**POSTER SESSION 3**

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**Cell growth, differentiation and stem cells - Heart**

**511** The role of the endocannabinoid system in modelling muscular dystrophy cardiac disease with induced pluripotent stem cells.

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Muscular Dystrophy (MD) is an umbrella term for genetic disorders affecting skeletal and cardiac muscle which arise due to abnormalities in the dystrophin gene. Unraveling underlying metabolic and structural abnormalities in cardiomyocytes (CMs) which in turn become predisposed to ectopic cell death and fibro-fatty replacement. The Endogenous Cannabinoid System (ECS) is a lipid signalling network present in the cardiovascular system and comprises G-protein coupled receptors (CB1 and CB2), endogenous ligands (anandamide and 2-arachidonylethanolamide) and regulatory proteins (fatty acid amide hydrolase and monoacylglycerol lipase). The ECS has an emerging function in stem cell survival and differentiation, MD skeletal muscle pathology, and cardiovascular diseases in general. Induced Pluripotent Stem Cell (iPSC) technology permits the reprogramming of somatic cells (e.g. fibroblasts) into pluripotent stem cells, which can be differentiated into cells from all three germ layers including CMs. In the present study we provide evidence that the ECS is involved in somatic cell reprogramming. Specifically, the CB1 receptor antagonist AM251 prevented the formation of iPS cell colonies (p<0.005, vs. control conditions, Newman-Keuls multiple comparison test, n=3). CMs derived from MD patients’ iPSCs (MD-CMs) displayed disease hallmarks such as lack of dystrophin expression, increased expression of Nup153 (a cardiomyopathy-associated protein; p<0.0009, vs. healthy CMs; Student’s unpaired t test, n=3) and increased CM cell death (p<0.0001, vs. healthy CMs, Student’s unpaired t test, n=3). Furthermore, we also provide evidence that the ECS is present in iPSCs and becomes dysregulated in MD-CMs. Our results highlight the dual functionality of the ECS in cell reprogramming and MD cardiac pathology which is of interest to cardiac disease modelling and novel drug discovery.

**513** Canonical wnt signaling reverses the ‘aged/senescent’ human endogenous cardiac stem cell phenotype

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**Background:** The adult human myocardium harbors endogenous, multi-potent cardiac stem cells (eCSCs). Manipulation of eCSCs ex-vivo and in situ has opened new therapeutic avenues for functional myocardial regeneration. However, as ageing/senescence of eCSCs determines their function and regenerative capacity, regulation of this parameter will impact the efficacy of these therapies, considering the advanced age of the majority of patients in need of regenerative therapy.

**Objectives:** Our aim is to determine the main factor(s) that determine the ‘aged’ human eCSC phenotype and investigate its potential reversibility.

**Methods:** eCSCs were isolated from the right atria appendage (~45,000/gram of tissue). The cloning efficiency was inversely age-related, single-cell derived eCSC clones obtained from young donors showed higher cloning efficiency compared to non-canonical Wnt signaling imparted a negative ‘ageing’ effect on eCSCs. Importantly, although the cloning efficiency was inversely age-related, single-cell derived eCSC clones obtained from young and old hearts were indistinguishable by their gene expression and differentiation potential, strongly suggesting that eCSC aging is a stochastic process.

**Conclusion:** eCSCs stochastically develop a senescent phenotype with age impacting their growth and differentiation potential. Manipulation of canonical and non-canonical Wnt signaling pathways reversed the ‘aged/senescent’ phenotype.
Purpose: Our aim was to investigate the role of transcriptional Hippo co-activators YAP and TAZ in cardiovascular diseases and cell culture models, and to test the effects of modulating the pathway on cardiomyocyte function and survival.

Methods: Human iPSC-CM were differentiated from embryonic stem cells (ESCs) or induced pluripotent stem cells (iPSCs) to recapitulate the native cardiac environment at various stages of cardiac maturation.

Results: Our results showed that YAP and TAZ genes are abundantly expressed both in iPSC-CM and adult ventricular cardiomyocytes. The time-dependent expression of NOS/sGC/cGKI during cardiac differentiation suggests that the chronic effect of pathway alteration could be beneficial for heart functions.

Conclusion(s): Our study presents a genome-wide transcription map of iPSC-CM, with low expression levels of pluripotency genes and high expression of liver-specific genes, closely related to PHHs, thus demonstrating the potential of these cells as a laboratory model for human liver.

517 Can NOS5/cGcGK1 pathway trigger the differentiation and maturation of mouse embryonic stem cells (ESCs)?

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Background: The role of nitric oxide synthetase (NOS)/soluble guanylyl cyclase (sGC) and cGMP-dependent protein kinase I (cGKI) pathway in adult cardiac cells is extensively studied. Indeed, physiological levels of NO generated by NOS5 and NOS3 or pharmacological treatments with NO-donor SNAP and inorganic nitrite may modulate cardiac contractility and modulate cell-to-cell interactions. The Ser/Thr phosphorylation (sGC/cGKI pathway) and cGMP-dependent protein expression and activity during cardiac maturation are acute and chronic effects of pathway alteration.

Methods: Undifferentiated mouse ESCs were differentiated by Embryoid Bodies formation (EBs). Cardiac maturation was followed for 21 days. Beating EBs were monitored starting from 7th-10th day. The analysis of NO expression was measured at d18 with immunofluorescence (NOS5) or real time PCR (NOS5) and the percentage of beating EBs at early stages of cardiac maturation (d5-d8).

Results: Q-PCR showed that during differentiation enzymes expression increased in a time-dependent manner and during cell culture. The peak of NOS5 expression was measured at d18 and then decreased. mGucy1b and mPrkgI were detected by real-time PCR (Q-PCR), western blot analysis and enzymatic activity were performed for protein evaluation.

Conclusion(s): The time-dependent expression of NOS5/cGcGK1 pathway during cardiac differentiation suggests a potential role of this pathway to trigger cardiomyogenesis showing a possible cyto-regenerative role of NO/cGMP/PKG-I signaling in the heart. Further functional data will clarify its role in cardiac maturation.

518 Introduction of external Ik1 to human-induced pluripotent stem cell-derived cardiomyocytes via Ik1-expressing HEK293

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Background: Human iPSC-CMs have provided an alternative to adult primary cells but they have shown an embryonic rather than adult phenotype in a number of characteristics including electrophysiology. One of the issues affecting the electrophysiology is the lack of the inward rectifying potassium channel, IK1, responsible for maintaining a stable resting membrane potential.

Purpose: This study examines the effects of the addition of Ik1 function to iPSC-CMs via co-culture with Ik1-expressing HEK293 cells.

Methods: Human iPSC-CMs obtained commercially (Cor-AU - Axogeen, and Pliorix - Plurionics) were prepared according to manufacturers’ protocols, and mixed with IK1-expressing HEK293.

Results: The density of IK1 was kept constant at 250-400 cells/well (varying in different cell lines according to manufacturers’ suggestions) to ensure a monolayer was formed. Ik1-expressing HEK293 were mixed with iPSC-CMs at decreasing densities from 1:10 to 3:100, immediately after thawing and then transferred to the plate and incubated at 37°C 5%CO2. Contractility measurements were taken daily for a maximum of 12 days. Data are expressed as mean ± SEM.
Results: A uniform monolayer developed using 25k-40k cells/well hiPSC-CMs in the presence of in- creasing densities of HEK293 cells. Contractility recordings from Cor-4U hiPSC-CMs showed that from day 3 onwards all cultures were spontaneously active. Higher densities of h1-expressing HEK293 (1:10) lead to an increase in interval time between beats of approximately 60% on day 9 (1972 ± 592 vs 1211 ± 44ms, p < 0.05). Time for relaxation was also significantly prolonged in 1:10 and 1:30 compared with control on day 9, 283% and 128% (875 ± 265 and 522 ± 135ms vs 229 ± 24ms, respectively, p < 0.001), respectively. Earlier and later culture times showed no sig- nificant difference in spontaneous contractile activity up day 12. In parallel, Pluricyte hiPSC-CMs were initially quiescent, becoming spontaneous at approximately day 4. Co-culture ratios of 1:10 and 1:30 did not show any spontaneous activity up to day 11.

Conclusions: Co-culture with h1-expressing HEK293 may provide a method of adding h1 conductance to a network of hiPSC-CMs but different sources of hiPSC-CMs respond differently. With Cor-4U, h1 higher densities of HEK293, such as 1:10, lead to a slowing of the spontaneous rate and slowing of relaxation time suggesting effects on the electrophysiology of the co-culture. Pluricyte cells responded differently suggesting a higher sensitivity to co-culture with h1 expressing HEK293 cells.

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Cell therapy of the heart studied using adult myocardial slices in vitro

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Introduction: Cardiac cell therapy is the introduction of stem cells in the heart to repair/replace damaged myocardium. In vivo studies have revealed that this therapy can induce arrhythmias, and the efficacy of improving myocardial function is still uncertain. Methods: hiPSC-CMs were cultured in vitro on 300 μm thick vibratome-cut slices prepared from adult dog left ventricular tissue. Viability and functionality were assessed by force measurements, histology and immunohisto-chemistry. Calcium transients were recorded by optical mapping. Results: hiPSC-CMs attached to the slices, within 24 hours formed electrical connection with the other grafted cells and beat spontaneously. Their beating activity however could not trigger the activation of the recipient tissue. Some cells, after 3 days in culture, could be paced with field stimulation at 1 Hz and contracted synchronously with the slice. When point stimulation was applied on a distant region of the slice, while the slice contracted, the signal did not propagate to the hiPSC-CMs, suggesting a lack of coupling with the recipient tissue. After 9 days in culture some hiPSC-CMs started to intertwine and aligned with the slices myocytes, but others did not and spread into a separate layer as with 2D culture. At this time point however, myocardial slices showed a significant degree of functional deterioration. Slice contractility decreased to 23% by day 6 and this was due to myocytes dedifferentiation and cell death.

Conclusions: Vibratome-cut slices are a viable platform to study cell therapy, particularly in the first few hours. Culture conditions need to be improved to better preserve myocardial slice structure and functionality for long term studies.

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Enhancement of the paracrine potential of human adipose derived stem cells when cultured as spheroid bodies

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Background: Ischemic heart disease remains a leading cause of mortality and morbidity worldwide. Cardiac cell therapy (CCT) is a promising therapeutic strategy to help in cardiac repair. Multiple cells have been proposed as candidates in CCT. Adipose tissue constitutes an important and accessible source of pluripotent mesenchymal stem cells (ASCs) that could improve cardiac function and volumes, mostly through a paracrine mechanism.

Study Aim: The objective of our in vitro study is to characterize and compare the secretion profile as well as the survival of ASCs when cultured under standard conditions (i.e. as a monolayer (ML)) versus in a three-dimension (3-D) structure (i.e. as a spheroid body (SB)). In vivo, the aim is to compare the anti-inflammatory potential of these two cell structures in peritonitis in a rat model.

Methods: Human ASCs (hASCs) were expanded in standard culture conditions in a monolayer form. ASCs were characterized according to both surface markers expression (assayed by immunofluor- escence) and their ability to maintain multilineage differentiation. Additionally, hASCs were cul- tured as 3-D structures as spheroid bodies (SBs), by using the hanging drop technique. Luminox and ELISA assays were conducted to quantify key immunomodulatory and angiogenic mediators to compare the two study groups. Western blots were used to study proteins involved in apoptosis.

Results: Human ASCs expressed similar surface markers as those documented for bone marrow MSCs in- cluding CD44, CD105 and CD90. Their ability to differentiate into adipogenic, chondrogenic and osteogenic lineage were unaltered. Paracrine activity of hASCs was enhanced when cultured as hASC-SBs. SBs secreted higher levels of immunomodulatory cytokines such as MCP-1, IL-6, IL-8 and IL-10, in a time dependent manner. Similarly, they exhibited greater pro-angiogenic potential as VEGF levels were increased also compared to hASC-MLs. Activation of caspase 3, shown by its cleaved form, was described in MLs under basic culture conditions and in response to TNFα stimulation, whereas cleaved caspase 3 was undetected in SB structures. Additionally, inflammation was reduced in both hASC-ML and hASC-SB treated groups of rats with induced peritonitis compared to the untreated group, with a slight efficacy of SBs over MLs.

Conclusion: hASC represent a promising cell source for stem cell therapy. Their paracrine, thera- peutic potential can be optimized. Our findings clearly showed that hASCs cultured as 3-D structures (i.e. as SBs) exhibit an improvement in both anti-inflammatory and angiogenic properties associated with resistance to apoptosis. Spheroid body formation thus represents an effective alternative to en- hance the therapeutic potential of hASCs.

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Mechanosensitivity of cardiomyocyte progenitor cells: the strain response in 2D and 3D environments

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Purpose: Cardiomyocytes progenitor cells (CMPCs) are a candidate source cell for cardiac regen- erative therapy. To assess their full potential for cardiac regeneration, it is essential to know if and how CMPCs sense and respond to the three-dimensional (3D) environment and mechanical stimuli pro- vided by the beating heart. Therefore, we study the response of cyclic strain of undifferentiated and predifferentiated human CMPCs in a 2D environment, as well as how CMPCs respond to unidirec- tionally constrained versus stress-free (unconstrained) 3D environments. The latter responses were studied using a hydrogel system that allows for interaction of the cells with a singular axial strain. Methods: To test mechanosensitivity of CMPCs in 2D and 3D environments, the response of LBTB CMPCs to uniaxial (cyclic) strain (10% with 0.5 Hz) was investigated. To represent the 3D environ- ment, undifferentiated CMPCs were cultured in unidirectionally constrained and stress-free collagen/ Matrigel hydrogels, where the confinement provides a static strain to the cells. The cellular mechan- osponse to the applied (cyclic) strain was quantified by cellular re-orientation away from the strain direction (strain avoidance) Next to cellular re-orientation, the effect of strain on cell differentiation was analyzed.

Results: We observe that while undifferentiated cells maintain their original orientation, upon early cardiomyogenic differentiation (predifferentiated) CMPCs exhibit a distinct strain avoidance response during 48hrs of cyclic straining in a 2D environment. In 3D unidirectionally constrained hydrogels, undifferentiated CMPCs retain their cardiomyogenic stem cell profile. CMPCs cultured in 3D collagen/ Matrigel hydrogels respond to static mechanical strains as expected by cell alignment.

Conclusions: Our results suggest that CMPCs respond to the presence of mechanical stimuli, in this research mediated by the application of uniaxial (cyclic) strain in 2D and 3D environments, suggesting that CMPCs are indeed mechanosensitive. Although in 2D environments, mechanosensitivity of the CMPCs is dependent on their differentiation status. Our findings provide the first understanding of the ability of human CMPCs to sense mechanical stimuli, which is the first initial step in mechanotrans- duction. Mechanotransduction is essential for optimal recruitment, migration, and mechanical integra- tion of progenitor cells into the injured myocardium. Therefore, the presented results can contribute to enhance efficacy of current treatments of cardiac disease, as well as to develop novel regenerative regeneration strategies.

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The effect of the vascular-like network on the maturation of the human induced pluripotent stem cell derived cardiomyocytes.

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Introduction: Stem cells and specifically induced pluripotent stem cell derived cardiomyocytes (iPSC-CMs) provide unique alternative to model human cardiomyocyte differentiation, the function of the cardiac cells and the pathology of severe cardiac diseases. However, the differentiated iPSCs-CMs are classified to resemble embryonic or fetal like cardiomyocytes due to their gene expression pattern, size, shape and mononuclear nature. Moreover, the lack of l-tubule network has been suggested to be a reason for the slow excitation-contraction coupling and calcium handling. The culture plat- forms that orientate the cardiac cells could have a positive effect on the overall maturation of the differentiated cardiomyocytes, e.g. patterned biomaterials could induce the orientation and fur- thermore the more mature phenotype of iPSCs.

Methods: We have utilized natural topography provided by the network formed by endothelial cells and fibroblasts in the maturation of iPSC-CMs. The iPSC-CMs have been differentiated and the beating differentiated cells have been plated to the aforementioned platform and the maturation state of the
cells is studied. The morphology and cell orientation have been assessed in addition to the cardiac specific gene functionality and the level of the structural gene expression.

Results: Our results show that iPSC-CMs were elongated and aligned with the vascular-like network and formed a synchronously beating cell construct. The electrical activity as well as calcium metabolisms was shown to be normal. In addition, the cells responded to pharmaceutical agents as expected. Furthermore, the iPSC-CMs cultured on the network expressed the genes of the cardiac structural proteins at the higher level than the control cells on gelatin coated cell culture plates.

Conclusions: The vascular-like network has beneficial effects on iPSC-CMs in terms of cell structure and alignment as well as sarcomere orientation and function. Our results suggest that iPSC-CMs cultured on these platforms present more mature phenotype compared to iPSC-CMs cultured on 2D gelatin coated cell culture plates and could therefore serve as more reliable cardiomyocyte model for disease modeling as well as cardiac safety and efficacy assessments.

