miR-19b as a potential candidate gene causative for human long QT.

WE-088

Early intravenous low/high doses of Metoprolol in myocardial infarction dogs on the effects of cardiac sympathetic activities and electrophysiological properties

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Objective :

Observed effects of early intravenous low/high doses of Metoprolol in myocardial infarction dogs on cardiac sympathetic activities and electrophysiological properties

Methods :

32 dogs were randomly divided into three groups. After establishing the MI model, the low-dose group was given metoprolol 0.6mg / kg iv, the high-dose group was given 1.6mg / kg, while the control group was injected with normal saline. Catecholamine levels in the coronary sinus blood, ERP and the incidence of VA were all measured.

Results :

1. NE and E were all increased compared with the values before ligation; Changes in the control group was the biggest; The low-dose and high-dose group performs no significant differences ($p > 0.05$);
2. ERP after MI was significantly shorter in all groups compared with the first measurement; The low and high dose group shortened approximately, there were no statistically differences; All exhibited uneven shortness of ERP among different regions, infarcted area was significantly shortened ($p<0.05$);
3. In control group there was 4 dogs induced PVT/VF, the low-dose group had 5, the high-dose group had 4. There was no significant difference among all groups ($p > 0.05$);

Conclusion :

Low and high dose of metoprolol performed similarly in reducing the catecholamine concentrations in dogs with anterior myocardial infarction, the same effects also observed in the reduction of regional ERP, but there were no differences in induced arrhythmias.

WE-089

Inhibition of small Conductance Ca^{2+}-activated K^+-Channels converts and prevents Reinduction of atrial Fibrillation in Pigs where Vernakalant fails

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Introduction:

Evidence has emerged that small conductance Ca^{2+}-activated K^+-channels (SK-channels) constitute a promising new atrial-selective target for treatment of atrial fibrillation (AF). Current antiarrhythmic therapy suffers from ventricular adverse effects and becomes less effective as the disease progresses. We therefore tested the antiarrhythmic properties of a new SK channel inhibitor in a porcine model of AF in a setting of remodelled atria that completely abolished efficacy of the marketed antiarrhythmic drug vernakalant.

Methods:

Eight pigs were subjected to atrial tachypacing (AT-P) until they developed sustained AF that could not be converted by vernakalant (4 mg/kg infusion over 10 minutes). In these pigs the efficacy of a new SK channel inhibitor, AP14145, was investigated.

The effects of AP14145 and vernakalant (constant rate infusion producing a clinically relevant plasma concentration of ~4000 ng/ml) on the effective refractory periods (ERP) in the atria and ventricles and the effects on acute burst pacing-induced AF were examined in open-chest experiments in anaesthetized pigs subjected to 7 days AT-P as well as sham operated control pigs.

In both sets of experiments AP14145 was given as bolus injections of 5 mg/kg, 8 mg/kg, and 8 mg/kg with 30 minutes intervals.

Results:

The time for the development of vernakalant-resistant AF was 17.6±5.2 days of A-TP. In 8/8 pigs, AP14145 converted vernakalant-resistant AF to sinus rhythm. 4 pigs converted after the low dose, 3 pigs after the middle dose and 1 pig after the maximal dose. Reinduction attempts (3xburst pacing) failed in all pigs after conversion with AP14145.

In open-chest experiments, vernakalant and AP14145 significantly prolonged atrial ERP by 68±31ms and 107±10ms, respectively in the AT-P pigs and by 49±32 ms and 100±19ms in the control pigs and significantly reduced AF-duration without affecting the ventricular ERP or blood pressure in pigs subjected to 7 days AT-P and control pigs.

Conclusion: SK current inhibition was effective even after some remodeling when vernakalant was no longer effective. This implies that SK inhibition may have advantages over current treatments and is therefore a promising concept for further development for treatment of AF.

WE-090

Comparing R_2CHADS_2 and $CHADS_2VASC$ Scores in Stroke Patients With Non-Valvular Atrial Fibrillation and renal failure.

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Introduction

Atrial fibrillation (AF) is the most common rhythm disorder in hospitalized patients. CHA_2DS_2-VASc and R_2CHADS_2 are the stroke risk assessment tool scores for patients with atrial fibrillation (2). Even though renal failure is independently associated with stroke (1), it has not been included in the $CHADS_2$-VASc risk stratification system, which is used for anticoagulation recommendation in non-valvular AF patients as endorsed by ACC/AHA. Our study retrospectively compared R_2CHADS_2 to CHA_2DS_2-VASc scores in stroke patients with a past medical history of non-valvular AF to assess differences in predicting stroke in patients with renal failure.

Methods

171 patients admitted over two years from one hospital with a diagnosis of atrial fibrillation and strokes were reviewed. Data variables included: age, medical record number, sex, race, renal function and any previously documented CHA_2DS_2-VASc scores. If the CHA_2DS_2-VASc and R_2CHADS_2 scores were not documented, they were calculated based on information within the medical record. GFR was calculated using the Chronic Kidney Disease Epidemiology Collaboration formula

Results

The median CHA_2DS_2-VASc score was 6 (range 2-9) and the median $R_2CHA_2DS_2$ score was 4 (range 2-8). The average GFR was 69.77 (range 6-108). A weak, but significant, correlation was found between renal function and CHA_2DS_2-VASc score ($r = -0.263$; $p = 0.0005$). A stronger and significant correlation was revealed between the R_2CHADS_2 and GFR ($r = -0.70$; $p < 0.00001$). CHA_2DS_2-VASc and R_2CHADS_2 scores also were significantly correlated ($r = 0.627$; $p < 0.00001$).

Discussion

The risk of stroke in patients with impaired renal function is high. Although CHA_2DS_2VASc and R_2CHADS_2 are significantly correlated to each other, using R_2CHADS_2 would be beneficial to assess stroke risk in patients with decreased renal function and non-valvular atrial fibrillation.

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WE-091

Characterization of Catecholaminergic Polymorphic Ventricular Tachycardia Using Patient-Specific Human Induced Pluripotent Stem Cells and a Transgenic Mouse Model Harboring the Mutation H2464D in the Cardiac Ryanodine Receptor.

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CPVT is a disease characterized by adrenergic-induced ventricular tachycardia caused by mutations in the cardiac Ryanodine Receptor (RyR2). We aimed to perform a characterization of the mechanisms of CPVT using recombinant RyR2 channels, human induced pluripotent stem cell-derived cardiomyocytes (hIPS-CMs) and a transgenic mouse model, all harboring the mutation RyR2-H2464D (HD), to determine mechanisms of arrhythmia and assess patient-specific therapeutic drugs. **Methods:** hiPSCs generated from human fibroblasts carrying the HD mutation (HD1 and HD2) and from his mother as a healthy control (HC) and a non-relative control (HNRC). HD mice were generated by homologous recombination. Ca^{2+}-dependence of [^{3}H]ryanodine binding served as an index of the activity of rRyR2. hiPS-CM single cells loaded with fluo-4AM were used to measure intracellular Ca^{2+} transients. Optical mapping measured Ca^{2+} handling in purified fluo-4AM loaded hiPSC-CM monolayers. Echocardiograms and surface ECGs in anesthetized mice evaluated the condition of the hearts. **Results:** HD mutation increases cytosolic Ca^{2+} sensitivity with respect to control (EC_{50}: HD-0.73±0.1 vs.WT-0.15±0.03 µM Ca^{2+}). Cytosolic [Ca^{2+}] (0.1 µM) increased the activity of HD higher than WT (P_o <0.01 WT and 0.236 HD). CaT amplitude (1.5±0.2 vs.2.2±0.4 F/F_0, n=4 and 5) and time-to-peak (101±44 vs. 181±40ms) were decreased in HD with respect to control and τ Increased in HD (420±24 vs. 287±26 ms,p<0.05). The incidence of monolayers with abnormal Ca^{2+} release after isoproterenol treatment was HD1 25%, HD2 45%, HC 0% and HNRC 0%. Heart rate and fractional shortening were not significantly different between WT and $HD^{+/-}$. Only $HD^{+/-}$ mice developed PVCs, bigeminy and bidirectional ventricular tachycardia after Epi/Caff challenge (n=6 for all groups). **Conclusions:** HD confers a gain of function to RyR2 channels; Ca^{2+} handling abnormalities in HD monolayers and arrhythmias in mice are due to higher Ca^{2+} sensitivity of RyR2 channels that is linked with the phenotype of the disease.

WE-092

Refractoriness in human atria: Time and voltage dependence of sodium channel availability

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Background: Refractoriness of cardiac cells limits maximum frequency of electrical activity and protects the heart from tonic contractions. Short refractory periods comprise excellent arrhythmogenic substrates and augmentation of refractoriness is therefore seen as the main mechanism of antiarrhythmic drugs. Excitability of cardiomyocytes depends on availability of sodium channels, a function involving both time- and voltage-dependent release from inactivation. The aim of this study was to characterize how sodium channel inactivation affects refractoriness and thereby human atrial electrophysiology.

Methods and Results: Steady-state activation and inactivation parameters of sodium channels measured *in vitro* in isolated human atrial cardiomyocytes were used to parameterise a new mathematical human atrial cell model. Action potential data were acquired from human atrial trabeculae muscle strips of patients in either sinus rhythm or chronic atrial fibrillation. The *ex vivo* measurements of action potential duration, effective refractory period and resting membrane potential were well-replicated in simulations using this new *in silico* model. Interestingly, the voltage threshold potential at which refractoriness was observed was not different between sinus rhythm and chronic atrial fibrillation tissues and was neither affected by changes in frequencies (1 vs. 3 Hz) nor by variations in action potential duration.

Conclusions: Our results suggest a preferentially voltage-dependent, rather than a time-dependent, effect with respect to refractoriness at physiologically relevant rates in healthy human atria. Hence, as the

resting membrane potential is hyperpolarized in chronic atrial fibrillation, this voltage-dependent dominance of excitability will profoundly increase the risk of re-initiating and maintaining arrhythmia in fibrillating atria.

WE-093

Carvedilol and its non-ß-blocking analog VK-II-86 prevent digitalis-induced Ca^{++} waves in cardiac myocytes.

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Background:

Cardiotonic glycosides inhibit the sarcolemmal Na^+/K^+-ATPase and cause an increase in intracellular Na^+, which reduces Ca^{++} extrusion through the Na^+/Ca^{++} exchanger. The result is an increase in sarcoplasmic reticulum (SR) Ca^{++} load and cardiac contractility. However, these compounds have associated arrhythmic effects due to the occurrence of spontaneous SR Ca^{++} waves as a result of SR Ca^{++} overload and CaMKII-dependent phosphorylation of RyR2.

Taking into account that Carvedilol and its non-β-blocking analog VK-II-86 are able to prevent spontaneous SR Ca^{++} waves, we hypothesize that Carvedilol and VK-II-86 would be able to prevent digitalis-induced SR Ca^{++} waves/spontaneous contractile activity without affecting inotropic response.

Methods and results: In rat cardiac myocytes, paced at 0.5 Hz and perfused in the presence of 50 µM Ouabain for 20 minutes, we observed an increase in cell shortening of 60±5% (n=15). We also observed spontaneous contractile activity as an index of SR Ca^{++} waves after stopping electrical stimulation. On average, Ouabain-treated cells presents a significantly higher number of non-stimulated events (NSE) compared with control cells (69±10 NSE/10min vs 11±4 NSE/10 min respectively)

In similar experiments performed in the presence of 1 µM Carvedilol, the frequency of NSE was significantly reduced to 24±4 events/10min (n=13). To confirm that the effect of Carvedilol was dependent on its capacity to reduce RyR2-mediated spontaneous Ca^{++} release instead of its β-blocking effect, we used Atenolol (a β-blocker without effects on RyR2 function) and VK-II-86. The presence of Atenolol did not significantly alter the frequency of NSE promoted by Ouabain. In contrast, VK-II-86 significantly reduced the frequency of NSE promoted by Ouabain (39±9 events/10min; n=14).

Additionally, VK-II-86 did not affect the development of the positive inotropic response and the increase in SR Ca^{++} load induced by Ouabain treatment.

Conclusions: We conclude that the combination of cardiac glycosides with VK-II-86 would improve cardiac contractility without increasing the risk of triggered-arrhythmias.

WE-094

Internal Pacemaker Cell Mechanisms Mediating Autonomic Nervous Regulation of the Heart Rate

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Introduction: Sinoatrial nodals pacemaker cells (SANCs) generate regular and spontaneous action potentials (APs) that control the rate of cardiac contraction in mammalians. The brain modulates SANC automaticity, via the autonomic-nervous system, by stimulating membrane receptors that activate (adrenergic) or inactivate (cholinergic) adenylyl cyclase (AC). However, there is a limited understanding of the underlying ionic and molecular mechanisms involved in the cross talk between these membrane receptors and the internal intrinsic mechanisms of SANCs. We hypothesize that AC-cAMP-PKA signaling is the major messenger between the autonomic-nervous system modulation to SANC function.

Methods: We modified an SANC computational model to include autonomic receptors stimulation and its resulting modulation of the level of intracellular AC-cAMP-PKA. We test the SANC function response to adrenergic receptor stimulation (by isoproterenol, ISO) or cholinergic stimulation (by carbachol, CCh). In addition, we perform new experiments on spontaneously beating SANC to assess the role of PKA on AP firing rate modulation in response to ISO and CCh.

Results: Similar to the experimental results, the model simulations showed a reduction of 26% in AP firing rate in response to CCh (100 nM) and an

increase of 22% in response to ISO (100 nM) with respect to the basal rate. Eliminating AC-cAMP-PKA signaling abolished the core effect of autonomic-receptor stimulation on the AP firing rate. Specifically, disabling the phospholamban modulation of the SERCA activity resulted in a significant reduced effect of CCh and a failure to increase the AP firing rate under ISO stimulation. The experiments on live SANC demonstrated the association between PKA activity and the AP firing rate.

Summary: We provide both experimental and theoretical evidences, that the autonomic nervous system mainly regulates SANC function via AC-cAMP-PKA signaling. The model predicts that the activation of the SERCA pump via phospholamban phosphorylation is a critical player within this regulatory process.

WE-095

An implanted dual-site pacing device mimics pacing-induced dyssynchrony and cardiac resynchronization therapy in freely moving rats

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Background: Patients with heart failure often exhibit electrical conduction disturbances leading to electromechanical dyssynchrony and poor outcome. Right ventricular (RV) pacing can also induce dyssynchrony and worsen outcome in a similar manner. Cardiac resynchronization therapy (CRT), in the form of biventricular (BIV) pacing, is a potent modality to treat dyssynchrony. However, critical issues such as a ~ 30% failure of CRT treatment mandate extensive additional research. Animal models currently relay on large mammals, which are expensive and not readily available. Our group developed a simple methodology for dual-site epicardial pacing in conscious freely moving rats. We previously demonstrated remarkable similarities to large mammalian findings by applying speckle-tracking echocardiography during different pacing modes.

Aims: (1) To precisely characterize the hemodynamic effects of ventricular pacing in the rat model. (2) To determine the electrophysiological and biochemical effects of RV vs. BIV tachypacing in conscious freely moving rats.

Methods and results: Two bipolar electrodes were implanted in the RV and LV of adult SD rats. Electrodes were exteriorized through the back. Following post-operative recovery, pressure-volume loop recordings were performed during pacing and ventricular function was evaluated. BIV pacing acutely enhanced systolic function compared with RV or LV pacing. As a single site, however, LV pacing improved systolic function considerably and similarly to BIV pacing. Three days of RV tachypacing (n=6), but not BIV tachypacing (n=6), induced dispersion of ventricular refractoriness between the RV and LV by 10.0±3.8 ms and prolonged the QT interval by 6.63±3.1 ms relative to control ($p<0.05$ for both). Biochemically, RV tachypacing increased p-JNK levels in the early-activated septum of the LV relative to the late-activated free wall.

Conclusions: This model mimics important electromechanical features seen in large mammalian hearts, and is therefore an attractive new tool to study the complex pathophysiology of ventricular dyssynchrony and CRT.

WE-096

Human Calmodulin Mutation associated with Idiopathic Ventricular Fibrillation causes CaMKII-dependent RyR2 Activation

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Background: Calmodulin (CaM) mutations have been associated with an autosomal dominant syndrome of sudden death that can present with clinical features of catecholaminergic polymorphic ventricular tachycardia (CPVT) or long QT syndrome (LQTS). CPVT-linked CaM mutations activate ryanodine receptor (RyR2) Ca release channels; whereas LQTS-CaMs have no effects on RyR2 channels but prolong the action potential by impairing L-type Ca current (LTCC) inactivation. A novel CaM mutation in CALM1 gene (F90L) was recently found in a family with Idiopathic Ventricular Fibrillation (IVF) but the mechanism by which this CaM mutation leads to IVF is not known. Here, we studied the regulation of RyR2 by F90L-CaM.

Methods and Results: Recombinant wild-type (WT) and mutant CaMs (F90L and N54I) were bacterially expressed and purified. Ca waves and sparks analyses were done in permeabilized murine cardiomyocytes incubated with WT or mutant CaMs using confocal microscopy. F90L-CaM increased Ca wave and spark frequency analogous to N54I, a previously reported CPVT-CaM mutant. However, single RyR2 channel measurements in lipid bilayers showed that unlike N54I-CaM, F90L-CaM does not activate RyR2 in a direct fashion. CaMKII inhibition using 1 µM AIP completely abolished the effect of F90L-CaM on Ca waves and sparks but did not prevent RyR2 activation by N54I-CaM. Accordingly, ablation of CaMKII phosphorylation site in RyR2 (S2814A) or introduction of a modification in RyR2 that mimics phosphorylated RyR2 (S2814D) also abolished F90L-CaM effect, confirming that CaMKII phosphorylation of RyR2 is needed for F90L effects. CaMKII activation by F90L-CaM was also confirmed by immunoblot. Western blots revealed that F90L-CaM, but not N54I-CaM, induced an increase in both CaMKII-Thr286 and RyR2-Ser2814 phosphorylation levels compared to WT-CaM.

Conclusion: In contrast to previously reported CPVT CaM mutants, the novel F90L-CaM evokes arrhythmogenic Ca disturbances by indirect activation of RyR2 via CaMKII, which may be a molecular mechanism underlying IVF.

WE-097

Prevailing action potential duration determines the electrical restitution curve

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Background: The dynamics of rate-dependent adaptation of action potential duration (APD), termed electrical restitution, are thought to be an important determinant of ventricular arrhythmia. APD prolongation is a hallmark of disease, such as heart failure; however, there has been little recognition of the apparent association between restitution kinetics and APD. Abnormal QT prolongation is associated with an increased risk of ventricular arrhythmia.

Objective- To test the hypothesis that restitution kinetics are determined by APD.

Methods and Results: Experiments were conducted in isolated hearts and ventricular myocytes from adult guinea pigs. Restitution curves were measured in control and following interventions that prolong (clofilium, veratradine, low $[Ca^{2+}]_e$) and shorten (catecholamines, rapid pacing) APD. Despite markedly different mechanisms of action, prolongation of the ventricular action potential was associated with a steepening of electrical restitution with all interventions (Figure 1A). By comparison, the slope of the restitution curve was reduced when APD was shortened. Isolated myocytes from animals with chronic transverse aortic constriction (TAC), a model of hypertrophy and heart failure, demonstrated prolongation of APD and augmented restitution kinetics (TAC vs. sham, Figure 1B). This phenotype was revered by application of a small outward current sufficient to normalise APD to sham values (TAC-INJ). The intrinsic geometrical relationship between the rate of repolarization and APD is shown to underpin these common effects, rather than specific effects on ion channel conductances.

Conclusions: APD is a major determinant of electrical restitution. Any factor that prolongs the action potential, irrespective of the underlying mechanism, will increase the slope of the restitution curve. This finding has implications for understanding of basic mechanisms of arrhythmia in conditions associated with QT prolongation.

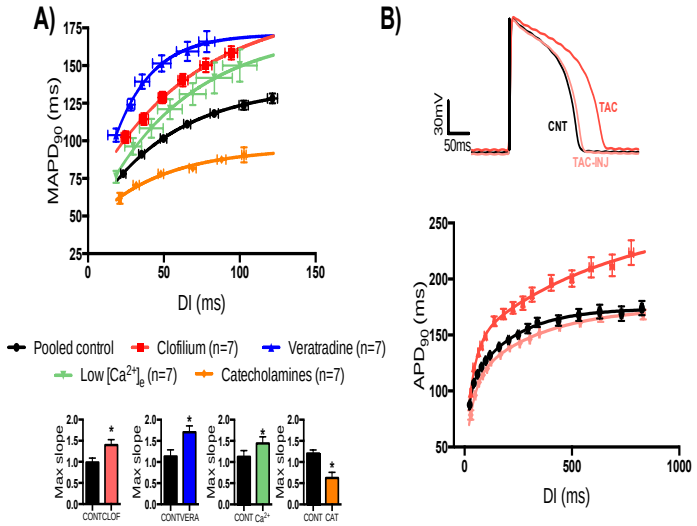

Figure 1. The influence of action potential duration on the electrical restitution curve. Data represent mean±SEM. Different form control; *P<0.05.

WE-098

Effective treatment of atrial fibrillation in isolated guinea pig hearts by combining established anti-arrhythmics and small conductance Ca^{2+} activated (SK) K^+ channel block

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Introduction: Atrial fibrillation (AF) is the most common sustained tachyarrhythmia. It is associated with increased morbidity and mortality and there is an unmet need in the current pharmacological treatment of AF due to low efficacy and severe side effects. The small conductance Ca^{2+} activated K^+ (SK) inhibition have been reported to exhibit anti AF effect ex vivo and in vivo. In recent years focus has been increasing on combining different anti-arrhythmia for synergistic antiarrhythmic effect.

Hypothesis: We hypothesized that the combination of SK channel block and established antiarrhythmics in sub-efficacious concentrations may prevent AF and have the possibility for a reduced proarrhythmic potential.

Method: Guinea pig hearts were placed in the Langendorff perfused model. AF was induced by addition of acetylcholine and burst pacing of the right atrium. Sub-efficacious concentrations of the SK channel blocker were tested alone and in combination with sub-efficacious concentrations of flecainide, ranolazine, amiodarone or dofetilide.

Results: The combination of SK blocker and flecainide, ranolazine, amiodarone or dofetilide reduced AF durations compared to the compounds as monotherapy. In higher concentrations at which monotherapy of flecainide and dofetilide cardiovert AF, a significant increase in QT interval was observed. This was not observed in combination therapy with SK channel blocker as the effective dose of the compounds could be reduced 3-fold.

Conclusion: Our data suggest that combination of SK blocker and flecainide, ranolazine, amiodarone or dofetilide at reduced doses may be an effective and safer way to treat atrial fibrillation.

WE-099

Unnatural Amino Acid Photo-Crosslinking of the I_{Ks} Channel Complex Demonstrates a KCNE1:KCNQ1 Stoichiometry of up to 4:4

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Background: The slow delayed rectifier current (I_{Ks}) provides repolarizing potassium current during the cardiac action potential. It is composed of KCNQ1, which forms the tetrameric voltage-gated ion channel, and KCNE1, a single transmembrane domain β-subunit. KCNE1 resides in the channels' exterior clefts and dramatically delays opening. While this channel complex was characterized almost 20 years ago, the stoichiometry between the α and β-subunits remains controversial. Several studies have reported either a strict ratio of 2 KCNE1: 4 KCNQ1 or a variable ratio up to 4:4. Here, we sought to clarify this issue using I_{Ks} fusion proteins, where KCNE1 was linked to one KCNQ1 (EQ) or two KCNQ1 subunits (EQQ), which produce channels with compulsory 4:4 or 2:4 stoichiometries, respectively. **Results and Conclusions:** Whole cell and single channel characterization of EQQ in mammalian cells demonstrated a hyperpolarized $V_{1/2}$ of activation, reduced conductance and shorter first latency of opening compared to EQ or wild type I_{Ks}. All of these differences were abolished by co-expression of EQQ with KCNE1-GFP. To confirm that these additional subunits can be integrated into the complex, the UV-crosslinking unnatural amino acid, p-benzoyl-L-phenylalanine (Bpa) was genetically incorporated into KCNE1-GFP at residue F57 using the amber stop codon (TAG) suppression system. Application of UV light to KCNQ1 + F75Bpa KCNE1-GFP complexes held at -90 mV, trapped channels in the closed state. The same UV-treatment of F57Bpa KCNE1 with EQQ was found to crosslink at half the rate of KCNQ1, which shows the association of the independent KCNE1 subunits into the unoccupied clefts in the EQQ channel complex. These findings differentiate the functionality of 2:4 KCNE1:KCNQ1 from a wild type channel complex and demonstrate that there is no intrinsic mechanism limiting the association of additional β-subunits up to four, confirming a variable stoichiometry model for I_{Ks}.

WE-100

Role of the NBCn1 Na^+/HCO_3^- Co-transporter in Mitochondria of Hypertrophic Hearts

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NBC Na^+/HCO_3^- cotransporter and NHE1 Na^+/H^+ exchanger have been associated with cardiac disorders and recently located in mitochondria of cardiomyocytes and coronary endothelial cells (CEC), respectively. Mitochondrial NHE1 (mNHE1) blockade delay the mitochondrial permeability transition pore (MPTP) opening and reduce mitochondrial-derived superoxide production, two critical events exacerbated in cells of the disease heart. Conversely, activation of the NBC isoform, NBCn1, prevented apoptosis in CEC subjected to ischemic stress. We characterize the role of these transporters in heart mitochondria of adult spontaneously hypertensive (SHR) and control (Wistar) rats. To examine the role of mNHE1 in mitochondria of SHR and Wistar rats, expression of mNHE1 in ventricular mitochondrial lysates was analyzed by immunoblots. mNHE1 expression increased by ~40% in hypertrophic SHR compared to control ($P<0.05$, n=4). To determine if the increased expression of mNHE1 in cardiac hypertrophy correlates with an increase transport activity of the exchanger, mitochondria were loaded with BCECF-AM and the maximal rate of pH_m change measured after addition of 50 mM Na^+, monitored by epifluorescence. Mitochondria of SHR showed greater changes in pH_m compared to Wistar rats, 0.10±0.01 vs. 0.06±0.01 ($P<0.05$, n=5). Additionally, mitochondrial suspensions from SHR and control myocardium were exposed to 200 □M $CaCl_2$ to induce MPTP opening (light scattering decrease, LSD) with the consequent mitochondrial swelling (MS). Surprisingly, SHR rats showed smaller LSD and a reduction in MS, 67±10% (n=26), compared to control, 100±8% (n=23). Blockade of the NBC with 1 □M S0859 significantly increased the MS in both, control 139±10% (n=7), and SHR 115±10% (n=7) mitochondria. Finally, NBCn1 Na^+/HCO_3^- cotransporter increased by ~70% its expression in SHR heart muscle mitochondria, compared to normal ($P<0.05$, n=5). Together our data suggest that the increased NBCn1 activity seem to play a compensatory role in hypertrophic hearts, protecting mitochondria from Ca^{2+}-induced MS and MPTP opening.

