duction. Morphological assessment using electron microscope revealed that mitochondria in the hearts of STZ-induced diabetic rats were swollen and the structure of cristae was collapsed; however, these changes were attenuated by the treatment with KN-93 and apocynin. This observation indicates that activation of CaMKII followed by NADPH oxidase-derived ROS increase contributes to mitochondrial dysfunction which may lead to cell death.
Conclusion: Increase in NADPH oxidase by activating CaMKII is a mechanism of ROS increase in the heart of diabetes mellitus. Up-regulation of ROS production facilitates mitochondrial malfunction and may result in cardiac dysfunction in diabetic patients.

239 The soluble guanylate cyclase activator cinaciguat improves cardiac dysfunction in diabetes mellitus

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Purpose: Patients with diabetes mellitus exhibit cardiovascular dysfunction along with increased oxidative stress and decreased nitric oxide – cyclic guanosine monophosphate (cGMP) signalling. It has been reported, that elevated intracellular cGMP-levels contribute to an effective cytoprotection against oxidative stress. In this study we investigated the effects of cinaciguat, a newly developed soluble guanylate cyclase activator on myocardial dysfunction in type-1 diabetic rats.
Methods: In male Sprague-Dawley rats diabetes was induced by a single ip. injection of streptozotocin (60mg/kg). In the treatment groups, cinaciguat (10mg/kg/d) was applied orally for 8 weeks. Rats of the control groups received vehicle for the same time. After the treatment left ventricular (LV) pressure-volume relations were measured by using a microtip Millar pressure-volume conductance catheter, and indexes of contractility (e.g. preload recruitable stroke work (PRSW)) were calculated. Blood plasma and myocardial tissue samples were collected for determination of cGMP-levels and immunohistochemical analysis, respectively. Myocardial gene expression analysis has been performed by quantitative real-time polymerase chain reaction (qRT-PCR).
Results: When compared to the non-diabetic controls, diabetic rats showed impaired left ventricular contractility (PRSW: 49.5±3.3 vs. 83.0±5.5mmHg; $p<0.05$) and a marked diastolic dysfunction (time constant of LV pressure decay, Tau: 17.3±0.8 vs. 10.3±0.3ms), which was significantly improved by cinaciguat (PRSW: 66.8±3.6mmHg, Tau: 14.9±0.6ms in the diabetes+cinaciguat group). The treatment had no hemodynamic effects in non-diabetic control animals. Diabetes was associated with increased myocardial immunoreactivity for transforming growth factor beta (TGF-b) along with enhanced gene expression for atrial natriuretic peptide (ANP), heat shock protein 70 (Hsp70) and matrix metalloproteinase-9 (MMP-9), which were all reversed after cinaciguat treatment. Efficacy of the therapy was reflected by markedly elevated levels of cGMP in the diabetes+cinaciguat group compared to control (44.2±11.4 vs. 16.1±2.0pmol/ml plasma).
Conclusions: Our results demonstrate that cinaciguat prevents diabetes-associated deleterious myocardial changes and improves diabetic cardiac dysfunction in our rat model. Pharmacological soluble guanylate cyclase activation might represent a novel therapy approach for diabetic cardiomyopathy.

240 Cx43 hemichannels of the inner mitochondrial membrane are constitutively active in heart failure

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Background: Ischemic preconditioning involves activation of multiple signaling pathways with mitochondrial ion channels as an end-effector. Recently, we demonstrated that connexin 43 hemichannels found in the inner mitochondrial membrane (mito-Cx43 hemi) are essential for cytoprotective signal transduction. Heart failure is a vulnerable disease state in which any additional myocardial damage may lead to acute circulatory failure. However, susceptibility to ischemic injury and the protective potential of IP in heart failure are unresolved.
Methods: We aimed to directly prove the presence of human mito-Cx43 hemi and to evaluate channel characteristics in failing versus non-failing myocardium. Furthermore we combined heart failure with ischemia-reperfusion injury in a mouse model. We used direct single-channel recordings of cardiac mitochondria to possibly identify a functional link between mito-Cx43 hemi and susceptibility to ischemic injury in murine and human heart failure.
Results: We identified single-channel currents, which were clearly distinct from mitoKATP channels, inhibited by the Cx43 mimetic peptide 43GAP27 which suppressed open probability and single-channel conductance. Single-channel properties thus supported the notion that we were recording mitochondrial Cx43 channels. Basal mito-Cx43 hemi activity was increased in both human and murine heart failure. A pharmacological preconditioning with diazoxide stimulated single-channels of failing and non-failing hearts to a similar maximal activity. Despite a reduced content and dephosphorylation of total Cx43 in failing hearts, mitochondrial Cx43 was increased and hyperphosphorylated.
Conclusion: For the first time we directly recorded human mitoCx43 hemichannels which were inhibited by 43GAP27. Our results indicate endogenous protective adaptation mediated via enhanced basal mito-Cx43 hemichannel activity through hyperphosphorylation of the mitochondrial Cx43 fraction in heart failure.