Transcriptional control and RNA species - Heart

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Gene expression regulation in heart failure: from pathology to bioinformatics

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Background: Heart failure (HF) syndrome results from abnormalities in multiple biological processes which independently contribute to the progression of the disease. Next-generation sequencing technologies revolutionized the analysis of the transcriptome, providing a panoramic view of all the transcriptional activity in a given sample and a powerful tool for the identification of new transcripts.

Purpose: RNA-sequencing (RNA-Seq) approach was employed to investigate the changes accompanying heart failure and to obtain the whole transcriptome of cardiac tissues from transplant recipients with advanced stage of HF. The knowledge of an expression network signature in end-stage HF diseased hearts may offer important insights into the complex pathogenesis of advanced cardiac failure, as well as it may provide potential targets for therapeutic intervention.

Methods: RNA from heart tissue explants from dilated cardiomyopathy (DCM) and restrictive cardiomyopathy (RCM) patients were analyzed. RNA-Seq data were analyzed using R software. Bioinformatics tests (edgeR and NOISeq-Bio) were employed and compared. Several public tools were used to effect in silico analysis of the specifically differenctially expressed genes (DEGs).

Results: The statistical methods adopted, generated different lists of genes both for the number of DEGs and for the function and/or the biological significance. Venny software was utilized to obtain a list with the common genes; particularly, 35 were detected as differentially expressed in failing hearts versus non-failing hearts. Moreover, DAVID functional analysis demonstrated that 5 cytoskeleton-related genes were differentially expressed in DCM. On the other hand, when hearts from RCM patients were compared with non-failing hearts, 19 differentially expressed cytoskeleton-related genes were found. Interestingly, genes encoding ACTA2 and ACTG2 have been associated with HF for the first time in this study. Noteworthy, NMUR1 gene, involved in modulating calcium channels, was particularly down-regulated in both DCM and RCM. Finally, several genes also encode for components of extracellular matrix, including ADAMTS4 and ADAMTS10, belonging to the extraplasMate gene list of common DEGs.

Conclusions: Our data revealed a new map of gene expression changes in the DCM and RCM cardiomyopathic genes and regulation of cell death pathways. The in silico analysis revealed for the T3 regulated miRNAs and with alterations in groups of proteins that play a key role in energy metabolism, quality control and regulation of cell death pathways. The in silico analysis revealed for the T3 regulated miRNAs and with alterations in groups of proteins that play a key role in energy metabolism, quality control and regulation of cell death pathways.

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The effect of ureaemia on the expression of miR-212/132 and the calcineurin pathway in the rat heart

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Background: The prevalence of ureaemia is continuously increasing in developed countries. Uremic cardiomyopathy characterized by left ventricular hypertrophy and diastolic dysfunction is a common cardiovascular complication of uremic patients. The pathogenesis underlying mechanisms are not clear. The overexpression of miR-212/132 has already been implicated in the development of left ventricular hypertrophy. In a mouse model of myocardial infarction, GDF15 deficiency results in increased incidence of cardiac remodeling and worsens patients prognosis. We previously reported a cardioprotective role of T3 in murine models of heart failure, whereas the protective role of T3 in uremic myocardium is unknown.

Purpose: The current study was set as to unveil putative uremic cardiomyopathy-targeted cardiac gene expression changes activated by T3 in the early post IR setting and dependent on the regulation of micro RNA molecules.

Methods: To this aim, miRNA profiling and mitochondrial proteome were performed in a model of cardiac IR. In vitro data were integrated in a mathematical model of cell remodeling by T3 with a brief hypoxia treatment. In this model, rats developing a low T3 state were treated with T3 (65g/Kg die) or vehicle for 48h. Therapeutic cardiac performance was evaluated through echocardiogram and the rats were sacrificed. Tissue from the LV peri-infarctual zone was used for miRNA profiling through next generation sequencing. In the same experimental model, mitochondria of the periinfarctual myocardium were purified from rats developing or not the low T3S and the proteomic profiling was performed through mass spectrometry.

Results: The presence of a post IR LT3S was associated to more serious impairments of cardiac and mitochondrial function and with altered expression of several miRNAs of critical importance for mitochondrial activity and cardiac remodeling, which was reverted by T3 treatment. Also we observed different remodeling of the mitochondrial proteome in the presence or absence of a LT3S, with alterations in groups of proteins that play a key role in energy metabolism, quality control and regulation of cell death pathways. The in silico analysis revealed for the T3 regulated miRNAs several predicted mitochondria targets well fitting with the proteomic results.

Conclusion: Our findings highlight a relationship between LT3S in the early post IR and poor cardiac and mitochondrial outcomes, while indicating a beneficial role for T3 treatment possibly through the regulation of miRNA-mediated cardioprotective circuits targeted to mitochondria.

Cytokines and cellular inflammation - Heart

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Lack of growth differentiation factor 15 aggravates adverse cardiac remodeling upon pressure overload in mice

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Introduction: Growth differentiation factor 15 (GDF15) is a distant member of the TGF-f family. Under homoostatic conditions GDF15 is not highly expressed, however, upon injury GDF15 levels robustly increase. GDF15 influences many processes including inflammation, apoptosis and fibrosis. In a mouse model of myocardial infarction, GDF15 deficiency results in increased incidence of cardiac remodeling changes.
Is there an association between low-dose aspirin use and clinical outcome in HFPEF?

Implications of modulating monocyte function and inflammatory mediator release

INTRODUCTION: We have previously reported an association between low-dose aspirin use and improved long-term outcome in chronic heart failure (HF) patients irrespective of ischemic heart disease. The majority of community-dwelling HF patients present with preserved ejection fraction (HFpEF), a syndrome characterized by inflammation, myocardial extracellular matrix remodeling and diastolic dysfunction. We hypothesized that low-dose aspirin has beneficial effects in HFpEF and that these benefits are likely related to effects on monocyte/macrophage function and cell–cell interactions in the blood.

METHODS: In a retrospective analysis of HFpEF patients under the care of a hospital-based HF disease management program, we identified 150 patients taking low-dose (75 mg/ml) aspirin and age- and sex-matched HFpEF controls not taking aspirin. Survival and hospitalizations were assessed over a 3-year follow-up period. From this cohort, we studied 26 HFpEF age- and sex-matched patients (14 aspirin, 12 non-aspirin) using primary monocyte isolation, monocyte qPCR, serum matrix metalloproteinase (MMP) and inflammatory marker assays. Subsequently, primary monocytes were isolated from 6 healthy volunteers and co-cultured with platelet releasate (PR, 16h) prepared from collagen-activated platelets from the same donor. Finally, primary monocyte/platelet aggregates were incubated with 10 μM aspirin in matrigel-coated invasion transwells (16h) to study the influence on monocyte migration.

RESULTS: Low-dose aspirin was associated with significantly higher overall survival and lower HF hospitalizations over the 3-year follow up period (HR 0.665, 95% confidence interval, 0.389-0.961). Serum MMP2 and IL-16 were significantly reduced in low-dose aspirin HFpEF versus matched HFpEF controls (n=14 per group). Monocyte incubation with PR caused cell activation with increased MMP1, MMP2, MMP9, and MCP1 release. Finally, healthy donor monocyte/platelet invasion was reduced by 50% with low-dose aspirin (p<0.01). Inflammatory cytokines (IL1, IL6, CCL17) were reduced in supernatants.

CONCLUSION: We demonstrate for the first time a retrospective association between the use of low-dose aspirin and better outcomes in HFpEF. We also show that aspirin use is associated with reduced monocyte/macrophage markers in vivo and reduced invasiveness of monocyte-platelet aggregates ex vivo. Antplatelet strategies to modulate monocytes may require further prospective evaluation in HFpEF.
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Expression of CD39 and CD73 on peripheral T-cell subsets in calcific aortic stenosis
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Mechanisms and involvement of the immune system into the pathogenesis of aorta valve calcification are still not fully investigated. The aim of the study was to identify possible participation of peripheral T-cell subsets divided using their differentiation status and involvement in adenosine regulation in pathogenesis of aorta valve calcification.

We examined 24 patients with severe calcific aortic stenosis (average flow gradient 48.3 (46.0-65.0)) and 16 healthy volunteers. Mean age was 63 (57.6-64) years. There were 14 patients with bicuspid (BAV) and 10 - with tricuspid aortic valve (TAV). We didn’t find significant differences in valve functioning measured using ejection fraction, maximal and average flow gradient on BAV and TAV.

The quantity of circulating CD39 and CD73 of peripheral naive (CD45RA+CD62L+), central memory (CD45RA+CD62L-), effector memory (CD45RA+CD62L-) and terminally differentiated CD45RA+positive effector memory (TEMRA, CD45RA+CD62L-+CD25+CD4+CD122+ (Th) and CD4+CD122+CD25+ (T cyt) cells were measured using multicolor flow cytometry. It was found that relative number of Naive T cyt (p=0.003) was decreased and the relative number of TEMRA T cyt (p=0.006) was increased in patients with calcification comparing with healthy donors. Meanwhile there were significant differences in number of Naive T cyt and CD39 (p=0.042), TEMRA T cyt (p=0.034), EM Th CD39+ (p=0.014).

Besides there were significant differences between T-cells subsets in patients with tricuspid and bicuspid aortic valve. Relative and absolute count of T cyt (p=0.04), absolute count of EM T cyt (p=0.01) and relative count of CD3-CD73 T cells (p=0.04) in patients with BAV were significantly lower than in those patients in TAV. Taking together achieved results are proving the hypothesis of participation of T-cell subsets (basically T-cytotoxic) in calcification of aorta valves. Besides taking into the account differences in expression of CD39 and CD73 on the T-cells subsets we assume the involvement of T-cells subsets (basically T-cytotoxic) in calcification of aorta valves. Besides taking into the account differences in expression of CD39 and CD73 on the T-cells subsets we assume the involvement of T-cells subsets (basically T-cytotoxic) in calcification of aorta valves. Besides taking into the account differences in expression of CD39 and CD73 on the T-cells subsets we assume the involvement of T-cells subsets (basically T-cytotoxic) in calcification of aorta valves. Besides taking into the account differences in expression of CD39 and CD73 on the T-cells subsets we assume the involvement of T-cells subsets (basically T-cytotoxic) in calcification of aorta valves. Besides taking into the account differences in expression of CD39 and CD73 on the T-cells subsets we assume the involvement of T-cells subsets (basically T-cytotoxic) in calcification of aorta valves.

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Mast cells in the atrial myocardium of patients with atrial fibrillation: a comparison with patients in sinus rhythm
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Background: Atrial fibrillation (AF) is one of the most frequent arrhythmias and its pathogenesis is believed to contribute to structural remodelling and perpetuation of arrhythmia. Mast cells (MC) have been found to be involved in AF. The role of MC in AF is still only partially explained. Various morphological and functional alterations have been associated with AF. Therefore, the aim of this study was to evaluate the role of MC in AF.

Methods: AF patients with atrial fibrillation undergoing an open heart surgery with atrial fibrillation compared to those in sinus rhythm (SR).

Results: In the AF group, MCs were significantly more frequent than in the SR group. When patients with AF and SR were compared. It is unlikely that these cells have any specific role in AF as an endogenous control. The Arg2 gene expression was also higher in M1 compared to unstimulated (9.98 ± 1.15 vs -12.37 ± 0.56 A.U. and 6.26 ± 7.4 A.U.), while the amplification of Fizz1 by qPCR was inefficient.

Conclusions: The Arg2 gene expression was also higher in M1 compared to unstimulated (9.98 ± 1.15 vs -12.37 ± 0.56 A.U. and 6.26 ± 7.4 A.U.), while the amplification of Fizz1 by qPCR was inefficient.

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Characterization of FVB/N murinic bone marrow-derived macrophage polarization into M1 and M2 phenotypes
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Background: In a range of pathophysiological conditions macrophage activation leads to changes in their functional and phenotypic profiles. Among the wide existing cellular phenotypic spectrum, the subpopulations of classical activated macrophages (M1) and alternatively activated macrophages (M2) represent the two extremes. In vivo, the process of macrophage polarization into the distinct phenotypes is complex and depends, among others, on the local microenvironment. In vitro, macrophage activation can be more easily controlled, however, M1 and M2 characterization varies widely in the literature due to incubation time, origin of the cells, animal species and stimuli. Purpose: To characterize the phenotypic profile of FVB/N murinic bone marrow-derived macrophages polarized into M1 and M2 subpopulations.

Methods: Bone marrow cells obtained from femurs and tibia of 8-10-week male FVB/N mice were cultured for seven days. The cells were divided into three groups: untreated, M1 and M2. The four-hour in vitro polarization into M1 phenotype was performed by using lipopolysaccharides and interferon-γ and into M2 phenotype, interleukin-4 (4 nM; 100 ng/ml). RNA was extracted and after DNase treatment, qPCR was used to analyze the differential gene expression of M1 marker gene (tumor necrosis factor-a (TNF-a), arginase-2 (Arg2), chemokine C-C motif ligand 2 (CCL2) and interleukin-6 (IL-6); as well as M2 markers mannose receptor (MR) and found in inflammatory zone protein (Fizt2). Glyceraldehyde 3-phosphate dehydrogenase was used as an endogenous control.

Results: In M1, both TNF-a and IL-1β gene expression increased compared to untreated (6.76 ± 0.48 and 6.26 ± 0.32 A.U., respectively). Moreover, both genes were more expressed in M1 compared to M2 (6.76 ± 0.48 vs -0.26 ± 0.56 A.U. and 6.26 ± 0.32 vs 0.14 ± 2.12 A.U., respectively). The Arg2 gene expression was also higher in M1 compared to untreated (9.98 ± 3.1 vs 2.27 ± 2.51 A.U.). For the CCL2 mRNA, a trend towards higher expression in M1 was found, without reaching significance. Intriguingly, the amplification of IL-6 gene was absent by qPCR. In M2, MR gene was more expressed compared to M1 (4.41 ± 1.15 vs -12.37 ± 7.4 A.U), while the amplification of Fiz2 by qPCR was inefficient.

Conclusions: The TNF-a, IL-1β and Arg2 mRNA expression profiles observed in M1 are consistent with the current literature on macrophage activation. To confirm that CCL2 gene is important for the characterization of this phenotype in our culture model, the trial number N must be increased. On the other hand, the IL-6 gene, although being a known marker for M1, appears to be inappropriate for this study. In M2, the MR expression profile finds support in the literature, however, changing the Fiz3 primer sequence is necessary to better understand the role of this protein in the characterization of M2 in our cell culture model.
542 The biological expression and thoracic anterior pain syndrome
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The Aim of the Work: The clinical study seeks involvement of oxidative stress and dyslipidemic syndrome in chest pain pathology anterior localized (TAP).

Material and Method: It was conducted in the Medical I and II Clinic CF Iasi and those who have been diagnosed with various disorders with common symptoms: chest pain earlier. The results were compared with the same data from a group of 70 healthy volunteers. Evaluation of patients was made by clinical, laboratory investigations routine (blood count, urea, creatinine, glucose, cholesterol, triglycerides, HDL-cholesterol, LDL-cholesterol, electrocardiography at rest, chest radiography, fundus examination, abdominal ultrasound, echocardiography transthoracic(s), determination of antioxidant enzymes SOD type, GPx or measurements of lipid peroxidation (MDA).

Results: Analysis of obtained data allowed the clinical characteristics of metabolic and biological readout status differs depending on the type of disease or age.

Conclusions: The oxidative hypothesis of dysvascular disease, in the context syndrome chest pain, show significant intensification of stress bio-oxidative compared with patients under 60 years, underpinned by the significant reduction of antioxidant enzymes and augmentation significant lipid peroxidation, resulting hypothesis radicals species that play a role in the pathology certainly painful.

Table 1

<table>
<thead>
<tr>
<th>Age</th>
<th>Patients under 65 years</th>
<th>Patients over 65 years</th>
</tr>
</thead>
<tbody>
<tr>
<td>sex (m/f)</td>
<td>57/40</td>
<td>66/88</td>
</tr>
<tr>
<td>smoking</td>
<td>1/1</td>
<td>3/1</td>
</tr>
<tr>
<td>hta degree association</td>
<td>44% Degree II, III</td>
<td>95% Degree II</td>
</tr>
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<td>obesity</td>
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<td>association</td>
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<td>100%</td>
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<td>dyslipidemia</td>
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</tr>
<tr>
<td>HTA</td>
<td>33.3%</td>
<td>16.6%</td>
</tr>
<tr>
<td>peripheral arterial disease</td>
<td>16.6%</td>
<td>0.08%</td>
</tr>
<tr>
<td>Stroke</td>
<td>25.0%</td>
<td>11.1%</td>
</tr>
</tbody>
</table>

Clinical features of patients with anterior chest pain (TAP)

545 The association of heat shock protein 90 and TGFβ receptor I is involved in collagen production during cardiac remodeling in aortic-banded mice
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The molecular interest of Hsp90 as a target in fibrosis is rising quickly, but there is still a lack of understanding of the role of Hsp90 in the fibrogenic process. TGF-β plays a crucial pathophysiological role in the maladaptive remodeling of the heart in response to pressure overload by triggering interstitial fibrosis and cardiomyocyte hypertrophy through the transforming growth factor β (TGF-β) signaling cascade. Hsp90 is considered to be a protein stabilizer known to bind many receptors and thus protecting the corresponding signaling. TGFβ receptors (TGFβRs) among other proteins, are clients of Hsp90. The molecular interest of Hsp90 as a target in fibrosis is rising quickly, but there is still a lack of studies understanding the role of Hsp90 on pressure overload models in response to aortic stenosis.