WE-101

Increased complex I dependent respiration and increased restriction for ADP in volume overload-induced atrial dilatation

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Background: Atrial dilatation is a typical consequence of cardiac failure caused by hemodynamic overload. The relations between structural, electrical, and contractile remodeling to oxidative phosphorylation (OXPHOS) and glycolysis are poorly understood.

Methods and results: The pieces of right atrium from 77 patients, detached in order to establish extracorporal circulation during heart surgery, were used for studies. We found that atrial dilatation was associated with impaired mitochondria as indicated by decreased rate of glutamate-dependent respiration. This decrease occurred at all functional states of mitochondria – nonphosphorylating and phosphorylating, either stimulated by excessive ADP or submaximally by endogenous ADP produced by ATPases of mitochondrial kinases. Functional coupling between the adenylate kinase system and OXPHOS was not affected in our dilatated atrium fibers, but the coupling between the kreatine kinase system and OXPHOS diminished by 17%. The significant increase of the Km^{ADP} in the absence and presence of creatine in dilatated fibers indicated that the diffusion restriction for ADP into the mitochondrial intermembrane space was due to all ADP transport pathways, including CrP shuttle. On contrary – the adenylate kinase aktivities increased and we observed also overexpression of HK2 in dilated human atria.

Conclusion: Despite inpaired complex I dependent respiration and increased diffusion restriction for ADP, no changes regarding adenylate and creatine kinase occurred. Cardiac energy dependence on glucose is enhanced in volume overload-induced atrial dilatation by functional coupling of HK2 with OXPHOS system in mitochondria.

WE-102

The effect of chronic continuous hypoxia on enzyme activities and membrane permeability of rat heart mitochondria

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Introduction: Adaptation to continuous normobaric hypoxia (CNH) increases cardiac ischemic tolerance. Cardiac mitochondria can play the role in this protection. We investigated the effects of CNH on the activity of selected mitochondrial proteins in spontaneously hypertensive rats (SHR), and in a novel conplastic strain SHR-mt^{BN}. Mitochondrial calcium retention capacity were measured exploring potential role of mitochondrial permeability transition pore (MPTP) in cardiac protection.

Methods: Rats were kept 21 days at CNH (inspired O_2 fraction 0.1). Left ventricular homogenate were used for determination of the enzyme activity of malate dehydrogenase (MDH), citrate synthase (CS), NADH-cytochrome c oxidoreductase, succinate-cytochrome c oxidoreductase and cytochrome oxidase (COX). Mitochondrial respiration were measured as oxygen consumption. Mitochondrial calcium retention capacity was determined fluorimetrically.

Results: Only MDH activity decreased in hypoxic SHR (23%). The respiratory pattern and respiratory control index were similar in mitochondria isolated from left ventricles of normoxic and hypoxic rats. Basic COX activity did not differ between the strains and was not affected by CNH. The reserve COX activity (measured with 0.02% lauryl maltoside) was significantly increased after adaptation to CNH in both strains (by 45 % in SHR and 38 % in SHR-mt^{BN}). We confirmed that the mitochondrial calcium retention capacity is lower in SHR hearts compared with control Wistar ones. However, we did not find any difference in this parameter between SHR and SHR-mt^{BN} strains.

Conclusions: Adaptation to CNH affected myocardial activity of some mitochondrial proteins in hypertensive strains, and increased the reserve COX activity in the left ventricle of SHR independently of mitochondrial genome. Heart mitochondria of SHR strain is more sensitive to calcium concentration than the control Wistar ones, but no difference between SHR and SHR-mt^{BN} strains suggests no effect of mitochondrial genome on MPTP properties.

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WE-103

Mice lacking the mitochondrial calcium uniporter have alterations in F_1F_0-ATP synthase

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Knockout (KO) of the mitochondrial Ca^{2+} uniporter (MCU) abrogates rapid mitochondrial Ca^{2+} uptake and permeability transition pore (PTP) opening. However, hearts from global MCU-KO mice were not protected from ischemic injury (Pan *et al*,Nat Cell Biol,2013). This study investigates the hypothesis that the lack of protection in the absence of MCU may be explained by alterations in PTP opening and/or mitochondrial protein complexes. To investigate whether pore opening occurs in MCU-KO, hearts were Langendorff-perfused in the presence of a cyclophilin D-independent pore inhibitor. PTP inhibition was protective against 30-mins of ischemia in WT hearts, but less so in MCU-KO. This suggests that pore opening is not a significant contributor to ischemic injury in MCU-KO. To better understand adaptations that occur in the MCU-KO heart that may alter ischemic cell death mechanisms, the cardiac proteome of WT and MCU-KO whole heart homogenates was compared using tandem mass tags (n=5 KO, 5 WT). Results indicate that 96 proteins were decreased and 112 proteins were increased by 1.3-fold or greater in MCU KO hearts in comparison to WT. Given the report that F_1F_0-ATP synthase is a component of the mitochondrial PTP (Giorgio *et al*,PNAS,2013), it was of interest that two ATP synthase proteins were altered in MCU-KO. ATP synthase subunit s was decreased 2.2-fold and F1 complex assembly factor 1 was decreased 1.6-fold in comparison to WT. Blue native PAGE experiments were performed to examine levels of ATP synthase monomers and dimers in isolated mitochondria.

Interestingly, the ratio of dimers to monomers was reduced in MCU-KO. Furthermore, in-gel trypsin digestion of ATP synthase followed by mass spectrometry analysis revealed that the composition of monomers and dimers differed between WT and MCU-KO. These results suggest that absence of MCU may alter ATP synthase subunit expression, as well as the formation of ATP synthase dimers.

WE-104

Blocking cell surface nucleolin in heart cells prevents uptake of immunogenic DNA

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Rationale

Cellular debris causes sterile inflammation after myocardial infarction. The human heart contains 25 per cent mitochondria and mitochondrial DNA (mtDNA) is a damage-associated molecular pattern that can trigger the immune system and induce injurious inflammation. It is not known if mtDNA can trigger inflammatory signaling pathways in the cardiomyocyte and how it is internalized to associate with its putative receptor, toll-like receptor 9 (TLR9). A better understanding of the post-infarction inflammatory response holds the promise of new treatments.

Objective

To understand if and how mtDNA induces inflammatory responses in cardiac cells and whether cell surface nucleolin is implicated in internalization of immunogenic DNA.

Methods and Results

The gene expression of the pro-inflammatory cytokines interleukin-1β, tumor-necrosis factor α and interferon α1 was upregulated by mtDNA, but not nuclear DNA (nDNA) in cardiomyocytes exposed to 40 minutes of non-lethal hypoxia and two hours of reoxygenation. In HEK293 cells, mtDNA induced NF-κB activity in normoxia. Furthermore, 40 minutes of hypoxia and 6-12 hours reoxygenation and CpG DNA synergistically induced TLR9-dependent NF-κB activity. In subcellular protein fractions, nucleolin was expressed in cardiomyocyte membranes and inhibition of cell-surface nucleolin with midkine inhibited the uptake of CpG DNA in cardiomyocytes and cardiac fibroblasts.

Conclusion

We show for the first time that isolated cardiomyocytes respond with an inflammatory response to mtDNA, but not nDNA. Nucleolin on the cell surface is a possible route for DNA internalization in cardiac cells. Cell-surface nucleolin might be a therapeutic target to reduce uptake of immunogenic DNA.

WE-105

Increased calpain-1 in cardiomyocyte mitochondria disrupts ATP synthase and promotes reactive oxygen species generation to induce dilated heart failure in mice

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Background: Calpain-1 has been shown to increase in mitochondria of the heart under pathological conditions including ischemia/reperfusion, diabetes and sepsis. Our recent study reported that mitochondrial calpain-1 promotes superoxide generation in cardiomyocytes and it may be implicated in myocardial injury and dysfunction. This study was to investigate whether and how increased calpain-1 in mitochondria induces adverse myocardial remodeling and heart failure.

Methods and Results: A novel line of transgenic mice over-expressing cardiomyocyte-specific and mitochondria-targeted calpain-1 was generated. Over-expression of mitochondria-targeted calpain-1 increased mitochondrial reactive oxygen species (ROS) generation in mouse hearts and induced adverse myocardial remodeling including cardiac hypertrophy, fibrosis and enlarged ventricular chambers, leading to heart failure and early death in transgenic mice, characteristic changes of dilated cardiomyopathy. These effects of mitochondrial calpain-1 up-regulation were attenuated by administration of mitochondria-targeted antioxidant mito-TEMPO. Increased mitochondrial calpain-1 also correlated with decreased ATP synthase activity in transgenic mouse hearts. In cultured cardiomyocytes, selective over-expression of calpain-1 in mitochondria induced superoxide generation, decreased ATP synthase activity and promoted apoptotic cell death, all of which were inhibited by up-regulation of ATP5A1 or mito-TEMPO.

Conclusions: Mitochondrial calpain-1 induces myocardial injury, remodeling and dysfunction possibly by disrupting ATP synthase and promoting ROS generation in cardiomyocytes. Thus, mitochondrial calpain-1 may be a therapeutic target for heart failure.

TH-001

Changes in cardiac adenosine A_3 receptor function and expression associated with essential hypertension

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Background: Essential hypertension is considered to be a multifactorial disorder and if not treated can contribute to the development of heart failure. As the adenosine receptors have a significant role in mediating vasodilation and cardioprotection, alterations in their structures or signalling pathways may be involved in the development of hypertension. This study measured the mRNA expression of adenosine A_3 receptors cardiac tissues and determined whether they could be altered with essential hypertension. We also investigated adenosine selective A_3 receptor agonist mediated vasodilator responses in coronary blood vessels using the isolated perfused heart preparation.

Methods: Male spontaneously hypertensive rats (SHR, 10 weeks) and age-gender matched Wistar rats were used in this study. Cardiac tissues and a range of blood vessels were collected and processed to isolate mRNA and adenosine A_3 receptor expression measured using real time PCR. Rat isolated hearts were set up in Langendorff mode and perfused with Krebs-Henseleit solution containing 8-phenyltheophylline (10 µM) an antagonist of adenosine A_1, A_{2A} and A_{2B} receptors to isolate adenosine A_3 receptor mediated coronary vasodilation.

Results: Adenosine A_3 receptor agonists APNEA and CL-IB-MECA induced coronary vasodilation in the presence of 8-phenyltheophylline (10 µM). Vasodilator responses to these agonist were attenuated in hearts from SHR when compared to control tissues ($p<0.05$). The mRNA expression of adenosine A_3 receptors was down-regulated in atria, left ventricle and thoracic aorta from SHR when compared to cardiac tissue from normotensive animals ($p<0.05$).

Discussion:.This study demonstrated decreases in the expression of adenosine A_3 receptors occurred in cardiac tissue and reduced adenosine A_3 receptor mediated coronary vasodilation in hearts from spontaneously hypertensive rats. Our findings with regard to changes in the adenosine A_3 receptor populations in hypertensive hearts suggest that adenosine A_3 receptor could play a role in physiopathology of essential hypertension.

TH-002

Physiological and pathological left ventricular hypertrophy of comparable degree is associated with characteristic differences of in vivo hemodynamics associated with distinct expression of mitochondrial regulators

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Background: Left ventricular (LV) hypertrophy is a physiological or pathological response of LV myocardium to increased cardiac load. We aimed at investigating and comparing hemodynamic alterations in well established rat models of physiological (PhyH) and pathological hypertrophy (PaH) by using LV pressure-volume (P-V) analysis.

Methods: PhyH and PaH were induced in rats by swim training and by abdominal aortic banding, respectively. Morphology of the heart was investigated by echocardiography. Detailed characterization of cardiac function was completed by LV P-V analysis. In addition histological and molecular biological measurements were performed. All data were normalized to the corresponding control group.

Results: Echocardiography revealed myocardial hypertrophy of similar degree in both models (LV mass index: +21.7±2.1% PhyH vs. +27.3±3.3% PaH, n.s.), which was confirmed by post-mortem heart weight data. In aortic-banded rats we detected subendocardial fibrosis. Reactivation of fetal gene program could be observed only in PaH model. PhyH was associated with increased stroke volume, whereas unaltered stroke volume were detected in

PaH along with markedly elevated end-systolic pressure values. Sensitive indices of LV contractility were increased in both models, in parallel with the degree of hypertrophy. Active relaxation was ameliorated in athlete's heart, while it showed marked impairment in PaH (time constant of LV pressure decay (τ): -7.7±2.6% PhyH vs. +37.0±11.1% PaH, p<0.01). Mechanical efficiency and ventriculo-arterial coupling were improved in PhyH, whereas remained unchanged in PaH. Myocardial gene expression of mitochondrial regulators showed marked differences between PaH and PhyH (peroxisome proliferator-activated receptor gamma coactivator 1-alpha (PGC-1α): +19.1±10.3% PhyH vs. -37.8±7.2% PaH, p<0.01).

Conclusions: We provided the first comparative hemodynamic characterization of PhyH and compensated PaH in relevant rodent models. Increased LV contractility could be observed in both types of LV hypertrophy, characteristic distinction was detected in diastolic function (active relaxation) and mechanoenergetics (mechanical efficiency), which might be explained by mitochondrial differences.

TH-003

Differential expression of plasmalogen lipids following modulation via dietary supplementation in a mouse model of reduced PI3K activity

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BACKGROUND: Phosphoinositide 3-kinase p110α (PI3K) is a critical regulator of physiological cardiac hypertrophy. Cardiac-specific dominant negative PI3K (DnPI3K) transgenic mice have smaller hearts and are more susceptible to cardiac insults. Increased PI3K activity is protective in response to various cardiac disease settings in mice. Plasmalogens are a class of glycerophospholipids enriched in the heart, previously shown to be protective against reactive oxygen species. A pilot study suggested that altered PI3K activity was associated with similar changes in plasmalogen levels.

AIM: To determine whether increased plasmalogens could restore heart size in DnPI3K mice.

METHOD: Plasmalogens were increased via dietary supplementation with 1% batyl alcohol (BA)/ kg of chow. Non-transgenic (Ntg)/DnPI3K mice began chow/1% BA dietary intervention at approximately 8 weeks old for 16 weeks (N=4-6 per group). Lipid analysis was performed using liquid chromatography electrospray ionisation tandem mass spectrometry. Data was analysed in Multiquant 2.1 software and specific lipid species normalised to total phosphatidylcholine (PC) levels.

RESULTS: Total cardiac PC and phosphatidylethanolamine (PE) plasmalogens were decreased in chow fed DnPI3K vs. Ntg mice (P<0.05). PC and PE plasmalogens with C18:0 fatty alcohol side chains were increased in Ntg and DnPI3K mice on 1% BA diet vs. chow fed mice (P<0.05). Interestingly, 1% BA supplementation increased expression of several C18:0 PC and PE plasmalogens species in the DnPI3K vs. Ntg mice. Morphological analysis however, revealed no change in heart weight to tibia length ratio amongst cohorts. The pathological marker, atrial natriuretic peptide increased 25 fold in chow fed DnPI3K vs. Ntg mice (P<0.05) and this trend was not altered with supplementation of 1% BA.

CONCLUSION: Under basal settings, increasing plasmalogens in DnPI3K mice does not restore cardiac size. However, differential modulation of some lipid species in BA fed DnPI3K mice suggests this approach may provide protection in a setting of cardiac stress.

TH-004

Proliferative and hypertrophic defects contributes to *LMNA* associated dilated cardiomyopathy

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Background;

LMNA is one of the leading causative genes of genetically inherited dilated cardiomyopathy (DCM). Unlike sarcomere related genes, *LMNA* encodes nuclear envelope proteins, lamin A and C, and does not have direct association with contractile function. However, mutation in this gene

also develops DCM. The underlying mechanisms of developing DCM with *LMNA* mutation still remain obscure.

Methods and Results;

To characterize *Lmna* mutant mice, we assessed cardiomyocyte number, size, nuclei counting and cell cycle activity. Both cell number and cell size were reduced, myocytes were immature and cell cycle activity, assessed by EdU incorporation to nucleus and phospho-histone H3 staining, was retarded in *Lmna* mutant mice. RNA-sequencing and pathway analysis revealed "proliferation of cells" had the strongest impact on *Lmna* mutant mice. Especially, *Cdkn1a,* which encodes cell cycle regulating protein p21, had significant relationship with *Lmna* mutation. Upregulation of p21 was observed not only RNA transcription level but also protein level by Western blot and immunostaining. DNA damage was more robustly detected in *Lmna* mutant mice by immunostaining. Furthermore, a repressed cardiomyocyte proliferating response after resecting apex of the neonatal heart was observed in *Lmna* mutant mice. In addition, *Lmna* mutant mice lacked the ability of compensatory hypertrophic response against pressure overload after administration of angiotensin II**.**

Conclusion;

These data strongly suggest that *Lmna* mutation damages DNA, which induces p53 and p21 activities and contributes to the reduction of cell proliferation as well as hypertrophic response in *Lmna* mutant mice. Inadequate response against cardiac injury and pressure overload stresses plays important roles in developing DCM with *LMNA* mutation.

TH-005

Targeting the L-type Ca^{2+} channel alters mitochondrial function and attenuates hypertrophic cardiomyopathy in a Troponin I mutant mouse model

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Hypertrophic cardiomyopathy (HCM) affects 1 in 200 of the general population. It is characterised by myocyte remodeling, disorganisation of cytoskeletal proteins and altered metabolic function. Mitochondrial function can be regulated by alterations in L-type Ca^{2+} channel (LTCC) activity, and the cytoskeletal network plays an important role in this response. We have previously demonstrated that the human HCM causing cardiac troponin I mutation Gly203Ser, leads to a *faster* LTCC inactivation rate and impaired functional communication between the LTCC and mitochondria in a mouse model of the mutation (*cTnI-G203S*). This results in a "hypermetabolic" mitochondrial state, which *precedes* development of HCM.

Application of a peptide derived against the cardiac alpha interacting domain (AID-TAT) *slows* the LTCC inactivation rate and *decreases* metabolic function in *wt* cardiac myocytes (*Viola et al JMCC 2009, Viola et al JAHA 2014*). Here we examined the efficacy of *in vitro* and *in vivo* exposure of *cTnI-G203S* to AID-TAT on mitochondrial function by assessing alterations in mitochondrial membrane potential (Ψ_m, JC-1 fluorescence) and mitochondrial oxygen consumption (flavoprotein autofluorescence).

We find that acute *in vitro* exposure of *cTnI-G203S* cardiac myocytes to AID-TAT normalises Ψ_m in response to activation of the LTCC with channel agonist BayK(-) (*cTnI-G203S*+AID-TAT=4.4±0.4% increase, n=6 versus *cTnI-G203S*=29.2±1.8% increase, n=15, $p<0.05$). AID-TAT also attenuated increases in flavoprotein autofluorescence in response to BayK(-) (*cTnI-G203S*+AID-TAT=4.0±0.3% increase, n=6 versus *cTnI-G203S*=24.4±6.5% increase, n=8,$p<0.05$). *In vivo* treatment of *cTnI-G203S* mice with AID-TAT via intraperitoneal (IP) injection (10µM 3x per week for 5wks) also normalised Ψ_m (*cTnI-G203S*+AID-TAT=22.1±2.6% increase, n=13 versus *cTnI-G203S*=35.8±4.7% increase, n=3, $p<0.05$), and flavoprotein autofluorescence in response to BayK(-) compared to untreated mice (*cTnI-G203S*+AID-TAT=17.9±2.5% increase, n=24 versus *cTnI-G203S*=44.0±10.1% increase, n=7, $p<0.05$). Treatment also prevented the development of HCM, as evidenced by changes on echocardiography. These data suggest that treatment of *cTnI-G203S* mice with AID-TAT restores mitochondrial function and prevents development of HCM.

TH-006

ProteoSeq – a proteotranscriptomics approach to decode alternative isoform expression in cardiac hypertrophy

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Background: Alternative protein isoform expression is a critical feature of the fetal genetic program associated with the early failing heart. Notable examples include differential expressions of sarco/endoplasmic reticulum Ca^{2+}-ATPase (SERCA2a/2b), cardiac sodium channel SCN5A, and titin. Advances in RNA-seq have led to the discovery of novel isoforms at the transcript level; however, without information on their physiological functions and disease relevance, the significance of these transcripts remains ambiguous and cannot be validated. Therefore, the characterization of protein isoforms encoded by alternative transcripts becomes a unique approach to ascertain the discovery of novel isoforms. To date, information on novel protein isoforms encoded by alternative transcripts in the cardiac proteome is scarce.

Methods: We developed a proteotranscriptomics approach, ProteoSeq, which combines transcriptomics datasets, cardiovascular disease models, mass spectrometry, and computational algorithms to characterize proteome-wide alternative isoform expression in the heart. We derived species- and tissue-specific splicing information from an ENCODE dataset (C57BL/6J mouse heart; ENCSR000BYQ) using a Bowtie-Tophat-rMATS pipeline, then translated the information into custom junction peptide databases to guide protein isoform discovery. Matching proteomics data were acquired in-house followed by a ProLuCID-DTASelect search.

Results: This new approach enabled the discovery of multiple novel splice junction peptides in the mammalian heart, presenting strong evidence for the corresponding alternative splicing events at the protein level. These isoforms belong to diverse pre-mRNA processing events from mutually exclusive exons, skipped exons, alternative 5' and 3' splice sites, to retained introns. Differential expressions of multiple novel protein isoforms are confirmed in models of cardiac hypertrophy (e.g., pyruvate kinase M1 to M2).

Conclusion: We demonstrate the utility of our proteotranscriptomics approach for identifying new alternative protein isoforms at a proteome scale. ProteoSeq is being implemented as a unified web-based platform for on-the-cloud multi-omics analysis. ProteoSeq is currently supported and beta-tested by 20+ laboratories globally.

TH-007

Folic acid reduces doxorubicin-induced cardiomyopathy by modulating endothelial nitric oxide synthase

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Aims: The use of doxorubicin (DOXO) as a chemotherapeutic drug has been hampered by cardiotoxicity leading to cardiomyopathy and heart failure. Folic acid (FA) is a modulator of endothelial nitric oxide (NO) synthase (eNOS), which in turn is an important player in diseases associated with NO insufficiency or NOS dysregulation, such as pressure overload and myocardial infarction. However, the role of FA in DOXO-induced cardiomyopathy is poorly understood. The aim of this study was to test the hypothesis that FA prevents DOXO-induced cardiomyopathy by modulating eNOS and mitochondrial structure and function.

Methods and results: Male C57BL/6 mice were randomised to a single dose of doxorubicin (20 mg/kg intraperitoneal) or sham. FA supplementation (10 mg/day per oral) was started 7 days before injection and continued thereafter. DOXO resulted in 70% mortality after 10 days, with the surviving mice demonstrating a 30%

reduction in stroke volume compared with sham groups. Pretreatment with FA reduced mortality to 45% and improved stroke volume (both $P<0.05$ vs. DOXO). These effects of FA were underlain by blunting of DOXO-induced cardiomyocyte atrophy, apoptosis, interstitial fibrosis, and impairment of mitochondrial function. Mechanistically, pretreatment with FA prevented DOXO-induced increases in superoxide production, by reducing the eNOS monomer:dimer ratio and S-glutathionylation, and attenuated DOXO-induced decreases in superoxide dismutase, eNOS phosphorylation and NO production. Furthermore, the protection effects of FA were abolished in eNOS-knockdown human microvascular endothelial cells.

Conclusions: Enhancing eNOS function and subsequently reducing oxidative stress with FA may be a novel therapeutic approach to attenuate DOXO-induced cardiomyopathy.

TH-008

The cardiopulmonary vascular system and the ventilatory reflex; scientific merits and clinical implications

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Introduction: The “ventilatory baroreflex” is not well explained phenomenon, where an increase in heart rate and decrease in blood pressure are associated with an increased tidal volume. We hypothesize that changes in lung blood pool and capillary pressure directly affect lung compliance and play a key role in mediating this “reflex”. The study investigated this hypothesis.

Methods: The pulmonary blood pool was modulated by inducing slowly progressing pneumothorax in mechanically ventilated rabbits (n=7), by continuous air injection into the pleural space. Hemodynamic parameters, tidal pressures and flows, $EtCO_2$ and SpO_2 were recorded. Tidal volume and respiratory system compliance were calculated.

Results & Discussion: The slowly progressing pneumothorax was associated with immediate progressive decline in the BP and compensatory increase in HR. A counterintuitive decrease in $EtCO_2$ was observed at the initial phase, concurrent with a gradual increase in the tidal volume (+14.6±5.3%) and respiratory compliance (13.7±5.2%). The respiratory rate and the inspiratory pressure were constant. Therefore, the increase in tidal volume resulted from a gradual increase in lung compliance. Only after 28 min the respiratory indices exhibited the reverse responses, when tension pneumothorax developed.

The initial phase mimics the ventilatory reflex. However, the counterintuitive increase in tidal volume and decrease in pCO_2 were not due to involvement of the central nervous system, as the rabbits were mechanically ventilated at constant inspiratory pressure. The effect appears with mild pneumothorax, demonstrating the high sensitivity of lung compliance to changes in lung circulation. The opposite occurs in heart failure where the pulmonary capillary blood pool increases, leading to smaller lung compliance and dyspnea.

Conclusions: The “ventilatory reflex” was observed in ventilated animal (without nervous pathway) and it is determined by a direct effect of the pulmonary circulation on lung compliance. Lung blood pool and capillary pressure are important determinants of the cardio-pulmonary "baroreflex".

TH-009

Heart failure assessment with a multiscale model

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Background: Heart failure (HF) produces mechanical and hemodynamic impairment. Mathematical models have analyzed the impact of HF on experimentally identified myocyte components, but their integration into a ventricular model forming part of a multiscale circulatory approach has not been properly addressed.

Objective: The aim of this study was to compare the experimental and multiscale model hemodynamic and regional contractile response to acute HF.