241 Glucagon-like peptide-1 receptor activation ameliorates cardiac steatosis and reverses pathological remodeling by quality control of mitochondria via cAMP/PKA axis

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Purpose: Glucagon-like peptide-1 receptor (GLP-1R) agonist exendin-4 facilitates cardiac contractility in systolic dysfunction model in rodents and patients with heart failure. However, the impact of GLP-1R on cardiac steatosis remains unclear.
Methods and Results: Male KK/Ay mice (16-week-old) were allocated into exendin-4 (24 nmole/kg/day, 40 days; KK-ex4) and vehicle group (KK-v). Male C57BL6 mice were fed with high fat diet for 3 months (DIO) and subjected to exendin-4 treatment at the age of 16 week old (DIO-ex4 and DIO-v). KK-ex4 exhibited decline in heart weight (-16.5% vs KK-v) without body weight loss. Oil-red-O staining revealed that KK-ex4 and DIO-ex4 reduced cardiac steatosis. Echocardiography revealed that systolic function of DIO-v was suppressed (-13.8% vs control), which was restored in DIO-ex4 with reduced LVPWd (-11.1% vs DIO-v) and IVSd (-30.0% vs DIO-v). Myocardial fibrosis and tissue oxidative stress detected by DHE staining were reduced in KK-ex4 and DIO-ex4 (0.45±0.10 fold and 0.58±0.10 fold vs vehicle). Transmission electron microscopy revealed restoration of irregular alignment and increase in number of mitochondria with normal cristae in KK-ex4 and DIO-ex4. Mitochondria-specific dyes (MitoTracker Red) revealed decline in oxidative activity of cardiac mitochondria both in KK-ex4 and DIO-ex4. The levels of myocardial PINK/Parkin, the surrogate indicators for damaged mitochondria, were reduced both in KK-ex4 and DIO-ex4.

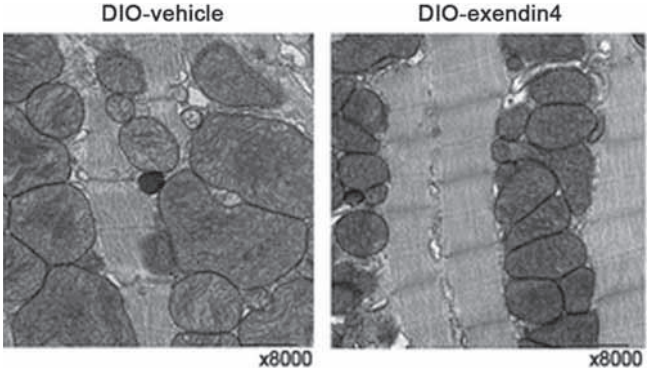

Figure 1. Typical mitochondria images by TEM

Conclusion: GLP-1R activation protects cardiac remodeling induced by steatosis through the restoring mitochondrial oxidative damage mediated by the activation of cAMP/PKA-dependent mechanisms.

242 Expression and physiological role of the novel adipokine nesfatin-1 in cardiomyocytes

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Purpose: Nesfatin-1 is a novel adipokine involved in the control of food intake and energy metabolism which shows anti-inflammatory properties. The role of this adipokine in cardiovascular physiology is unknown. In previous studies we determined that nesfatin-1 is expressed in human, rat and mouse heart. Our aim now is to study the effect of this adipokine in cardiomyocytes and the possible regulation of nesfatin-1 cardiac synthesis by diet and inflammatory mediators.
Methods: Real-time PCR was used to determine nesfatin-1 mRNA levels in cultured neonatal cardiomyocytes of Sprague Dawley rats treated with TNF-α, dexamethasone and insulin. In heart tissue of rats fed with high fat diet for 16 weeks, we used real-time PCR to determine nesfatin-1 cardiac mRNA levels and an ELISA to determine nesfatin-1 plasma levels. Cardiomyocytes were treated with nesfatin-1 and confocal microscopy was used to study the glucose transporter Glut-4 movilization. Finally, western blot was used to identify possible transducing signalling molecules (Erk $\frac{1}{2}$, AMPK and AKT) after nesfatin-1 treatment in cardiomyocytes.
Results: Cardiomyocytes treatment with 0.1-20 ng/ml TNF-α for 6-48 h induces an increase of nesfatin-1 mRNA levels with a maximum stimulatory effect at 20 ng/ml for 24 h (p=0.0159; Fold-Change (FC)=1.16, n=5). Treatment with 0.1-100 nM dexamethasone for 6-48 h also increases of nesfatin-1 mRNA levels with a maximum stimulatory effect at 100 nM for 24 h (p=0.0079; FC=2.457, n=5).