Purpose: We postulated that the association of Hsp90 and TGFβRII is critical in collagen production during damaging myocardial fibrotic events.

Methods: Left ventricle pressure overload was induced by transverse aortic constriction (TAC group). Mice (n = 5 to 12 per group) were euthanized at 1 h, 3 h, 7 h, 14 h or 2 weeks after TAC surgery. Levels of expression of TGFβRI and Hsp90 and Col1 were determined by qPCR and Western blot. Cardiac fibroblasts were isolated from hearts of long-term TAC-treated mice and functional rebinding assay was used to determine Hsp90 activity levels in presence/absence of specific inhibitors (SB43152 and 17AAG).

Results: Hsp90 was shown to play a critical role in TGFβ signaling by stabilizing the TGFβ signaling cascade. Hsp90 is considered to be a protein stabilizer known to bind many receptors and thus protecting the corresponding signaling. TGFβ receptors (TGFβRs) among other proteins, are clients of Hsp90. The molecular interest of Hsp90 as a target in fibrosis is rising quickly, but there is still a lack of studies understanding the role of Hsp90 on pressure overload models in response to aortic stenosis.

Conclusion: Our studies propose that the formation of Hsp90-TGFβRII complex is involved in collagen production and further suggest it could be a good target to reduce the damaging myocardial fibrosis.

546 Loss of the inhibitory Galphao protein in the rostral ventrolateral medulla of the brainstem leads to abnormalities in cardiovascular reflexes and altered ventricular excitability
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The heart is controlled by the sympathetic and parasympathetic limbs of the autonomic nervous system with inhibitory signaling mechanisms recruited in both limbs. This study aimed to determine the role of inhibitory heterotrimeric G proteins in the central mechanisms underlying autonomic control of the heart.

Methods: Mice with conditional deletion of inhibitory heterotrimeric G protein GaIo in the rostral ventrolateral medulla oblongata were generated to determine the effect of specific GaIo deletions on autonomic control and electrophysiological properties of the heart.

Results: GaIo deletion in the presynaptic area of the rostral ventrolateral medulla (RVLm) was not associated with changes in HR or the arterial blood pressure (BP) or rest (home cage, normal behaviour). However, exposure to stressful conditions (novel environment, hypoxia or hypercapnia) in these mice was associated with profoundly exaggerated heart rate responses and an increased baroreflex gain when studied under urethane anaesthesia. This was associated with a reduced ventricular effective refractory period and lower ventricular tachycardia threshold. This phenotype was reversed by systemic administration of a beta-adrenoceptor blocker atenolol, suggesting that GaIo loss in the RVLm increases central sympathetic drive.

Conclusions: The data obtained suggests that GaIo-mediated signalling within the presynaptic circuits of the RVLm contributes to the autonomic control of the heart. GaIo deficiency in the RVLm is associated with exaggerated cardiovascular responses to stress, altered cardiovascular reflexes and electrical properties of the heart.
membrane AC to stimulation. Revealed disturbances in ESAC activity in patients with CHF reflect the disturbance of the endothelial nitric oxide synthase (eNOS) pathway, which is known to be a causative or a progressive factor in many vascular defect associated diseases. Emerging evidence suggests an important role for Fzd5 signaling in angiogenesis. In previous studies, Fzd5 knock-out mice showed a lethal deficiency in placenta and yolk sac angiogenesis, but the exact molecular mechanism behind this observation still lacks clarification. In our study, we tried to decipher the function of Fzd5 in endothelial cells, and its involvement in regulating angiogenesis. In vitro experiments revealed that the modulation of VEGF-induced tubule formation and migration can be affected by knockdown of Fzd5 in HUVECs. These results indicated that thrombin is a potent hypertrophic factor of the atrial myocardium and that DTIs and PAR1 inhibitor could prevent the atrial remodeling and AF substrate formation.

**549 Direct thrombin inhibitors inhibit atrial myocardium hypertrophy in a rat model of heart failure and atrial remodeling**

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Atrial ﬁbrillation (AF) is associated with a high risk of stroke due to thrombin formation in poorly con- tracted atria. In addition to its role in thrombus formation, thrombin has pleiotropic effects through the activation of protease-activated receptor-1 (PAR-1). Here we examined the involvement of the thrombin pathway in the atrial remodeling associated with heart failure (HF) and the effects of direct thrombin inhibitor (DTI) on this remodeling process. This study was conducted in a rat model of HF due to myocardial infarction and associated with atrial dilation and susceptibility to AF. Animals were treated immediately or one month post-MI with either vehicle control, 25 mg/kg/d dabigatran or 6 mg/kg/d of another DTI, S35972. Two months treatment with DTIs reduced both left atria dilation and the duration of burst pacing-induced AF whereas treatments had no effect on venous dilation and systolic dysfunction. The vitamin K antagonist, Warfarin, had no effect on both atrial and venous remodeling. The increase in hypertrophic markers such as brain natriuretic peptide and β-myosin heav- y chain, of the transcription factor NFATc3 observed in vehicle-treated HF rats was suppressed by DTIs. PAR-1 antagonist reproduced the effect of DTI on atrial dilation and AF susceptibility. In an atrial explant culture model, 10nM thrombin upregulated hypertrophic markers and plasmaminogen activator inhibitor-1 through PAR-1 and the Rho/Rho kinase pathway. These results indicate that thrombin is a potent hypertrophic factor of the atrial myocardium and that DTIs and PAR1 inhibitor could prevent the atrial remodeling and AF substrate formation.

**550 Tissue factor / TFVIIa transactivates the IGF-1R by a Src-dependent phosphorylation of caveolin-1**

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**Background:** The receptor tyrosine kinase IGF-1R is transactivated and translocated to the nucleus in response to the factor TF/TFVIIa complex. This occurs in several cell types including monocytic and smooth muscle cells. Caveolae are well-characterized cell membrane signaling compartments, but their role in TFI signaling is poorly understood.

**Purpose:** To clarify the mechanism behind the TF-induced phosphorylation of the IGF-1R, we uti- lized TF-expressing cancer cells to investigate the interaction between IGF-1R and caveolin-1 (Cav1), the principal protein of caveolae.

**Methods:** Prior incubation with FVIIa, PCI prostate or MDA-MB-231 breast cancer cells were trea- tled with 500 nM simvastatin, Cav1 siRNA, a peptide corresponding to the Cav1 scaffolding domain, or Src-family inhibitors. The phosphorylations of IGF-1R and Cav1 were determined using the Duo-Link In Situ proximity ligation assay (PLA) or by WB on fractionated cell lysates.

**Results:** FVIIa treatment (10 and 100 nM) increased the phosphorylation of the IGF-1R after 30 min- utes and induced a nuclear translocation of the receptor after 2 h. Incubation with simvastatin for 72 h resulted in a hyperphosphorylation of the IGF-1R owing to downregulation of Cav1 translocation. The IGF-1R was similarly activated by Cav1 siRNA knockdown. Additional experiments showed that pre- treatment with the Cav1 scaffolding domain peptide completely abolished the effects of FVIIa regard- ing IGF-1R phosphorylation and nuclear translocation. The formation of the TFVIIa-Cav1 complex did not alter Cav1 protein levels but induced a Src-dependent phosphorylation of tyrosine 14 on Cav1 after 10 minutes. Src completely abolished the transactivation of the IGF-1R by TF/FVIIa.

**Conclusions:** We found the Cav1 scaffolding domain to prevent IGF-1R phosphorylation in resting cell and could connect TF/FVIIa, Src, and Cav1 to the activation and nuclear translocation of the IGF-1R. These results also emphasize the importance of Src-family kinases in diseases characterized by aberrant TF expression such as cancer and atherosclerosis.

**551 Notch signaling is differently altered in endothelial and smooth muscle cells of ascending aortic aneurysm patients**

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**Background:** Notch signaling is differently altered in endothelial and smooth muscle cells of AoA patients. This cor- responses to the hypothesis that Notch-dependent differentiation of SMC is governed by endothelial cells. We suppose that alterations of key Notch pathway elements in HAEC population may cause an impairment of SMC differentiation in patients with thoracic aortic aneurysm.

**552 Frizzled 5 expression is essential for endothelial proliferation and migration**

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**Background:** Angiogenesis is the process in which endothelial cells and pericyclal cells create new vessels under the influence of a broad spectrum of stimuli. This process is tightly regulated and imbalances in signal- ling can be a causative or a progressive factor in many vascular defect associated diseases. Emerging evidence suggests an important role for Frizzled/Wnt signaling in angiogenesis. In previous studies, Frizzled 5 (Fzd5) was described to be indispensable in embryonic vascular development. Frizzled 5 knock-out mice show a lethal deficiency in placenta and yolk sac angiogenesis, but the exact molecular mechanism behind this observation still lacks clarification. In our study, we try to decipher the function of Fzd5 in endothelial cells, and its involvement in regulating angiogenesis.

**Methods:** Endothelial tubule formation and migration was studied in a model system, in which human umbilical vein endothelial cells (HUVECs) and pericytes were cocultured in a collagen matrix. Short inter- ference RNA (siRNA) based knockdown of Fzd5 in HUVECs resulted in significant reduction of endothelial tubule formation. The involvement of endothelial apoptosis as a causative factor for the poor angiogenic phenotype in the Fzd5 knockdown condition was excluded based on TUNEL staining. However, endothelial proliferative capacity of AoA patients was severely inhibited after knockdown of Fzd5. In order to define the molecular cause for the inhibition of angiogenesis after knockdown of Fzd5, known downstream Fzd5 transcription cascades were studied. No alterations were observed in various parameters of the canonical Wnt/β-catenin pathway and the non-canonical Wnt/Ca2+ pathway after knockdown of Fzd5. qPCR analysis of Fzd5 siRNA treated endothelial cells did however show a significant up-regulation of both Fstl and Ang2, two important factors in vascular regression. The up-regulation of Ang2 could be suppressed by a com- bined knockdown of Fzd5 and the transcription factor Erta-1. These data indicate that the degenerative effect of Fzd5 silencing on vascular structure formation in vitro strongly resembles with inhibition on endothelial proliferation and migration. Wnt/β-catenin signaling and Wnt/Ca2+ signaling are not affected by knockdown of Fzd5 in HUVECs. Instead, there could be a role for the transcription factor Erta-1 in Fzd5 signal transduction, as it is involved in the up-regulation of Ang2 after knockdown of Fzd5.

**553 Modulation of vascular function and ROS production by novel synthetic benzopyran analogues in diabetes mellitus**

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**Background:** Mitochondria have emerged in the past decade as major therapeutic targets in cardio-vascular pathology. We have previously demonstrated, in isolated rat heart mitochondria, that novel synthetic benzopyran analogues derived from a BMS-191095, a selective mKATP opener, modulated mitochondrial respiratory function and decreased generation of reactive oxygen species (ROS) in a dose-dependent manner. Whether the compounds have an effect on vascular function in diseased vessels is not known.

**Purpose:** The present study was purposed to assess the effect of three benzopyran analogues on the vascular reactivity and HGO2 production in aortic rings isolated from rats with streptozotocin-induced diabetes mellitus (DM) and mammaries harvested from coronary ar- tery disease patients with and without DM subjected to by-pass grafting.

**Methods:** The effect of KL-1487, KL-1492, KL-1507 (10μM) on endothelium-dependent relax- ation (EDR, assessed in the organ bath-system) and HGO2 production (determined by ferrous oxida- tion xylene orange assay) have been studied in diabetic vs. non-diabetic mice and human vascular segments. Results: We found an important decrease in EDR in diabetic vessels whereas HGO2 gener- ation was significantly increased in both humans and rats. Incubation of vascular segments with all inves- tigated compounds attenuated HGO2 production, reduced contractility and partially restored EDR. Conclusion: The novel benzopyran analogues KL-1487, KL-1492, and KL-1507 might be useful in improving vascular function in clinical conditions associated with high oxidative stress and endothelial dysfunction such as coronary artery disease and diabetes.

**Extracellular matrix and fibrosis - Heart**

**554 Cardiac fibroblasts as inflammatory supporter cells trigger cardiac inflammation in heart failure**

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**Abstracts S99**
Purpose: Cardiac remodeling and inflammation are hallmarks of cardiac failure and correlate with outcome in patients. However, the basis for the development of both remains unclear. We have previously reported that cardiac inflammation triggers transdifferentiation of fibroblasts to myofibroblasts and increase cardiac collagen deposition, one key pathology in cardiac remodeling. Furthermore, our findings reveal that cardiac fibroblasts are chemosensitive sentinel cells activated by increasing stretch intensities and are able to recruit inflammatory cells into the cardiac tissue, a process known to aggravate prognosis of patients. Here, we investigate the role of fibroblasts in the inflammatory process as well as the cross-talk between fibroblasts and inflammatory cells.

Methods and Results: We address the role of fibroblasts as inflammatory supporter cells in heart failure. Using endomyocardial biopsies from patients with heart failure we created a primary human cardiac fibroblast cell culture system to stimulate the primary fibroblasts we used the flexercell system with increasing stretch intensities or with increasing stretch frequencies. We found that not only increasing stretch intensities mimicking cardiac dilation induce activation of fibroblasts but also increasing stretch frequencies. Both types of mechanical activation lead to up-regulated chemokine production and triggers typical inflammatory pathways in vitro. Furthermore, we investigated the composition of the extracellular proteome of human cardiac fibroblasts using mass spectrometric analysis of the cell culture supernatant. We clearly demonstrate that besides ECM proteins different chemokines could be identified. Next, we used this conditioned medium derived from cardiac fibroblasts to perform co-culture experiments to investigate the cross-talk between fibroblasts and inflammatory cells.

Conclusion: Cardiac fibroblasts serve as supporter cells for cardiac inflammation. Due to different stimuli such as increased mechanical stretch mimicking dilation, increased stretch frequencies mimicking tachycardia, fibroblasts secrete cytokines and chemokines. This might be important in different forms of heart failure and therefore may be one general mechanism specific for fibroblasts. Furthermore, inflammatory cells are further modulated by proteins secreted by activated fibroblasts which shows the close association between fibroblasts and inflammatory cells.

557
A role for galectin-3 in calcific aortic valve stenosis
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Background: Aortic stenosis (AS) is a chronic inflammatory disease, and calcification plays an important role in the progression of the disease. Galectin-3 (Gal-3) is a proinflammatory molecule involved in vascular osteothesis in atherosclerosis.

Purpose: To study whether Gal-3 mediates valve calcification in AS.

Methods: Blood samples and biopsies from aortic valves (AVS) from 80 patients undergoing aortic valve replacement were studied by histological and molecular analysis. Valvular interstitial cells (VICS) isolated from adult human AVS were differentiated in the presence or absence of the pharmacological inhibitor of Gal-3, modified citrus pectin (MCP). In addition, AS rats were treated with MCP (100 mg kg-1 day-1) in the drinking water for 6 weeks.

Results: Gal-3 was spontaneously expressed in the AVS of patients with AS. Positive correlations were found between valvular Gal-3 protein levels and calcification markers. Valvular Gal-3 colocalized with osteogenic markers such as BMP-2, Runx2 and SOX-9. In vitro, MCP treatment decreased the expression of osteogenic markers in differentiated VICS. In rats, MCP treatment prevented the increase in Gal-3 protein levels, as well as the enhanced osteogenic markers found in the AV of AS rats.

Conclusion: Gal-3 appears to play a central role in the process of calcification in AS. Gal-3 could be a novel therapeutic approach to delay the progression of AV calcification in AS.

558
Omega-3 polysaturated fatty acids - can they decrease risk for ventricular fibrillation?
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Background: Reports, including ours, indicate that lower omega-3 (ω-3) index accompanied by cardiac fibroelastosis and myocardial connexin-43 remodeling and enhanced extracellular matrix production to the adrenergic β1 receptors (b1-AAB) are implicated in development of heart failure and inflammation, gaps in coronary artery disease.

Methods: Blood samples and biopsies from aortic valves (AVS) from 80 patients undergoing aortic valve replacement were studied by histological and molecular analysis. Valvular interstitial cells (VICS) isolated from adult human AVS were differentiated in the presence or absence of the pharmacological inhibitor of Gal-3, modified citrus pectin (MCP). In addition, AS rats were treated with MCP (100 mg kg-1 day-1) in the drinking water for 6 weeks.

Results: Gal-3 was spontaneously expressed in the AVS of patients with AS. Positive correlations were found between valvular Gal-3 protein levels and calcification markers. Valvular Gal-3 colocalized with osteogenic markers such as BMP-2, Runx2 and SOX-9. In vitro, MCP treatment decreased the expression of osteogenic markers in differentiated VICS. In rats, MCP treatment prevented the increase in Gal-3 protein levels, as well as the enhanced osteogenic markers found in the AV of AS rats.