Methods: The left ventricle (LV) was based on a validated contractile human myocyte and the remaining chambers were defined as elastic structures. Electrically simulated

preload and afterload were coupled to heart chambers, integrating a closed circulatory circuit. HF effect in the myocyte decreased K1 and Ito channel conductances by 49% and 36% and SERCA2a activity by 24%, and increased sodium-calcium exchanger conductance by 100%. Right ventricular elastance was decreased by 30%. Halothane (H) 3-4% was used to elicit HF in open-chest sheep (n=23) instrumented with LV piezoelectric crystals (wall thickness, WT), Swan Ganz catheter (cardiac output, CO) and ventricular and carotid artery catheters for intraventricular and arterial pressure (AP) assessment.

Results: The hemodynamic performance of the model in normal conditions was: mean AP (MAP): 82 mmHg, CO: 4.3 L/min and ejection fraction: 65%. In sheep experiments, MAP, CO and systo-diastolic WT fraction (WTF) dropped to 74.2 ± 10.2%, 73.0 ± 17.5% and 71.0 ± 27.1%, respectively after 15 min H ($p<0.01$ vs. 100% baseline). Model simulated HF gave comparable results: 75.6%, 73.8% and 80.3% for MAP, CO and WTF, confirming suitable HF effect on the myocyte.

Conclusion: The model shows adequate coupling between myocyte-derived left ventricular chamber and the circulatory loop, and would be useful to predict the contractile and hemodynamic response to changes in myocyte variables.

TH-010

The specific inhibition of the cardiac electrogenic sodium/bicarbonate cotransporter (NBCe1) leads to cardiac hypertrophy

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The Na^+/HCO_3^- cotransporter (NBC) regulates cardiac intracelular pH (pH_i). There are two isoforms of NBC in the cardiomyocyte, the electrogenic NBCe1 (2 HCO_3^-: 1 Na^+) and the electroneutral NBCn1 (1 HCO_3^-: 1 Na^+). Both isoforms incorporate Na^+ into the cell but the NBCe1 does it more efficiently because contributes with half of Na^+ per HCO_3^-. The increase of Na^+ enhances intracellular Ca^{2+} leading to cardiac hypertrophy (CH). We have previously demonstrated in CH models that while the activity of NBCe1 is reduced, that of the NBCn1 is increased. Due to the absence of specific pharmacological inhibitors we were unable to demonstrate if this phenomenon was cause or consequence of CH. We developed an interference RNA (shNBCe1) cloned in a lentiviral vector to study the effect of the specific inhibition of NBCe1 in CH. In western-blot assay we found a reduction of expression of NBCe1 in cells transduced with the shNBCe1 (cont: 100±5 %, n=4 vs shNBCe1: 15±2 %, n=4, $P<0.05$). We used confocal microscopy to study the expression of NBCe1 in transduced neonatal myocytes and we found a significantly decrease of NBCe1 expression. Furthermore, we found an increase of cell size (cont: 14330±350 AU, n=68 vs shNBCe1: 18570,61±611 AU, n=66, $P<0.05$). In parallel experiments, the lentivirus was injected into the rat anterolateral wall of the left ventricle. After 30 days of injection, we obtained the mass ventricle index (MVI) by echocardiography, showing an increase of MVI in rats hearts injected with the shNBCe1 (shNBCe1:1.85±0.07 mg/g, n=2 vs cont: 1.57±0.03 mg/g, n=2). In addition, NBCe1 activity was investigated in cardiomyocytes isolated from these rats, using intracellular fluorescent measurements of BCECF-AM, to monitor pH_i. Membrane potential depolarizing pulses (potassium pulse) were applied by extracellular addition of 45 mM K^+, to study NBCe1 activity in isolation. Cardiomyocytes transduced with shNBCe1 showed a decrease of NBCe1 activity, indicating consisting reduction of NBCe1 expression and function. Overall, these results suggest that the development of CH involves, at least in part, the decrease of NBCe1 expression and function.

TH-011

The Role of Profilin-1 in Hypertrophic Signalling of Adult Cardiomyocytes

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Background: Hypertrophy is characterized by altered protein abundance and increased cell size. Pathological changes generally associate with increased amounts of natriuretic peptide A (ANP) and B (BNP). Previously, we found that the actin-binding protein profilin-1 is an essential component of the hypertrophic signalling response in neonatal rat ventricular cardiomyocytes and its effects were mediated by pERK1/2. However, this work was limited by the use of developing cardiomyocytes, rather than

stable adult cells, and therefore it was difficult to distinguish between the consequences of normal cell growth, and physiological and/or pathological hypertrophy. This study reports the functional and mechanistic role of profilin-1 in the hypertrophic signalling response of stable adult rat cardiomyocytes.

Methods and results: Overexpression of profilin-1 was accomplished using adenoviral transfection. The functional effect of profilin-1 on contractility was measured utilizing a video edge-detection system and data acquisition software (Ion Optix). Morphologically, increased levels of profilin-1 resulted in enlarged cell size. However, these changes were not accompanied by increased transcript levels of ANP and BNP. In addition, utilizing the specific inhibitors PD98059 and rapamycin, we found that profilin-1 induced cell enlargement was regulated by both ERK1/2 and mTOR ($p=<0.01$ and $p=<0.001$ respectively). Functionally, increased levels of profilin-1 resulted in enhanced contractility without changing relaxation times. However, the increase in contractile force was regulated by mTOR ($p=<0.01$) but not ERK1/2.

Conclusion: Our results show that profilin-1 is an essential mediator of the hypertrophic signalling response in stable adult rat cardiomyocytes, and influences cell size through both ERK1/2 and MTOR, and cell contractility through mTOR. Taken together, these data suggest that profilin-1 is a key mediator of physiological hypertrophic signalling.

TH-012

Moderate-intensity physical activity reduces systemic inflammation and maintains cardiorespiratory function following $PM_{2.5}$ exposure during exercise in rats

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Background: Exposure to fine particulate matter (PM) during outdoor activities in populated cities in Asia, Central and South America is increasing. Following excess PM exposure, the risk of cardiorespiratory complications and events is significantly increased.

Aims: The purpose of the current study is to 1) examine the beneficial effects of moderate levels of PA on functional and biochemical markers of the cardiorespiratory system, 2) establish the detrimental effects of a single, daily PM exposure event on cardiorespiratory function and 3) determine if exercising during daily PM exposure increases the deleterious effects caused by PM exposure due to increased inhalation of particulates.

Methods: Four groups were used: control (CON), physical activity (PA), $PM_{2.5}$ exposed and PA combined with $PM_{2.5}$ exposure (PA+PM) (n=16 per group). Both PA and PM exposure were initiated when the animals reached 8 weeks of age, for 8 weeks. **Results:** PA alone did not alter body weight, markers of inflammation or BP compared to control animals. However, there was a significant decrease in epididymal fat pad mass. The PM exposed rats were hypertensive, showed increased systemic inflammation and oxidative stress without pathological changes in the cardiac action potential or impaired vascular function. PA was able to decrease systemic inflammation in PM exposed animals, including reduction in IL-6 serum levels, however this did not translate to an improvement in blood pressure or vascular reactivity. Smooth muscle relaxation in the trachea from the PA+PM tissues was not significantly different to CON and PA groups but was significantly higher than the PM group. **Conclusions:** The current study showed that while there is an increased CVD risk associated with PM exposure, engaging in PA during exposure events imposes no increased risk with exercise providing a protective mechanism against some of the biochemical signaling changes caused by inhaled PM.

TH-013

Osteopontin Regulates the Inflammatory and Fibrotic Response of Transgenic Mice Expressing Cardiac Specific Active Na+/H+ Exchanger Isoform 1

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Background: Heart failure is increasing in incidence and prevalence around the world. As a result, the need for new therapeutic advances is urgent. We have previously shown that elevated cardiac specific NHE1 activity induced cardiac hypertrophy both *in vivo* and *in vitro.* This overexpression of active NHE1 elicited modulation of gene

expression in cardiomyocytes including an up regulation of myocardial osteopontin (OPN) expression. To determine the role of OPN in inducing NHE1 cardiomyocyte hypertrophy, we developed an *in vitro* and *in vivo* model expressing active NHE1 in the presence of silenced OPN.

Methods: *In vitro*, H9c2 cardiomyocytes were characterized for parameters of cardiomyocyte hypertrophy in the presence of active NHE1 and OPN siRNA. *In vivo*, we evaluated by echocardiography, histology and qRT-PCR the cardiac phenotypes and function of the transgenic mice expressing active NHE1 or active NHE1 cross breed with OPN knockout mice ($OPN^{-/-}$).

Results: Our data showed that expression of active NHE1 resulted in a remodeled cardiac phenotype both *in vitro* and *in vivo*. *In vitro*, OPN siRNA regressed the hypertrophic effect. However, *in vivo*, the decrease in FS (%) and EF (%) demonstrated in NHE1 expressing transgenic mice was not reversed in the presence of $OPN^{-/-}$. Interestingly, transgenic mice expressing NHE1 demonstrated an upregulation of fibrosis and inflammatory mediators (CD44 and IL-6), all of which were regressed in the presence of $OPN^{-/-}$.

Conclusions: We have developed an interesting comparative model of active NHE1 transgenic mouse lines which express a dilated hypertrophic phenotype expressing CD44 and fibrosis, an effect which is regressed upon knocking out OPN. Despite the regression CD44 and the fibrotic response in NHE1-$OPN^{-/-}$, the cardiac function as assessed by echocardiography was not reversed.

TH-014

Characterization of the role of inhibitory G protein, adenylyl cyclase isoforms and phosphodiesterases to regulate β-adrenoceptor-evoked inotropic responses.

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Background: Our data indicate that inhibitory G protein (G_i) exerts intrinsic receptor-independent inhibitory activity upon adenylyl cyclase (AC). The two major subtypes of AC in the heart are AC5 and AC6. The aims were to determine if intrinsic G_i inhibition is AC subtype selective and whether there is a differential role of AC5 and AC6 to mediate β_1-adrenoceptor-(β_1AR) and β_2AR-evoked inotropic responses. In addition, To determine if there is an interplay between G_i and phosphodiesterases 3 and 4 (PDE3,4).

Methods: β_1AR- and β_2AR-mediated changes in contractility were measured ex vivo in left ventricular myocardium from wild type (WT), AC5 or AC6 knockout (KO) mice with or without pertussis toxin (PTX) pretreatment to inactivate G_i.

Results: Adrenaline potency (EC_{50}) to evoke a β_1AR-mediated inotropic response (IR) was increased in AC6KO versus WT and AC5KO and also by PDE4 inhibition only in AC5KO with no change in the maximal IR. Preliminary data suggest PTX increases adrenaline potency in WT and unveils a β_2AR-IR not observed in WT even after PDE3,4 inhibition. A β_2AR-IR is also observed after prior PDE4 inhibition only in PTX-treated ventricle. Unlike WT, a β_2AR-IR is observed in AC5KO after only PDE 3,4 inhibition and in AC6KO after only PDE4 inhibition.

Conclusion: These data are consistent with prior data indicating G_i exerts a tonic inhibition upon AC since both β_1AR-IR and β_2AR-IR are enhanced by PTX. Further, neither β_1AR-IR nor β_2AR-IR appear dependent upon either AC5 or AC6. At least β2AR-IR appears primarily regulated by PDE4. In contrast, the regulatory role of PDE4 seen upon β_1AR-IR in AC5KO appears absent in AC6KO. Together, these data indicate a complex interplay amongst G_i, AC isoforms and PDEs.

TH-015

Frequency of renal artery lesions in patients with hypertension

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Aim: To identify the frequency of renal arteries lesions, renal hypertension (RHT) in patients with essential hypertension (EHT)

Materials and methods: From 1986-2015, in Bakulev SCCVS were examined 2050 patients of age 5-84 years with persistent hypertension, who were treated in hospitals and leading clinics in Moscow with a diagnosis of "Essential hypertension"

Results: Hemodynamic hypertension was diagnosed in 9.7% patients. RHT was detected in 5.5% patients. In 20 patients due to occlusion of the renal artery and long-term hypertension, had renal scarring and reduced renal morphometric indicators, they underwent nephrectomy epi- sub phrenic splanchnicganglionectomy. Renal artery stenosis was detected in 54 patients. The blood pressure normalized in 92% patients who had a 5-year history of EHT, following renal artery angioplasty. The blood pressure normalized in only 65% patients who had a 10-year history of EHT due to irreversible changes in the kidneys. Dissecting aortic aneurysm with the discharge of one of the renal arteries was detected in 0.8% patients. Only 40% patients were fit for surgical interventions and they underwent successful reconstruction of aorta and arteries with satisfactory antihypertensive effects.

Conclusion: The use of highly informative diagnostic methods (CT, MRI) contributed to reduction of serious complications in patients with EHT and improved the results of treatment. Late surgical interventions were ineffective in 25- 35% patients with RHT. Timely restoration of circulation in 85-95% of cases lead to normalization of blood pressure.

TH-016

Secondary (symptomatic) high blood pressure following aortic lesions

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Aim: To reveal coarctation of the aorta, coarctation syndrome (congenital hypoplastic, stenotic nonspecific aortoarteritis, thoraco-abdominal aortic dissecting aneurysm of the aorta) in patients with essential hypertension (EHT)

Materials and methods: From 1986-2015 were examined 2050 patients of age group 5-84 years with persistant hypertension, who were treated in hospitals and leading clinics in Moscow for essential hypertension (EHT).

Results: Coarctation of the aorta was detected in 2.5% patients. In 53.3% patients aged 20-60 years coarctation of the aorta complicated aneurysm of the thoracic aorta due to prolonged hypertension, which was not diagnosed for many years. Correction of coarctation and aortic aneurysm led to normotension only in 4 (25%) patients. Coarctation syndrome with aortic stenosis and its branches due to nonspecific aortoarteritis and congenital hypoplasia was detected in 1.0% patients with a 10-year history of hypertension. All patients underwent surgical interventions. Elimination of coarctation syndrome brought about normotension in only 55% patients. Dissecting aortic aneurysm was detected in 0.8% patients. Only 45% patients were fit for surgical interventions and they underwent successful reconstruction of aorta and arteries with satisfactory antihypertensive effects

Conclusion: The volume of medical care to patients with hypertension in the Russia is unsatisfactory frequency of EHT is much less (29%), in contrast to that mentioned in the literature (70-80%). During an extensive survey, it was observed that 71% of specialized clinics in Moscow managed to find out the exact cause of hypertension and put a correct diagnosis.

TH-017

Conserved epigenomic basis in mouse and human heart aging

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Aging is a major risk factor for cardiovasulcar diseases, which leads to deterioration of many physiological functions (e.g. cardiac dysfunction). Although some genes (e.g miR-34a) have been implicated in heart aging and its associated cardiac dysfunction, but the global epigenome reconfiguration during mouse and human heart aging remain unclear. Here we profiled transcriptomics, chromatin state, DNA methylation/hydroxymethylation and

chromatin accessibility dynamics across young, middle-aged and old mouse and human hearts. By RNA-seq approach, we found a coordinated downregulation of genes related to calcium-ion binding and surprisingly, upregulation of genes related to Systemic Lupus Erythematosus (SLE), implying the common immunological foundation of cardiac aging and SLE. Moreover, we identified cardiac-aging specific enhancer transition via ChIP-seq profiling and unravelled the common transcription factor (TF) footprinting on these enhancers by DNase-seq and demystified the correlation of these TF footpriting and methylation/hydroxymethylation pattern by Whole Genome Bisulfate Sequencing (WGBS) and Oxidative Bisulfite Sequencing (oxBS-Seq) at single base resolution level. Taken together, this comprehensive dataset will not only expand our understanding of the complex process of cardiac aging at epigenome-wide level and but will also direct the future in-depth functional and translational study in the field.

TH-018

Transferring an *in vitro* model of pathological cardiac hypertrophy from rat to human engineered heart tissue

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Background

Previously, we established an *in vitro* hypertrophy model based on rat engineered heart tissue (rEHT). In this system, afterload enhancement (AE) was induced by mechanical reinforcement of the silicone posts to which the EHTs were attached. We found that AE resulted in diminished contractile function, cardiomyocyte enlargement, increased fibrosis, and activation of the fetal (hypertrophic) gene program. The aim of the current study was to perform similar experiments on EHTs, made from human induced pluripotent stem cell derived cardiomyocytes (hiPSC-CMs), in order to more closely model the situation in patients.

Methods

Three different hiPS-cell lines from healthy donors were differentiated to cardiomyocytes using a growth factor-based two-step protocol, which yields up to 90% α-actinin-positive cells. Following the common mesodermal induction, cardiomyocytes were generated via inhibition of wnt-signaling and in parallel hiPSC-fibroblasts were differentiated from the same cell lines using wnt-activation and FGF-treatment. The cardiomyocytes were then used to generate fibrin-based human EHTs (hEHTs), or were supplemented with 5% hiPSC-fibroblasts to get isogenic multicellular constructs. After three weeks of cultivation, the spontaneously beating hEHTs were subjected to AE for 7 days.

Results

Video-optical analysis of the AE-hEHTs (n=47) revealed lower contractile forces (-24%) and longer relaxation times (+10%) compared to control hEHTs (n=51), but, in contrast to rat EHTs, no cardiomyocyte hypertrophy, fibrosis or fetal gene program. Fibroblast-containing multicellular hEHTs showed faster development, higher maximal forces (+35%, n=23) and more pronounced AE-induced dysfunction (35% lower forces, n=11). However, results were not completely consistent across different cell lines or different hypertrophic stimuli (AE or 50 nM endothelin-1).

Conclusion

AE in pure hiPSC-CM EHTs leads to contractile dysfunction, but without consistent hypertrophy. AE-induced functional impairment was more pronounced in fibroblast-supplemented multicellular EHTs. Future experiments are required to investigate their hypertrophic and fibrotic response and causes of inconsistencies between cell lines and stimuli.

TH-019

Tenascin-C promotes fibrosis and impairs cardiac function under pressure overload

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Background: Extensive reorganization of the extracellular matrix involving altered

activity of matrix metalloproteinases (MMPs) is responsible for an excess of fibrous connective tissue and cardiac dysfunction in the failing heart. The two extracellular matrix proteins Tenascin-C (TN-C) and the extracellular matrix metalloproteinase inducer CD147 (EMMPRIN) have been identified as possible regulators for MMPs. However, the roles of TN-C and CD147 levels on cardiac remodelling during left ventricular hypertrophy (LVH) have not yet been studied. Therefore, the purpose of this study was to assess the influence of these two proteins under pressure overload in a TN-C knockout (KO) model of transverse aortic constriction (TAC).

Methods:

TAC or sham surgery was performed in TN-C-KO or wild type (WT) animals, respectively. After four and ten weeks cardiac function was evaluated by magnetic resonance imaging (MRI; Medspec 3T MR) before animals were sacrificed and histologic and immune-histochemistry analyses were made.

Results:

After 10 weeks WT-TAC animals showed significantly more cardiac hypertrophy: heart weight (196±8 vs. 152±13mg), ventricular myocytes size (550±25 vs. 300±27µm^2), septum thickness (1.59±0.08 vs. 1.2±0.04) and fibrosis (17±3 vs. 5±2% of LV) were significantly higher as compared to KO-TAC hearts (all $p<0.01$). Similarly MRI evaluation revealed significantly impaired cardiac function (EF 44.5±3.1 vs. 66.9±4.3; $p<0.01$) and significantly higher expression of MMP-9 (22.4±3.1 vs. 14.5±1.2 % of LV, $p<0.05$), MMP-2 (15.5±1.2 vs. 12.2±1.1 % of LV, $p<0.05$) in WT-TAC. There is a tendency towards higher CD147 levels in WT-TAC mice. These findings correlated significantly with the level of fibrosis ($p<0.01$).

Conclusion:

TN-C is a key regulatory factor during cardiac remodelling in the pressure overloaded heart, leading to increased expression of MMPs, which results in more ventricular hypertrophy and fibrosis as well as impaired cardiac function.

TH-020

Tenascin-C in the murine geriatric heart after myocardial infarction

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Introduction: Aging is associated with a higher incidence, mortality, and complication rate of myocardial infarction (MI). Tenascin-C (TNC) is a glycoprotein produced in the infarction border zone. Previous studies discussed TNC as prognostic marker for outcome after MI.

Methods: In male geriatric (OM, age: 18 months) and young (YM, age: 11 weeks) OF1 mice MI was induced by permanent LAD ligation. In SHAM groups the procedure was performed without LAD occlusion. 32 days after MI, cardiac MRI was used for hemodynamic evaluation. TNC plasma and septum tissue concentrations were assessed by ELISA (IBL 27767).

Results: In a 2-way ANOVA MRI examination showed significant effects of age and of MI vs. SHAM on ejection fraction, stroke volume heart weight ratio, cardiac output heart weight ratio, end-systolic, and end-diastolic left ventricular volumes. Moreover, MI had a significant effect on stroke volume. No significant effects of age and of MI vs. SHAM were found on heart rate and cardiac output. Furthermore, no significant interactions between the two factors were found in any parameter.

TNC plasma concentration was significantly increased in mice with MI at all time points, and significantly decreased in geriatric mice 3 and 7 days after MI compared to young mice after MI (3 days: OM: 4.52±0.94µg/ml, YM: 11.11±3.46µg/ml; 7 days: OM: 4.22±1.92µg/ml, YM: 9.03±4.09µg/ml). Additionally, geriatric mice after MI showed decreased TNC septum tissue concentrations (7 days: OM: 0.114±0.043ng/mg, YM: 0.217±0.064ng/mg).

Conclusion: We have successfully implemented a geriatric mouse model of MI with common signs of heart failure. Confirmed by MRI, we found significant hemodynamic differences between MI and SHAM groups, and also between OM and YM. We could find first evidence for age

dependent differences in TNC production. These alterations should be respected in clinical studies examining the prognostic role of TNC in MI and heart failure.

TH-021

Beta-2 Microglobulin Contributes to Myocardial Fibrosis during Pressure Overload

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Background: Plasma Beta-2 microglobulin (β2M) level is inversely associated with glomerular filtration rate (GFR) and ejection fraction (EF) in patients with chronic kidney disease. However, the effects of β2M in cardiovascular diseases are unclear.

Methods: Serum β2M level in patients and healthy individuals was measured by ELISA. Pressure overload mice model was built by transverse aorta constriction (TAC) to induce chronic heart failure. Cultured cardiomyocytes or cardiac fibroblasts were stretched to examine the β2M level in culture medium at different time-points (3,6,12, 24hours).

Results: Serum β2M level was significantly higher in patients (n=216) with chronic heart failure than in healthy individuals (n=162) ($P < 0.001$). After TAC, β2M level in serum or heart tissue increased progressively in time-dependent manner. In vitro, mechanical stretch induced an increase in secretory β2M from cardiomyocytes but not fibroblasts Exogenous β2M (from 0.5ng/ml to 3ng/ml) didn't activate ERK, regarded as a hypertrophic response, in cultured cardiomyocytes, but it caused the upregulation of fibrotic genes col1 and col3 in cultured fibroblasts with concentration-dependent manner. β2M-treated-fibroblasts also increased migratory capability compared to those without β2M by a scratch assay. Cardiac fibroblasts when treated with conditioned-medium of mechanical stretch-cardiomyocytes displayed the higher expression of fibrotic gene. But these effects were significantly inhibited in fibroblasts treated with conditioned medium of siRNA-β2M-cardiomyocytes subjected to mechanical stretch. In vivo, exogenous β2M greatly promoted cardiac fibrosis by Masson's trichrome staining and decreased cardiac contractility by hemodynamics analysis at 3 week after TAC. However, β2M didn't induce cardiac hypertrophy or fibrosis in sham mice. Further analysis indicated that β2M increased the expression of TGF-β mRNA, the level of p-ERK, p-p38 and p-smad2/3 in cultured fibroblasts.

Conclusion: Cardiomyocyte secretory β2M was increased during pressure overload that acts on fibroblast to promote cardiac fibrosis. TGF-β, MAPK or smad signaling may be involved in the progress.

TH-022

The Role of Calcium-Sensing Receptor in Human Peripheral T Lymphocytes on the different stages of Acute Myocardial Infarction

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Background：

Acute myocardial infarction (AMI) is an inflammation disease which seriously affects the human health. Calcium-sensing receptor (CaSR）in T lymphocytes is involved in inflammation reaction. But, the relation between AMI and CaSR in T lymphocytes is not very clear.

Methods:

In this study, we collected human peripheral blood T lymphocytes from AMI patients in different stages of PCI (percutaneous coronary intervention) (on the onset of AMI, the first day after PCI, the third day after PCI, and the fifth day after PCI) to identify the expressions of CaSR and related signal transduction pathway proteins, the levels of Th-1 type and Th-2 type cytokines in plasma, the lymphocytes apoptosis rate and number. At the same time, related laboratory indicators, drinking or smoking history and medical history such as hyperlipidemia, hyperglycemia or hypertension were recorded.

Results:

The results showed that cTnI, hs-CRP, LDL-C and FBG levels and the incidence of hypertension, hyperlipidemia and diabetes increased significantly in AMI group compared with the normal group. And, the expressions of CaSR, P-ERK1/2, P-JNK (subgroup of MAPKs), P-p65 (subunit of NF-κB), Caspase-12 and the secretions of all the cytokines were increased on the onset of AMI, continued to increase greatly on the first day after PCI. But, from the third day after PCI, all the indicators began to decline. Meanwhile, the neutrophils to

lymphocytes ratio (NLR) increased and the apoptosis rate of all the CD^{3+}, $CD^{3+}CD^{4+}$ and $CD^{3+}CD^{8+}$ T lymphocytes increased, and the change trend was consistent with the expressions of proteins.

Conclusion:

These results indicated that CaSR in the human peripheral blood T lymphocytes were involved in the AMI onset and progression, which probably was related with the NF-κB and MAPK signaling pathways.