On the other hand, 0.1-100 nM insulin treatment for 6-48 h decreases nesfatin-1 mRNA levels with a maximum stimulatory effect at 100 nM for 24 h (p=0.0159; FC= -0.6962, n=5). Treatment of cardiomyocytes with 10-1000 nM nesfatin-1 for 5-30 minutes induces Glut-4 movilization from the cytoplasm to the plasma membrane (p=0.0007; FC=1.125, n=216 cells) and an Erk $\frac{1}{2}$ phosphorilation (p=0.0156; FC=3.133, n=7) with a maximum stimulatory effect at 1000 nM for 10 minutes. In rats fed with high fat diet for 16 weeks nesfatin-1 cardiac mRNA levels are higher compared with control (p=0.0111, FC =2.201, n=7) and there is a positive correlation with body weight (Spearman's rho (Rho)= 0.715, Sig. =0.004, n=14) and the fat percentage (Rho= 0.692, Sig. =0.006, n=14). There is also a positive correlation between plasma nesfatin-1 levels and body weight (Rho= 0.582, Sig. =0.037, n=13).
Conclusions: In cardiomyocytes nesfatin-1 regulates glucose homeostasis. Cardiac levels of this adipokine are modified by inflammatory and metabolic status. Our work provides the first evidences about a potential role of nesfatin-1 in a paracrine/autocrine system at cardiac level.

NON PHARMACOLOGICAL TREATMENT IN HYPERTENSION

247 Adherence to the Mediterranean diet and albuminuria levels in adolescents: Emerging data from the Lyceum Leontio Albuminuria (3L) Study

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Purpose: Mediterranean diet has favorable effects on the cardiovascular system, while albuminuria is associated with atherosclerosis progression. The aim of the study was to investigate the relationship of dietary habits with urinary albumin excretion, expressed as the albumin to creatinine ratio (ACR), in a cohort of adolescents
Methods: A total of 365 adolescents 12-17 years of age [212 males, aged 13.9 years, office blood pressure (BP)=115/67 mmHg] that were included in the Lyceum Leontio Albuminuria (3L) study were considered for analysis. In all participants ACR values were determined in a morning spot urine and for each adolescent a questionnaire was completed on lifestyle and socio-economic characteristics. Moreover, the Mediterranean Diet Quality Index for children and adolescents (KIDMED) was estimated and accordingly subjects were divided into those with optimal ($\geq$7), average (4-7) and low ($<$4) score.
Results: Only 6.8% of the participants had optimal KIDMED score, whereas 51.2% had an average and 42% had a low score. Participants with at least average KIDMED score (n=187) compared to those with low KIDMED score (n=153) had higher body mass index (22.2 vs 21.4 kg/m^2, p=0.043) and waist circumference (77.6 vs 75.4 cm, p=0.044), spent more frequently time for sports activities outside school (75.2% vs 58%, p=0.001), reported less consumption of foods outside home (3% vs 14%, p$<$0.001) and less hours of watching television (1.75 vs 2.05 hours, p=0.013). Moreover, those with at least average compared to those with low KIDMED score exhibited higher systolic BP (117 vs 114 mmHg, p=0.039), whereas had lower heart rate (84 vs 87 bpm, p=0.014) and ACR levels (12.6 vs 20.5 mg/g, p=0.015). In the total population, ACR was associated with age (r=-0.11, p=0.044), body mass index (r=-0.131, p=0.016), systolic BP (r=-0.144, p=0.008) and KIDMED score (r=-0.111, p=0.041).
Conclusion: In adolescents there is an inverse relation of KIDMED score with albuminuria and those who adhere to the Mediterranean diet exhibit lower levels of ACR. However, the paradoxical associations of both ACR and KIDMED score with obesity markers and BP levels suggest distinct mechanisms of albuminuria development in adolescents.