Conclusion: Gal-3 appears to play a central role in the process of calcification in AS. Gal-3 could be a novel therapeutic approach to delay the progression of AV calcification in AS.

560
Endocardial fibroelastosis is secondary to hemodynamic alterations in the chick model of hypoplastic left heart syndrome
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Background: Endocardial fibroelastosis is a diffuse thickening of the ventricular endocardium, causing myocardial deformation and presenting as an unexplained heart failure in infants and children. One of the postulated causes is persistent and increased wall tension in the ventricles. Its frequent association with hypoplastic left heart syndrome (HLHS) as well as aortic stenosis or atresia led us to hypothesize that abnormal hemodynamic loading could be an important factor in its pathogenesis.

Purpose: To test our hypothesis in a chick model of HLHS induced by left atrial ligation (LAL) at embryonic day (ED) 4.

Methods: At EDB and 12, modifications of myocardial architecture and fibrosis were studied by histology and immunofluorescence microscopy, and the amount of collagen was quantified by image analysis and mass spectrometry (MS).

Results: Histology with H&E, Alcian Blue staining did not reveal any significant amount of fibrosis in neither control nor LAL hearts with the exception of the cardiac skeleton and valves. Hypoxygen tissue staining revealed an increased extent of hypoxic regions, normally limited to the septum, in the ventricular myocardium of LAL hearts at ED8. Immunohistochemistry with Collagen I antibody clearly demonstrated a significant thickening of the subendocardial fibrous tissue in LAL hearts. MS showed a significant increase in Collagen I and V in LAL hearts.

Conclusion: We conclude that abnormal hemodynamic loading leads to myocardial fibrosis, stimulating collagen production in the subendocardium. Therefore, EFE in HLHS clearly appears to be a secondary effect of abnormal hemodynamics.

561
Dynamics of serum levels of matrix metalloproteinases in primary anterior STEMI patients
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Purpose: To study the dynamics of serum levels of MMP-2, 3, 9 in patients (pts) with primary anterior STEMI.

Methods: 21 pts with primary anterior STEMI (mean age 60 ± 7 years) were enrolled. Blood samples were drawn on the 1st (T1), 3rd (T2), 7th (T3), 14th (T4) days of STEMI and 6 month after STEMI (T5). The serum levels of MMP-2, 3, 9 were determined by the immunoassay (ng/ml). The serum levels of MMP-2 were decreased in T1 and normalized to T5. The levels of MMP-3 were decreased in T1 to T4. The levels of MMP-9 decreased from T1 to T5. At T5 the levels of MMP-3 were not changed.

Conclusions: These findings suggest multiple cardio-protective effects of omega-3 intake that can contribute to decreased susceptibility of the hypertensive rat heart to lethal arrhythmias.

559
Serum levels of elastin derived peptides and circulating elastin-antielastin immune complexes in sera of patients with coronary artery disease
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Background and Aims: Elastin and collagen are the main proteins of vascular wall. An important factor in the development of vascular wall alterations is degradation of the elastic fiber major protein — elastin. Elastin peptides from this degradation are present in the circulation and are a stimu-}
Deletion of the alpha-7 nicotinic acetylcholine receptor changes the vascular remodeling induced by transverse aortic constriction in mice.

Methods: We used wild type mice (WT) and knockout mice with deletion of alpha7 nicotinic acetylcholine receptor (α7nAChR) at 10 weeks of age. Mice were divided into the following groups: WT SHAM, WT TAC, α7 SHAM and α7 TAC. Seven days after TAC, mice were sacrificed and the ascending aorta was isolated for analysis.

Results: The vascular cross-sectional area (VCSA) was increased in WT TAC (0.32 ± 0.005mm²) and α7 TAC (0.31 ± 0.02mm²) groups when compared to their respective controls WT SHAM (0.26 ± 0.02mm²) and α7 SHAM (0.28 ± 0.012mm²). A similar pattern was also observed for the area of the lumen, in the values of WT TAC (43 ± 6.56%) and α7 TAC (38.4 ± 2.41%) groups were larger when compared to their controls WT SHAM (28 ± 0.71%) and α7 SHAM (29 ± 3.61%). While the WT TAC group had a significant increase in the deposition of collagen type I (7.33 ± 0.89μm²) and III (2.97 ± 0.75μm²) when compared to SHAM, the deletion of α7nAChR inhibits this process maintaining the level of both types of vascular collagen as in SHAM and α7 TAC operated groups. Regarding the density of cells, the α7 TAC group had the highest values.

Conclusion: The results demonstrate that the TAC promotes a positive vascular remodeling in the proximal aortas of both WT and α7nAChR knockout mice. In response to TAC, the vascular deposition of collagen and the density of cells is influenced by this receptor. Further studies are needed to understand the mechanism involved in these processes.

565 Extracellular matrix remodelling in response to venous hypertension: proteomics of human varicose veins

Introduction: Extracellular matrix (ECM) remodeling has been implicated in a number of vascular conditions, including venous hypertension and varicose veins. However, to date no systematic analysis of matrix remodeling in human veins has been performed.

Purpose: To assess and provide mechanistic insight into ECM changes in varicose veins.

Methods: Varicose saphenous veins removed during phlebectomy and normal saphenous veins obtained during coronary artery bypass surgeries were collected. Gene expression analysis was performed on RNA extracted from venous tissues and cultured human saphenous vein smooth muscle cells, while sections were processed for histological and immunohistochemical analysis. Matrix proteome was enriched from varicose vein tissues and subjected to proteomics analysis by liquid chromatography tandem mass spectrometry (LC-MS/MS).

Results: The proteomics analysis revealed the presence of more than 150 ECM proteins, of which 75 had not been previously detected in human venous tissue and 34 showed significant differences between normal and varicose saphenous veins. ECM in varicose veins was characterised by a loss of several small leucine-rich proteoglycans, aggrecan and a compensatory increase in collagen I and laminin. Chymase and trypsin, two serine proteases commonly associated to mast cells, were among the up-regulated proteins. Using immunohistochemistry, however, chymase expression was localised to smooth muscle cells in varicose veins. The effect of chymase and trypsin on the venous ECM was explored by incubating normal saphenous veins with recombinant enzymes. Proteomics analysis revealed extensive ECM degradation after digestion with trypsin. In comparison, chymase was less potent and degraded predominantly basement membrane-associated proteins. When human saphenous vein smooth muscle cells were stimulated with transforming growth factor beta (TGF-β), tumor necrosis factor-a (TNF-a) or angiotensin II (Ang II), a number of ECM genes differentially expressed in varicose veins, including mmp13,changed in response to TGF-β and TNF-a but to a lesser extent to Ang II.

Conclusion: The present proteomics study provides unprecedented insights into the degradation of structural and regulatory components of the venous ECM in varicose veins.

568 Microtubule-associated protein RPE/EB family member 1 modulates sodium channel trafficking and cardiac conduction

Introduction: Microtubule-associated protein RPE/EB family member 1 (EB1) encoded by the gene MAPRE1 is part of a protein network which binds microtubules at their (+)-end extremities underneath the cell membrane. EB1 has been shown to regulate trafficking of connexin43 (Cx43), and that of connexin40 in cardiac myocytes, at the intercellular discs. Furthermore, EB1 is removed from intercalated discs in cardiac hypertrophy, heart failure and in the setting of Cx43 mutations. Recent studies have also demonstrated that EB1 is implicated in the subcellular localization of sodium channel in neurons. We here investigated the effects of EB1 on cardiac sodium channel function and its modulation effect on cardiac conduction.

Methods and Results: eQTL experiments performed on an F2 population of mice of two separate inbred strains carrying a sodium channel mutation (Scn5a79BdnfΔ1-7) showed a strong negative correlation between the expression of the MAPRE1 gene and QRS duration on the surface ECG, suggesting a functional impact for EB1 on ventricular conduction. Co-immuno precipitation experiments confirmed the physical interaction between EB1 and the major cardiac sodium channel Nav1.5. Over-expression of MAPRE1/EB1 in HEK293 cells together with SCNA5A shows a significant lead in increased so- dium current density without affecting kinetic properties, indicating an increased membrane trafficking of the Nav1.5 protein.

Conclusion: Our study demonstrates the functional role for EB1 in cardiac conduction and we highlight its direct regulation of the cardiac sodium channel Nav1.5. We recently produced lenti- viruses in order to knock-down and overexpress EB1 in order to characterize its modulatory effect on ion currents and action potential parameters in hiPSC-CM using the dynamic clamp technique.

569 Investigation of electrophysiological abnormalities in a rabbit athlete’s heart model

New Zealand white rabbits were randomized into a ‘Sedentary’ and an ‘Exercised’ (Ex’) group (n=7). Animals of the ‘Ex’ group were trained during a 12-week long treadmill-running protocol. Electrocardiography and resting ECG recording were performed under ketamine anaesthesia. At the end of the training protocol, proarrhythmic sensitivity were tested with dofetilide (50 μM) on Langendorff-perfused rabbit hearts. ECG repolarization parameters and sinus variability of ECG parameters were evaluated. Tissue samples were taken from the left ventricle and messenger RNA (mRNA) expression level of TGF-β, fibronectin-1, collagen-I, III, MMP-2 and TIMP-2 were quantified with RT-qPCR to determine the collagen metabolism.

Results: Electrocardiography on the 12th week showed significant increase in the internal end-diastolic diameter of the left ventricle (LVdID) in the ‘Ex’ group (17.4 ± 0.3 vs. 14.7 ± 0.8 mm, p<0.05) compared to the ‘Sedentary’ group. Resting heart rate was significantly lower (198 ± 4 vs. 253 ± 8, p<0.05), PQ, QT, RR, Tpeak-Tend interval and variability parameters of the RR and Tpeak-Tend intervals in vivo were significantly greater in the ‘Ex’ group. Dofetilide tended to increase the QTc interval in the ‘Ex’ group, however, there was no difference in the incidence of proarrhythmia between the two groups. RT-qPCR showed significantly greater mRNA expression of TIMP-1 in the ‘Ex’ group.

Conclusion: The increased LVdID and the decreased heart rate are characteristics of the exercise-induced athlete’s heart. Increased parasympathetic tone of the autonomic nervous system was manifested by the extended PQ and RR intervals and their variability parameters. Greater vari- ability and repolarization parameters may indicate the sensibility of the athlete’s heart to arrhythmia. Increased TIMP-1 indicated structural remodelling in our model. Further investigations are warranted. This work was supported by OITKA (PD 105888) and Bolyai fellowship of Farkas Attila.

570 Upregulation of expression of multiple genes in the atrioventricular node of streptozotocin-induced diabetic rat

Introduction: Diabetes mellitus is a serious global health problem and there is clear evidence of the negative influence of diabetes on the prevalence, severity, and prognosis of cardiovascular disease. The electrical conduction system is frequently compromised in diabetic heart. Prolongation of the QT interval and QRS complex correlates with an increased incidence of sudden cardiac death in diabetic patients. Atrial fibrillation is prevalent and there is a higher incidence of atrioventricular block in diabetic patients compared to non-diabetic individuals. In vivo and in vitro studies have demonstrated a reduced heart rate in the streptozotocin (STZ) – induced diabetic rat. Diabetes can increase the duration of the sinoatrial node action potential and the duration of the atrioventricular node action potential. Increased action potential duration, reduced action potential firing rate, upstroke velocity and rate of diastolic depolarization have been demonstrated in atrioventricular node (AVN) cells from STZ rats. Recent studies have demonstrated a reduction in peak L-type Ca2+ current, faster time- dependent inactivation, a negative shift in the voltage dependence of activation, and an increase in the voltage dependence of inactivation in STZ AVN cells. Recent studies have also demonstrated a significant increase in the AVN gap junction protein activity in STZ rat AVN cells.

Purpose: The aim of the present study was to investigate changes in the expression of genes encoding cardiac proteins that underlie the generation and propagation of electrical activity of the AVN in the diabetic heart.

Abstracts

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Methods: Diabetes was induced in male rats with STZ (60 mg/kg bodyweight) and age-matched controls. Rats and experiments were performed 12 weeks after treatment. Real-time RT-PCR techniques were used to measure the expression of genes.

Results: Diabetes was characterized by a 5-fold increase in blood glucose in STZ versus controls. The presence on the control group showed no significant changes. RT-PCR analysis revealed a significant decrease in the expression of HHb in the diabetic group. This was also observed in the expression levels of NCX1, TRPC1, and CAV3, which were significantly lower in the diabetic group.

Purpose: The aim of this study was to characterize the effect of racemic and levo-methadone on the action potential (AP) profile detected in single human ventricular cardiomyocytes at different driving rates (0.2, 0.5, and 1 Hz).

Methods: Single cardiomyocytes were obtained from ventricular samples of patients undergoing septal myectomy or heart transplantation. APs were recorded from cells using the perforated patch technique.

Results: Racemide methadone (0.1-10 μM) reduced AP duration (APD) in a concentration-dependent manner. The effect was not prevented by naloxone (1 μM), an opioid receptor antagonist, thus excluding the involvement of cardiac opioid receptors in the reduction of APD. Similarly to the racemic form, levo-methadone (0.1-10 μM) reduced APD of human ventricular cardiomyocytes, showing properties comparable to those of the racemide form. Other AP parameters, including amplitude and maximal diastolic potential were not modified either by racemide and levo-methadone.

Conclusions: In our experimental setting racemic and levo-methadone are able to reduce APD of human ventricular cardiomyocytes. Reduction is particularly pronounced in the early phase of AP repolarization, suggesting that, beyond Hb, other channels, such as NaVA15, are likely to be affected by methadone. Latter effect probably counterbalances and overcomes the reduction of repolarization due to Hb channel blockade.

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Acute temperature changes on the chick embryonic heart function

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Background: The function of the chick embryonic heart is highly affected by the temperature. Changes in the kinetics of ion channels and pumps are crucial for generation and propagation of electricity.

Purpose: We analyzed the effects of acute temperature changes on the beating rate, conduction properties and calcium transients in the chick embryonic heart in vitro and in vivo.

Methods: The effects of temperature changes (34°C, 37°C, 40°C) on calcium dynamics in isolated ED4 chick hearts in vitro was investigated by high-speed calcium optical imaging. Experiments were performed in vitro and comparison of in vitro measurements. Artificial stimulation experiments were performed in vitro and in vivo to uncover conduction limits of heart segments.

Results: Decrease in temperature from 37°C to 34°C led in vitro to 23% drop of the heart rate and unchanged amplitude of Ca2+ transients, compared to 25% acceleration in vivo. Increase in temperature from 37°C to 40°C led in vitro to 20% and in vivo to 23% increase of the heart rate, and a significant decrease in amplitude of Ca2+ transients (atrrium -35%, ventricle -38%). We observed in vitro wide spectrum of arrhythmias, of which the most common was atrioventricular block (67%). In vivo, we found variability of atrioventricular block locations. Pacing experiments in vitro and in vivo suggested that the atrioventricular blocks were likely caused by relative tissue hypoxia and not by the tachycardia itself.

Conclusions: The pacemaker and atrioventricular canal are the most temperature-sensitive segments of the embryonic heart. We suggest that the critical point for conduction is the connection of ventricular trabecular network to the atrioventricular canal.

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Vasogeneses, angiogenesis and arteriogenesis

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Clinical improvement and enhanced collateral vessel growth after monocyte transplantation in mice

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Monocytes are the most important mediators in arteriogenesis. Previous results by our group demonstrated the enormous potential of monocyte monocyte transplantation (different mouse strain) for improvement of collateral vessel growth. This phenomenon seemed to be due to a considerable host vs. graft reaction. To prove this hypothesis and in forecast to introduce this new method into clinical practice, we performed transplantation of human monocytes in a mouse model. Therefore we ligated the right femoral artery of BalbC-mice and injected human monocytes 24 hours after surgery via the tail vein. Perfusion was measured by Laser-Doppler-Perfusion Imaging (LDP) prior, as well as 7,14 and 21 days post ligature. We calculated a perfusion index (ligature/diluted ligature, PI). Additionally, we performed a clinical score, which included behavior, wound healing, signs of inflammation and mobility of the ligated extremity. A low score represented a good clinical outcome with less impairment. Furthermore, histological examination of hind limb muscle was done to examine perivascular cell infiltration, density of vessels and cell-cell interactions.