Key Words: Calcium-sensing receptor; Acute myocardial infarction; Lymphocyte ; Signaling pathway; Cytokine

TH-023

Effect and regulation mechanism of exogenous catestatin on blood pressure and cardiac function in renal hypertensive rats

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AIM: To examine the effect and mechanism of catestatin (CST), a small molecular active peptide, on blood pressure and cardiac function in renal hypertension induced by the method of 2-kidney 1-clip (2K1C) in rats. **METHODS:** Forty male SD rats were randomly divided into two groups: control group (n=10) and 2K1C renal hypertension group (n=30). Six weeks after 2K1C operation, 2K1C renal hypertension group were randomly subdivided into three groups: 2K1C group, 2K1C+CST group (80μg/100g weight), 2K1C+NS group (0.9%NS). Cardiac function was measured by left ventricular catheterization and blood pressure was measured by femoral artery catheterization. The ratio of left ventricular weight/body weight (LVW/BW) was calculated as left ventricular mass index. The levels of histamine (His), epinephrine (E) and CST in plasma were measured by ELISA assay. Calcium receptor-like receptor (CRLR) gene expression level in left ventricular tissue was tested by real-time PCR. **RESULTS:** ①The blood pressure of rats in 2K1C renal hypertension group was increased gradually from the 3rd week after 2K1C operation, and reached maximum in the 6th week. ② Systolic function and diastolic function parameters in 2K1C group were higher than those in Control group. The systolic function and diastolic function parameters in 2K1C+CST group were significantly lower than those in 2K1C+NS group. ③Compared with Control group, the pressure volume loop (PVL) in 2K1C group was to the right and its area was increasingly shifted under the effect of pressure load. However, the PVL in 2K1C+CST group was left and its area was decreasingly shifted, when compared with those in 2K1C+NS group. ④The level of CST in 2K1C group was lower than those in Control group. The content of His and E in 2K1C group was higher than those in Control group. However, an application of CST significantly increased His, when compared with 2K1C+NS group. ⑤The results from real-time PCR showed that levels of CRLR gene expression in left ventricle in 2K1C group were lower than those in Control group. However, an application of CST obviously decreased CRLR gene expression, when compared with those in 2K1C+NS group. **CONCLUSIONS:** Exogenous catestatin can significantly lower the blood pressure of renal hypertensive rats, which may be related to the decrease of left ventricular systolic function and promote the release of histamine.

TH-024

The effect of genes involved in monogenic human cardiomyopathies in a polygenic model of cardiac hypertrophy

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Background: Cardiac hypertrophy (CH) is the main risk factor for heart disease after age. Genetic factors are known to be involved, but their contribution is still poorly understood. We hypothesise that genes implicated in monogenic human forms of CH might also be involved in the more common polygenic forms of the disease.

Aims: Our aim was to use the hypertrophic heart rat (HHR), a unique normotensive polygenic model of CH, to investigate mRNA expression of genes previously described to be associated

with monogenic forms of dilated and hypertrophic cardiomyopathy in humans.
Methods: We measured the expression of 37 transcripts with the TruSeq Targeted RNA expression kit using the MiSeq Desktop sequencer (Illumina) in left ventricles of HHR and its sister control strain, the normal heart rat (NHR), at five ages (2 days old, 4-, 13-, 33- and 50 weeks old).
Results: We found only one gene (*Ttr*) differentially expressed in all age groups (FDR<0.1; *P*<0.05). *Ttr* is involved in cardiac amyloidosis, infiltrating cardiovascular structures, leading to hypertrophy. However, in animals older than 13 weeks old, when CH is established in the HHR, we found four genes upregulated (*Actc1, Ankrd1, Cav3 and Fhl2*). These genes are involved in a variety of muscle development pathways, growth and contractibility. Interestingly, *Ankrd1* (fold change 1.3-2.47) has been described to be upregulated in the failing myocardium of dogs and in the left ventricles of patients with CH. *Fhl2* is associated with cardiomyopathy in rats but seems to not be essential in cardiac development in mice.
Conclusion: Our results show that genes involved in monogenic forms of human CH may also influence polygenic forms of the disease and deserve further investigation.

TH-025

Assessment of miR-669f in the development of pulmonary arterial hypertension and right ventricular hypertrophy

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Background: Pulmonary arterial hypertension (PAH) is a proliferative vascular disease with a poor prognosis resulting in right ventricular hypertrophy (RVH) and RV failure. The pathology of PAH involves vascular cell remodeling including pulmonary arterial endothelial cell (PAEC) dysfunction and pulmonary arterial smooth muscle cell (PASMC) proliferation. Recently, (miRNAs have emerged as a new class of post-transcriptional regulators of genes having a key role in vascular remodeling. However, the function of miRNAs in the development of PAH and RVH remain elusive. Here, we investigate that miR-466a/-669f-cluster is a pathogenic niche regulating the pulmonary vascular remodeling and inhibition of miR-669f prevents RVH by restoring BMPRII and PPAR□ □level.
Method/Result: We identified a panel of novel dysregulated miRNAs and miRNA clusters in the RV and lungs of MCT treated WT mice. Among them; we discovered miR-466a/-669f cluster is critical for the development of PAH. We confirmed our finding using MCT and hypoxia-induced mouse models and observed significant upregulation of miR-466a/-669f cluster in the RV and lungs. To screen the potential target genes for miR-669f in an unbiased fashion, we transfected mouse PAEC with miR-669f mimic and inhibitor and followed by the stimulation with TGF□1, separately and confirmed BMPRII and PPAR□ are the *bona-fide* target for miR-669f. The *in vitro* studies showed that TGF□1 stimulation significantly enhanced the expression of mature miR-669f and reduced the level of BMPRII and PPAR□ in rodent PAEC. The in vivo inhibition of miR-669f showed a promise in attenuating PAH.
Conclusion: Our findings provide evidence that miR-669f displays a critical role in the pathogenesis of vascular remodeling leading to the development of PAH and RVH by directly targeting BMPRII and PPAR□, and that inhibition of miR-669f reversed the remodeling process. We conclude that miR-669f could be a triggering factor in PAH and may providing new mechanistic information for therapeutic benefit.

TH-026

CARDIAC APOPTOSIS IN THE PREDIABETIC HEART: CaMKII, Ca MISSHANDLING AND MITOCHONDRIA DYSFUNCTION

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The mitochondria are a well-known intermediate of apoptosis, which is one of the more important steps leading to heart failure (HF). This disease occurs more frequently in people with type 2 diabetes than in the general population. However, cardiac apoptosis has not been previously evaluated at the prediabetic state. Since CaMKII is involved in cardiac apoptosis and Ca2+ mishandling, the aim of the present study was to evaluate the presence of cardiac apoptosis in a prediabetic model (PM) induced by a fructose-rich diet (FRD) in rats or mice and the putative link with CaMKII activity and mitochondria dysfunction. FRD rats showed decreased contractility (echocardiography) and increased CaMKII (P-CaMKII 191.6±18.3%), and ROS (185.4±28.6%) with respect to control diet (CD) rats (100%). Moreover, the apoptotic ratio Bax/Bcl2 increased in FRD vs CD rats (273.6±39.7%) as well as TUNEL positive nuclei. Mitochondria from FRD rats showed significant more swelling (DO 0.34±0.05 CD vs 0.53±0.03 FRD), enhanced mitochondria membrane depolarization and mitochondria Ca2+ content than CD rats. Moreover, myocytes from FRD rats significantly increased sarcoplasmic reticulum (SR) Ca2+ leak vs CD myocytes. In Wild Type (WT) mice, collagen type III increased in FRD (27.06±5.24%) with respect to CD (13.33±1.23%) hearts. FRD SR-AIP mice (which express the CaMKII autocamtide inhibitory peptide [AIP] at the SR membranes) showed less TUNEL positive nuclei and no change in collagen type III than FRD WT mice. Co-treatment with tempol, a membrane permeable ROS scavenger, decreased apoptosis, collagen type III as well as SR Ca2+ leak in FRD WT mice. Moreover, mitochondria swelling could be also prevented in S2814A mice, which ryanodine receptor (RyR2) cannot be phosphorylated by CaMKII. The results would indicate a causal link between CaMKII activation by increased ROS, SR Ca2+ leak produced by CaMKII-dependent phosphorylation of RyR2 and mitochondria damage induced by Ca2+ overload.

TH-027

Glycoprotemics reveals decorin fragments with anti-myostatin activity in human atrial fibrillation

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Background. Myocardial fibrosis is a feature of many cardiac diseases. We used proteomics to profile glycoproteins in the human cardiac extracellular matrix (ECM).

Methods and Results. Left atrial specimens from patients who developed postoperative atrial fibrillation (AF) were compared to patients who maintained sinus rhythm (SR). Out of more than 100 ECM proteins identified, the levels of the small leucine-rich proteoglycan (SLRP) decorin were reduced in patients with postoperative AF. Within its protein core, eighteen different fragmentation sites were identified using mass specrometry. In contrast, no fragmentation was observed for biglycan, the most closely related SLRP. Decorin processing differed between human ventricles and atria and was altered in disease. Atrial appendages from patients in persistent AF had higher levels of decorin harboring a unique cleavage site not found in atrial appendages from patients in SR. This cleavage site preceded the N-terminal domain of decorin that controls muscle growth via altering the binding capacity for myostatin. A synthetic peptide corresponding to this region dose-dependently inhibited the response to myostatin in cardiac myocytes, where phosphorylation of AMPK and SMAD2 (i.e. downstream targets of myostatin) resulted affected. The same effect was observed in and in perfused mouse hearts. Notabily, myostatin expression was decreased in hearts of decorin null mice. In contrast, C-terminal fragmentation of decorin, important for the interaction with connective tissue growth factor (CTGF), was reduced in patients with persistent AF.

Conclusion. This proteomics study is the first to analyze the human cardiac ECM. Novel processed forms of decorin core protein uncovered in human atrial

appendages can regulate the local bioavailability of anti-hypertrophic and pro-fibrotic growth factors and may impact on the manifestation or perpetuation of cardiac arrhythmias.

TH-028

CARDIOPROTECTIVE EFFECT OF IGF-1 UPON THE HYPERTROPHIED MYOCARDIUM OF THE SPONTANEOUSLY HYPERTENSIVE RATS (SHR): A KEY ROLE ON CARDIAC Na+/H+ EXCHANGER (NHE-1) ACTIVITY AND OXIDATIVE STRESS

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Oxidative stress and NHE-1 hyperactivity are interrelated phenomena that play a key role in pathological but not in exercise-induced cardiac hypertrophy (CH). We have demonstrated that IGF-1, released during exercise training, through AKT inhibits NHE-1 and that a swimming routine transformed pathological into physiological CH in the SHR. Therefore, we hypothesize that IGF-1 by preventing NHE-1 hyperactivity and oxidative stress could be responsible for the cardioprotective effect of training in SHR. NHE-1 activity in cardiomyocytes (proton efflux mmol/L/min) monitored by BCECF-AM epifluorescence was significantly reduced by IGF-1 (2.03±0.47, n=7), effect prevented by AG1024, an antagonist of IGF-1 receptor (3.71±0.9, n=7) and by the AKT inhibitor MK2206 (4.01±0.65, n=12). Similarly, IGF-1 significantly reduced H2O2 production in cardiomyocytes loaded with DCF-DA (IGF-1: -3.63±1.1; n=7, IGF-1 + AG1024: 6.06±3.4, n=7; control: 5.12±2.5, n=12, AU after 10 min incubation). The antioxidant action of IGF-1 was accompanied by a significant increase in the activity of superoxide dismutase (SOD) catalase (IGF-1: 20±1.5, n=7 and 44.9±3.6 N=5 vs. control: 14.5±1.6, n=5 and 34±2.3, n=7, U/mg, respectively). Interestingly the beneficial effects of IGF-1 correlated with higher cardiac contractility revealed by an increase in cardiomyocyte shortening (IGF-1: 145.8±14, n=5 vs. control: 96.8±5, n=3, % at 10 min respect to time 0, $p<0.05$). Since the bioactive peptide apelin, up-regulated by training, may increase cardiac contractility and was proposed to exert antioxidant effects, we quantified its mRNA abundance and that of its receptor APJ in our experimental conditions finding that IGF-1 significantly increased both (IGF-1: 251±48 and 184±29 vs. control: 100±6.2 and 100±15.9, apelin and APJ respectively).

In summary, our results suggest that the inhibition of NHE-1 hyperactivity as well as the antioxidant effect of IGF-1, probably by apelin-mediated increase in SOD and catalase activity, represent beneficial cardiac adaptations leading to the physiological phenotype in the SHR subjected to exercise training.

TH-029

Polycystin-1 regulates L-type calcium channel stabilization during mechanical stretch in cardiomyocytes

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Deregulation of LTCC protein levels has been reported in cardiac hypertrophy and ischemic heart disease; however, the underlying mechanisms are unknown. Mechanical stretch is a common factor in both pathologies. Polycystin-1 (PC1) is a mechanosensor and a G-protein coupled receptor, GPCR (Gi/o) expressed in cardiomyocytes. We hypothesized that, in cardiomyocytes, PC1 regulates LTCC protein levels in response to mechanical stretch.

Methods: Mechanical stretch was induced *in vitro* using cyclic mechanical stretch (MS) or hypo-osmotic solution (HS) in neonatal rat cardiomyocytes control or with siRNA against PC1 (siPC1). We measured the protein levels of $Ca_V\alpha_{1C}$ LTCC subunit and p-AKT in the presence of AKT inhibitor, pertussis toxin, βARk. Also, we overexpressed a mutated c-terminal of PC1 (mct-PC1) in order to avoid the interaction between the Gi protein and the ct-PC1.

Results: $Ca_V\alpha_{1C}$ protein levels increased after MS or HS and these increments were blunted in polycystin-1 knockdown (siPC1) cardiomyocytes. Changes in $Ca_V\alpha_{1C}$ mRNA were not detected, suggesting that PC1 stabilizes LTCC during mechanical stretch.

AKT, necessary to $Ca_V\alpha_{1C}$ and $Ca_V\beta_2$ binding, was activated after HS but blunted in siPC1. $Ca_V\alpha_{1C}$ protein increment also was prevented by AKT inhibitor (10 µM). AKT activation and $Ca_V\alpha_{1C}$ increment induced by HS were blunted in presence of pertussis toxin (Gi/o inhibitor) or Gβγ subunit inhibitor (βARk). Finally, overexpression of mct-PC1 inhibited the increased of $Ca_V\alpha_{1C}$ protein levels and AKT activation by HS.

Conclusion: PC1 is involved in LTCC stabilization during mechanical stretch in cardiomyocytes possibly through its GPCR (Gi) activity.

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TH-030

A mechanism of calmodulation of the human cardiac sodium channel

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The human cardiac sodium channel ($Na_V1.5$) is responsible for the initial upstroke of the action potential and essential to heart function. Genetic mutations causing channel dysfunction are associated with the life threatening cardiac conditions Brugada and Long QT syndromes. Despite much investigation, successful treatment options for patients suffering from $Na_V1.5$ dysfunction are lacking. In-depth understanding of the molecular mechanisms of channel function and regulation provides a powerful means to identify and develop novel therapeutic targets and improvements to existing treatments. To this end, we have undertaken studies of the binding of the Ca^{2+} sensing regulatory protein calmodulin (CaM) to the $Na_V1.5$ channel inactivation gate. We discovered a previously unrecognized high affinity interaction and generated a high-resolution structural model using a combination of X-ray crystallography, NMR spectroscopy and small angle X-ray scattering. Ca^{2+}-activated CaM is found to bind to two independent sites on the channel inactivation gate in an unanticipated domain configuration. The structure enabled predictions of the mechanism of mal-function for cetain disease associated mutations contained within the $Na_V1.5$ inactivation gate. Our predictions were tested using NMR analyses, which confirmed perturbations of the interaction with CaM. Our results combined with data from previous studies provides a rationale and molecular mechanism for Ca^{2+} CaM modulation (Calmodulation) of $Na_V1.5$, and sets the stage for evaluating the therapeutic potential of targeting this key regulatory interaction.

TH-031

Tenascin-C deficiency attenuates abdominal aortic aneurysm progression

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Purpose: Tenascin-C (TNC) is a matricellular protein produced by vascular smooth muscle cells and fibroblasts in various remodeling processes. In numerous cardiovascular pathologies high TNC levels are associated with unfavorable outcomes. TNC production has also been found in abdominal aortic aneurysms (AAA). The aim of the study is to evaluate whether TNC deficiency could attenuate AAA formation.

Methods: We compared male AJ TNC -/- and AJ wildtype (WT) mice. After laparotomy and preparation of the infrarenal aorta, AAA were induced by periaortal $CaCl_2$ 0,5M application for 15 minutes. In the sham-operated groups the same procedure was performed, however aortas were incubated with saline solution. The aortic diameter was measured before AAA induction and before organ harvesting after 3 and 10 weeks. The main parameter was the ratio of the diameters.

Results: TNC knockout (KO) mice with AAA showed significantly lower diameter ratios than the wildtype group 3 weeks (TNC KO: 1.39±0.25, WT: 1.67±0.22 $p<0.05$) and 10 weeks (TNC KO: 1.51±0.47, WT: 1.98±0.55 $p<0.05$) after AAA induction. No significant changes in diameter ratios were found in sham groups (3 weeks: TNC KO: 0.92±0.08, WT: 0.96±0.22, n.s., 10 weeks: TNC KO: 1.05±0.16, WT: 0.94±0.10, n.s.). Additionally, WT mice with AAA showed a more disrupted Elastin structure than TNC KO mice 10 weeks after AAA induction.

Conclusions: In our study we found first evidence that TNC deficiency is associated with reduced AAA formation. To identify possible causal pathways immunohistological and molecular biological assessments will be conducted.

TH-032

Critical transcriptional regulation of stress-response kinase JNK2 in CaMKIIδ gene expression in the aging atrium

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Introduction: Stress-response c-Jun N-terminal kinase (JNK) is implicated in a wide range of physiological and pathological cellular processes. We recently revealed that JNK isoform 2 directly activates CaMKII, a pro-arrhythmic molecule, which enhances atrial arrhythmogenicity in the aged heart. Cardiac CaMKII delta isoform (CaMKIIδ) is known to regulate Ca handling proteins and promotes pathogenesis of cardiac arrhythmias. Here, we assess the role of JNK2 in CaMKIIδ gene expression in the aged atrium.

Methods and Results: We found that CaMKIIδ protein expression (immuoblotting) markedly increased in human atria with increasing age as well as in HL-1 atrial myocytes treated with JNK activator anisomycin. However, either a JNK2 specific inhibitor JNK2I-IX or overexpression of inactivated dominant-negative JNK2 (Adeno-JNK2dn) completely attenuated this anisomycin-induced CaMKIIδ up-regulation (compared to Adeno-LacZ-infected controls), whereas overexpression of Adeno-JNK1dn did not. JNK2-induced up-regulation of CaMKIIδ was further confirmed in HL-1 atrial myocytes co-infected with Adeno-MKK7D-JNK2, but not in the cells co-infected with Adeno-MKK7D-JNK1. Moreover, dramatically up-regulated CaMKIIδ mRNA (quantitative qPCR) was exhibited in human atria with increasing age and in HL-1 atrial myocytes treated with anisomycin. It is known that JNK regulates target gene expression via its downstream transcriptional factors including c-Jun and ATF2. We found that activated JNK was associated with a substantially increased phosphorylation of c-Jun but unchanged ATF2 in both aged atrium and anisomycin-treated HL-1 atrial myocytes. Cross-linked chromatin-immunoprecipitation (XChIP) assay showed significantly increased binding of c-Jun to CaMKIIδ promoter in the presence of anisomycin. Moreover, transcriptional activity of CaMKIIδ promoter in CaMKIIδ promoter vector transfected HEK293 cells was significantly elevated in response to anisomycin challenge assessed by luciferase reporter assay.

Conclusion: We discovered a critical role of JNK2 in up-regulating CaMKIIδ expression. This JNK2 isoform-specific regulation occurs through the activation of CaMKIIδ promoter, which is modulated by JNK downstream transcriptional factor c-jun in atrial myocytes.

TH-033

Up-regulation of 5-Hydroxytryptamine receptor signaling in coronary arteries after organ culture

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Background : 5-Hydroxytryptamine (5-HT) is a powerful constrictor of coronary arteries and is considered to be involved in the pathophysiological mechanisms of coronary artery spasm. But mechanism of enhancement of coronary artery contraction to 5-HT is unclear during the development of coronary artery disease. Organ culture of intact blood vessel segments has been suggested as a model for the phenotypic changes of the smooth muscle cells in cardiovascular disease in recent studies.

Methods : The objectives of the present study were to characterise the 5-HT receptor-induced vasoconstriction and quantify the 5-HT receptor signaling expression levels in cultured rat coronary arteries. **Results :** The results demonstrated that the cumulative application of 5-HT produced a concentration-dependent vasoconstriction in fresh and 24 h-cultured rat coronary arteries without endothelium. 5-HT induced markedly higher contractions in cultured coronary arteries than in fresh coronary arteries. U46619- and $CaCl_2$-induced contractions were comparable in two groups. 5-HT stimulates 5-HT_{2A} receptor and PLC cascade to induce coronary vasoconstriction. Calcium influx through L-type calcium channels (LCC) and non L-type calcium channels contributed the coronary artery constrictions induced by 5-HT. Vasoconstraction induced by

thapsigargin was augmented in cultured coronary arteries compared with fresh coronary arteries. The decrease in Orai1 expression significantly inhibited 5-HT-evoked coronary arterial cell Ca^{2+} entry. 5-HT_{2A} receptor, Orai-1 and Stim1 expression levels were augmented in cultured coronary arteries compared with fresh coronary arteries. **Conclusions:** Upregulation of 5-HT_{2A} receptor signaling pathway elicits the enhancement of vasoconstriction induced by 5-HT in cultured coronary arteries.

TH-034

Target identification of curcumin on ischemic blood flow and anticancer activities by network analysis and biological approaches

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We investigated the angiogenic effects of curcumin on an ischemia and lung cancer model. Unilateral femoral arteries of C57BL/6 mice were disconnected on one side of the mouse and LLC cells were xenografted on the opposite side. Angiogenic effects and underlying mechanisms associated with curcumin were investigated. Molecular targets, signaling cascades and binding affinities were detected by Western blot, 2-DE, computer simulations and SPR techniques. Curcumin promoted post-ischemic blood recirculation and suppressed lung cancer progression in inbred C57BL/6 mice via regulation of the HIF1alpha/mTOR/VEGF/VEGFR cascade oppositely. Inflammatory stimulation induced by neutrophil elastase (NE) promoted angiogenesis in lung cancer tissues, but these changes were reversed by curcumin through directly reducing NE secretion and stimulating alpha1-AT and IRS-1 production. Curcumin had opposite effects on blood vessel regeneration under physiological and pathological angiogenesis, which was effected through negative or positive regulation of the HIF1alpha/mTOR/VEGF/VEGFR cascade. Curcumin had the promise as a new treatment modality for both ischemic conditions and lung cancer simultaneously in the clinic.

TH-035

The role of mast cell tryptase in the progress of atherosclerosis

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Atherosclerosis is by far the most frequent underlying cause of coronary artery disease and is associated with high morbidity and mortality. Accumulated mast cells in atherosclerotic plaques secrete a high level of tryptase that may participate in the pathogenesis of atherosclerotic disease by diverse pathways. In our study, we found that tryptase might promote foam cell formation by suppressing LXRalpha activation via PAR-2/LXRalpha/LXRalpha target genes signaling pathway. The addition of tryptase into THP-1-derived macrophages increased both intracellular lipid accumulation and total cholesterol level. These effects were resisted by APC366, a selective inhibitor of mast cell tryptase. Tryptase dramatically resisted 22RHC induced activation of LXRalpha protein expression, which can be reversed by SAM-11 (a PAR-2-specific neutralizing antibody) and reduced LXRalpha, ABCG1, ABCA1 and SREBP-1c mRNA levels and ABCG1 protein level, which were all blocked by APC366. PAR-2 agonist also redeemed 22RHC stimulation to activate LXRalpha, ABCG1 protein expression, and mRNA levels of LXRalpha and its target genes in THP-1-derived macrophages. In addition, tryptase promotes plaque haemorrhage distinctively because 50% of the ApoE-/- mice in the tryptase overexpression group had plaque haemorrhage, while only 10% in the siRNA group did. Hematoxylin and eosin(HE) staining showed that the mouse cervical artery plaque area was much larger in the tryptase overexpression group and there was greater artery stenosis. The immunohistochemistry of the cervical artery plaque showed that plasminogen activator inhibitor-1 (PAI-1) expression was the lowest while tissue plasminogen activator (tPA), CD31, CD34 and VEGF was the highest in the tryptase overexpression groups. This observation was completely contrary to what was observed in the siRNA group. Thus, regulating tryptase expression

in MCs may provide a potential target for atherosclerosis treatment.

TH-036

Intermedin 1-53 attenuates vascular calcification in rats with chronic kidney disease by upregulation of alpha-Klotho.

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Deficiency in α-Klotho is involved in the pathogenesis of vascular calcification. Since intermedin1-53 (a calcitonin/calcitonin gene related peptide) protects against vascular calcification, we studied whether intermedin1-53 inhibits vascular calcification by upregulatingα-Klotho. A rat model of chronic kidney disease (CKD) with vascular calcificationinduced by the 5/6 nephrectomy plus vitamin D3 was used for study. The aortas of rats with CKD showed reducedintermedin content but an increase of its receptor, calcitonin receptor-likereceptor, and its receptor modifier, receptor activity-modifying protein 3. Intermedin1-53 treatment reducedvascular calcification. The expression of α-Klotho was greatly decreased in the aortas of rats with CKD but increased in the aortas of intermedin1-53-treated rats with CKD. *In vitro*, intermedin1-53increased α-Klotho protein level in calcified vascular smooth muscle cells. α-Klothoknockdown blocked the inhibitory effect of intermedin1-53 on vascular smooth musclecell calcification and their transformation intoosteoblast-like cells. The effect of intermedin1-53 to upregulate α-Klotho andinhibit vascular smooth muscle cell calcification was abolished by knockdown of its receptor or its modifier protein, or treatmentwith the protein kinase Ainhibitor H89. Thus, intermedin1-53 may attenuate vascularcalcification by upregulating α-Klotho via the calcitonin receptor/modifying protein complex and protein kinase Asignaling.