248 Influence of potassium intake on blood pressure of subjects with different levels of sodium consumption

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Background: Increase of potassium (K) intake has been recommended as a non pharmacologic strategy to reduce blood pressure (BP) levels and hypertension incidence. However, the benefits of such strategy in subjects with different patterns of sodium (Na) intake have not been evaluated. In this study we investigated in a population-based study the influence of the K intake on BP of individuals consuming low ($<$6 g/day) or high ($>$6 g/day) salt diets.
Methods: A random sample (N=1,661) of the adult (25-64 y) population of Vitória, Brazil, was recruited to investigate cardiovascular risk factors. BP, anthropometry and biochemical variables (fasting blood) were obtained according to standard methods during a programmed visit to the University Hospital. Urine was collected for 12 h (7 pm to 7 am) in the night previous to the hospital visit to assess Na, K and creatinine excretion. Individuals with inadequate urine collection (n=54), plasma creatinine $>$1.4 mg/dL (n=52) or under use of any hypertensive medication, including diuretics (n=270) were excluded from the present analysis. Variables are shown as mean$\pm$standard deviation. Statistical significance was set atp$<$0.05.
Results: Data refer to 1,285 subjects (613 men, age = 43$\pm$10 y). Hypertension (bloodpressure $\geq$140/90 mmHg) was found in 31%, obesity (BMI$\geq$30 kg/^{2}) in 15.5% and diabetes (fasting glicemia $>$125 mg/dL) in 5.3%. Mean urinary Na and K excretion was 99$\pm$57 mEq and 23$\pm$15 mEq/12 h, giving a mean daily consumption of 211 mEq of Na and 78 mEq of K. Higher K intake was associated with individuals older and with higher BMI and urinary Na excretion. Systolic and diastolic BP mean levels were stable along quartiles of urinary K excretion. However, significant (P$<$0.001) decrease was observed for the systolic and diastolic BP adjusted for age, BMI and 12h Na and creatinine excretion. Interestingly, the association between K excretion (as surrogate of K intake) was lost in the subgroup of subjects (N=182) consuming $<$6 g salt/day ($<$1.20 g Na/12-h urine). In subjects consuming $>$6 gsalt/day, the adjusted systolic and diastolic BP were, respectively, 7 mmHg and 3 mmHg lower in those in the upper urinary K excretion quartile ($>$28 mEq/12h) as compared with those in the lowest one ($<$14 mEq/12h).
Conclusion: Our data confirm based in data obtained in the general population that increasing the K intake may partially inhibit the hypertensive effect of high salt diets. This strategy seems to be more efficient in highr salt consumers.
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249 Dietary omega-3 acid ethyl esters enhance omega-3 index, attenuate myocardial arrhythmogenic substrate and protect from malignant arrhythmias in a model of human essential hypertension

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Background and Purpose: Hypertension-induced myocardial remodeling is known to be associated with increased propensity to malignant arrhythmias that can be attributed to impairment of cell-to-cell synchronization due to alterations in electrical coupling protein, connexin-43 (Cx43). Omega-3 fatty acids (omega-3) exert cardioprotective and antiarrhythmic effects in both experimental and clinical setting. We tested our hypothesis that omega-3 intake can protect of hypertensive rats from malignant arrhythmias via protection of intercellular communication ensured by Cx43 channels.
Design and Methods: Experiments were conducted on male spontaneously hypertensive rats (SHR) at early (3month) and late (12 month) stage of diseases as well as age-matched normotensive Wistar rats. Untreated rats were compared with animals supplemented by omega-3 (EPA+DHA ethyl esters, Vesterlanes, Norway, 30 mg/day) for two month. Blood pressure, body, heart and left ventricle weights were monitored. Plasma and red blood cells (RBC) fatty acids profile (omega-3 index) was estimated by gas chromatography. Left ventricular tissues were taken for examination of Cx43 distribution (using immunostaining and electron microscopy) and expression (using immunoblotting). Expression of protein kinases C (PKC), which phosphorylates Cx43 was examined as well. Langendorff-isolated heart preparation was used to test inducible VF.
Key results: Comparing to healthy rats the omega-3 index was lower in old SHR (0.7% vs 2.5%) and increased due to omega-3 intake in both groups to 2.3% and 4.8%. Young SHR rat hearts exhibited LVH associated with enhanced distribution of Cx43 on lateral surfaces of the cardiomyocytes, while old SHR exhibited both lateralization and severely disordered distribution of Cx43 at the area of fibrosis. Total Cx43 expression was increased in young and decreased in old SHR hearts but phosphorylated (functional) forms of Cx43 were suppressed in both SHR groups. In contrast, omega-3 intake diminished arrhythmogenic substrate, i.e. abnormal Cx43 distribution and attenuated significantly abnormal Cx43 expression and phosphorylation. The latter was linked with enhanced PKCe expression. Consequently, omega-3-treated SHR were less prone to inducible VF comparing to untreated rats.
Conclusions: Results indicate that hypertensive rats benefit from omega-3 fatty acids supplementation due to an increase of omega-3 index, alleviation of Cx43-related arrhythmogenic substrate and suppression of malignant arrhythmias.
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250 AT1 receptor blockade attenuates insulin resistance and myocardial remodeling in rats with diet-induced obesity

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Interactions between angiotensin-II type 1 (AT1) receptor and insulin has classically resulted in insulin resistance and cardiac remodeling in obesity. This study evaluated the influence of AT1 receptor blocker losartan on insulin receptor/phosphatidylinositol 3-kinase (PI3-kinase) pathway and myocardial remodeling in rats with diet-induced obesity.