P decreased from preoperative 0.97 ± 0.08 to 0.19 ± 0.05 in all groups post ligature. Already one week after ligature PI increased in mice which had received human monocytes (HMuO 0.39 ± 0.12) as well as in the BalbC-control group (BalbCCo 0.35 ± 0.17). At day 14 in HMuo was 0.57 ± 0.07 vs. BalbCCo 0.50 ± 0.15 (ns.). A difference in perfusion became significant at day 21: HMuo 0.65 ± 0.12 vs. BalbCCo 0.50 ± 0.14 (p<0.05). Histological evaluation of the tail showed significant more collateral arteries in the adductory muscles within the ligated site after HMuo transplantation (B 3.5 ± 1.3) in comparison to the BalbCCo (6.3 ± 1.5; each n=9; p<0.05). Muscle tissue in the lower leg was histological examined for capillary density and hypoxia (HIF) as a measure for angiogenesis. The gastrocnemius muscle of mice which did not receive human monocytes showed an increase in capillary density as marker for ischemia: BalbCCo 1000 ± 305 vs. 629 ± 154 Cap/mm² HIF (n=9; p<0.01). The promotion of collateral vessel growth after xenogenic monocyte transplantation resulted in a better clinical score, as well: The score decreased in HMuo from initially postoperative 6 up to 2.3 vs. 1.7 BalbCCo at day 7. At day 14 the condition of mice improved even more: HMuo 1.17 vs. 4 BalbCCo. The most important clinical difference between the two groups was found after 21 days: HuMo 0.17 vs. 3.3 BalbCCo
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The role of HIF-1 alpha, VEGF and obstructive sleep apnoea in the development of coronary collateral circulation

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Introduction: Intermittent hypoxia (IH) in obstructive sleep apnoea (OSA) confers cardioprotection by enhancing coronary collateral circulation (CCC) development, thereby decreasing myocardium vulnerability to hypoxia and ischemia. The exact mechanism is as yet unclear. By better understanding of the physiology, one may attempt to replicate these adaptive mechanisms in non-OSA ischemic heart disease (IHD) patients to better augment CCC.

Purpose: The study objective was to assess whether Hypoxia Inducible Factor-1α (HIF-1α) and Vascular Endothelial Growth Factor (VEGF) play a role in the development of CCC in patients with OSA.

Methods: Methodology: A total of 44 patients with reported collaterals on angiography were selected as cases, with 21 patients not having a CCC recruited as controls. All patients underwent ambulatory polysomnography to test for the presence of OSA. Blood samples for HIF-1α (HIF-1α ELISA Kit, Antibodies-online Inc, Atlanta, GA, USA) and VEGF (Human VEGF ELISA Kit, KHO0111, Invitrogen Corporation, Carlsbad, CA, USA) were collected. The development of CCC was classified according to the Rentrop Score, with the cardiologists interpreting the angiograms blinded as to whether patients were cases or controls.

Results: HIF-1α increased with increasing Rentrop Score (p=0.04), in all patients. VEGF levels were however not significantly higher [p=0.03]. HIF-1α levels in moderate and severe OSA patients were significantly higher with higher Rentrop Scores (p=0.02). Patients without or mild OSA patients showed no difference with Rentrop Scores (p=0.49). VEGF levels did not significantly differ with Rentrop Score in none of the patient subgroups (no or mild OSA [p=0.23] and moderate or severe OSA [p=0.29]). A separate analysis did not reveal any significant difference between diabetic and non-diabetic patients for HIF-1α (p=0.00) and VEGF (p=0.34) in the absent or mild OSA subgroup. There was also no significant difference in the moderate and severe OSA subgroup for both HIF-1α (p=0.825) and VEGF (p=0.454).

Conclusion: This is the first study to date that links OSA, CCC, and plasma HIF-1α and VEGF levels. Augmented HIF-1α in moderate/severe OSA patients might be an important mediator in the development of CCC, but not in patients with mild OSA.

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Initiating cardiac repair with a trans-coronary sinus catheter intervention in an ischemia/reperfusion porcine model

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Objective: We analyzed the potential of a trans-coronary sinus catheter intervention activating endothelium to induce angiogenesis and the potential of temporary coronary venous pressure elevation (VICSO) to initiate cardiac repair in an ischemia/reperfusion model.

Material and Methods: 32 open chest pigs were divided sham-operation (n=3); 4 hours Infarct and 1 hour reperfusion (controls), 4 hours VICSO in the intact heart (VICSO-A, n=10), VICSO (started 15 min. after ischemia (VICSO-B, n=15). Specimens were taken from LAD region (infarct), adjacent zones Border1 and 2. Circumflex region remote R, Right ventricle RV. VEGFR1, 2 positive arteries and veins were calculated as percentage of total number of vessels, p38 positivity was measured as percentage of total amount of pixels and K67 expression was calculated as total number of cells using confocal-microscopy.

Results: VEGFR1 was significantly upregulated in arteries and veins in both interventional groups as compared to controls (p<0.05). VEGFR2 expression in arteries was significantly upregulated in arteries of both VICSO groups as compared to control (p<0.05). Significant upregulation could further be found in veins of VICSO groups as compared to control and sham-operated animals (p<0.05). p38 was significantly downregulated in myocardial tissue of pigs from VICSO A group in comparison with control pigs (p<0.05). K67 was significantly upregulated in VICSO A in comparison with controls (p<0.05).

Conclusions: Significant upregulation of angiogenic proteins stimulates a creating of new coronary vasculature as a result to temporarily blocking venous drainage, thus activating endothelium. Furthermore the downregulation of p38 is construed as shortage of myocardial damage, usually leading to apoptosis. Whereas upregulation of the proliferating marker K67 indicates that a trans-coronary sinus catheter intervention enables cell cycle reentry.

In conclusion, this study substantiates the concept that the PCI catheter displays beneficial effects on pathologically affected myocardium by exciting neoangiogenesis and cardioprotection leading to structural repair of the damaged heart.

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Early adaptation of pre-existing collaterals after acute arteriolar and venular microoclusion: an in vivo study in chick chorioallantoic membrane

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Introduction: After arteriolar occlusion, outward remodeling of pre-existing arteriolar collaterals occurs within days and increases collateral stress has been generally accepted as the driving force. However, knowledge is lacking on arteriolar collateral adaptation at the early stage post occlusion (PO) and on venular collateral adaptation.

Purpose: To address two questions: (1) What are the morphological and hemodynamic changes of pre-existing arteriolar and venular collaterals from immediately after occlusion up to 24 h PO? (2) What are the differences in those changes between arteriolar and venular collaterals?

Methods: White leghorn chicken eggs were cracked open on embryonic day 3 (E3) and the content was transferred into petri dishes for the development of chick chorioallantoic membrane (CAM). On E4, a 4-vascular segment ‘collateral unit’ was chosen at an arteriolar or venular anastomosis, a unit consisting of the two consecutive vessels from each side of the ‘anastomotic point’. The vessel adjacent to the collateral unit was occluded via micro-irradiation. Video recordings were made before occlusion, repeatedly during the first 2 h PO, hourly from 3 to 12 h PO and 24 h PO. Vessel diameter and blood flow velocity of all collateral unit vessels were measured offline from the video recordings. Blood flow rate and wall shear rate (WSR) were calculated.

Results: Arteriolar and venular collateral diameters did not show a significant increase over 23 hours in the control group ([P=0.05]). After occlusion, diameter of both arteriolar and venular collaterals decreased and lasted for several minutes and then increased continuously until reaching the maximal (arterioles: 3.4±0.1, venules: 2.3±0.4). Parallel, WSR showed an initial increase (arterioles: for 5 h PO, venules for 1h PO) and then a gradual decrease to the starting values ([P=0.05]). Maximal collateral enlargement (arterioles: 60%; venules: 100%) occurred in the smallest segment before occlusion and maximal WSR increase (arterioles: 230%; venules: 400%) occurred in the second smallest arteriolar and smallest venular collateral segment before occlusion.

Conclusions: In the CAM, collateral enlargement occurs several minutes after the occlusion, suggesting vasodilatory metabolites accumulated during this time might initiate the enlargement. In contrast, later collateral adaptation might mainly be driven by an increase in WSR. The differences between arteriolar and venular collateral units in diameter and WSR changes over time as well as the hypothesized cause-effect relations might be useful to develop therapeutic schemes.

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EDH-type responses to the activator of potassium SKCa2.3 and KCa3.1 channels

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Background: Endothelium-derived hyperpolarization (EDH) type relaxation is NO- and prostacyclin (PGI2)-independent hyperpolarization, with activation of small (SKCa2.3) and intermediate conductance (KCa3.1) calcium-activated potassium channels (KCa). Cardiovascular diseases with endothelial dysfunction, e.g. hypertension, are related with impairment of KCa2.3 and KCa3.1. Their activation increases coronary flow in diabetic rats, reduced systemic blood pressure in hypertensive mice, normotensive dogs and pigs.

Methods: Influence of SKCa2.3 and KCa3.1 type responses after the occlusion, suggesting vasodilatory metabolites accumulated during this time might initiate the enlargement. In contrast, later collateral adaptation might mainly be driven by an increase in WSR. The differences between arteriolar and venular collateral units in diameter and WSR changes over time as well as the hypothesized cause-effect relations might be useful to develop therapeutic schemes.

Endothelium

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Cardiovascular Research Supplements
Examined 31 men (mean age 56.7, SD 10.7 years) of protected vascular function of arterial compliance and level of leptin in patients with coronary heart disease in combination with hepatic steatosis depend on body mass index (BMI).

Methods: Consecutive performed in all patients.

Results: We studied the levels of leptin. The reactive hyperemia test for assessment of endothelial dysfunction was done in the sample with nitroglycerin and in normal geometry. Correlation depend-

Conclusion: Thus, in patients with coronary heart disease in combination with hepatic steatosis, endothelial dysfunction is not observed.

Cigarette smoke extract augments atheroprotective effects of high laminar flow on endothelial function

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Background/Introduction: Tobacco smoking and local hemodynamic forces are key stimuli in the development of endothelial dysfunction and atherosclerosis. High laminar flow has an atheroprotective effect on the endothelium. This leads to a reduced response of endothelial cells to cardiovascular risk factors compared to regions with disturbed or low laminar flow. The molecular mechanisms controlling the atheroprotective effect of high laminar flow and its effect on the cardiovascular risk factor of smoking is not well understood.

Purpose: We hypothesize that the atheroprotective molecular mechanisms of high laminar flow could be used to prevent the development of endothelial dysfunction by tobacco smoking. Therefore, we exposed human endothelial cells to cigarette smoke extract (CSEaq) under different flow conditions and studied gene expression, monocyte adhesion and wound healing.

Methods/Results: Primary human endothelial cells were stimulated with increasing dosages of CSEaq for 24-48h. CSEaq reduced cell viability in a dose-dependent manner. The major mediator of cell apoptosis was inhibited by CSEaq in a time-dependent manner. Induction of the NRF2 system by CSEaq was not further regulated by high laminar flow. In contrast, proatherosclerotic low laminar flow had no effect on eNOS expression and NO release compared to high laminar flow. Proinflammatory adhesion molecule ICAM1, VCAM1, SELE, and CCL2 were increased by CSEaq. Low laminar flow induced VCAM1 and SELE compared to high laminar flow. High laminar flow improved endothelial wound healing. This protective effect was inhibited by CSEaq in a dose-dependent manner. Low laminar flow did not affect wound healing compared to static conditions. Low and high laminar flow decreased adhesion of primary monocytes to endothelial cells. Interestingly, monocyte adhesion was increased by CSEaq under low laminar flow, which was not evident under high laminar flow.

Conclusions: In conclusion, our data suggest novel molecular mechanisms that underlie the association between tobacco smoking and the development of endothelial dysfunction. In contrast to low laminar flow, high laminar flow mediates protective effects on tobacco smoke-induced endothelial inflammation and wound healing.

The prognostic value of anti-connective tissue antibodies in coronary heart disease and asymptomatic atherosclerosis

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Aims: Since a low-grade immune inflammation may play a role in the pathogenesis of coronary heart disease (CHD) the contribution of anti-connective tissue antibodies in this process should be assessed.

Methods: We studied the association of plasma anti-connective tissue antibodies levels with acute coronary syndrome (ACS) manifestation in chronic CHD and in asymptomatic atherosclerosis (AA). Baseline levels of plasma antibodies against collagen, chondroitin-sulfate and hyaluronic acid were measured in 147 pts with chronic CHD and in 120 individuals with AA. The incidences of ACS in both cohorts during the 5 year period were registered. Statistical analyses include weighted Cox-regression model and ROC-analysis for detection the most informative predictive test.

Results: The association of ACS manifestation with elevated levels of antibodies against chondroitin sulfate was more prominent in chronic CHD (HR=2.57 95% CI: 1.09-5.99). On the other hand in AA the manifestation of ACS was associated with high levels of anti-collagen (HR=5.7 95% CI: 1.6-15.11) and anti-chondroitin sulfate antibodies (HR=4.95 95% CI: 1.54-14.02). According to ROC-analyse the elevated levels of anti-collagen antibodies was more predictive in AA (AUC 0.789).

Conclusions: Anti-connective tissue antibodies levels reflect the manifestation of ACS in chronic CHD and in AA. Evaluation of these antibodies might be used for diagnostic and prognostic purposes.

Novel potential properties of bioactive peptides from Spanish dry-cured ham on the endothelium

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Background: Bioactive peptides (BP) showing angiotensin I converting enzyme (ACE) inhibitory capacity have been widely pursued for the management of hypertension and are believed to exert beneficial physiological effects on the endothelium.

Purpose: To test ACE inhibitory capacity of a protein hydrolysate containing characterized BP from Spanish dry-cured ham. Besides, we aimed to evaluate other beneficial properties in the endothelium.
(protein expression and antioxidant activity). Methods. EA.hy 926 endothelial cells were cultured and transiently transfected with a human ACE ORF mammalian expression plasmid containing ACE DNA using Trans-it® X2 reagent (Mirus®). Transfected cells were treated with cured ham hydrolysate (500 μg/mL). ACE activity was tested in cell lysates by spectrophotometry. The effect of BP on cell proliferation and viability was assessed by MTT assay alone and with H2O2 300 μM. Potential mechanisms of action of BP over endothelial function were quantified by RT-PCR after treatment with TNFα 100 ng/mL or H2O2 300 μM and/or BP.

Results: BP do not affect cell proliferation but significant ACE inhibition was observed at 50 μg/mL. BP prevented the TNFα-induced ICAM-1 mRNA expression. H2O2 decreases cell viability and BP significantly reversed the H2O2-damage at 37.5 and 100 μM. In the presence of H2O2 they also increased expression of ANOTS mRNA although BP alone present an opposite effect. Increased expression of antioxidant enzymes mRNA such as superoxide dismutase and catalase and reduced NADPH oxidase were found incubating with H2O2 and BP together.

Conclusions: BP from Spanish dry-cured ham have important implications over the endothelium and may protect it from oxidative and inflammatory damage. These results implicate that BP may display clinical relevance.

**Lipids**

592 Intermediate density lipoprotein is associated with monocyte subset distribution in patients with stable atherosclerosis

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Background: Intermediate density lipoprotein (IDL) consists mainly of chylomicron remnants and very low density lipoprotein (VLDL) remnants that are thought to be proinflammatory lipoprotein particles. Atherosclerosis is considered to be an inflammatory disease of the vessel wall in which monocytes and monocyte-derived macrophages are crucially involved. Circulating monocytes can be divided according to their surface expression pattern of CD14 and CD16 into at least three subsets with distinct inflammatory and atherogenic potential. The aim of this study was to investigate whether IDL is associated with proinflammatory monocyte subsets.

Methods: We included 90 patients with stable coronary artery disease (CAD). Monocyte subsets were identified as classical monocytes (CD14++ CD16–), intermediate monocytes (CD14++ CD16+ – IM) and non-classical monocytes (CD14+ CD16+ + NCIM) by flow cytometry. Lipoprotein subclass fractions were measured by an electrophoresis method on polyacrylamide gel.

Results: IDL correlated significantly with the proinflammatory IM (r=0.24; p<0.05) whereas VLDL and low density lipoprotein (LDL) were not associated with monocyte subsets. IDL was not associated with CD14 (r=0.18; p=0.09) and NCIM (r=0.16; p=0.13) but correlated significantly with the acute phase protein C-reactive protein (r=0.40; p<0.01). The association of IDL with IM was independent of cardiovascular risk factors and statin treatment. Patients with IDL median (38mg/dL) showed a significant higher proportion of IM as compared to patients with IDL<38mg/dL (5.6 vs. 4.1; IQR 2.6-6.2%)

Conclusion: In conclusion, we provide a potential link between elevated levels of IDL and a proinflammatory distribution of monocyte subsets in patients with stable atherosclerotic disease. This possible proatherogenic role of IDL warrants further studies.

593 The characteristics of dyslipidemia in rheumatoid arthritis

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Introduction: Rheumatoid arthritis (RA) and dyslipidemia as the manifestation of atherosclerosis have the same mechanisms of development that causes cardiovascular complications. Purpose is to evaluate the characteristics of dyslipidemia in women depending on the duration of RA.

Materials and Methods: The study included 201 women, 33% of them had early RA lasting less than 1 year. The RA was diagnosed by the criteria ACR/EULAR 2010. Statistical analysis of the results was performed with “Statistics” 10.software.