TH-037

Impact of High Salt Independent of Blood Pressure on PRMT/ADMA/DDAH Pathway in the Aorta of Dahl Salt-Sensitive Rats

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Objectives: The objectives of this study were to investigate the
impact of a high salt diet on the PRMT/ADMA/DDAH (protein arginine methyltransferases;
dimethylarginine dimethylaminohydrolase) pathway in Dahl salt-sensitive (DS) rats and SS-13BN consomic (DR) rats, and to explore the mechanisms that regulate ADMA metabolism independent of blood pressure reduction. **Methods:** 8-weeks-old male Dahl salt-sensitive (SS) rats and SS-13BN (13BN) rats were randomly divided into five groups: SS normal diet group (NaCl 0.3%, SN group), SS high-salt diet group (NaCl 8%, SH group), high salt diet (8% NaCl) and hydralazine (10 mg/kg/d) intragastric administration (SH + HYD group), 13BN normal diet group (containing NaCl 0.3%, BN group), 13BN high-salt diet group (containing NaCl 8%, BH group). The plasma concentration of ADMA and NOx were determined, mRNA and protein expression of PRMT-1, mRNA expression and activity of DDAH, mRNA and protein expression of eNOS in aortic tissue were detected with RT-qPCR and Western blot. **Results:** Plasma levels of nitric oxide (NO) in DS rats given a high salt diet and subjected to intragastric administration of hydralazine (SH + HYD group) were lower than those given a normal salt diet (SN group). There were significant decreases in expression and activity of dimethylarginine dimethylaminohydrolase (DDAH) and endothelial NO synthase (eNOS) in DS rats given a high diet (SH group) in comparison to the SN group. The activity of DDAH and expression of eNOS in the SH + HYD group decreased more significantly than SN group. The mRNA expression of DDAH-1 and
DDAH-2 were lowest in the SH group. The results suggest that salt, independent of blood
pressure, can affect the PRMT-1/ADMA/DDAH system to a certain degree and lead to
endothelial dysfunction in Dahl salt-sensitive rats.

Keywords: endothelial dysfunction; asymmetric dimethylarginine; dimethylarginine; dimethylaminohydrolase; endothelial nitrite oxide synthase; oxidative stress

TH-038

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Stomatin is an important lipid raft-associated protein which interacts with membrane proteins and plays a role in the membrane organization. In order to know the effect of glucocorticoid (GC) on the expression of stomatin in vivo and in vitro, and the mechanism and significance of regulation of stomatin by GC, in this study, we at first examined the mRNA levels of stomatin in heart, lung and cerebral cortex of rat underwent sham surgery or adrenalectomy(ADX) with or without supplementation of Dex, a synthetic GC. We found that adrenalectomy resulted in significant decrease of stomatin mRNA in all above tissues, and treatment of ADX rats with Dex significantly increased the levels of stomatin mRNA of heart and lung, but did not in cerebral cortex. These results indicate that GCs up-regulate the expression of stomatin in vivo in a tissue-specific manner. Dex also up-regulated expression of stomatin in A549 cells, which was mediated by its receptor(GR). The reporter gene activity determined by luciferase assay showed that up-regulation of stomatin expression by Dex occured at transcriptional level . furter deletion and mutational studies demonstrated that a GC response element (GRE) within the promoter region mainly contributed to the induction of stomatin by Dex. Moreover, we found that Inhibiting stomatin expression by stomatin siRNA significantly decreased dense of peripheral actin ring in dex treated A549 cells. Taken all together, these data indicated that GC significantly up-regulated the expression of stomatin in vivo and in vitro , which could stabilize membrane-associated actin in A549 cells.

TH-039

HIP-55 function in endocytosis

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Clathrin-depended receptor internalization is a complex and delicate process, which is tightly regulated by an intricate complex protein network. So far, there are still much unknown about how the multiple components of clathrin-coated structures are spatially and temporally organized and regulated. In this work, we studied the regulatory role of HIP-55, an adaptor protein, in clathrin-depended receptor internalization. We found that HIP-55 was colocalized precisely with clathrin on cell membrane and shared similar dynamics with clathrin during the early assembly and late invagination of coated-structures by duel-color live-cell total internal reflection fluorescence microscopy (TIRFM) imaging. HIP55 knockdown decreased the density of dynamin on cell membrane. As a consequence, HIP55 depletion inhibited the internalization of transferrin and EGF, and impaired EGFR and TGF-β receptor signal transduction. Our results showed that HIP-55 acted as a linker protein by binding to dynamin, to recruit dynamin to clathrin-coated pits for fission.

TH-040

LncRNA Hand2-AS1, Hand2, and MiR-138-5p Crosstalk to Participate in VSMC Phenotypic Switch

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Vascular smooth muscle cell (VSMC) phenotypic switch is a common pathological feature of vascular remodeling diseases. Long non-coding RNAs (lncRNAs) have many important regulatory functions, but the functions in VSMC phenotypic switch are largely unkown. Here, we identified that Hand2 (heart and neural crest derivatives expressed 2) gene and lncRNA Hand2 antisense RNA 1 (Hand2-AS1) are co-expressed, and their expression levels are significant decreased in dedifferentiated VSMC by RNA-seq and qRT-PCR analysis. By using both gain-of-function and loss-of-function approaches, we found Hand2 promote VSMC phenotypic switch by regulating SM22, a differentiated VSMC maker gene. Furthermore, we demonstrated that lncRNA Hand2-AS1 binds to the Hand2 gene promoter, and increases Hand2 expression at transcriptional level. MiR-138-5p inhibits Hand2 expression by targeting its 3′-untranslated region. LncRNA Hand2-AS1 is a competitive endogenous RNA, blocks

miR-138-5p to targeting Hand2, and increases Hand2 expression at post-transcriptional level. In summary, our findings provide a novel mechanism that one lncRNA can regulate one target gene from both transcriptional and post-transcriptional level, our results indicate lncRNA Hand2-AS1, Hand2, and miR-138-5p can form a regulation loop to participate in VSMC phenotypic switch.

TH-041

Epac is an essential component of the cAMP-mediated cardioprotection and acts synergically with PKA

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Background. Acute β-adrenergic stimulation and subsequent elevation of cAMP level are implicated in cardioprotection against ischaemia/reperfusion (I/R) induced by heart conditioning. However, cAMP signalling involves activation of both protein kinase A (PKA) and guanine nucleotide exchange protein (Epac). In this study, we aimed at identifying the involvement of PKA and Epac in cardioprotection.

Methods. Langendorff perfused adult rat hearts were used either for protein determination, isolation of cardiomyocytes or subjected to 30 min global ischemia and 2 h reperfusion. Cardiac tissue, H9C2 myoblasts and isolated cardiomyocytes were used to optimise conditions and validate changes in PKA & Epac expression and activity. The effect of cell-permeable cAMP analogue, an activator of both PKA and Epac (8-Br-cAMP-AM; 8-Br), on haemodynamic function was investigated in the presence or absence of an inhibitor of PKA (H-89) or Epac (ESI-09). In cardioprotection studies, 8-Br was introduced to the heart prior to ischaemia and compared to the effect of activation of either PKA (6-Bnz-cAMP-AM; 6-Bnz) or Epac (CPT-2′-O-Me-cAMP-AM; CPT). Functional recovery, lactate dehydrogenase (LDH) release and infarct size were used to assess I/R injury.

Results. Simultaneous inhibition of PKA and Epac by 8-Br increased baseline haemodynamic function and induced a marked cardioprotective effect (complete recovery of haemodynamic function, 3.5-fold reduction of infarct size and 3-fold reduction of LDH release vs. control). These effects were abolished by selectively inhibiting PKA and Epac using H-89 and ESI-09. Both PKA activation alone (6-Bnz) or Epac activation alone (CPT) increased baseline haemodynamic function but could not confer significant protection. However, the cardioprotective effect of 8-Br could be mimicked by using a mixture of PKA and Epac activators.

Conclusion. Cell permeable cAMP analogues that simultaneously activate both PKA and Epac confer marked protection against I/R injury. Activation of either PKA or Epac alone has little cardioprotective effect.

TH-042

Temporal Phosphoproteomics to Investigate the Mechanotransduction of Vascular Smooth Muscle Cells in Response to Cyclic Stretch

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Background: Vascular smooth muscle cells (VSMCs) are exposed to mechanical cyclic stretch *in vivo*, which play important roles in maintenance of vascular homeostasis and regulation of pathological vascular remodeling. Reversible protein phosphorylation is crucial for intracellular signaling transduction. However, the dynamic phosphorylated profile induced by cyclic stretch in VSMCs is still unclear.

Methods and Results: Using the stable isotope labelling by amino acid in cell culture, VSMCs were labeled and exposed to 10% physiological cyclic stretch *in vitro* at 1.25 Hz for 0 min, 15 min, 30 min, 1 hr and 6 hr, respectively. Using TiO_2 beads and liquid chromatography tandem mass spectrometry, the temporal phosphoproteomic profiles in response to cyclic stretch were then detected. Bioinformatics analysis including fuzzy c-means clustering, functional classifications, and Ingenuity Pathway Analysis were applied to further reveal the potential mechanotranduction networks. The results indicated that protein kinase C (PKCs) family, Rho-associated coiled-coil containing protein kinase 1 (ROCK1) and Akt may participate in cyclic-stretch induced VSMC functions. Cyclic stretch repressed the expression of ROCK1, while it had no significant effect on the phosphorylation of PKCα/βII, PKCζ/λ and PKCδ/θ. PKCθ was

activated first at short time-phase (15 min and 30 min), and again at long time-phase (6 hr, 12 hr and 24 hr). The activation of p-PKCμ was immediate and short-term, similar to p-Akt.

Concultion: Our present *in vitro* work hence revealed that cyclic stretch activates complex mechanotransduction networks, suggesting that novel mechanoresponsive molecules, i.e., PKCθ, PKCμ, and ROCK1, may participate in the mechanotransduction and modulation VSMC functions.

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TH-043

Involvement of BK Channel in Differentiation of Vascular Smooth Muscle Cells Induced by Mechanical Stretch

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Background: The differentiation of vascular smooth muscle cells (VSMCs), which are exposed to mechanical stretch in vivo, plays an important role in vascular remodeling during hypertension. Here, we demonstrated the mechanobiological roles of large conductance calcium and voltage-activated potassium (BK) channels in this process.

Methods and Results: In comparison with 5% stretch (physiological), 15% stretch (pathological) induced the de-differentiation of VSMCs, resulting in significantly decreased expressions of VSMC markers, i.e., α-actin, calponin and SM22. The activity of BK channels, assessed by patch clamp recording, was significantly increased by 15% stretch and was accompanied by an increased alternative splicing of BK channel α-subunit at the stress axis–regulated exons (STREX). Furthermore, transfection of whole BK or STREX-deleted BK plasmids revealed that STREX was important for BK channels to sense mechanical stretch. Using thapsigargin (TG) which induces endoplasmic reticulum (ER) stress, and xbp1-targeted siRNA transfection which blocks ER stress, the results revealed that ER stress was contribute to stretch-induced alternative splicing of STREX.

Conclusion: Our results suggested that during hypertension, pathological stretch may induce the ER stress in VSMCs, which affects the alternative splicing and activity of BK channels, and subsequently modulates VSMC differentiation.

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TH-044

Functional and morphological improvements mediated by long-term β-arrestin biased agonism of the AT1R in familial dilated cardiomyopathy

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Background: Biased agonism of the angiotensin II type 1 receptor (AT1R) has been shown to improve cardiac contractility and promote cardioprotection. Recent work by our laboratory has indicated that these effects may be due to changes at the level of the myofilaments. We hypothesized that β-arrestin signaling would increase myofilament Ca^{2+}-response and may be of therapeutic value in the context of familial dilated cardiomyopathy (DCM).

Methods: We tested a DCM-linked mouse model expressing a mutant form of sarcomeric tropomyosin (Tm-E54K). We treated these mice for three months with either TRV120067 (TRV067), an angiotensin receptor modulator that blocks G-protein responses while stimulating β-arrestin mediated responses, or losartan, an angiotensin receptor blocker. At the end of the treatment protocol, we assessed cardiac function using echocardiography, the myofilament Ca^{2+}-response of detergent-extracted fiber bundles, and proteomic approaches to understand changes in post-translational modifications of proteins that may explain functional changes.

Results: We found that Tm-E54K mice treated with TRV067 had improved cardiac function and morphology whereas losartan-treated mice had no functional improvements but did have some improvement in left-ventricular wall

dimension. Myofilaments of TRV067-treated Tm-E54K mice had an improved Ca^{2+}-sensitivity of tension and normalized maximal tension generation, which were depressed in untreated controls. We attributed these changes to an increase in myosin light chain (MLC2v) and MYPT1/2 phosphorylation that was seen only in TRV067-treated mice. Western blots revealed these functional changes were due to an activation of ERK1/2-RSK3 signaling, which we show, for the first time, directly increases MLC2v phosphorylation. Morphological improvements were attributed to downregulation of β-catenin signaling, which was also found in losartan-treated Tm-E54K mice.

Conclusions: Improvements in cardiac function due to biased agonism of the AT1R are due to changes in the myofilament Ca^{2+}-response and long-term biased ligand therapy may be a viable approach for the treatment of familial DCM.

TH-045

TOR pathway regulates calcium handling in heart tissue through eIF-4E and 4E-BP

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The target of rapamycin (TOR) pathway regulates growth, survival and aging. It senses environmental cues to control metabolism, protein synthesis and autophagy, and its dysregulation has been implicated in cardiac diseases. Protein synthesis is the best characterized process controlled by TOR. Initiation of translation occurs when the eukaryotic initiation factor (eIF4E) promotes mRNA recruitment to the ribosome. This step takes place when eIF4E recognizes the cap structure of mRNAs. The eIF4E-binding protein (4E-BP) inhibits cap recognition by associating with eIF4E. TOR phosphorylates and inhibits 4E-BP, thus promoting translation. Changes in expression of eIF4E and 4E-BP alter cardiac stress-response during aging, but the molecular mechanisms associated to cardiac calcium handling remains not understood. In this report, we studied the effect of genetic up and downregulation of eIF4E and 4E-BP on cardiac calcium handling using *Drosophila melanogaster* as genetic model. We assessed the intracellular calcium level by registering the fluorescent signal of a cardiac reporter system (TinC-Gal4-UAS-GCaMP3) in semi-intact preparation of 7 days-old flies. Overexpression of 4E-BP incremented the Ca^{2+}-transient amplitude (125%) and relaxation (100%), and the sarcoplasmic reticulum (SR) calcium load (20%). These effects were linked to a higher SR Ca^{2+} reuptake through the Ca^{+2}-ATPase pump (SERCA). Downregulation of 4E-BP prevented these changes. Accordingly, interference of eIF4E mimicked the effects of 4E-BP overexpression on cardiac performance. Likewise, a 48 hs period of starvation provoked an increment in the amplitude of Ca^{2+}-transients and SR-Ca^{2+} load. TOR inactivation, and therefore 4EBP derepression, on flies overexpressing eIF4E is consistent with the phenotypes observed in flies overexpressing 4E-BP. eIF4E downregulation and TOR inactivation mimicked these effects. Altogether, our results provide evidence for a critical role of the TOR pathway, via eIF4E and E-BP,on cardiac Ca^{2+} handling, SERCA activity and contractility.

TH-046

NOX2 activity induces lateralization, S-nitrosylation and opening of connexin/pannexin hemichannels, causing arhythmogenesis and apoptosis in dystrophic cardiomyopathy

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Background

Duchenne dystrophy is a fatal progressive genetic disease that causes cardiomyopathy. One of the features of this disease is oxidative stress, which derives mainly from NADPH oxidase (NOX) in the dystrophic heart. It has been shown that oxidative stress interferes with connexin 43 (Cx43) location to the intercalated discs; and hemichannels formed by connexins (Cx) or pannexins (Px) constitute a potential pathway for dissipation of ionic gradients and tissue damage.

Aims

Here we tested the hypothesis that increased oxidative stress due to increased

NOX activity causes S-nitrosylation, lateralization and deregulation of Cxs and/or Pxs, increasing cell permeability, causing myocytes apoptosis, decreased inotropism increased arrhythmogenicity in *mdx* mice, a model of Duchenne disease.

Results

Hearts from 2 and 10 months of age *mdx* mice presented increased NOX activity and oxidative stress, reduced contractility and higher number of arrhythmic episodes. At the cellular level, *mdx* hearts presented a larger number of apoptotic cells and increased degree of fibrosis, as compared with controls. All these conditions were more severe at 10 month of age, and were reversed to control when *mdx* animals were treated chronically 1 month with NOX inhibitor apocynin.

While total cardiac Cx43 content was unchanged, dystrophic hearts showed higher presence of Cx43 at lateral membranes in 2- and 10-month *mdx* mice. Hemichannels opening, evaluated using ethidium permeability was substantially higher in *mdx* hearts and this condition was normalized when mice were treated by apocynin or acutely, using hemichannel blockers carbenoxolone (for Cx) and probenecid (for Px). In addition, *mdx* hearts exhibit increased S-nitrosylation of Cx43 and Px1 that was reversed by apocynin.

Conclusions

These results suggest that, in Duchenne disease, increased NOX activity deregulates Cx43 distribution and S-nitrosylation, causing hemichannels formation and/or activation, which may contribute to increased apoptosis and cardiac dysfunction.

TH-047

HGF/Met tyrosine kinase receptor in heart physiology and pathophysiology

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Our work has mainly investigated the role of HGF/Met tyrosine kinase receptor signaling in the heart during physiological and pathological conditions. Targeting HGF/Met activation in neonatal heart *in vivo* modulates the gene expression program involved in cardiomyogenesis. Furthermore, sustained activation of Met pathways in postnatal cardiomyocytes *in vivo* strongly increases the heart growth. Our research has also extended to the influence of Met activation in the heart protection against injury. Importantly, we have shown that Met stimulation by HGF protects cardiac cells from hypoxic damage both *in vivo*, in a mouse model of myocardial infarction, and *in vitro*, in cardiomyoblast cells cultured in low oxygen tension. Recently, we have shown that HGF protects cardiac cells from antracycline-mediated cardiotoxicity, showing that the induction of ROS-triggered apoptosis and autophagy is attenuated by HGF. In addition, our approach get closer to the clinic, with the development of original tools for therapeutic application.

TH-048

Translocase of the outer mitochondrial membrane 22 is a novel substrate for p38 alpha Mitogen Activated Protein Kinase

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TOM22 is a key component of the outer mitochondrial membrane pore complex responsible for the import of precursor proteins from the cytosol into their final position in the mitochondrial matrix. Little is known about its regulation and phosphorylation in mammals. p38 alpha MAPK is a stress activated kinase and a member of the MAP kinase family. We identified TOM22 as a novel substrate of p38 alpha MAPK. We identified the p38 alpha phosphorylation site and confirmed it by mutation of the serine 15 residue to alanine in a recombinant protein and tested in an *in vitro* kinase reaction. Wildtype TOM22 was phosphorylated by p38 alpha MAPK but the mutant lacking the phosphorylation site was not. Isolated perfused rat hearts were subjected to either 10 mins ischemia or were perfused as control hearts. Following homogenisation, both sample and control hearts were phosphorylated with an analogue sensitive form of p38 alpha MAPK, mutated to allow use of an ATP analogue, to label substrates of the kinase. Substrates were isolated by the covalent capture method. TOM22 was

phosphorylated by p38 alpha MAPK in both ischaemic and control heart samples. A rabbit polyclonal antibody raised against the phosphorylation site, did not detect differences in the phosphorylation levels between control, ischaemic or ischemic and p38alpha inhibitor SB203580 treated Langendorff perfused mouse hearts. There was a reduction in phosphorylation of TOM22 detected by western blotting in lysates from mitochondria expressing non-active p38 alpha MAPK compared to wildtype expressing p38 alpha MAPK. Further work is underway to investigate the functional significance of this phosphorylation.

TH-049

NFAT and MEF-2 control the Expression of Calsequestrin-2 in rat Cardiomyocytes

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Calsequestrin-2 (*CASQ2*) is the main Ca^{2+}-binding protein inside the sarcoplasmic reticulum of cardiomyocytes. The proximal *CASQ2* gene promoter is highly conserved, containing a TATA-Box, and binding sites for MEF-2 (Myocyte Enhancer Factor-2) and SRF (Serum Response Factor) transcription factors. Previously, we demonstrated that MEF-2 and SRF binding sites within this region are functional in neonatal rat cardiomyocytes. The calcineurin/NFAT pathway is functional in cardiomyocytes. NFAT (nuclear factor of activated T-cells) transcription factor regulates the expression of muscle specific proteins, such the β-myosin heavy chain gene. In this work, we investigated if NFAT regulates *CASQ2* gene expression. Sequence analysis of the human *CASQ2* gene promoter revealed potential NFAT binding sites at -1869 bp and -230 bp. Functional assays in neonatal rat cardiomyocytes with two *hCASQ2* promoter constructs (-3102/+176 and -288/+176) showed that the inhibition of NFAT dephosphorylation with Cyclosporine A (CsA) or with INCA-6 reduced the luciferase activity of both *hCASQ2* promoter constructs up to 50%. CsA and INCA-6 also reduced the CASQ2 mRNA levels. Additionally, NFATc1 and NFATc3 over-expressing cardiomyocytes showed a 2-3-fold increase in luciferase activity of both *hCASQ2* promoter constructs that was prevented by CsA treatment. However, EMSA and site-directed mutagenesis experiments failed to demonstrate a direct interaction between NFAT and *CASQ2* gene promoter. Mutation of the -133bp MEF2 site prevented trans-activation by NFAT overexpression. Chromatin Immunoprecipitation assays revealed NFAT and MEF-2 enrichment within the -288 bp to +76 bp of the *hCASQ2* gene promoter, suggesting that NFAT interacts with MEF2 at the -133 bp site. Taken together, these data demonstrate that the Ca^{2+}-calcineurin/NFAT pathway modulates expression of the *CASQ2* gene in cardiomyocytes. Funded by CONACyT grant 164413 to A.Z.-H., and doctoral scholarship 57838 to R.E-A.

TH-050

Proteins Secreted Preferentially in Response to ER Calcium Dysregulation Protect Cardiac Myocytes from ER Stress-induced Cell Death

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Protein secretion is important for normal cell-cell communication. Many secreted proteins are synthesized and folded in the endoplasmic reticulum (ER). A number of diseases, including neurodegenerative and heart disease, are thought to alter the ER in ways that impair ER protein folding, which causes ER stress. However, the impact of ER stress on secreted proteins, i.e. the secretome, has not been examined. Accordingly, we studied how ER stress affects the secretome of neonatal rat ventricular myocytes (NRVM), a well-established model system for studies of cardiac myocyte protein secretion. To mimic the effect of heart disease on ER protein folding, NRVM were treated with

either tunicamycin (TM) or thapsigargin (TG), which inhibit ER protein glycosylation or decrease ER calcium, respectively. The identities of proteins in NRVM-conditioned medium (CM) were determined using proteomics. Twenty-four different proteins known to be synthesized in the ER were identified in control NRVM-CM. The levels of most of these proteins, none of which are ER stress-inducible, were decreased in response to TG or TM. Interestingly, three ER-resident, ER stress-inducible chaperones, Grp94, Grp78 and Crt were secreted only in response to TG. Moreover, TG was a potent mediator of cardiac myocyte death in high culture media volumes, but not in low volumes. Addition of recombinant Grp94, Grp78 and Crt to high culture media volumes decreased TG-mediated cardiac myocyte death. Thus, TG, which mimics the effects of heart disease on ER calcium in cardiac myocytes causes the secretion of select ER stress-inducible chaperones, which protect against cell death upon ER calcium dysregulation.

TH-051

Proximal Endoplasmic Reticulum Stress Response Element is essential for *SERCA2* gene basal and Thapsigargin-induced Transcription

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The cardiac sarco/endoplasmic reticulum Ca^{2+}-ATPase (SERCA2a) is vital for the proper contractile function in the heart. Decreased levels of SERCA2a mRNA and protein are found in animal models of cardiac hypertrophy and patients with heart failure; however, the molecular mechanisms mediating its altered expression have not been fully elucidated. The SERCA2 specific inhibitor thapsigargin (Tg) increases cytoplasmic calcium concentration, induces endoplasmic reticulum stress (ERS) and has been associated with increased SERCA2a expression in cardiomyocytes. In this work, we show that Tg increased 2-3-fold SERCA2a mRNA, protein, as well as the transcriptional activity of a human *SERCA2* gene promoter construct containing the proximal 259 bp of the promoter sequence and 323 bp of 5´-UT region in primary cultures of neonatal rat cardiomyocytes. Since Tg induces ERS, we mutated three conserved DNA binding sites (CCAAT, GGC and CCACA) present within the ERS response element (ERSE) located in the proximal *SERCA2* promoter (-60 to -78 bp) and assessed the response of the mutated constructs to Tg. The CCAAT and CCACA mutated constructs showed lower basal activity compared to the wild-type construct and did not respond to Tg treatment, whereas the activity of the GGC mutant did not show any change. Furthermore, by EMSA and super-shift assays, we showed the interaction of the CCAAT sequence with NF-Y transcription factor present in nuclear extracts from neonatal rat cardiomyocytes and observed that Tg treatment decreased DNA interaction with this factor. These results demonstrate that the ERSE present in the proximal *SERCA2* gene promoter is essential for basal transcriptional activity and also necessary for the response to ERS. Funded by CONACyT grant 164413 to A.Z.-H., and doctoral scholarship to J.F.-M.

TH-052

HYPEROSMOTIC STRESS PROMOTES NO RELEASE IN THE RAT MYOCARDIUM

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Tissue osmolarity is tightly regulated under physiological conditions. However, in different pathological situations as states of severe dehydration, hyperglycemia, hyperlipidemia and diabetes, cardiomyocytes undergo osmotic shrinkage and it is associated with alterations in calcium handling, negative inotropic effects (NIE) and apoptosis.

Nitric oxide (NO) synthesized by the nitric oxide synthase (NOS) has been well defined as a second messenger and as a regulator of cardiac function. In a previous study we showed that hyposmotic swelling promotes NO release and that this NO provides contractile support.

The aim of this study is to evaluate whether membrane deformation produced by hyperosmotic stress also promotes NO release and to examine the underlying mechanisms involved.

We observed that superfusing rat cardiac myocytes, loaded with the NO sensor (DAF-FM), with a hyperosmotic solution (HS:440 mOsm) results in a decrease of cell volume (26%±1.95; n=21) and a

significant increase in fluorescence of DAF-FM (10%±2.55; n=22) compared to myocytes superfused with an isosmotic solution (IS: 309 mOsm; n=10). When cells are superfused with HS+L-NAME (inhibitor of NOS), HS+Nitroguanidine (NG: inhibitor of NOS1) or HS+Wortmaninn (WT: inhibitor of NOS3) cell volume decreases in absence of NO release suggesting that NOS1 and NOS3 are responsible for NO release during hyperosmotic stress.