Results: Dyslipidemia was revealed in patients with early RA in 68% (RA in 65%). In addition, the increased cholesterol blood–lower than 61% in early RA (100% in RA) was always associated with the disorder of other components of the lipid profile. In early RA the mean value of total cholesterol was higher in 0.2 and a maximum value in 1.4 times than in RA (3.2 (5.5) 10.2 and 3.5 (3.7) 2.2mmol/L) (p<0.05). Increased level of triglycerides (TG) in early RA was 1.5 times more frequent (51% (RA-39%)), the mean value 1.9 mmol/L in early RA (1.6 mmol/L in RA). Increased level of low-density lipoproteins (LDL) was 1.8 times more frequent in early RA-62%, (34% in RA), the mean value 3.2 mmol/L in early RA, (2.6 mmol/L in RA). Increased high-density lipoproteins (HDL) level was 2.3 times more frequent in early RA-55% and (24% in RA) (p<0.05). Atherogenic coefficient was 63% in early RA, (53% in RA) (p<0.05). Erythrocyte sedimentation rate (ESR) was 1.3 times more frequent in early RA-81%, (60% in RA). CRP (9%), and reactive protein C (rPCR) was 3.7% in early RA, (61% in RA) (p<0.05). CRP was 1.2 times higher in early RA than in RA, the mean value in early RA was 2.3mm/L (19mg/L in – RA). The correlation between total cholesterol and CRP (r=0.23, p<0.05), LDL and CRP (r=0.21, p<0.05) were revealed.

Conclusions: Thus, dyslipidemia in early RA presents the following characteristics: increased blood atherogenicity (the increase of LDL in 1.8 times, TG in 1.5 times, the decrease of HDL in 2.3 times), the average TBC was higher in 0.2 times and a maximum value in 1.4 times in early RA (p<0.05). There is a correlation between the level of TBC, LDL and the markers of systemic inflammation (p<0.05). The contribution of chronic immune-inflammatory processes in the development of dyslipidemia is observed more frequently in early RA in 1.2 times (p<0.05).
Amiodarone versus esmolol in the periprocedural period: an in vitro study of coronary artery bypass grafts

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Background: Atrial fibrillation (AF) is a major concern after coronary artery bypass grafting (CABG) surgery. Beta blockers and amiodarone are indicated in both prophylaxis and treatment of AF in the periprocedural period.

Methods: Sixty five vascular rings (32 IMA, 32 RA and 32 SV graft samples) obtained from 40 CABG patients were compared in terms of vascular functionality with atherosclerosis. The percentage of vasodilatation responses with esmolol was 48.9 ± 2.28% for IMA, 49.7 ± 3.03% for RA and 41.9 ± 4.05% for SV. The log half maximal effective concentration (log EC) values for IMA, RA and SV were -5.810, -5.500 and -5.440, respectively. On the other hand, the percentage of vasodilatation responses with amiodarone were 71.6 ± 5.18% for IMA, 58.6 ± 5.87% for RA and 65.0 ± 4.09% for SV. The log EC values for IMA, RA and SV were -5.290, -5.090, and -4.840, respectively. In both groups, IMA samples had more pronounced vasodilatation than RA and SV graft samples.

Conclusion: This study demonstrates that both amiodarone and esmolol can safely be used in the periprocedural period. Although amiodarone has a class III indication for the prophylaxis of AF in CABG patients, present data suggests that it may be the drug of choice due to its more favorable effects on graft patency in this specific patient population.
Atherosclerosis is characterized by acute and chronic vascular inflammation and formation of coronary thrombus, leading to an Acute Coronary Syndrome (ACS). However, the relationship between bacterial infection and acute myocardial infarction (AMI) has not yet been completely clarified and more research in this field is warranted.

**Purpose:** The aim of this study is to detect bacterial DNA in thrombus aspirates and peripheral blood samples of patients who presented Acute Coronary Syndrome with ST segment elevation (STEMI) treated with Primary Percutaneous Coronary Intervention (PPCI).

**Methods:** We studied 109 consecutive patients with STEMI from whom removal of thrombus with aspiration catheters was obtained. Bacterial DNA detection was performed by probe-based real-time PCR using the LightCycler 480. We used 12 probes for the detection of Aggregatibacter actinomycetemcomitans, Chlamydia pneumoniae, viridans group streptococci, Porphyromonas gingivalis, Fusobacterium nucleatum, Tannerella forsythia, Treponema denticola, Helicobacter pylori, Mycoplasma pneumoniae, Staphylococcus aureus, Prevotella intermedia, and Streptococcus gordonii. After the baseline vascular US, mice were randomized into four treatment groups: ESBP-Dex, ESBP, low dose dexamethasone or saline, delivered by four weekly intraperitoneal injections. One week after the application designed to measure in-vivo vessel wall anatomy and motion in small-animal models. Following the baseline vascular US, mice were randomized into four treatment groups: ESBP-Dex, ESBP, free dexamethasone or saline, delivered by four weekly intraperitoneal injections. One week after the application designed to measure in-vivo vessel wall anatomy and motion in small-animal models.

**Results:** Four different species have been detected in the aorta and in the wall of the aorta. The most frequent bacterial DNA found was from viruses group streptococci (6 patients, 55%), followed by DNA from Porphyromonas gingivalis (9.9%) and another patient Prevotella intermedia (0.9%). The bacterial DNA was not detected in the peripheral blood samples of any patient.

**Conclusions:** Bacterial DNA from four species have been detected in the thrombus aspirates of patients with STEMI. No bacterial DNA was detected in peripheral blood. This fact suggest that such bacteria could be latently present in plaques and might have a role in the plaque instability and subsequent thrombus formation leading to an Acute Coronary Syndrome in these patients.

**603 Novel E-selectin binding polymers reduce atherosclerotic lesions in ApoE(-/-) mice**

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**Background:** Atherosclerosis is characterized by acute and chronic vascular inflammation and leukocyte infiltration that result in plaque formation, instability, and rupture. E-selectin is the adhesion molecule expressed on activated endothelium that recruits leucocytes to the inflammation site, making it a therapeutic target to interfere with the development and progression of atherosclerosis.

**Purpose:** We aimed to test the hypothesis that E-selectin-targeted polymers with and without the anti-inflammatory drug would prevent inflammation and plaque progression.

**Methods and Results:** To target and modulate vascular inflammation we used novel N-(2-hydroxypropyl)-meth-acrylamide (HPMA) polymers conjugated with peptides that bind E-selectin with high affinity, and without dexamethasone (1mg/Kg ESBP-Dex, ESBP). Five-month-old APOE(-/-) mice were fed a high-fat diet (HFD) for 8 weeks. Plaques growth was assessed by vascular ultrasound (US) 4 weeks after onset of HFD. We used a novel Vevo Vasc software application designed to measure in-vivo vessel wall anatomy and motion in small-animal models. Following the baseline vascular US, mice were randomized into four treatment groups: ESBP-Dex, ESBP, free dexamethasone or saline. Delivery of 1 mg/kg/d of dexamethasone was delivered by 4 weekly intraperitoneal injections. One week after the final injection, we performed a second vascular US and harvested the aorta for histological analysis. We found that both ESBP and ESBP-Dex selectively targeted atherosclerotic lesions and reduced wall thickness of the ascending aorta (Figure). The addition of dexamethasone to E-selectin binding polymers did not increase their therapeutic effect (Figure).

**Conclusions:** E-selectin binding polymers reduce the growth of atherosclerotic lesions. We suggest a novel nanomedicine-based strategy to treat atherosclerosis and to stabilize the vulnerable plaque.

**604 Differential expression of the plasminogen receptor Plg-RKT in monocyte and macrophage subsets - possible functional consequences in atherogenesis**

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Human monocytes can be divided into a classical (CM, CD14++ CD16-), a non-classical (NMC, CD14+ CD16+), and an intermediate subset (IM, CD14+ CD16+) whereby CM are mainly phagocytes, NMC patrol along the endothelium and IM present proinflammatory properties and are associated with inflammatory diseases such as atherosclerosis. Similar to monocytes, macrophages exhibit distinct heterogeneity. M1 macrophages secrete inflammatory cytokines, reactive oxygen species and matrometallatoenzymes and are possibly involved in plaque vulnerability and destabilization whereas M2 macrophages are anti-inflammatory and linked to plaque stabilization. The plasminogen receptor Plg-RKT might contribute to plaque rupture as it is used together with the receptor of the urokinase plasminogen activator (uPA) uPAR by cells like monocytes and macrophages, to activate plasminogen to plasmin which is then used to degrade extracellular matrix. Here we aimed to analyze the expression of the Plg-RKT on monocyte and macrophage subsets. PMBCs were isolated from whole blood samples of healthy donors and were stained with fluorochrome-labelled antibodies against CD 14, CD 16, CD45 and Plg-RKT and uPAR and were analyzed by a flow cytometer. Cells were also incubated with FITC labelled plasminogen and stained and measured as described before via flow cytometry. The same experiments were performed with murine blood samples. However, to identify mouse monocyte subsets, CD11b and Ly-6C antibodies were used. Plg-RKT levels were also measured on macrophage subsets via flow cytometry. IM express the highest levels of Plg-RKT compared to CM (p < 0.0005) and NMC (p < 0.005). In addition, IM also bind the highest amounts of plasminogen indicating that they have a higher plasminogen activation capacity in comparison to the other two subsets. IM, in addition, have the highest amounts of uPAR compared to CM (p < 0.05) and NMC (p < 0.05). Interestingly, there seems to be a gender dependent difference in Plg-RKT levels with cells isolated from female donors having higher levels of Plg-RKT as compared to male cells. Ly-6C high expressing mouse monocytes are also able to bind higher amounts of plasminogen in comparison to Ly-6C low expressing monocytes (p < 0.05). M1 macrophages express significantly more Plg-RKT compared to M0 (p < 0.00005) and M2 (p < 0.005). Based on our data one might speculate that besides their inflammatory capacity IM as well as M1 macrophages might also be involved in processes requiring matrix degradation such as plaque destabilization in atherogenesis.

**605 Apelin-13 treatment enhances the stability of atherosclerotic plaques**

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**Background:** Atherosclerosis remains one of the main causes of worldwide death and morbidity. For this reason, substantial efforts have been made to identify novel approaches to improve the management of this disorder. Apelin is an endogenous peptide family having an essential role in cardiovascular homeostasis and pathological alterations. Recent studies demonstrated the contribution of the Apelin system to the development of atherosclerosis. However, such reports revealed contradictory results, and to date, it is difficult to accurately define the beneficial or deleterious role of Apelin in atherogenesis.

**Purpose:** To investigate the actions of Apelin-13 treatment on atherosclerotic plaques composition, focusing on features of plaque vulnerability.

**Methods:** Apolipoprotein E gene-deleted mice were fed with a Western-type diet for 11 weeks. Atherosclerotic plaque formation was induced in the carotid artery by a shear stress modifier device, which expose the vessel to distinct patterns of shear stress inducing plaques with distinct compositions. Mice were treated with Apelin-13 (2 mg/Kg/day) or vehicle for the last 3 weeks of the protocol. Results: Apelin-13 treatment did not change the atherosclerotic plaque size in the aorta. Similarly, it did not alter the lipid content of low shear stress- and oscillatory shear stress-induced plaques in the carotid. However, Apelin-13 ameliorated plaque stability by increasing intraplaque collagen content, which was associated with a reduction in MMP-9 expression. Furthermore, Apelin decreased cell infiltration (neutrophil and macrophage) and intraplaque reactive oxygen species production. Interestingly, Apelin-13 treatment reduced total cholesterol, LDL levels and free fatty acid serum levels, while HDL, triglycerides serum levels were not significantly changed.

**Conclusion:** Apelin-13 treatment for 3 weeks did not alter the lesion size, but significantly enhanced the stable phenotype of atherosclerotic plaques and improves serum lipid profile. These results indicate that activation of Apelin system decreases plaque vulnerability.

**606 Mast cells are increased in the media of coronary lesions in patients with myocardial infarction and favor atherosclerotic plaque instability**

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**Objectives:** Mast cells (MCs) may play an important role in plaque destabilization and atherosclerotic coronary complication. Here we have studied the presence of MCs in the intima and media of unstable and stable coronary lesions at different time points after myocardial infarction (MI).

**Methods:** Coronary arteries were obtained at autopsy from patients with acute MI (up to 5 days old, n=27) and with chronic MI (5 - 14 days old, n=18) as well as sections from controls without cardiac disease (n=10). Herein, tryptase-positive MCs were quantified in the intima and media of both unstable and stable atherosclerotic plaques.
Results: In the media of both acute and chronic MI patients, the number of MCs was significantly higher than in controls. This was also found when evaluating unstable and stable plaques separately. In patients with chronic MI the number of MCs in unstable lesions was significantly higher than in stable lesions. This coincided with a significant increase in the relative number of instable plaques in patients with chronic MI compared with control and acute MI.

Conclusion: The presence of MCs in the media of both stable and unstable atherosclerotic coronary lesions after MI suggests that MCs may be involved in the onset of MI and, on the other hand, that MI triggers intra-plaque infiltration of MCs especially in unstable plaques, possibly increasing the risk of re-infarction.

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Association of neutrophil to lymphocyte ratio with presence of isolated coronary artery ectasia

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Objectives: Coronary artery ectasia (CAE) has been defined as a dilated artery luminal diameter that is at least 1.5 times greater than the diameter of the corresponding normal artery. Isolated CAE without significant coronary artery stenosis and isolated CAE has more pronounced inflammatory symptoms. Neutrophil to lymphocyte ratio (NLR) is widely used as a marker of inflammation and an indicator of cardiovascular outcomes in patients with coronary artery disease. We examined a possible association between NLR and the presence of isolated CAE.

Study design: In this study, 113 patients who underwent coronary angiography for suspected or known ischemic heart disease were evaluated. Our study population consisted of 83 CAE patients and 30 age- and gender-matched subjects who proved to have normal coronary angiograms. Baseline neutrophil, lymphocyte and other hematologic indices were measured routinely prior to the coronary angiography.

Results: Patients with angiographic isolated CAE had significantly elevated NLR when compared to the patients with normal coronary artery pathology (2.79 ± 1.70 vs. 1.98 ± 0.56, p=0.008). However, there was no statistical difference between both groups as regard to age, gender and common risk factors including hypertension, diabets, smoking and family history of premature CAD.

Conclusion: Neutrophil to lymphocyte ratio is a readily available clinical laboratory value that is associated with the presence of isolated CAE.

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HMW-AGES application acutely reduces ICaL in adult cardiomyocytes

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Background: Several studies have shown that advanced glycation end products (AGEs) are associated with adverse cardiac outcome. Growing evidence show that high molecular weight AGEs (HMW-AGES) play a role as important as the well characterized low molecular weight AGEs (e.g, pentosidine and carboxymethyllysine). Despite their suggested deleterious involvement in chronic situations, HMW-AGES might also have deleterious effects acutely. However, to date, their effects at the cardiomyocyte level remains unknown.

Purpose: In this study, we investigated whether HMW-AGES acutely alter ICaL.

Methods: HMW-AGES were prepared by incubating 7 mg/ml bovine serum albumin (BSA) with 90 mM glycolaldehyde dimers in phosphate buffered saline (PBS) (pH 7.4) for 5 days at 4˚C. The BSA-modified AGES sample was validated by SDS-PAGE and fluorescence spectrometry. Single cardiomyocytes from the left ventricle of adult male rats were obtained by enzymatic dissociation through retrograde perfusion of the aorta. Perfusate alone (200 μg/ml) was used as control. Currents were measured in ncells and normalized to cell capacitance. Experiments were performed at room temperature. Data are expressed as mean ± SEM.

Results: The prepared BSA-modified AGES display high molecular weight and fluorescent proteins, characteristic for advanced glycation molecules. After 4 minutes HMW-AGES application, peak ICaL measured at +10 mV significantly decreased (-5.12 ± 1.01 pA/pF at baseline, ncells=11, p<0.05). In comparison, 200 μg/ml BSA used, a control, did not affect ICaL amplitude (-5.45 ± 0.54 pA/pF vs. -4.69 ± 0.48 pA/pF at baseline, ncells=7). Application of HMW-AGES did not affect the voltage-dependence of ICaL, which remained bell-shaped and displayed a maximal current at +10 mV, significantly reduced by 33 ± 3% (ncells=4). BSA application in comparison, did not have any effect on Ca2+-currents (ncells=5).

Conclusion: Our data demonstrate that HMW-AGES acutely alter ICaL and suggest a possible role in altered excitation-contraction coupling in rat cardiomyocytes.
As a result of the large size of cardiac myocytes in mammals, excitation-contraction coupling is dependent on spatially distributed calcium release sites working on the calcium-induced calcium release (CICR) principle. This is enabled by a network of t-tubules that spread the sarcoplasmic reticulum and thus the excitation signal throughout the cell volume. This morphological feature develops postnatally at early stage when myocytes undergo rapid growth and structural remodeling.

This work was aimed at comparison of changes in morphology and growth of t-tubules with the development of L-type calcium current amplitude in isolated rat cardiomyocytes.

Ventricular myocytes of neonatal and young rats (ages 2-21 days) were compared with the myocytes of 28 days old rats. Calcium currents recorded in isolated ventricular myocytes using 80 ms depolarizations from -50 to 0 mV in whole-cell patch-clamp mode were analysed for changes in the amplitude and kinetics. The morphology of t-tubules was assessed in the isolated myocytes as well as in intact myocardial tissue using the plasma membrane-specific fluorescent probe FM 4-64 and laser-scanning confocal microscopy.