Supporting the involvement of NOS1 and NOS3 in hyperosmotic stress-induced NO release, Western blot analysis showed an increase in NOS1 and NOS3 activity (pNOS1 and pNOS3) in hearts perfused with hyperosmotic solution compared with hearts perfused with isosmotic solution.

These results suggest that NOS1 and NOS3 promote NO release during hyperosmotic stress. This NO release could impact on altered cell function observed in pathological situations associated with hyperosmotic stress.

TH-053

Role of DBC1 protein in the regulation of hypertension

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Lifestyle changes have determined an increase in the incidence of noncommunicable diseases which have become the first cause of death worldwide. Among them is arterial hypertension, a silent and invisible killer. In Uruguay the prevalence of hypertension in the adult population has increased from 30 % in 2006 to 39 % in 2015.

Vascular injury is one of the main consequences of maintained hypertension. The cellular and metabolic mechanisms involved in vascular injury are complex. The renin-angiotensin-aldosterone system plays a main role since angiotensin II (ANGII) is involved in the generation of inflammation, fibrosis and apoptosis; all of them observed in arterial hypertension. However, the molecular mechanisms that underlay these processes are not completely elucidated.

Our group has been working in the role of Deleted in Breast Cancer -1 (DBC1) protein, a sirtuin 1 inhibitor, in the physiopathology of cardiovascular diseases. DBC1 knock out (KO) mice are protected against atherosclerosis in experimental obesity. Within this context, we aim to evaluate whether DBC1 plays a relevant role in hypertension. We treated C57Bl6 mice with ANGII to induce hypertension by means of an osmotic pump (1mg/kg/day). We measured blood pressure non-invasively twice a week and isolated the tissues after 28 days of treatment for molecular biology analysis. Our preliminary results show that DBC1 expression in renal and vascular tissues is induced by treatment with ANGII in vivo. Surprisingly, DBC1KO mice are more sensitive to ANGII, reaching higher values of systolic blood pressure than wild type mice. Our results suggest that DBC1 plays a main role in cardiovascular diseases, although we still have to understand deeply the mechanisms involved.

TH-054

The adenosine signalosome requires ROS activation to mediate cardioprotection

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Background: Mitochondria are central actors in cardioprotection against ischemia-reperfusion injury (IRI), which depends upon activation of the mitochondrial ATP-sensitive potassium (mitoK$_{ATP}$) channel and inhibition of the mitochondrial permeability transition (MPT) to prevent cell death and reduce myocardial infarct. MitoK$_{ATP}$ opening is mediated by signalosome, a multi-protein complex that buds off from the cell membrane as a lipid raft comprised of the internalized G$_i$-protein coupled receptor (GPCR), its attached ligand agonist, and the entire downstream signaling pathway. Cardioprotection by ischemic preconditioning (IPC) occurs by way of endogenous adenosine signaling. As ATP decreases during ischemia, it is degraded to adenosine, which moves to the extracellular space to activate adenosine receptors (ADOR) and trigger the GPCR signaling cascade. This process can be mimicked pharmacologically.

Aims: To characterize the signaling components and activation requirements of the ADOR signalosome.

Methods: The *ex vivo*, Langendorff-perfused rat heart was used to generate signalosomes, which can readily be isolated and purified from the perfused heart and which cause mitoK_{ATP} opening and MPT inhibition in mitochondria isolated from untreated hearts. This permits study of a signaling unit in its naturally organized state with preserved functionality.

Results: We show that ADOR activation forms a unique signalosome that contains the expected PI3K-to-PKG pathway but is initially inactive and requires reactive oxygen species (ROS) before it can accomplish its downstream signaling effects. The ROS signal activates a signalosomal PKCε that is upstream of ADOR and triggers the PI3K-to-PKG pathway to confer cardioprotection by ischemic preconditioning, ischemic postconditioning, K_{ATP} channel openers (e.g., diazoxide), and GSK-3β inhibition.

Conclusions: ADOR signaling is identical to signaling by other GPCR, but the ADOR signalosome requires both receptor activation and ROS to complete the signal. The signalosome mechanism is a general mechanism of cell signaling that is probably utilized by all receptors and all cell types.

TH-061

SWITCHABLE CARDIAC L-TYPE CA^{2+} CHANNEL TRANSCRIPT BY MINERALOCORTICOID PATHWAY.

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Regulation of expression of the ubiquitous L-type Cav1.2 Ca^{2+} channels (encoded by the CaCNA1C gene) is critically involved in the pathogenesis of serious neurological, retinal, cardiac, vascular and metabolic disorders, in which the mineralocorticoid hormone, aldosterone, *via* its cognate receptor (MR), plays pivotal, yet elusive, roles. MR-related extrarenal actions can be attributed to Cav1.2 deregulation, notably in cardiac and vascular cells that express this transcriptional factor. However, underlying molecular mechanisms remain unexplained. Here, we show that aldosterone induces expression of the cardiac long N-terminal Cav1.2 isoform (Cav1.2-LNT) through MR transactivation on the most proximal CaCNA1C gene promoter (P1). In cardiomyocytes aldosterone increased in dose-dependent manner Cav1.2-LNT expression at both mRNA and protein levels, correlating with enhanced dose-, time- and MR-dependent P1-promoter activity, through MR recruitment to specific DNA binding elements. The *in vivo* relevance of this regulation is confirmed in transgenic mice harbouring the luciferase reporter gene under the control of the P1-promoter. Moreover, aldosterone enhanced the functional expression of the Cav1.2-LNT in rat coronary smooth muscle cells increasing vascular tone. These results identify cardiac CaCNA1C gene as a new specific mineralocorticoid target gene, unravelling new insights into the molecular mechanisms associated with MR activation.

TH-062

Meis1 regulates sympathetic target-field innervation: consequences for autonomic nervous system induced sudden cardiac death

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Background:

Sudden cardiac death (SCD) are among the leading causes of premature death in the general population. Genome-wide association studies have recently identified the transcription factor Meis1 as a risk factor for SCD. Recent studies demonstrated a function of Meis1 in shaping heart morphology and in cardiomyocytes proliferation. The autonomic nervous system is a major regulator of cardiac functions and its imbalance is a source of dysrhythmias. Here, we hypothesise that Meis1 is implicated in cardiac nervous system development.

Methods & Results:

We report that specific Meis1 inactivation in mouse sympathetic neurons (HtPACRE/Meis1LoxP/LoxP) leads to SCD independently of cardiac structural defect. We showed that Meis1 is implicated in the development of cardiac sympathetic neurons, in particular in NGF/TRK1 trafficking. Using telemetric system, we record electrocardiograms in baseline condition, and after either pharmacological testing of autonomic nervous system or treadmill exercise. By heart rate variability analysis, we show that mice developed impaired sympatho-vagal regulation of cardiac rhythm. Mice exhibited atrial and/or atrioventricular conduction defects that led to spontaneous bradycardia and desynchronization, concomitant with a high occurrence of sinus arrests. Pharmacological testing revealed that mutant mice were intolerant to carbamylcholine injection which induces death in 40% of HtPACRE/Meis1LoxP/LoxP mice and, as well as to exercise tests on treadmill. During exercise, the RR decreased by 45% in WT mice ($p<0.01$, $n=8$), whereas a non-significant and delayed 13% decrease in the RR interval was observed in mutant mice. The maximal RR decrease in WT mice was 77±8 vs.104±3ms in mutant mice. During the recovery phase, 3 out of 4 mutant mice developed ventricular fibrillations and died.

Conclusion:

Mutant mice presented profound alterations in the sympatho-vagal regulation of cardiac functions that are independent of cardiac structural phenotype, arguing for an essential role of the transcription factor Meis1 in the sympathetic nervous system development and function.

TH-063

Extracellular matrix metalloproteinase 9 (MMP-9) and Tissue endogenous inhibitor (TIMP-1) has significantly associated with cardiovascular dysfunction (CVD) defined by echocardiography

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Sepsis is a pathophysiological interaction complex of different processes (infectious, inflammatory, hemodynamic, organ dysfunction, impaired tissue perfusion). Recent data suggest that the annual cost of hospital care for patients with septicemia is $ 14 billion in the United States. MMPs have been involved in the CVD in animal models of sepsis. However its role in humans has not been clearly defined.

Objective:

Establish the association between MMP-9 and TIMP-1 with the CVD from the echocardiographic context in septic patients.

Methodology:

An analytic observational study of prospective cohort was performed, that include 5 health Centers of the city of Bucaramanga. Sepsis was defined according to the International Conference on Sepsis of 2001, MMP-9 and TIMPs- 1 was quantified by immunoassay of systemic blood samples, echocardiograms were performed within the first 24 hours of study entry. A bivariate analysis was performed to define the association between MMP-9, TIMPs-1 with echocardiographic variables.

Results:

A significant relationship between MMP-9 with left ventricular diastolic diameter LVDD ($P <0.03$) and left ventricular systolic diameter LVSD ($P <0.001$) and cardiac ejection fraction of the left ventricle LVEF ($P <0.01$) was found. The TIMP-1 was significantly related to left atrial volume LAV ($P <0.01$), the E / A ratio ($P <0.05$) and LVEF ($P <0.001$). Tables 1 and 2.

Conclusion:

MMP-9 and its endogenous inhibitor seems to be important as CVD biomarkers on the stage of sepsis. More studies to define the true extent of these markers and its prognostic, diagnostic and monitoring value are missing.

TH-064

Interpretation of arrhythmia generation induced by sarcoplasmic reticulum Ca^{2+} loss using a human myocyte mathematical model

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Background. Contraction in cardiac myocytes is produced by the release of Ca^{2+} from the sarcoplasmic reticulum (SR) through ryanodine receptor channels (RyR2) by Ca^{2+}-induced Ca^{2+} release (CICR)[1]. There are also spontaneous diastolic Ca^{2+} discharges that are increased when RyR2 are altered and this situation may trigger arrhythmias[2]. Experimental data showed that transgenic mice carrying a mutation that represents a constitutive pseudophosphorylation of RyR2 (S2814D) exhibit spontaneous action potentials (SAP) and that the intensity of these events decreased until reaching the level of delayed afterdepolarizations (DAD) when Ca^{2+} reuptake by the SR-Ca^{2+}-ATPase (SERCA2a) was increased in mice with mutated RyR2 and phospholamban (PLN, a SERCA2a inhibitory protein) ablation (SDKO).

Methods. To analyze the mechanisms involved in these arrhythmic events, a human myocyte mathematical model[3] was used to represent both experimental conditions. Basal conditions and a proarrhythmogenic stress were simulated. The model was developed in MATLAB, and ODE15s solver was used to solve the system of differential equations.

Results and Conclusions. The model reproduced the arrhythmic events. Simulations showed that in S2814D conditions, the enhancement in diastolic Ca^{2+} leak increased Ca^{2+} concentration in the dyadic cleft (DC) that surrounds RyR2 which is exchanged by Na^{+} through the Na^{+}-Ca^{2+} exchanger (NCX) working in forward mode. Na^{+} entrance depolarizes the membrane to the threshold level of Na^{+} channels giving rise to an action potential. In SDKO conditions, the increased Ca^{2+} reuptake produces lower NCX activity resulting in membrane depolarization below the threshold needed to generate SAP; in this situation only DAD appeared. Simultaneous representation of ionic fluxes in the myocyte using model-derived data allowed us to explain the differences in the arrhythmic events observed in both experimental conditions.

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TH-065

Molecular and Functional Characterization of Novel Mutation in the Cardiac Ryanodine Receptor Gene (RYR2) in a Patient With Long QT Syndrome

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Background: Long QT syndrome is characterized by prolongation of the QT interval in the ECG, syncope and sudden death. Mutations in 16 genes that encode ion channels or associated proteins account for ~80% of all cases, however 20% of the cases still remain genetically unknown. Further, mutations in cardiac ryanodine receptor (RYR2) have been implicated in arrhythmia syndromes such as cathecolaminergic ventricular tachycardia (CPVT). Methods and Results: We identified a novel RyR2 mutation in the cardiac ryanodine receptor (RyR2) R2920Q, in a patient with family history of sudden death, syncope and prolongation of the QT interval (QTc, 525 ms). 80 cardiogenes were simultaneously sequenced using Haloplex design on a MiSeq device (Illumina). All novel or low frequency variants predicted to be damaging were confirmed by Sanger sequencing. To study the mechanism by which RyR2 might cause RYR dysfunction to lead LQT, we engineer the R2920Q and the in the mRYR and expressed in HEK-293 cells and human IPS-derived cardiomyocytes (hIPS-CM). The recombinant protein obtained from HEK-293 cells showed that [3H]ryanodine binding of RyR2-R2920Q has increased Ca2+ sensitivity compared to RyR2-WT with no difference in protein expression. Monolayers of hIPS-CM expressing mRYR2-R2920Q or -WT were loaded with a voltage sensitive dye and subjected optical recordings of action potential at 1 Hz pacing. hIPS-CM expressing mRYR2-R2920Q showed an AP90 of 232 compared to 197 or 185 ms in hIPS-CM expressing mRYR-WT and non-transfected, respectively. Monolayers of hIPS-CM expressing the mRYR- constructs were confirmed by RT-PCR using mouse primers and immunolabeling. Conclusion: We

identified novel mutation in RyR2 in a patient with LQT syndrome that resulted in abnormal RYR function; these results are not surprising since abnormal Ca2+ handling has been reported in animal model of LQT. To elucidate the mechanisms by which RyR2-R2920Q is linked to LQT and not CPVT remain unknown.

TH-066

C543 is the reactive cysteine responsible for increased human L-type calcium channel protein function following glutathionylation

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The development of cardiac hypertrophy is associated with oxidative stress and altered calcium homeostasis. The L-type calcium channel (LTCC) is the major route for calcium influx into cardiac myocytes. We have previously demonstrated that oxidative stress is associated with persistent glutathionylation of the LTCC that results in an increase in intracellular calcium and protein synthesis consistent with the development of myocyte hypertrophy. We searched for the reactive cysteine on the $Ca_v1.2$ (alpha) subunit of the channel responsible for modulating channel function during oxidative stress. Human long and short N terminal (NT) isoforms of $Ca_v1.2$ were expressed in HEK cells**.** Cysteines were mutated to a serine or an alanine. The channel protein was purified by histidine tag purification and incorporated in liposomes for functional analysis by patch-clamp technique.

Exposing the long NT isoform to 2mM oxidised glutathione increased P_o from 0.026±0.008 to 0.088± 0.014 without altering the magnitude of the current or the current–voltage relationship (n=6) while1mM reduced glutathione decreased P_o from 0.029±0.007 to 0.010±0.007 (n=5; $p<0.05$). Similarly oxidised glutathione significantly increased P_o of the short NT isoform that lacks the first 46 amino acids of the N terminus (n=16) and following truncation of the C terminus (n=7). However mutation of 3 cysteines in cytoplasmic loop I-II attenuated the effect of glutathione on open probability and altered protein folding assessed by thermal shift assay. Specifically we find that C543 is critical for conferring sensitivity of $Ca_v1.2$ to glutathione and is responsible for modifying channel function and posttranslational folding.

TH-067

Voltage and Calcium Dynamics in Atrial-like and Ventricular-like Cardiomyocytes derived from Human Embryonic Stem Cells by Optical Mapping

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Human embryonic stem cell-derived cardiomyocytes (hESC-CMs) are important *in vitro* models of human cardiac physiology due to their ability to recapitulate the corresponding electrical phenotype. Using simultaneous voltage and calcium optical mapping, the relationship between this electrical activity and the subsequent calcium response was investigated.

We have generated atrial- and ventricular-like cardiomyocytes from hESCs using established differentiation protocols that employ activin A and BMP4 signaling for mesoderm induction followed by Wnt inhibition for cardiac specification. The atrial-like cardiomyocytes (CMs) were generated with a differentiation protocol that also added retinoic acid.

Atrial- and ventricular-like CMs were labeled using the voltage-sensitive dye RH-237 and the calcium indicator dye Rhod-2, which were imaged concurrently on a single CMOS camera. Clusters of CMs were spontaneously active at various independent rates, and responded uniformly to electrical field stimulation. This facilitated an examination into the rate dependencies of the action-potential (AP) profiles and calcium transient dynamics. As expected, atrial voltage dynamics were significantly faster than the ventricular dynamics, in which ventricular durations were twice that of the atria. Atrial- and ventricular-like CMs had distinct calcium dynamics, with atrial-like CMs demonstrating more rapid repolarization,

which was associated with elevated calcium levels at the end of the AP. In ventricular-like CMs, the more prolonged AP was associated with a correspondingly prolonged calcium transient. Voltage and calcium dynamics in both atrial- and ventricular-like CMs were slowed by the addition of 100 nM dofetilide, which also resulted in the development of early and late-after depolarizations.
Our preliminary data have demonstrated clear differences in the atrial-and ventricular like CM populations when generated by targeted differentiation strategies. The patterns and responses observed are consistent with those seen and expected *in vivo*.

TH-068

Isolation of cardiac myocytes from human heart

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Background: The investigation of single cardiac myocytes from healthy and diseased hearts of various species is a valuable tool to explore cardiac physio/pathophysiology. The application of cell isolation to human donor tissue has been proofed to be difficult due to the limited amount of human tissue (mainly human right atrial appendages during cardiac surgery). Another limitation is the low viability of cardiomyocytes after isolation. In this study, we present a method to obtain single cardiac myocytes from different regions of human heart.

Methods and results: Human hearts rejected for transplantation were obtained from Bordeaux hospital. This protocol was approved by the Agence de la Biomédecine. Left atrial (LA) and ventricular (LV) myocytes were obtained by enzymatic dissociation. The ventricles and right atrium were removed and used for other studies (*e.g.* high resolution optical mapping). LA was cannulated by the circumflex artery and mounted into a Langendorff perfusion system after suture of the leaky atrial branches. LA was perfused with a Ca^{2+}-free solution (~10 min), then collagenase and protease solution (0.08 mM Ca^{2+}) and recirculated for ~25 min. Enzymes were washed out with a 0.2 mM Ca^{2+} solution. LA was separated into 4 regions: Endocardium, Epicardium, roof and pulmonary vein; LV myocytes were also obtained. Cells were re-suspended into a 1.8 mM Ca^{2+} solution by steps. Ca^{2+} transients were recorded (Fura-2, field stimulation) using an IonOptix system and cell membrane was stained with di-8 ANEPPS and visualized under confocal microscopy. Ca^{2+} tolerant myocytes were obtained from the 4 LA regions and LV. Human cardiac myocytes respond to electrical stimulation and Ca^{2+} transient can be recorded. Analysis of functional and structural data will be presented.

Conclusion: Isolation of single cardiac myocytes from human samples is a tedious task, but we present data showing reliable method to obtain functional and structural insights.

TH-069

Characterization of electrophysiological properties of right ventricular tissue in human using optical mapping

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Introduction: Spatial dispersion of action potential (AP) repolarization plays an important role in arrhythmogenesis. Although the mechanisms underlying tissue-dependent electrotonic modulation have been studied in various animal species there is limited information in humans. In this study, we investigated electrotonic modulation by the activation sequence and the site of pacing in human right ventricular tissues.

Methods: Three human hearts rejected for transplantation were obtained from Bordeaux hospital. This protocol was

approved by the University ethic committee. High-resolution optical mapping experiments were performed in coronary-perfused right ventricle (RV). Potentiometric dye was dissolved in DMSO and further diluted in Tyrode solution (95% O2-5% CO2). RV were paced at 1Hz on 4 different sites of the endocardial (Endo) or epicardial (Epi) surfaces (base, apex, right or left) and action potential duration at 80% repolarization (APD), activation time (AT) and repolarization time (RT) were calculated.

Results: APD range from 225 to 300 msec in the three human RV. Changing pacing site induced significant differences in APD, with the longest APD observed when stimulation originated from the base of the RV. Similar results were observed for AT and RT. In addition, transmural (Epi vs Endo) APD heterogeneity was observed. Linear correlation analysis showed no relation between APD and AT in all 3 preparations.

Conclusion
We have demonstrated that optical mapping of human heart will provide opportunities for elucidation of arrhythmia mechanisms in human. Analysis revealed a pronounced heterogeneity of the APD in RV, which is strongly modulated by the activation sequence and pacing site. Such heterogeneity and dispersion of electrophysiological characteristics are crucial to reveal understanding and treatment of cardiac arrhythmia.

TH-070
IL-1β production induces cardiac arrhythmias in diabetic mice
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Diabetes causes a multitude of secondary disorders, which have a severe prognostic impact in heart disease and arrhythmias. The latter are probably due to inflammation and pathologic signaling. Herein, we investigated origin and mechanisms underlying the onset of these arrhythmias using a combination of genetic and pharmacological tools. We demonstrate that TLR2 mediates the production of IL-1β, which in turn induces arrhythmias. In fact, IL-1β induces longer action potential as a consequence of a decrease in potassium current (Ito). Additionally, IL-1β increase calcium sparks in cardiomyocytes. Thus, our study assigns a critical role to the diabetes-induced inflammation process in one of the major secondary fatalities associated with this widespread disease. We further demonstrate that blocking the IL-1β receptor can therapeutically treat diabetes-induced ventricular arrhythmias.

TH-071
Cardiac electrical remodelling study on a type 2 diabetes experimental model
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AIM: The aim of the present study was to investigate the mechanisms underling the cardiac electrical remodeling on type 2 diabetes (T2D) mice model.
BACKGROUND: T2D is the most prevalent form of diabetes and it represents about 90% of the diabetic cases all over the world. As a consequence of the lifestyle and feeding, this syndrome has turned into one of the largest health problem worldwide and it is associated with an increase of premature appearance of several disorders such as cardiovascular complications which; can evoke cardiac electrical disturbances, as arrhythmias.
METHODS: c57bl/6 adult mice were fed with a high fat diet (HFD) (45% energy from fat) and on the second week its were injected intreperitoneally (2 doses of 40mg/kg) of streptozotocin separated 24h each other to induce T2D model. Control group received a standard chow (4,15%

energy from fat) and a vehicle (citrate buffer pH 4.5). Weight and blood glucose levels and electrocardiogram recording, were measured weekly. Intraperitoneal glucose tolerance test (IPGTT) and insulin tolerance test (IPITT) were carried out at the end of the study (6 weeks).

RESULTS: After 6 week a T2D was established, observing an average value of 158.77 mg/dl and 108.3 mg/dl of glucose in the T2D and control group respectively. Additionally the metabolic tests of glucose homeostasis were different between studied groups. Both groups showed similar body weight. However, both QT and QTc intervals of T2D group were longer than control group.

CONCLUSION: In the present work we showed that the combination of HFD (45%) with low dose of streptozotocin (40 mg/Kg/2 times) was able to induce a T2D mice model. It reproduced the typical metabolic and cardiac electrical disturbance of this disease.

TH-072

Modeling CPVT1 through patient-specific induced pluripotent stem cell-derived cardiomyocytes reveals aberrant mechano-biological and intracellular calcium handling properties associated with beta-blocker resistance.

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Catecholaminergic polymorphic ventricular tachycardia (CPVT) is a highly lethal inherited arrhythmogenic disorder predominantly caused by mutations in cardiac ryanodine receptor gene (*RYR2*). Human induced pluripotent stem cells (hiPSCs) offer a unique opportunity for disease modeling.

Aims: The goals were to derive functional cardiomyocytes (CMs) from CPVT patient via hiPSCs and test whether the novel CPVT1 mutation is associated with abnormal intracellular Ca^{2+} handling properties in CMs.

Methods: Human iPSCs were generated from dermal fibroblasts from a young athletic female diagnosed CPVT and carrying a novel heterozygous point mutation RyR2-D3638A. Following molecular characterization, healthy control (HC)- and CPVT-hiPSCs were differentiated into CMs. Using confocal microscopy and atomic force microscopy, their intracellular Ca^{2+} handling and mechano-biological properties were studied in resting and stress conditions.

Results: HC- and CPVT-hiPSCs expressed pluripotency markers (OCT4, NANOG, SSEA4) and had normal karyotype. Derived CMs via embryoid body (EB) formation showed typical cardiac markers such as cardiac troponin T and I, and α-actinin. At rest, there was no significant difference in any property of the spontaneous Ca^{2+} transients between HC- and CPVT-hiPSC CMs while CPVT-EBs exhibit higher beat rate. Significant differences in the kinetic properties of Ca^{2+} transients as well as in the mechano-biological properties were observed under stress in agreement with the arrhythmias only induced under stress. Furthermore, we revealed that the CPVT hiPSC-CMs exhibit partial resistance to the beta-blocker drug metoprolol similarly to the clinical observations of the CPVT proband.

Conclusions: Our results indicate that hiPSC-CMs can provide a suitable tool for CPVT disease modeling including resistance to beta-adrenergic receptor inhibition. In stress conditions, the novel RyR2-D3638A mutation may cause sarcoplasmic reticulum Ca^{2+} leak that cannot be fully prevented by standard beta-adrenergic receptor blockade.

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TH-073

Melatonin protects against low potassium induced ventricular fibrillation: role of melatonin receptors activation and connexin-43.

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Background: Hypokalemia, a common electrolyte abnormality in clinical practice, enhances the propensity for ventricular fibrillation (VF). Melatonin up-regulates the gap junction channels protein, connexin-43 (Cx43), rendering the heart more resistant to electrically-induced VF. We hypothesized that melatonin may protect against low potassium induced VF in part by affecting Cx43 through the activation of its membrane receptors.
Methods: Isolated rat hearts underwent 10 min of Krebs-Henseleit perfusion (4.5 mEq/L K^+) followed by K^+-deficient (1 mEq/L) perfusion in presence of 100 µM melatonin, a melatonin receptor blocker (luzindole 5 µM), luzindole+melatonin or the vehicle of the drugs. Low K^+ perfusion was maintained 25 min unless VF occurred earlier. Two min VF was followed by normokalemic perfusion aimed to restore sinus rhythm. Incidence of arrhythmias and heart function were registered and analyzed using BiolabF software. Ventricular tissue analysis was performed for Cx43 expression and distribution.
Results: Melatonin was the only treatment that reduced the incidence of low K+-induced VF from 100% (vehicle 15/15; luzindole+melatonin 10/10; and luzindole 8/8) to 69% (9/13) (P=0.0349 vs vehicle by Fisher test) and delayed the occurrence of VF to 12 min (9-25 IQR) from 7 min (5-12 IQR) in vehicle group (P=0.041). Luzindole and luzindole+melatonina developed VF at a median of 6 min (4-11 IQR) and 8 min (6-14 IQR), respectively. resulted in a faster recovery of sinus rhythm restitution (P=0.047). Melatonin, luzindole or luzindole+melatonin did not affect heart rate, PR and QT intervals as well as the incidence of transient arrhythmias. The levels of total Cx43 was not changed by any treatment, however, melatonin prevented dephosphorylation and abnormal topology (lateralization) of Cx43.
Conclusions: Our results suggest that acute treatment with melatonin protects against low potassium induced VF in part due to prevention of abnormal expression and distribution of myocardial Cx43 mediated by melatonin receptors activation.

TH-074

Restoration of p21-activated Kinase Activity Attenuates Atrial Arrhythmia in a Dog Model of Atrial Fibrillation

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Introduction: The mechanisms underlying the genesis of atrial fibrillation (AF) are not fully understood. Activation of the small GTPase Rac1 through production of reactive oxygen species (ROS) is believed to contribute to the development of an AF substrate. We identified Pak1 as an endogenous negative regulator of Rac1 and hypothesized that stimulation of Pak1 activity attenuates atrial arrhythmia by antagonizing ROS induced changes in Ca handling. **Method:** Tissue and isolated myocytes (left superior pulmonary vein, LSPV) were obtained from dogs with persistent AF (atrial tachypacing, 600bpm, 3 weeks) or sinus rhythm (SR) and changes in Pak1 expression were quantified by western blotting. Changes in [Ca]i (fluo-4/AM) or ROS (fluorescent2',7'-dichlorofluorescein, DCF) were monitored by confocal microscopy in isolated atrial myocytes (AMs). AMs from WT and Pak1-/- mice were used to determine the mechanism by which a decrease in Pak1 enhances arrhythmic activity. **Results:** For the first time we demonstrate that Pak1 is down regulated in the atria of the canine AF model (adjusted density: SR: 85.6±7.2 % vs. AF 50±8.1%, n=3; p<0.05) and that this decrease is mimicked in an in vitro AF model (HL-1 + AngII(24h); Ctrl: 109±5.7% vs. AngII: 78.9±6.6%; p<0.05). ECGs in conscious mice revealed increased atrial arrhythmic events in Pak1-/- mice and an increased number of delayed after depolarizations during Ctrl and AngII stimulation in isolated Pak1-/- AMs. On a cellular level Rac1 stimulation by AngII (1microM) induced exaggerated ROS production in Pak1-/- AMs (DCF(au): WTAngII: 4572±487, n=20 vs. Pak1-/-AngII: 11231±838, n=16, p<0.05) and an enhanced increase in [Ca]i (F/F0: WTAngII: 3.4±0.4 n=6 vs. Pak1-/-AngII: 4.1±0.4 n=9,

p<0.05). In isolated WT AMs the AngII induced increase in ROS and DADs were attenuated by stimulation of Pak1 activity with the sphingosine 1 phosphate receptor agonist FTY720 (200 nM) and in canine AMAFs prevented the AngII induced increase in DADs that was based on spontaneous Ca release. **Conclusion:** In AF ROS production is enhanced by down-regulation of Pak1, an endogenous negative regulator of Rac1. Restoring Pak1 activity could be a therapeutic strategy to attenuate ROS induced arrhythmia and remodeling.

TH-075

Protein factor damage like vector prediction of acute coronary syndrome complicated by acute heart failure

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Introduction. In the world of cardiac mortality in the share accounted for about 60%. PAPP-A - marker reflecting processes scratch in the atherosclerotic plaque.

The purpose of the comparative analysis PAPP-A acute coronary syndrome, the impact of PAPP-A and prognosis.

Material and methods. The study included 71 patients with acute coronary syndrome, the average age of 57. The plasma was determined PAPP-A. The blood sampling was carried out at the time of admission. The control group of 20 healthy individuals. A comparison group of 40 patients with hypertension and coronary heart disease with stable forms.

Results. PAPP-A infarction acute phase STEMI was the highest 27.75±11.75 and close to the mortality rate for 27.7±7.1. PAPP-A infarction acute phase non STEMI were slightly below 22.12±7.69, but very significantly higher (p<0.05) than in patients with unstable angina-8.22±3,16. Increased IGF-I in all cases of myocardial infarction. The negative correlation between PAPP-A and the cases of death from acute coronary insufficiency-the levels of PAPP-A the highest. All patients with myocardial infarction at admission had decorated complications of acute period. PAPP-A is associated with increased severity of acute heart failure. Several lower concentration of PAPP-A in Killip-IV (n=5) 24,20±12,09, than Killip-III (n=32) 26,31±11,27, probably due to the small number of patients with Killip-IV (n = 5) in this study.3 patients - death during the day. In patients who died concentration of PAPP-A was made 26-26-27 IU/L.

Conclusion. PAPP-A is not only a marker of atherosclerotic instability

plaques, but probably the marker of massive damage to the coronary arteries, which leads to the increase of acute heart failure. That is, a significant increase in PAPP-A is a poor prognostic sign indicating massive necrosis of cardiomyocytes and is associated with poor outcome.

TH-076

The Mitochondrial Calcium Uniporter is a therapeutic target in the hypoxia/reoxygenation injury

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Introduction: The alteration of the intracellular Ca^{2+} homeostasis and energy production are important pathogenic mechanisms in HF. These mechanisms are leading by mitochondrial Ca^{2+} overload carried out by the MCU. For a long time, the molecular characterization of the MCU was limited, but this situation changed recently and allows to reveal in greater detail their involvement in the pathophysiology of myocardiac diseases.

Methods: Specific siRNA targeting MCU was used to transiently silence the MCU expression in cardiac myoblast. The MCU mRNA expression was measured using qRT- PCR and the protein levels of the MCU and its regulatory proteins were determined by W. blot analysis. Later, MCU silenced cells was exposed to hypoxia/reoxygenation protocol. Necrosis, apoptosis, $\Delta\psi_m$ and mPTP were determined by flow cytometry and confocal microscopy. In addition, MCU, MICU1 expression was measured from samples of human HF-LV tissues at the time of heart orthotopic transplantation or the LVAD insertion in patients with HF.

Results: MCU expression decreased by 65% with a consequent decrease in mitochondrial Ca^{2+} transport. MCU silencing

effects reduced the hypoxia/reoxygenation injury in myoblasts decreasing necrosis and apoptosis by 30 and 20%, respectively *vs* control after 3 hours of reoxygenation, with a reduction in caspases 3, 7 activity. In the human tissue, MCU expression was significantly elevated in HF compared with non-failing left ventricular samples. In addition, the mitochondrial protein MICU1 witch interacts with the uniporter pore-forming subunit MCU was 2-fold over-expressed.

Conclusions: The hypoxia/reoxygenation injury reduction suggest that MCU has a main role in post-ischemic cardiac dysfunction. Morover, the overexpression of MCU and MICU1 could be mediated mitochondrial calcium overload and cardiac dysfunction in HF. Overall, the pharmacological inhibition of MCU or MCU knockdown could be a therapeutic approach used to prevent calcium overload, which induces injury in several pathologies such ischemia/reperfusion, cardiac arrhythmias and HF.

TH-077

Carbonic anhydrase inhibition by benzolamide attenuates myocardial ischemia/reperfusion injury via p38MAPK-dependent mechanism

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Carbonic anhydrase (CA) catalyze the hydration of CO_2 to H+ and HCO_3^-. During ischemia-reperfusion CO_3H^--dependent transporters participate of the intracellular pH (pHi) regulation, leading to Ca^{2+} overload. The involvement of CA in reperfusion injury has not been elucidated yet. Isolated rat hearts were submitted after 20-min stabilization to the following protocols: 1.-Ischemic control (IC): 30 min of global ischemia (GI) and 60 min of reperfusion (R); 2.- BZ: the CA inhibitor benzolamide (5 µM) was administered during the initial 10 min of R. To examine the participation of p38MAPK, SB202190 (10 µM) was perfused simultaneously to BZ. Infarct size (IS) was measured by TTC staining technique. Left ventricular developed pressure (LVDP), +dP/dtmax, left ventricular end diastolic pressure (LVEDP) and -dP/dtmax served to assess myocardial function. The p38MAPK expression was measured. The changes of pHi in papillary muscle by immunofluorescence were also determined. BZ decreased the IS (6.3 ± 0.6 % vs 32 ± 2 %, $p < 0.05$) and improved postischemic recovery of myocardial function. At the end of R LVDP was 69 ± 4 % vs. 15 ± 4 %; +dP/dtmax: 75 ± 5 % vs. 19 ± 5 %; LVEDP: 23 ± 3 vs. 52 ± 5 mmHg; -dP/dtmax: 72 ± 5 % vs. 17 ± 5 %, $p < 0.05$). The p38MAPK level increased after BZ treatment (189 ± 3 % vs. 53 ± 1 %, $p < 0.05$). BZ annulled pHi recovery from sustained intracellular acidosis (JH+ at pHi 6.8 in control was 0.102 ± 0.004 mmol/L x min-1). SB attenuated all the effects detected by BZ. The present data demonstrate that CA inhibition by BZ protects the heart against reperfusion injury through a p38MAPK-dependent pathway and suggest that an attenuation of Ca^{2+} overload could be the responsible mechanism.

TH-078

Phospholamban ablation rescues reperfusión arrhythmias in hearts with Ca/calmodulin kinase II constitutive phosphorylation of ryanodine receptors, but not myocardium infarction.

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CaMKII-dependent phosphorylation of ryanodine receptors (RyR2) at the onset of reperfusion has been previously associated with an increase in cardiac damage and Ca-triggered arrhythmias (Di Carlo et al., 2014, Said et al., 2011). However, whether increasing SR Ca^{2+} uptake/load would also protect against cardiac damage and Ca^{2+}-triggered arrhythmias or exacerbate them, is unknown and difficult to predict, since the decrease in SR Ca^{2+} uptake was associated with a decrease in cytosolic Ca^{2+} but produced an increase in SR Ca^{2+} leak. Hypothesis: Increasing SR-Ca^{2+} uptake by ablation of phospholamban (PLN) rescues reperfusion arrhythmias but fails to protect against cardiac damage in a

mice model with constitutive CaMKII pseudo-phosphorylation of RyR2 (S2814D mice), linked to reperfusion arrhythmias and exacerbated infarct size. We developed SDKO mice by crossbreeding PLNKO with S2814D mice. At baseline, S2814D and SDKO mice have structurally normal hearts without arrhythmias. However, after a period of global ischemia (15 minutes) only S2814D mice developed ectopic beats (6/7 vs. 1/7 in SDKO mice, $P<0.05$). In contrast, hearts from SDKO exacerbate infarct size (23.2 ± 0.9 % of risk area, n=5) after a short ischemic period (15 min), not only with respect to S2814D hearts (10.8± 2.2%, n=5), but also when compared to PLNKO hearts (14.3 ± 2.0 %, n=6). Conclusions: Improving SR Ca^{2+} uptake by PLN ablation prevents the arrhythmic events triggered by CaMKII-dependent increase in SR Ca^{2+} leak but exacerbates cardiac damage. The results underscore the benefits of increasing SERCA2a activity on reperfusion arrhythmias but reveal a detrimental effect of increasing SR Ca^{2+} uptake on cardiac injury.

*similar contribution to the present work

TH-079

The use of synthetic wine to delineate the cardioprotective components in red wine

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Background: Moderate and chronic consumption of red wine protects against cardiovascular disease. Wine is a complex matrix containing multiple molecules whose concentrations can vary from one bottle to another. Therefore, the delineation of the cardioprotective components in wine such as alcohol, resveratrol and melatonin is very challenging when using commercially available red wine.

Aim: We used synthetic wine whose composition is well characterized to explore whether the presence of alcohol, resveratrol and melatonin (as found in commercial wine) contributes to the cardioprotective effect of chronic moderate (2 glasses wine/day) consumption of red wine.

Methods: The drinking water of male Long Evans rats was supplemented with synthetic wine (12% v/v) with/without resveratrol (100µg/L) and/or melatonin (0.075µg/L) to a final concentration corresponding to the concentration found in 2 glasses of wine per day. After 14 days of treatment, hearts were perfused on the Langendorff system and subjected to 30 minutes global ischemia (I) followed by 60 minutes of reperfusion (R). Functional parameters were recorded throughout the experiments and infarct size was measured at the end of the protocol. Functional recovery (heart rate x left ventricular developed pressure) was expressed as a percentage of baseline value.

Results: Control hearts subjected to I/R presented a functional recovery of 11±2%. Pre-treatment with synthetic wine with/without melatonin or resveratrol did not improve functional recovery (15±6%,12±1%, 19±4% respectively, n.s. vs control). However, addition of both melatonin and resveratrol in synthetic wine improved functional recovery to 32±5% ($p<0.01$ vs control). Additionally, synthetic wine enriched with both melatonin and resveratrol significantly reduced the plasma total antioxidant capacity compared synthetic wine only ($p<0.01$, Trolox equivalent: 1.1±2.9µmol/mL vs. 15.2±3.6µmol/mL).

Conclusion: In conclusion, our data strongly suggest that the presence of melatonin and resveratrol in wine is required for cardioprotection with chronic moderate consumption of wine.

TH-080

Simulated ischemia does not mimic stop flow ischemia in perfused mouse hearts

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Introduction: Cardiac ischemia is a pathological condition in which the blood supply to the myocardium is interrupted. This loss of circulation leads to the impairment of cardiac mechanical and electrical function. Still, the role of Ca2+ underlying these dysfunctions is not fully understood. To identify the ionic alterations that occurred during ischemia, several laboratories appealed to the use of ischemia-like conditions (hypoxia-metabolic inhibition-acidification), in isolated myocytes. However, whether simulated

ischemia (SI) actually mimics stop-flow ischemia (SFI) at the cellular level, has not been previously explored and is the aim of the present work. **Methods**: Hearts from Balb/c mice were perfused (Langendorff technique), at constant flow/temperature. Left-ventricle developed pressure, LVDP, and LV-end-diastolic pressure, LVEDP, were assessed with a latex balloon connected to a pressure transducer. Cytosolic Ca2+ was assessed in Rhod-2-loaded hearts in a pulsed-local-field fluorescence microscope. Action potentials (AP) were registered with microelectrodes. Phosphorylation of phospholamban (PLN), known to occur at the onset of reperfusion, was assessed by western blot. Hearts were submitted to 15min of either SFI or SI (pH 6.2, absence of glucose, N2 instead of O2). **Results**: SI produced a milder mechanical dysfunction than SFI. Similarly, Ca2+ transient and AP amplitude were lower during ischemia in SFI than in SI. Upon reperfusion, the mechanical recovery of LVDP was significantly more pronounced in SI than in FR-hearts (LVDP: 48.5±6.2 vs. 11.9±6.6% of preischemic value; LVEDP: 29.0±5.5 vs. 56.6±3.1mmHg, respectively), whereas PLN phosphorylation by CaMKII at early reperfusion was higher in SFI than in SI hearts (812±46 vs. 155±26%). No changes were observed in PLN-Ser16-phosphorylation in any of these conditions. **Conclusion**: SI generates a milder alteration of mechanical and Ca2+ handling when compared to SFI. The findings indicate that SI results have to be interpreted with caution and underscore the use of SFI to assess intracellular Ca2+ during ischemia/reperfusion.

TH-081

Reversible redox modifications of ryanodine receptor ameliorate ventricular arrhythmias in the ischemic-reperfused heart

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Background: Previous results from our laboratory showed that phosphorylation of ryanodine receptor-2 (RyR2) by Ca-calmodulin-dependent kinase II (CaMKII) was critical but not the unique event responsible for ventricular arrhythmias following ischemia/reperfusion (I/R). Oxidative stress is a prominent feature of I/R injury and both, CaMKII and RyR2 are subject to oxidation. **Aim**: The present study was designed to elucidate the contribution of redox changes in CaMKII and RyR2 on reperfusion arrhythmias. **Methods:** Isolated perfused rat hearts were subjected to I/R (20/30min) in the presence or absence of inhibitors of NADPH oxidase (Apocynin) and nitric oxide synthase (L-Name) and a free radical scavenger (MPG). Contractile and electrical parameters were recorded to detect ventricular premature beats (VPBs). CaMKII oxidation and S-nitrosylation, S-glutathionylation and free thiol levels (mBB) of RyR2 were assessed together with glutathione content (GSH) and ROS production (DHE).

Results: CaMKII was oxidized in early reperfusion but this modification had no consequences in enzyme activity or RyR2 phosphorylation. In addition, I/R induced an increase in the reversible RyR2 oxidations: S-glutathionylation and S-nitrosylation. Accordingly, free thiols on RyR2 decreased. Pre-treatment with Apocynin and L-Name selectively abolished S-glutathionylation and S-nitrosylation of RyR2, respectively, and increased VPBs in I/R: (I/R: 44±14, Apocynin: 58±6 and L-Name: 64±16 VPBs, $p<0.05$). Conversely, MPG diminished VPBs (24±5) with the simultaneous prevention of both reversible RyR2 oxidations. The discrepancy in the effects of the drugs could be explained by their differential ability to influence the nitroso/redox balance: only MPG was effective to preclude the I/R-induced reduction in GSH levels. This result was confirmed by the decreased I/R-induced ROS production and the recovery of RyR2

free thiol level towards pre-ischemic values elicited by the scavenger.

Conclusions: Oxidation of RyR2 contributes to arrhythmogenesis in I/R. The selective suppression of S-glutathionylation and S-nitrosylation of RyR2 in an oxidative environment allowed us to unmask a protective role of these redox alterations which counterbalanced pro-arrhythmogenic oxidations of RyR2.

TH-082

Extracellular HSP27 and TLR4 exaggerate functional injury in aging hearts following ischemia

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Background: While cardiac functional recovery is poor in the elderly following cardiac surgery with obligatory global myocardial ischemia/reperfusion (I/R), the underlying mechanism remains incompletely understood. We found recently that human and mouse myocardium releases HSP27 during global I/R, and extracellular HSP27 plays a role in post-ischemic inflammatory response in adult mouse hearts.

Objectives: The aim of this study was to determine the role of extracellular heat shock protein (HSP) 27 and Toll-like receptors (TLRs) in cytokine production and functional injury caused by global I/R in aging hearts.

Methods and Results: Isolated hearts from aging (18-24 months) and adult (4-6 months) mice were perfused by the Langendorff system and subjected to global normothermic I/R (20 min/120 min). Augmented release of HSP27 in aging hearts preceded greater production of cytokines (MCP-1, KC, IL-6 and TNF-alpha) and worse functional recovery. Anti-HSP27 suppressed the inflammatory response and markedly improved functional recovery in aging hearts. Perfusion of recombinant HSP27 to aging hearts resulted in greater cytokine production and contractile depression. TLR2 KO and TLR4 deficiency, particularly the latter, markedly reduced cytokine production and contractile dysfunction in aging hearts exposed to recombinant HSP27. Interestingly, aging hearts displayed enhanced NF-kappaB activation following TLR4 stimulation.

Conclusion: Enhanced myocardial inflammatory response to global I/R in aging mouse hearts is due, at least in part, to augmented myocardial release of HSP27. Extracellular HSP27 up-regulates myocardial cytokine production and depresses cardiac contractility through TLR2 and TLR4. Augmented HSP27 release and enhanced myocardial TLR4 responsiveness jointly play an important role in the greater inflammatory response and worse post-ischemic functional recovery in aging hearts.

TH-083

Non-nuclear estrogen receptor activation reduces cardiac ischemic-reperfusion injury in mice with cardiac specific ablation of ER-alpha

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Introduction-Steroid hormone receptors, ERα and ERβ, function as regulated transcription factors. However, recent data indicate that estrogen can also elicit effects through binding to estrogen receptors (ERα, ERβ and GPR30) at the plasma membrane and initiate kinase signaling. We investigated the hypothesis that non-nuclear ER activation reduces cardiac ischemia-reperfusion injury in mice. We also addressed the role of cardiac ERα signaling using a cardiac-specific ERα knock out mouse.

Results-We treated ovariectomized wild type mice with estrogen or an estrogen-dendrimer conjugate (EDC), which has been demonstrated in mice to be a non-nuclear selective ER modulator, or dendrimer control for two weeks. Ischemia-reperfusion injury was evaluated in isolated Langendorff perfused hearts. Two weeks of treatment with estradiol significantly decreased infarct size and improved post-ischemic contractile dysfunction (40.4±2.5% vs. 62.9±5.8% for infarct and 44.7±4.0% vs. 27.0±2.7% for post-ischemic functional recovery). Similarly, EDC treatment significantly decreased infarct size

(40.9±3.6% for EDC vs 63.8±4.7% vehicle) and increased post-ischemic functional recovery (48.8±3.0% EDC vs. 28.6±2.5% vehicle) compared to vehicle-treated hearts. To test if ERα was involved in cardioprotection, we generated cardiac-specific ERα knockout mice. In these mice, EDC treatment significantly decreased infarct size (20.1±1.9% vs. 51.2±7.8% dendrimer) and improved functional recovery (65.8±4.2% vs. 36.8±5.2% dendrimer) compared to vehicle-treated ERα knockout mice. Interestingly, EDC protection was significantly higher in ERα knockout compare to the wild-type hearts. Moreover, treatment with a ICI 182 780, a selective inhibitor of ERα and ERβ and an activator for GPR30 significantly blocked the EDC mediated cardioprotection.

Conclusion-These results indicate that EDC is effective in providing cardioprotection during ischemia-reperfusion injury in mice, by a mechanism that does not require cardiac ERα or GPR30. Thus, EDC could be utilized clinically to provide cardiovascular benefit without the classical steroid hormone side effects, such as uterine and breast cancer.

TH-084

Xenon administration at reperfusion protects against myocardial infarction in the *in vivo* mouse heart: insight into the mechanism

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Xenon is a noble gas with favourable physical, chemical, and pharmacological properties to serve as an ideal anaesthetic. In previous studies, it was demonstrated that volatile anaesthetics offer specific protection against myocardial reperfusion injury. We investigated whether xenon, administered at the onset of reperfusion, protects the mouse heart from reperfusion injury *in vivo*. Moreover, as its mechanisms of action are still uncertain, we are exploring whether xenon protection works by preventing ROS production.

C57BL6/J male mice (8-10 weeks) were subjected to 30 min occlusion of the left anterior coronary artery followed by 120 min reperfusion. During the last 15 min of ischaemia and the first 10 min of reperfusion, mice were treated with inhaled 70% xenon/30% oxygen, whilst control mice inhaled 70% nitrogen/30% oxygen. Infarct size was determined at the end of the reperfusion period by using triphenyltetrazolium chloride staining. Xenon reduced infarct size from 40.8% ± 3.3% of the area at risk in controls to 27% ± 1.5% (**p<0.01). Further work is being carried out to assess changes in the levels of hydrogen peroxide within mitochondria by using the well-established hydrogen peroxide probe, MitoB, *in vivo* and Amplex Red assay *in vitro*. The effect of xenon on the activity of mitochondrial Complex I *in vitro* is also being examined.

TH-085

PKG-dependent inhibition of endoplasmic reticulum stress contributes to protective effects of vasonatrin peptide against myocardial ischemia/reperfusion injury in diabetic rats

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Aims: Diabetes mellitus (DM) increases morbidity/mortality of ischemic heart disease. Although the ability of the natriuretic peptides to modulate cardiac function and cell proliferation has been recognized, their effects on myocardial ischemia/reperfusion (MI/R) injury is still unclear. This study was to investigate the effects of the artificial synthetic natriuretic peptide — vasonatrin peptide (VNP) on MI/R injury in diabetic rats, and underlying mechanisms. **Methods:** The high-fat diet-fed streptozotocin (HFD-STZ) induced diabetic rats were subjected to MI/R (30 min/4 h) and VNP treatment (100 μg/kg, i.v., 10 min before R). In vitro study was performed using H9c2 cardiomyocytes subjected to hypoxia/reoxygenation (H/R, 3 h/6 h) and incubated with or without VNP (10^{-8}mol/L). **Results:** The diabetic state aggravated MI/R injury and showed more severe myocardial functional impairment than normal state. VNP treatment (100 μg/kg, i.v., 10 min before R) significantly improved ± LV dP/dtmax and LVSP, and decreased infarct size, apoptosis index, caspase-3 activity, serum CK and LDH levels (n=8, P<0.05). Moreover, VNP inhibited endoplasmic reticulum (ER) stress by suppressing GRP78 and CHOP, and consequently increased Akt and ERK1/2 expression and phosphorylation levels

(n=3, *P*<0.05). These effects were mimicked by 8-Br-cGMP (1 mg/kg, i.p., 20 min before R), a cGMP analogue, whereas inhibited by KT-5823 (0.5 mg/kg, i.p.), the selective inhibitor of PKG (*P*<0.05). Pretreated DM rats with TUDCA (50 mg/kg, i.p.), an inhibitor of ER stress, couldn't further promote the VNP's cardioprotective effect. Additionally, gene knockdown of PKG1α with siRNA blunted VNP's inhibition of ER stress and apoptosis, while overexpression of PKG1α resulted in significantly decreased ER stress and apoptosis in H/R H9c2 cardiomyocytes (n=6, *P*<0.05). **Conclusions:** We demonstrated that VNP protects diabetic heart against MI/R injury by inhibiting ER stress via cGMP-PKG signaling pathway.

Keywords: Natriuretic peptide; Diabetes; Myocardial ischemia/reperfusion; Vasonatrin peptide

TH-086

Ischaemic preconditioning protects the heart against ischaemia-reperfusion injury without affecting ischaemic succinate accumulation and metabolism

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Ischaemia-reperfusion (IR) injury occurs when blood supply to an organ is disrupted and then restored, and underlies many disorders, notably myocardial infarction and stroke. While reperfusion of ischaemic tissue is essential for survival, it also initiates cell death through generation of mitochondrial reactive oxygen species (ROS). Recent work has revealed a novel pathway underlying ROS production at reperfusion *in vivo* in which the accumulation of succinate during ischaemia and its subsequent rapid oxidation at reperfusion drives ROS production at complex I by reverse electron transport (RET). Pharmacologically inhibiting ischaemic succinate accumulation or slowing succinate metabolism at reperfusion has been shown to be cardioprotective against IR injury. Here, we aimed to establish if ischaemic preconditioning (IPC), as part of its cardioprotective mechanism, acts via manipulating succinate kinetics during IR in an *in vivo* mouse model. Mice were subjected to 30 min occlusion of the left anterior descending coronary artery followed by 1 min reperfusion with or without an IPC protocol of 3 cycles of 5 min ischaemia, 5 min reperfusion prior to sustained ischaemia. The left ventricle was then rapidly isolated and analysed by mass spectrometry based-metabolomics to determine the effect of IPC on myocardial succinate levels. Data revealed that IPC had no effect on ischaemic succinate accumulation with both control and IPC mice having increases in succinate of 2.79 and 2.54 fold respectively compared to normoxia. Analysis of hearts after only 1 min reperfusion revealed that succinate was rapidly metabolised returning to near pre-ischaemic levels. IPC had no significant effect on succinate metabolism at reperfusion. These findings suggest that IPC does not affect ischaemic succinate accumulation or its metabolism at reperfusion. Further work is being carried out to determine if IPC affects RET-mediated ROS production downstream of succinate by inhibiting the re-activation of complex I at reperfusion.