Isotropy of sarcolasmic reticulum was observed around day 9 (D9) in the form of short membrane invaginations. These progressed into tubular elements with mostly longitudinal orientation and by D14 formed a loose network containing also transversal elements. After D17, a semi-regular tubular system developed. The calcium homeostasis undergoes significant changes during I/R, not only in the ischemic area, but also occurs in the remote area. These calcium changes may contribute to the development of cardiac hypertrophy and failure via Ca2+ overload.

Hibernation, stunning and preconditioning

618 Volatile anesthetic preconditioning attenuates ischemia-reperfusion injury in type II diabetic patients undergoing on-pump heart surgery
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Background: Conflicting evidence is existing that whether diabetic myocardium is afforded by ischemic preconditioning (IP) or not. Several in vitro experimental studies, volatile anesthetic drugs are known to mimic the ischemic preconditioning by reducing intracellular calcium sparks.

Methods: Total 60 patients were recruited. 30 Diabetic Mellitus (DM) patients were randomly selected to 2 groups (15 patients per each group): DM patients with no AP and DM patients with AP. Another 30 non-DM patients were randomly grouped into non-DM with AP and non-DM without AP. Patients of the AP group received 1% MAC sevoflurane for 5 min, interspersed by 5 min washout for three times prior to establishing the CPB. TnI and CK-MB were measured as marker of myocardial injury. Tissue sample from atrial trabecula harvested for Western blot analysis of total and phosphorylated PKC, FTO and STZ3, eNOS, Akt activation.

Results: There were no significant differences regarding preoperative demographic data. Peak of TnI defined at 5 hour postoperatively in the DM group without preconditioning: 2.1 ± 0.3 ng/ml vs. 1.65 ± 0.65 ng/ml in the DM + Sevo group (p<0.05). In the non-DM group, the group without AP were also observed higher TnI level as 1.6 ± 0.62 ng/ml vs 1.2 ± 2.45 ng/ml. Total amount of CK-MB released was higher in the group without AP than groups with AP (39.2 ± 3.63 vs. 3.45 ± 2.41 ng/ml). Non-DM patients group showed similar results (p<0.02). In the Western Blot, only phosphorylation of PKC and total STZ3 showed some differences. Levels of postoperative inflammation, postoperative stay in the ICU were significantly longer in non-AP groups, as well as length of hospital stay.

Conclusion: This study showed that sevoflurane-induced preconditioning is provides better cardioprotection and cardiac function postoperatively. The diabetic patients still benefit from preconditioning of volatile anesthetics. However, larger randomized trials are needed to carry out to clarify the protocol used for AP and follow-up checkups for long-term outcome.

619 The effect of early and delayed phase of remote ischemic preconditioning on ischemia-reperfusion injury in diabetic rats
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Introduction: Phenomenon of remote ischemic preconditioning (RIP) is an alternative strategy of protection which is induced by short episodes of ischemia and reperfusion applied to tissue or organ distant from the heart that results in effective cardiac protection against ischemia-reperfusion (IR) injury. It is well known from experimental studies that hearts of animals with acute diabetes mellitus (DM), except increased sensitivity to ischemia, may also exhibit increased resistance to IR injury. However, the relation between the impact of DM and individual phases of RIP is not clear.

Objectives: To explore the impact of early (1-RIP) and delayed phase (2-RIP) of remote ischemic preconditioning on IR injury in isolated heart of healthy rats and pathologically altered diabetic rats.

Materials and Methods: We used male Wistar rats; 8-days acute DM was induced by a single dose of 50 mg/kg (i.p.). Diabetic and healthy rats were subjected to RIP induced by 3 cycles of 5-min ischemia / 5-min reperfusion of cuf occlusion of the right hind limb. Delayed phase was investigated 24h after the last ischemic impulse. Isolated hearts were perfused according to Langendorff immediately after RIP or its delayed phase. After 15 min stabilization, the hearts were subjected to 30 min global ischemia followed by 3 h reperfusion for evaluation of the infarct size (IS, expressed in % of area at risk, AR), contractile function (recovery of LVDP in % of baseline values) and indexes of contraction and relaxation (+dP/dtmax, -dP/dtmax).

Results: In diabetic hearts, IS was decreased by 17.8 %, and LVDP recovery was improved by 56.8 % as compared with non-diabetic hearts. In non-diabetic healthy hearts, both 1-RIP and 2-RIP also attenuated postischemic stunning and lethal injury. Early phase significantly reduced ISAR to 11.7 ± 3.2 % and delayed phase to 17.8 ± 18.6 % vs. ISAR 33 ± 3 % in controls (p<0.05). In addition, LVDP recovery was increased to 70 ± 11.3 % and 83.4 ± 4 %, in 1-RIP and 2-RIP, respectively. On the other hand, when RIP applied in diabetic rats, both 1-RIP and 2-RIP did not confer any additional protective effect.

Conclusion: The results indicate that RIP provides an effective protection against IR in healthy myocardium, but does not have any positive impact in the diabetic myocardium. The latter indicates that...
620 Post-conditioning with 1648-thioate leads to attenuation of the inflammatory response and remodeling with less fibrosis and better left ventricular function in a murine model of myocardial infarction

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Background: Development of ischemic cardiomyopathy has been associated with inflammation and toll-like receptor (TLR) signaling. It has been shown that post-conditioning (PCON) is able to attenuate inflammation and fibrosis in myocardial infarction.

Purpose: The purpose of this study is to investigate whether PCON with the synthetic Cpg-containing TLK ligand 1648-thioate (Cpg) can modulate the development of inflammation and remodeling in reperfused murine myocardium.

Methods: Thirty min of LAD-ligation followed by reperfusion was conducted in 12 weeks old male C57Bl/6j mice. Mice where treated with Cpg ip. 5 min. before reperfusion. Control group received PBS; stam group did not undergo ischemia. After 3, 7 and 28 days M-mode echocardiography and Pitllar II: left ventricular (LV) pressure volume catheter measurements were performed. Hearts were excised and harvested for immunohistochemical analysis. Gene expression (Tagman® RT-qPCR) was measured after 6 and 24 hrs reperfusion.

Results: Apoptosis markers Caspase 3 and 8, and matrix metalloproteinase (MMP-9) were not induced in Cpg PCOn group compared to high induction in PBS PCon, indicating lesser degree of apoptosis and extracellular matrix degeneration. However, proinflammatory chemokines CCL2, CCL3 and CCL4, and cytokines TNF-alpha and IL-1beta were significantly up-regulated in Cpg PCOn group compared to PBS PCOn after 6 hrs. Interestingly, this peak of inflammatory activation was accompanied by significant induction of anti-inflammatory IL-10. Further, after 3 and 7 days significantly lower macrophage density (stained with MAC-2) was observed in the ischemic myocardium of Cpg PCOn mice compared to PBS PCon, suggesting that anti-inflammatory and other mechanisms mitigate proinflammatory activity. Total LV collagen area using picrosirius red protein stain was significantly attenuated in Cpg PCOn mice after 7 and 28 days compared to PBS PCOn mice. Cpg PCOn mice showed significantly lower level of ventricular dysfunction than PBS PCOn.TplR1 mRNA expression was increased in Cpg PCOn hearts, while TPLR4 and 9 were not induced. The specific role of the early inflammatory peak, followed by less macrophage infiltration, has to be further elucidated.

Conclusion: Our study suggests a cardioprotective mechanism of Cpg PCOn in modulation of remodeling and subsequent development of LV dysfunction in a murine model of reperfused myocardial infarction. This mechanism seems to evolve TLR-modulation being associated with early chemokine and cytokine action.

621 Maturation-related changes in response to ischemia-reperfusion injury and in effects of classical ischemic preconditioning and remote preconditioning

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Background: Effect of aging on tolerance to ischemia/reperfusion (IR) injury and adaptation mechanisms is known in older human and animal hearts. However, the onset of unfavorable changes has not been explored in details. Results concerning the effectiveness of classical ischemic preconditioning (IPC) also remain controversial since some studies demonstrated preservation of IPC-induced cardioprotection even in the elderly. Although remote ischemic preconditioning (RIPC) has been shown to protect young and aged human hearts from IR injury, little is known with respect to RIPC protection in animal hearts and age-dependency of this phenomenon.

Purpose and Methods: We aimed to study the changes in myocardial function, response to ischemia and changes in adaptation mechanisms related to both, IPC and RIPC, in the hearts of juvenile (5 months), younger adult (3 months) and mature adult (6 months) male Wistar rats, in Langendorff-perfused hearts exposed to 30-min I/10-min R without or with prior IPC. IPC was induced by one cycle of 5-min I/5-min R, in perfused hearts. RIPC (3 cycles of 5-min I/5-min R) was applied on the hind limb of anesthetized rats (pressure cuff inflation (200 mmHg)/deflation). We measured infarct size (IS, %), left ventricular (LV) pressure-volume catheter measurements were performed. Hearts where excised and harvested for immunohistochemical analysis.

Results: Maturation did not affect heart function, however, improved cardiac injury caused to lethal IR injury (IS increased by 40% and 65%, respectively, vs. juvenile group) and promoted arrhythmogenicity. IPC reduced occurrence of arrhythmias, IS and improved LVDP recovery in younger animals, while efficacy of attenuation was smaller in the mature ones. RIPC also reduced occurrence of arrhythmias, IS and improved LVDP recovery in younger animals. However, different from IPC, cardioprotective effect of RIPC was preserved even in the mature adults.

Conclusion: Early maturation already starts to impair the resistance of rat hearts against IR injury and causes gradual loss in IPC efficiency. On the other hand, RIPC appears more effective and easily performed clinically relevant cardioprotective intervention.

Mitochondria and energetics

624 Phase changes in myocardial mitochondrial respiration caused by hypoxic preconditioning or periodic hypoxic training

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Purpose: The heart muscle is often lacking of oxygen in pathological conditions, however, myocardial hypoxia is also used as preconditioning or therapeutic factor. Nevertheless, basic patterns and sequences of metabolic processes occurring in the heart tissue during various hypoxic conditions are poorly understood. The aim was to determine impact of hypoxic preconditioning or periodic hypoxia on dynamics of energy metabolism in left and right heart ventricles.

Materials and Methods: Wistar rats were subjected to hypoxic preconditioning (640 m, 3 hours) or periodic hypobaric hypoxia (PHH, "lifting" at 5600 m in barochamber, 6 sessions of 1 hour in every 72 hours).

Results: The dynamics of oxygen consumption (VO2), and body temperature (Tb) was studied during 3 weeks. In the left and right ventricle samples from urethane narcotized rats, mitochondrial respiration was estimated by Chn, mRNA and protein expression was assessed by RT PCR and Western blot.

Conclusion: Four phases of physiological changes were found in both experimental conditions, after preconditioning and during PHH. The first phase, hypometabolic, was associated with low ATP production, and accompanied by decreasing of VO2, Tb, V1/V4, ATPho and increasing of FAD-dependent substrate oxidation. The second, transition phase was observed by 5-7 days after start, it was characterized by shift of myocardial metabolism to activation. In the third phase, hypermetabolic (7–12 days or more), the recovery of metabolism and high ATP production, increased VO2, decreased V4, increased NAD-dependent substrate oxidation, V3/V4, ATPho was found. The fourth, phase of adaptation, was characterized by normalization or reduction of VO2 and mitochondrial respiration. These results were correlated with changes in the expression of HIF-1α and HIF-3α mRNA. In the hypometabolic phase, the antioxidant protection was increased due to induction of MnSOD protein, and membrane-associated mechanisms of cardiotriggeration were raised through the induction of caveolin-3. In the hypometabolic phase, kinase Akt and Akt-dependent mechanisms of myocardial protection were stimulated promoting antiapoptotic and prohypertrophic effects.

Conclusion: Thus, common regularities in the phase changes of the mitochondrial metabolism in the left and right ventricles in the PHH and during the recovery period after hypoxic preconditioning were found. The sequence of phases as a hypometabolic, transient, hypometabolic and adaptive has been established. The hypometabolic and hypermetabolic phases are associated with various mechanisms of myocardiaprotection due to target gene and protein induction.

625 Desmin mutations depress mitochondrial metabolism

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Purpose: Our previous data demonstrated reduced mitochondrial calcium in muscle cells carrying aggregate-prone desmin mutations. We speculated that decreased mitochondrial calcium might affect mitochondrial respiration parameters, e.g. oxygen consumption rate (OCR).

Materials and Methods: We applied Cell Mito Stress Test (Seahorse Bioscience, USA) to evaluate cell respiration. This experiment allows measuring oxygen consumption in living cells and assessing mitochondrial respiration parameters in real-time mode. Four key parameters were estimated according to the manufacturer's protocol— basal OCR, ATP-linked (non-phosphorylating) OCR, maximal OCR, and non-mitochondrial OCR. Each experiment consisted of six experimental groups corresponding to (i) control cells and cells transduced via LV encoded one of exogenous desmin (ii) Des WT, (iii) Des L165P, (iv) Des A357P, (v) Des L370P, (vi) Des D399Y. Obtained data were non-parametrically evaluated using statistical software, with p<0.05 considered significant.

Results: All cell types had similar bioenergetic profiles: decreasing OCR after oligomycin application, rapid OCR raise after FCCP application, and drop of OCR after rotenone/antimycin A application. Significantly, it was only maximal OCR that declined in the presence of desmin mutations; all other parameters did not show any significant difference between cells expressing various forms of desmin. Relative increase of OCR after FCCP application was 1.95±0.09 for non-transduced cells, 2.36±0.09 for Des WT, 1.85±0.12 for Des L165P, 1.81±0.11 for Des A357P, 1.95±0.11 for Des L370P and 1.87±0.12 for Des D399Y. Thus, Des L345P and A357P, being the most prominent aggregate-prone mutations, resulted in the most prominent decline in maximal OCR in comparison with Des WT, while other mutations decreased maximal OCR but not as dramatically. Furthermore, we found that only cells expressing mutant desmin had relative increase of maximal OCR less than one, implying lack of spare respiratory capacity in some of these cells.

Conclusion: We found that in the presence of desmin mutations maximal OCR was decreased in comparison to cells harbouring Des WT and spare respiratory capacity rate declining as well. Thus, we assumed that desmin mutations might confine mitochondrial respiration parameters.

626 Methyline blue modulates mitochondrial function and monoamine oxidases-related ROS production in diabetic rat hearts

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Background: Mitochondrial dysfunction and reactive oxygen species (ROS) generation are critical events in the pathophysiology of type II of diabetes mellitus (DM), the most severe metabolic disease. We have recently reported that monoamine oxidases (MAOs), mitochondrial enzymes with 2 isoforms, A and B, contribute to the oxidative stress in experimental diabetes. Methylene blue (MB) is a redox-drug with widely reported protective effects at mitochondrial levels that also inhibits MAO activity. The present study was purposed to characterize the effects of MB (0.1 μM) on mitochondrial respiration, calcium sensitivity, and MAO-related ROS production in rat heart mitochondria (RHM) isolated from diabetic rats.
Methods: Mitochondrial respiratory function was assessed by high-resolution respirometry whereas ROS production and calcium retention capacity of isolated RHM were measured spectrophotometrically.

Results: In RHM respiration on both complex I and II substrates (glutamate/malate and succinate + rotenone, respectively) a significant increase in all bioenergetic parameters was found in treated versus non-treated mitochondria. No changes in sensitivity to Ca + induced opening of the mitochondrial permeability transition pore were found in the presence of MB. Interestingly, MB elicited a significant increase H2O2 release (Amplex Red assay) in the presence of CI substrates, but had no effect in the presence of the CI substrate.

Conclusions: In diabetic rat hearts, metylene blue improved mitochondrial respiratory function regardless the substrates used and elicited a dichotomous, substrate-dependent effect on ROS production. MAO inhibitors mitigated the MB-dependent increase in ROS production for complex I (but not complex II)-supported respiration.

627 Dxorubicin modulates the real-time oxygen consumption rate of freshly isolated adult rat and human ventricular cardiomyocytes

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Introduction: As cancer survival rates improve, cardiotoxicity as a result of the agents used to treat neoplastic disorders has become increasingly clinically relevant. Despite our current knowledge, a full understanding of the early steps and functional changes that lead to cardiotoxic dysfunction remains to be determined. Here, we investigate the acute effect of doxorubicin (DOX) on the real-time oxygen consumption rate (OCR) of ventricular cardiomyocytes.

Methods and Results: Adult rat ventricular cardiomyocytes (ARVC) were isolated using a Langendorff perfusion system and enzymatic digestion. A Seahorse Bioscience XFp instrument was used to measure the OCR of ventricular cardiomyocytes in real-time. To assess the use of ARVC and adult human ventricular cardiomyocytes in a Seahorse assay the Mito Stress Test (Seahorse Bioscience) was performed with compounds that have a known effect on the cellular OCR. The standard Mito Stress Test profile was observed in both cell types in response to the compounds oligomycin, FCCP and a mix of rotenone and antimycin A, indicating that the Seahorse assay is valid to assess the real-time OCR in these cells. To test the acute effect of DOX on the OCR of ventricular cardiomyocytes, following four baseline measurements of the OCR over 20 min, DOX was serially injected into the microchamber at increasing concentrations of 1, 3, 10 and 30 μM. The OCR values are corrected for total protein concentration and normalised to baseline measurements. Acute injection of 1 μM DOX resulted in a significant concentration-dependent increase in the real-time OCR of ARVC (1 μM DOX 0.974 ± 0.009 vs. control 0.952 ± 0.015; 3 μM DOX 1.18 ± 0.033 vs. control 0.954 ± 0.038; 10 μM DOX 1.64 ± 0.136 vs. control 0.964 ± 0.057; P < 0.001; 30 μM DOX 2.44 ± 0.348 vs. control 1.01 ± 0.079; P < 0.001, n=6). Serial DOX injections resulted in a similar concentration-dependent increase in the OCR of freshly isolated adult human ventricular cardiomyocytes (n=3).