TH-087

Hypothyroidism reduces cardiac stunning with a mitochondrial regulation of sarcoreticular Ca^{2+} leak: a mechano-energetical study

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Hypothyroidism (HypoT) may be a cardiac risk for angor. In this work, its influence on the energetics during cardiac stunning by ischemia-reperfusion (I/R) was studied in rats. HypoT was induced by drinking methimazole (0.02%) for 15 days. Isolated hearts were perfused inside a calorimeter at 37°C to measure left ventricular pressure (LVP, in mmHg) and total heat flow (Ht, in mW) during exposition to 20 minutes I and 45 minutes R. Protocols were done in HypoT and euthyroid (EuT) rats. HypoT improved the postischemic contractile recovery (PICR) to 92±5% vs 69±6% in EuT ($p<0.05$) and reduced diastolic contracture (ΔLVEDP) without changing muscle economy (P/Ht). When ischemic

hearts were reperfused with Krebs-36 mM Na^+-caffeine 10 mM (to release SR Ca^{2+} minimizing the NCX efflux) the initial rise of contracture was decreased in HypoT (+27.3±1.6) vs EuT (44.1±4.8 mmHg) without changing the area under curves (AUC-ΔLVP and AUC-Ht). When hearts were pretreated with 10 µM clonazepam (Clzp, mNCX inhibitor), PICR and P/Ht were reduced to 36.7±6.4% and 38.4±7.4% respectively, and ΔLVEDP was increased to 86±15 mmHg in HypoT. Contrarily, Clzp cardioprotected EuT hearts. Cyclosporine A (Cys-A, 0.2 µM) slightly improved PICR and P/Ht but increased ΔLVEDP in HypoT hearts pretreated with Clzp.

Isolated cardiomyocytes loaded with Fluo-4 (free cytosolic Ca^{2+}) or with Rhod-2 (free mitochondrial Ca^{2+}) were exposed to Krebs-36 mM Na^+-caffeine 10 mM with or without Clzp. F/Fo-Fluo-4 increased and fell more quickly in HypoT than EuT cells. F/Fo-Rhod-2 increased and decreased more quickly in HypoT than in EuT, and Clzp raised it.

Results suggest that: a) HypoT reduced the stunning, b) mitochondria were more sensitive in HypoT than in EuT, contributing through the mNCX, to regulate the SR store and leak to cytosol, c) blocking the mNCX in HypoT becomes in contractile dysfunction (not in EuT) with mPTP opening.

TH-088

The way of administration makes a difference in the effects of genistein on cardiac stunning: mechano-energetical study

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Although genistein (Gen) could prevent cardiovascular diseases, its effects on cardiac ischemia are contradictory. A previous work showed sex and temperature-dependence on Gen effects, participating the inhibition of tyrosin-kinases (TK), blockade of Ca^{2+} influx and mitochondrial uptake, and increase of SERCA activity (Colareda et al. 2016). Now, we compared the effects of administering 5 mg/kg Gen via IP 24 h before the experiment (Gen-IP), with those of perfusing 20 µM Gen before stunning (Gen-BS). Two models of stunning were assessed: no-flow ischemia/reperfusion (I/R) and hypoperfusion/reperfusion (Hip/R). In both cases, isolated rat hearts were perfused at 6 ml/min inside a calorimeter at 37°C to measure left ventricular pressure (LVP, in mmHg) and total heat flow (Ht, in mW) throughout the experiment.

During Hip/R Gen-BS did not change the post-ischemic contractile recovery (PICR, 23.5±8.3%), reduced total muscle economy (P/Ht) and increased the diastolic contracture (ΔLVEDP). Contrarily, in male rats Gen-IP improved PICR (86.4±12.6%) and P/Ht, but also increased ΔLVEDP during Hip/R. Perfusing 10 µM clonazepam (Clzp, mNCX inhibitor) improved PICR (55.5±11.1%) and P/Ht during Hip, and reduced ΔLVEDP. Nevertheless, addition of Gen to Clzp before Hip/R reduced PICR (19.9±2.7%) and increased ΔLVEDP (+41.4±8.4 mmHg during R). Ciclosporine-A (Cys-A) reduced this dysfunction except the diastolic contracture, suggesting that Clzp+Gen induced a Ca^{2+} overload that triggered the mPTP activation.

In the severe stunning by I/R, administration of Gen-IP also improved PICR (50,8±4,9%) and P/Ht, more in male than in female rat hearts, although it increased ΔLVEDP in both. These effects were not modified by ortho-vanadate 15 mg/kg.

Results suggest that there is an agonistic interaction between Gen and endogenous estrogen on receptors: Gen is more cardioprotective in males, which had low exposition to estradiol, than in females. The inhibition of TK don´t participate in the in vivo cardioprotection, but Gen increased the SR leak.

TH-089

Depression And Risk Of Cardiovascular Diseases In Men Aged 25-64 Years: Who Program Monica –Psychosocial

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Objectives: To examine the relationship between depression symptoms and the risk development of arterial hypertension (AH), myocardial infarction (MI) among men aged 25-64 years.

Methods: Within the framework of program WHO MONICA-MOPSY representative

sample of male population aged 25-64 years one of Novosibirsk district was examined in 1994. Total sample was 657 persons. Depression symptoms were measured with the use of the MONICA - psychosocial Interview Depression scale. The incidence of new cases of AH, MI was revealed over 14-year of follow-up. Cox - proportional regression model was used for an estimation of hazard ratio (HR).

Results: Prevalence of depression in cohort of men with AH was - 28.9%, with MI- 65.8%. The risk of AH within 5 years in group of men with high level of depressive symptoms, in compared with those with low depressive symptoms was 6.7 times higher, 10 years HR=4.2, 14 years HR=2.1.The risk of MI within 5 years HR=2.26, 10 years HR=2.4, 14 years HR=2.6 (p for all <0.05). Most frequently of cardiovascular diseases occurred in men with higher negative psychosocial factors, i.e. widowers, divorced, those with primary and not-completed secondary school education and those engaged in hard and moderate manual labor as well as pensioners.

Conclusion: Depression is a predictor of cardiovascular diseases in middle-age men. The risk of development of cardiovascular diseases in group of men with depression was 2.5- 6 times higher than without it.

TH-090

Ticagrelor prevented reperfusion arrhythmias in dysmetabolic rats

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BACKGROUND: Ticagrelor (TICA) is a potent inhibitor of the P2Y12 receptor. In addition, TICA increases adenosine concentration by interfering with its cellular reuptake. Higher tissular levels of adenosine during ischemia and reperfusion protects against ischemia-reperfusion injury. In this study, we used TICA to prevent cardiovascular remodeling and ventricular arrhythmias in Langendorff-perfused hearts from SHR rats fed with fructose to induce metabolic syndrome.

METHODS: Male WKY and SHR were separated into two groups whether receiving tap water or 10% (w/v) fructose solution during all 6 weeks and designated as fructose-fed rat (FFR) or fructose-fed hypertensive rat (FFHR), respectively. These four groups were divided into vehicle or TICA: (30 mg/kg intraesophageal for 6 weeks) (n=8 each group). Metabolic variables and systolic blood pressure were measured weekly through the 12 weeks. Cardiac and vascular remodeling were evaluated by macroscopic and microscopic measurements. Reperfusion ventricular arrhythmias were determined in Langendorff-perfused hearts after 10 min of regional ischemia. Connexin-43 and PKC-epsilon expression was determinate by western blot.

RESULTS: The FFHR experimental model presented metabolic syndrome criteria, vascular and cardiac remodeling. Chronic treatment with TICA reduced ventricular fibrillation incidence in all groups. Connexin 43 and its phosphorylated form by PKC-epsilon were reduced in the pathological models. TICA reversed changes in this pathway.

CONCLUSIONS: We conclude that FFHR model increases arrhythmogenesis and TICA protects against these changes. TICA preserve phosphorylated connexin 43, possibly stabilizing the intercellular communication and this effect is mediated by PKC.

TH-091

Administration of anabolic steroid during adolescent phase promote long-term increase in the susceptibility to myocardial ischemia/reperfusion injury: involvement of cardiac renin-angiotensin system and k_{atp} channel

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Anabolic steroids (AS) abuse between adolescents has been raising among occidental nations. Given that myocardial infarction is the most frequent cardiovascular report associated with AS abuse, we hypothesized whether administration of AS during pre/pubertal phase of Wistar rats promotes long-term increase in the susceptibility to ischemia/reperfusion (IR) injury in adulthood. Rats were treated with testosterone propionate (TP) (AS group, 5

mg.kg^{-1}, starting in the 26º postnatal day [PND], 5 days per week for 5 weeks) or vehicle (control group, peanut oil 10%, v:v). Rats were euthanized (82º PND) and heart, liver, lung, kidneys and testis were collected. Isolated-perfused hearts were submitted to IR protocol. Left Ventricle (LV) end diastolic pressure (LVEDP), LV systolic pressure (LVSP), LV developed pressure (LVDP), maximal (+) and minimal (-) first derivatives of pressure (dP/dt) were measured. Area of infarct was delimited with triphenyl tetrazolium. The expression of key genes associated with cardiac hypertrophy was analyzed through quantitative real time polymerase-chain reaction. Protein expression of Angiotensin-II type 1 receptor (AT1R) and Kir6.1 was analyzed via Western Blot. NADPH oxidase-dependent hydrogen peroxide production was analyzed through spectrofluorometry. TP significantly increased cardiac weight (P<0,001) and index (P<0,001), whereas testicular weight was reduced (P<0,001). Infarct size was increased by TP (P<0,05). TP impaired the recovery of LVDP, LVEDP, +dP/dt and –dP/dt in the reperfusion period. Myosin heavy chain β (βMHC) mRNA expression was enhanced in the AS group (P<0,01), likewise βMHC/αMHC ratio (P<0,001). AT1R expression was up-regulated (P<0,05) by TP, whereas Kir6.1 was down-regulated (P<0,01). Nox activity did not change between groups. Chronic administration of AS promotes long-term increase in the susceptibility to IR injury with abnormalities in the expression of cardiac AT1R and ATP-sensitive potassium channel.

TH-092

Novel software tools for crowdsourcing cardiac protein knowledge in Gene Wiki

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Background: Mitochondrial and sarcomeric biology are integral to our understanding of cardiac physiology and pathophysiology. A wealth of knowledge is available to experienced biomedical scientists, but its access and comprehension remain elusive to many, particularly the general public. The Gene Wiki project, an effort within Wikipedia, was established to bridge this gap and transform scientific knowledge from esoteric to common knowledge. However, many mitochondrial and sarcomeric proteins remain poorly represented and inadequately annotated.

Aims: The Cardiac Gene Wiki team at UCLA aims to build high-quality, interconnected Gene Wiki pages for genes expressed in cardiac muscle in order to inspire and facilitate crowdsourced annotation by experts in the scientific community as well as citizen scientists.

Methods: Cardiac-expressed genes were clustered into functional subproteomes, with an emphasis on core cardiac mitochondrial and sarcomeric proteins, comprising a total of 620 gene pages. Two tools were developed to enhance productivity and prioritize crowdsourcing efforts. The article assessment tool reports the quality of gene pages within Wikipedia by examining the presence of biologically relevant content and the number of semantic web links and peer-reviewed references. Secondly, a curation tool was designed to streamline PubMed database searches, a time-intensive aspect of scientific writing, by conducting simultaneous searches of user-defined keyword combinations.

Results: At the outset of this effort, the assessment tool revealed that only 5 of the 556 core mitochondrial proteins and 5 of the 64 sarcomeric proteins had relatively complete pages, while two-thirds were either missing pages or were grossly incomplete. To date, the Cardiac Gene Wiki team has improved over 400 articles and added 3,813 references, 11,163 Wiki links, and 2,682kB of content.

Conclusions: Together, these tools and crowdsourcing initiatives support the aggregation of unstructured knowledge from the biomedical literature and its organization into a structured, user-friendly format for a broad community of users.

TH-093

CTRP3 promotes the production of mitochondrial reactive oxygen species in vascular smooth muscle cells

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Background: C1q/tumor necrosis factor-related protein-3 (CTRP3) is an adipokine with modulation effects on metabolism and inflammation. This study aimed to explore the effect of CTRP3 on reactive oxygen species (ROS) production in vascular smooth muscle cells (VSMCs) and its underlying mechanism. **Methods:** Primary VSMCs were obtained from rat aorta and identified. Cellular ROS and mitochondrial membrane potential (MMP) in VSMCs were detected by confocal microscopy. ERK1/2 phosphorylation was measured by western blot analysis. **Results:** CTRP3 (2 μg/mL) significantly increased the levels of intracellular ROS, ERK1/2 phosphorylation and mitochondrial membrane potential in VSMCs. The CTRP3-induced ROS production was markedly inhibited by preincubation with N-acetyl-L-cysteine (NAC, 5 mmol/L), the ROS scavenger, or carbonyl cyanidem-chlorophenylhydrazone (CCCP, 10 μmol/L), an uncoupler of oxidative phosphorylation. However, diphenyleneiodonium (DPI, 10 μmol/L), the inhibitor of NADPH oxidase, or allopurinol (100 μmol/L), the inhibitor of xanthine oxidase, had no effect on CTRP3-induced ROS production. In addition, NAC significantly suppressed CTRP3-induced ERK1/2 phosphorylation, but blocking ERK1/2 signaling by U0126 (10 nmol/L), an ERK1/2 upstream kinase inhibitor, had no effect on CTRP3-induced ROS production. **Conclusion:** CTRP3 promoted mitochondrial ROS production, which in turn activated ERK1/2 signaling molecule in VSMCs. (This work was supported by grants 81470398 and 81100192 from National Natural Science Foundation of China).

TH-094

Cystathionine-gamma-lyase/hydrogen sulfide inhibitinging smooth muscle cells proliferation through regulating mitochondrial morphology in diabetic rat.

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BACKGROUND: Molecular gas hydrogen sulfide (H_2S) reduces the proliferation of vascular smooth muscle cells (VSMCs). Reactive oxygen species (ROS) overproduction induced by hyperglycemia and high glucose is involved in VSMC proliferation, which may cause mitochondrial fragmentation. Whether exogenous H_2S reduces ROS production, inhibits mitochondrial fragmentation, and decreases VSMC proliferation is unclear. **METHODS AND RESULTS:** The morphological and ultrastructural alterations of the mesenteric secondary artery loop in diabetic rats, changes in the H_2S concentration and the relaxation were determined. Additionally, the expression levels of CSE and Cyclin D1 in the mesenteric arteries of rats were examined by western blotting. The intracellular calcium concentration, the expression of p-CaMK II (phospho-calmodulin kinases II), CSE activity, the concentration of endogenous H_2S and the proliferation of cultured VSMCs from rat thoracic aortic smooth muscle cells(RASMCs) were measured by using confocal microscope, western blotting, MTT and BrdU, respectively. The VSMC layer thickened, the H_2S concentration dropped, the relaxation of the mesenteric secondary artery rings weakened, and the expression of CSE decreased whereas the expression of Cyclin D1 increased in diabetic rats compared with the control group. Exogenous H_2S (100 μM NaHS) reduces ROS production in the cytoplasm and mitochondria. Higher mitochondrial fusion-fission protein expression levels for dynamin-related protein 1 (Drp 1) in diabetic rats. When RASMCs proliferate with a high glucose treatment, the mitochondria become small spheres with a short rod-shaped structure, whereas NaHS, a mitochondrial division inhibitor and DrpsiRNA prevent VSMC proliferation and maintain mitochondria as stationary and randomly dispersed with fixed structures.**CONCLUSIONS:** Exogenous H_2S aid in inhibiting mitochondrial fragmentation and affect VSMCs proliferation by decreasing Drp 1 expression.

TH-095

Mitochondrial DAMPs in sterile inflammation after acute myocardial infarction

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Background: Acute myocardial infarction results in necrosis and initiation of a local sterile inflammation activated by Damage-Associated Molecular Patterns (DAMPs). Based on the endosymbiotic theory, mitochondria are of bacterial origin, displaying bacterial traits in their DNA and proteins. Moreover, the cardiomyocyte volume consists of 30% mitochondria. Our research group has recently shown that mitochondrial DNA induces cell death and activates the innate immune system in cardiomyocytes. In this study, we hypothesize that mitochondrial constituents in general, or *N*-formyl-peptides are detrimental for cardiac cells.

Methods: Cardiac mitochondria were isolated from C57BI6 male mice and this debris was utilized as agonists for isolated adult mouse cardiomyocytes and cardiac fibroblasts. The cardiomyocytes were stimulated with increasing concentrations of mitochondrial debris or the *N*-formyl-peptide receptor (Fpr) agonist fMLP. Cardiomyocytes and cardiac fibroblast were also exposed to 40 minutes hypoxia (1% O_2) and 2h re-oxygenation. Cell death was investigated with High-Throughput microscopy. The cardiac fibroblasts were exposed to mitochondrial debris and sampled in time intervals, and expression of cytokines was measured by qPCR.

Results: Cardiomyocytes exposed to normoxic conditions showed a significant increase in cell death when stimulated with 100µg/ml mitochondrial debris compared to control. In addition, hypoxic conditions increased cell death of cardiomyocytes at lower concentrations of mitochondrial debris (1 and 10µg/ml). Cardiac fibroblasts exposed to 10µg/ml mitochondrial debris showed a significant increase in Interleukin-6 mRNA expression after 1h compared to control.

Absolute quantification of *Fpr* genes revealed no expression in cardiomyocytes or cardiac fibroblasts. However, the receptors were expressed in mRNA extracted from PBS perfused mouse hearts possibly indicating presence of resident macrophages. Cardiomyocytes stimulated with fMLP showed no significant decrease in viability compared to control.

Conclusion: Mitochondrial debris reduced the viability of the cardiomyocytes, and gave an inflammatory response in cardiac fibroblasts. This response appears not to be mediated through Fpr.

TH-096

Alpha-MHC MitoTimer mouse: in vivo mitochondrial turnover model reveals remarkable mitochondrial heterogeneity in the heart.

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In order to maintain an efficient, energy-producing network in the heart, dysfunctional mitochondria are cleared through the mechanism of autophagy, which is closely linked with mitochondrial biogenesis; these, together with fusion and fission comprise a crucial process known as mitochondrial turnover. Until recently, the lack of molecular tools and methods available to researchers has impeded in vivo investigations of turnover. To investigate the process at the level of a single mitochondrion, our laboratory has developed the MitoTimer protein. Timer is a mutant of DsRed fluorescent protein characterized by transition from green fluorescence to a more stable red conformation over 48 hrs, and its rate of maturation is stable under physiological conditions. We fused the Timer cDNA with the inner mitochondrial membrane signal sequence and placed it under the control of a cardiac-restricted promoter. This construct was used to create the alpha-MHC-MitoTimer mice. Surprisingly, initial analysis of the hearts from these mice demonstrated a high degree of heterogeneity in the ratio of red-to-green fluorescence of MitoTimer in cardiac tissue. Further, scattered solitary mitochondria within cardiomyocytes display a much higher red-to-green fluorescence (red-shifted) relative to other mitochondria in the cell, implying a block in import of newly synthesized MitoTimer likely due to lower membrane potential. These red-shifted mitochondria may represent older, senescent mitochondria. Concurrently, the cardiomyocytes also contain a subpopulation of mitochondria that display a lower red-to-green fluorescence (green-shifted) relative to other mitochondria, indicative of germinal mitochondria that are actively engaged in import of newly-synthesized mito-targeted proteins. These mitochondria can be isolated and sorted from the heart by flow cytometry for further

analysis. Initial studies suggest that these mice represent an elegant tool for the investigation of mitochondrial turnover in the heart.

TH-097

Oncotic and apoptotic mechanisms of toxic cardiomyocyte injury: role of mitochondria and gene expression

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Mechanism of chemotherapy-induced cardiotoxicity was studied in primary cultures of cardiomyocytes (CMC) derived from mouse embryonic stem cells (ES) (Reach Bio LLC, Seattle, WA) exposed to sanguinarine (Sang) and doxorubicin (Dox). CMC exposed to Sang, 4 uM and 33 uM, for 2 hours, or Dox, 2uM and 20uM, for up to 24 hours displayed the morphologies of typical apoptosis (shrinkage) at the lower doses and oncosis (swelling) at the higher doses, in most CMC, respectively. In CMC loaded with the cationic green fluorochrome rhodamine 123 (rh 123), mitochondrial membrane potential at 2 hours was maintained in apoptotic CMC but was markedly reduced in oncotic CMC. To identify genes altered in oncosis vs. apoptosis, high density microarray analysis on RNAs prepared from CMC was performed using Illumina Beadchips. Sang altered the expression of 2514 probes at the higher oncosis-inducing dose and 1643 probes at the lower apoptosis-inducing dose ($p < 0.001$), indicating the differential involvement of multiple biochemical and signaling pathways. With high dose Sang, perforin, a cytolytic protein found in CD8 T cells and NK cells, was induced more than 11-fold. Silencing of perforin gene by RNA interference demonstrated salvage of CMC confirming the involvement of perforin in Sang-induced oncosis. Compared to low dose Sang (n=4), high dose Sang (n=8) changed expression of 286 genes of canonical pathways after 1 hour ($p < 0.01$, with a false discovery rate of 0.05), particularly mitochondrial genes: citrate cycle ($p = 5.0 \times 10^{-4}$)(6/30 genes), mitochondrial function ($p = 5.1 \times 10^{-4}$)(12/130 genes), and oxidative phosphorylation ($p = 3.55 \times 10^{-3}$) (11/150 genes). Thus, CMC exhibit a biphasic injury response to low and high dose Sang and Dox characterized by apoptosis and oncosis with differential gene expression and rate of mitochondrial impairment.

TH-098

Monoamine oxidases are major contributors to mitochondrial ROS formation and dysfunction, and cardiac damage in diabetic cardiomyopathy

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Recent studies highlight the important role of monoamine oxidases (MAOs) in the oxidative stress and cardiovascular damage. Reactive oxygen species (ROS) and inflammation play a major role in the pathogenesis of diabetes, but so far the involvement of MAO in these processes has been overlooked. Thus, we investigated whether MAOs contribute to high glucose (HG) and inflammation induced oxidative stress as well as mitochondrial dysfunction in vitro and cardiac damage in type 1 diabetes (T1D) in vivo. Neonatal rat ventricular myocytes (NRVMs) displayed a significant increase in mitochondrial ROS formation and loss of mitochondrial membrane potential when exposed to HG. Moreover, co-treatment with HG and interleukin-1β (IL-1β), a pro-inflammatory cytokine found to be elevated in diabetes, further increased mitochondrial ROS levels. MAO inhibitor pargyline reduced ROS formation in both conditions, suggesting that HG and IL-1β induce oxidative stress in a MAO-dependent manner. Interestingly, mitochondrial ROS formation was accompanied by upregulated endoplasmic reticulum (ER) stress markers in IL-1β treated cardiomyocytes and pargyline treatment prevented it, suggesting that mitochondrial ROS generated by MAO is responsible for triggering ER stress in these conditions. Furthermore, in an in vivo model of streptozotocin-induced T1D, oxidative stress, fibrosis and ER stress markers were upregulated in the heart and diastolic stiffness, a marker of diastolic dysfunction, was increased. Pargyline administration to these mice prevented these events, indicating that MAO contributes to cardiac damage in diabetes. In conclusion, we demonstrated that

pharmacological inhibition of MAO is able to prevent HG and IL-1β induced mitochondrial ROS formation and dysfunction in vitro, as well as diastolic dysfunction, oxidative stress and fibrosis in an in vivo model of T1D. Furthermore, we show that ER stress occurring in these conditions is MAO-dependent, suggesting an important role of these flavoenzymes in coordinating the interplay between mitochondrial dysfunction and ER stress in diabetes.

TH-099

Factors controlling MAO-dependent oxidative stress in myocytes and non-myocytes of the heart

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Background: Monoamine oxidases (MAOs) are mitochondrial enzymes producing H_2O_2. As MAO inhibitors (iMAO) protect the heart in experimental models of cardiac injury, the molecular mechanisms underlying MAO activation was evaluated by (i) the availability of MAO substrates under stress conditions and (ii) their main cellular sources in the whole heart.

Methods and results: Mass spectrometry (MS) was used to identify and quantitate potential MAO substrates. We exploited two protocols of oxidative stress by means of (i) H_2O_2 perfusion or (ii) post-ischemic reperfusion in the absence and the presence of iMAO in mouse Langendorff model. The iMAO pargyline caused a relevant increase in the heart content of N1-methyl-histamine (NMH) in both protocols. Histidine-decarboxylase and histamine-N1-methyltransferase that are involved in NMH production are found in heart. The basal MAO substrate content was measured by MS in isolated cardiomyocytes and NMH was found to be the most abundant. Furthermore, upon histamine addition to cardiomyocytes, we measured an increase in ROS level that was inhibited both in the presence of a histamine-2-receptor (H2R) specific inhibitor, and pargyline, suggesting that H2R stimulation increase histamine effect without excluding MAO activity although the signaling pathway remains to be clarified. To investigate non-cardiac sources for MAO substrates under oxidative stress conditions we focused our attention on the synaptic terminals that innervate heart and commonly represent a pivotal source of neurotransmitters. Mice were denervated by 6-hydroxydopamine injection, hearts were subjected to the I/R protocol and the MS analysis showed no relevant differences upon these treatments suggesting that synaptic terminals did not represent a major sources of MAO substrate. **Conclusion**: Histamine appears to promote MAO activity through both receptor and non-receptor pathway. In fact besides the intracellular generation of NMH, MAO-induced ROS formation results from H2R activation.