Conclusions: Serial injections of DOX resulted in an acute concentration-dependent increase in the real-time OCR of freshly isolated adult rat and human ventricular cardiomyocytes. This acute response indicates that DOX has an immediate effect on metabolic function in isolated ventricular cardiomyocytes.

Cardiomyopathies and fibrosis

630 Effects of genetic or pharmacologic inhibition of the ubiquitin/proteasome system on myocardial proteostasis and cardiac function

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Purpose: The Ubiquitin Proteasome System (UPS) and the autophagy/lysosome system (ALS) mediate the removal of intracellular misfolded/unfolded proteins, and are essential for cardiomyocyte survival. Therefore we aim to study how cold temperatures affect cardiac hypertrophy and whether this phenotype is reversed after thermo-neutral conditions.

Methods: Studies in vivo were performed in two-month old wild-type (wt) mice. Animals were subjected to chronic cold exposure (+4°C) for three weeks (CC). After this period animals were put into thermo-neutral conditions (30°C) for one week (CD). Cardiac samples from both groups were obtained and analyzed.

Results: At trophic mutant DSG2 to the intercalated disks was demonstrated for Tg-HWT mouse cardiomyocytes, but not for both Tg-HQ10 and Tg-HQ3131 cardiomyocytes which showed a cytoplasmatic localization of the truncated form of the protein. Whereas Tg-HWT mice had no detectable histologic, morphologic, or functional cardiac changes, the Tg-HQ3131 mice hearts displayed cardiomyocytes loss and biblendicular fibrous tissue replacement, similar to those of ACM patients. Ultrastructural analysis revealed reduction of desmosome number, density, and length at the cardiac intercalated disks, together with swollen cisternae of endoplasmic reticulum, suggesting partial retention of the mutant DSG2, and mitochondrial damage in Tg-HQ mice when compared to Tg-HWT. Despite the unchanged levels of total beta-catenin, Tg-MQ mice at 3-, 6- and 12-months of age displayed reduced production of active beta-catenin, together with decrease expression of c-Myc, Foxo and Cdc42-D1 down-stream targets, and reduction of glycogen synthase kinase 3-beta levels.

Conclusions: These findings suggest that inhibition of Wnt-beta-catenin signaling is involved in the pathogenic mechanism of DSG2-related ACM since early stages of the disease.

632 Cold-induced cardiac hypertrophy is reversed after thermo-neutral deacclimatization

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Introduction: Of the four seasons, winter has the highest mortality and morbidity from cardiovascular complications. Chronic exposure to cold is known to cause hypertension and cardiac hypertrophy, although cold-induced cardiac hypertrophy is independent of elevations in blood pressure. Therefore we aim to study how cold temperatures affect cardiac hypertrophy and whether this phenomenon is reversible after return to a thermo-neutral temperature.

Methods: In RHM respiring on both complex I and II substrates (glutamate/malate and succinate + rotenone, respectively) a significant increase in ROS production for complex I (but not complex II)-supported respiration.

Results: In RHM respiration on both complex I and II substrates (glutamate/malate and succinate + rotenone, respectively) a significant increase in all bioenergetic parameters was found in treated versus non-treated mitochondria. No changes in sensitivity to Ca + induced opening of the mitochondrial permeability transition pore were found in the presence of MB. Interestingly, MB elicited a significant increase H2O2 release (Amplex Red assay) in the presence of CI substrates, but had no effect in the presence of the CI substrate.

Conclusions: These data improve our understanding on the role of the muscle specific ubiquitin li-Sep 2016. Cardiovascular Research Supplements
634 Atrial epicardial adipose tissue derives from epicardial progenitors

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Background & Aims: The accumulation of the adipose tissue (AT) in the sup- and sub-epicardium of the atrial myocardium is associated with a high risk of atrial fibrillation. Here we addressed the cellular origin of atrial AT.

Methods: Human right atrial specimens obtained during cardiac surgery were used for histological, biochemical and molecular expression assays. To study the ability to differentiate into adipocyte, progenitor-derived cells (EDPCs) were cultured in adipogenic medium from 3 weeks. In order to determine the cellular origin of atrial adipose tissue, we studied a lineage tracing Wt1-Cre-Rosa-tdT mice model. We found that a number of adipocytes that compose the atrial EAT could derive from aEDPC through an EMT process.

Conclusion: Atra EPDC have the ability to differentiate into adipocyte and to contribute to the accumulation of EAT.

635 Caloric restriction ameliorates cardiac function, sympathetic cardiac innervation and beta-adrenergic receptor signaling in an experimental model of post-ischemic heart failure

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Introduction: Restricted diets are effective interventions to enhance cardiovascular function and metabolic profile and are known to improve life span. IF (Intermittent fasting) dietary regimen has a cardioprotective effect in a rat model of myocardial infarction (MI) when diet is started before MI induction. Chronic heart failure (HF) is associated with reduced cardiac sympathetic innervation and with upregulation of G protein-coupled receptor 2 (GR2), which contributes to dysfunction of beta-adrenergic receptor (beta-AR) signaling and to decrease cardiac inotropic reserve.

Purpose: To test the effects of a long-term restricted diet, started late after MI on cardiac function, sympathetic innervation and beta-AR signaling in an experimental model of post-ischemic HF.

Methods: Two-months-old male Wistar-Kyoto rats (n=40) were randomly assigned to left ascending coronary artery ligation to induce MI or sham operation. Four weeks later, a time point when HF was established, HF rats were further randomized to a one year IF dietary restriction or ad libitum diet (standard diet). Thus, our final animal population consisted in 4 groups: Sham normal diet, Sham IF diet, HF normal diet and HF IF diet.

Results: One year of IF diet resulted in a significant reduction in body weight (p<0.001) and heart weight (p<0.05) when compared to groups treated with normal diet. At the end of the study period, echocardiography revealed that HF animals that underwent to restricted diet resulted in improved systolic function and ameliorated left ventricular remodeling compared to HF rats fed with normal diet. Consistently, invasive hemodynamic showed a significant improvement in cardiac inotropic reserve in HF IF rats compared to HF normal diet animals. Importantly, IF diet was associated with a significant increase of cardiac sympathetic innervation, as assessed by confocal microscopy, and with an improved cardiac beta-AR density in HF rats when compared to HF rats treated with standard diet. Accordingly, IF diet resulted in a dramatic reduction of cardiac GR2K2 recruitment to the plasma membrane.

Conclusions: We have demonstrated for the first time that IF, started when HF was already established, ameliorates cardiac function and inotropic reserve in an experimental model of HF. At the molecular level, we have found that IF diet significantly improved sympathetic cardiac innervation and beta-AR signaling in HF.
637 Epigenetic therapy reduces cardiac hypertrophy in murine models of heart failure
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Background: Heart failure with preserved ejection fraction (HFrEF) is one of the leading causes of global morbidity and mortality. HFrEF is driven by pathological remodeling in the heart where there is hypertrophy of cardiomyocytes (cardiac hypertrophy) and an increased accumulation of extracellular matrix proteins in the interstitium (fibrosis). Recent evidence suggests that epigenetic processes such as DNA methylation are involved in the pathogenesis of cardiac remodelling. Inhibition of DNA methylation may yield a novel therapeutic avenue for the treatment of HFrEF.

This study investigated the therapeutic potential of the DNA methyltransferase inhibitor, 5-azacytidine (Saza) to inhibit pathological hypertrophy in the heart using preclinical models of HFrEF, the transaortic constriction (TAC) model and the Angiotensin II (AngI) infusion model.

Methods: Wild type C57BL/6J mice underwent surgical creation of the aortic arch or implantation of a subcutaneous osmotic pump infusing 1000 ng/kg/min angiotensin II (AngII) to induce pressure overload. Sham surgery was used as the TAC surgical control group and a saline infusion pump was used as the AngII infusion control group. TAC and AngII mice were treated with 5aza or placebo for 9-10 days after surgery with intervals of 2-3 days between injections. Cardiac structure and function was examined in vivo using non-invasive echocardiography.

Results: Echocardiographic analysis revealed that TAC and AngII mice treated with Saza displayed a significant reduction in the interventricular septal wall and left ventricular posterior wall thickness compared to mice which received placebo treatment. Reduction in both models, even when Saza treatment was initiated in the TAC model after cardiac hypertrophy was established.

Conclusion: Therapeutic options for HFrEF patients are limited. Inhibition of DNA methylation using Saza shows therapeutic potential by reducing cardiac hypertrophy in preclinical models of heart failure and seems to have a beneficial effect even in the setting of established cardiac hypertrophy.

638 Imbalance of the VHL/HIF signaling in WT1+ Epidermal Progenitors results in coronary vascular defects, fibrosis and cardiac hypertrophy
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Background: Epicardial progenitors (EPs) of the mammalian heart express Wilm’s tumor 1 tumor (WT1) and contribute to coronary-vascular interstitial fibroblasts and marginally to cardiomyocytes. WT1 is re-expressed in the adult epicardium upon cardiac injury and has been associated with potential regenerative capacity. Thus, there is a great interest in understanding the signals governing their biology.

Purpose: Our goal is to study the influence of embryonic hypoxia in the biology of WT1+ EPs and Epicardial Derived Cells (EDCs).

Methods: We have generated several conditional hypoxia gain and loss of function models (GOF/LOF) in WT1+ EPs and Epicardial Derived Cells (EDCs).

Results: We have identified a signaling pathway that is involved in the development of coronary vascular defects, fibrosis and cardiac hypertrophy. This pathway involves the imbalance of the VHL/HIF signaling in the epicardium, leading to ventricular hypertrophy, fibrosis, coronary defects and inflammation, demonstrating that epicardial VHL/HIF axis is important for proper cardiovascular development and homeostasis.

639 Diastolic dysfunction is the first stage of the developing heart failure
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Background: Heart failure with preserved ejection fraction (HFrEF) is one of the leading causes of global morbidity and mortality. HFrEF is driven by pathological remodeling in the heart where there is hypertrophy of cardiomyocytes (cardiac hypertrophy) and an increased accumulation of extracellular matrix proteins in the interstitium (fibrosis). Recent evidence suggests that epigenetic processes such as DNA methylation are involved in the pathogenesis of cardiac remodelling. Inhibition of DNA methylation may yield a novel therapeutic avenue for the treatment of HFrEF.

This study investigated the therapeutic potential of the DNA methyltransferase inhibitor, 5-azacytidine (Saza) to inhibit pathological hypertrophy in the heart using preclinical models of HFrEF, the transaortic constriction (TAC) model and the Angiotensin II (AngI) infusion model.

Methods: Wild type C57BL/6J mice underwent surgical creation of the aortic arch or implantation of a subcutaneous osmotic pump infusing 1000 ng/kg/min angiotensin II (AngII) to induce pressure overload. Sham surgery was used as the TAC surgical control group and a saline infusion pump was used as the AngII infusion control group. TAC and AngII mice were treated with 5aza or placebo for 9-10 days after surgery with intervals of 2-3 days between injections. Cardiac structure and function was examined in vivo using non-invasive echocardiography.

Results: Echocardiographic analysis revealed that TAC and AngII mice treated with Saza displayed a significant reduction in the interventricular septal wall and left ventricular posterior wall thickness compared to mice which received placebo treatment. Reduction in both models, even when Saza treatment was initiated in the TAC model after cardiac hypertrophy was established.

Conclusion: Therapeutic options for HFrEF patients are limited. Inhibition of DNA methylation using Saza shows therapeutic potential by reducing cardiac hypertrophy in preclinical models of heart failure and seems to have a beneficial effect even in the setting of established cardiac hypertrophy.

640 Colchicine aggravates cosacavirus B3 infection in mice
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Background: There is a clinical need for an immunosuppressive therapy that can treat myocarditis patients in the presence of an active viral infection. Colchicine is an immunosuppressive agent that downregulates the immune response and was found to be a safe and effective treatment option for paediatric patients, despite the fact that it is also commonly caused by viral infection. The aim of this study was to investigate the effects of colchicine in a mouse model of acute cosacavirus B3 (CVB3)-induced myocarditis.

Methods: Four groups of C57BL/6J mice were included. Control mice (n=8), mice infected with CVB3 (100 000 PFU, n=10), mice with colchicine administration (2 mg/kg ip, n=5) and mice with combined CVB3 infection and colchicine administration (n=10). After three days, the heart, pancreas and spleen were harvested and evaluated using immunohistochemical analysis and CVB3 qPCR.

Results: Mice were terminated at day 3 post-infection as colchicine treatment rapidly resulted in severe illness and mortality in CVB3-infected mice. Colchicine significantly decreased the number of macrophages in the heart and increased in the spleen (p<0.01) but significantly increased the number of neutrophils (p<0.01). In the pancreas, colchicine caused complete destruction of the acini in the CVB3-infected mice and also significantly decreased macrophage (p<0.01) and increased neutrophil numbers (p<0.01). In the spleen, colchicine treatment of CVB3-infected mice caused massive apoptosis in the white pulp and significantly inhibited the virus-induced increase of megakaryocytes in the spleen (p<0.01). Finally, we observed that colchicine significantly increased CVB3 levels in both the pancreas and heart.

Conclusion: Colchicine treatment in CVB3-infected myocarditis has a detrimental effect as it causes complete destruction of the exocrine pancreas and enhances viral load in both heart and pancreas.

Arterial and pulmonary hypertension

642 Osteopontin as a marker of pulmonary hypertension in patients with coronary heart disease combined with chronic obstructive pulmonary disease
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Background/Introduction: Comorbidity of coronary heart disease (CHD) and chronic obstructive pulmonary disease (COPD) worsens both diseases, leading to the development of complications and, consequently, to a large social and economic burden. Progression of pulmonary hypertension in these patients is one of factors causing mortality. Relationship between Osteopontin (OPN) levels and pulmonary hypertension development in comorbid patients is still debated.

Methods: The aim of this study was to evaluate the relationship between osteopontin plasma concentrations and pulmonary hypertension levels in patients with coronary heart disease combined with chronic obstructive pulmonary disease.

Purpose: The aim of this study was to evaluate the relationship between osteopontin plasma concentrations and pulmonary hypertension levels in patients with coronary heart disease combined with chronic obstructive pulmonary disease.

Methods: Eighteen patients with known CHD combined with moderate to severe COPD and in regular treatment were enrolled in a 6-month period study. All patients were evaluated by plasma osteopontin level (ELISA kit, Enzo Life Science), spirometry, echocardiography. The study was approved by our Institutional Ethic Committee. Patients signed an informed consent to take part to the study.

Results: All patients were matched by sex, age (mean age 63.3±5.3), and the severity of the disease. All patients were divided into two groups depending on the development or absence at them pulmonary hypertension. There was no considerable difference between parameters spirometry in both groups. Osteopontin level were directly related to pulmonary artery pressure (r=0.25, P<0.001) and inversely to 6-minutes walk test distance (r=-0.32, P<0.01). Median OPN value was 43 ng/ml, OPN levels >43 ng/ml remained statistically significant predictor of PH in patients with CHD and COPD (HR = 2.73, 95% CI 1.06-6.83, P = 0.008).

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Conclusion(s): These findings indicate that OPM may be a predictor for pulmonary vascular remodeling and could be a novel biomarker to improve risk stratification of developing PH in patients with CHD and COPD. Our results call for validation of our findings in large prospective cohort trials.

643 Myocardial dynamic stiffness is increased in experimental pulmonary hypertension partly due to incomplete relaxation

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Increased myocardial stiffness may cause diastolic dysfunction and explain right atrial dilatation in experimental pulmonary hypertension (PH). We aim to demonstrate that dynamic stiffness of right ventricular papillary muscle is increased in experimental PH, and that increased stiffness is related to basal oxygen consumption. PH is induced in 15 male Wistar rats (60 mg monocrotaline/kg, c.s.), the 5 controls are untreated. A right ventricular papillary muscle is mounted in an oxygen chamber at 37°C (Wong and colleagues, Am. J. Physiol H191:70, 2010). Stiffness is measured by muscle cross-sectional area/0.925 L0 to allow comparison between muscles. The contribution of cross-bridge cycling to stiffness is estimated using 10μM blebbistatin. Basal oxygen consumption is measured during length changes without stimulation. Static stiffness is 67 ± 17 kPa (mean ± SD) in control and 102 ± 32 kPa in PH (P=0.006). Dynamic stiffness is smaller than static stiffness: 40 ± 19 kPa and 74 ± 37 kPa, respectively (P=0.001). Blebbistatin has no effect on dynamic stiffness in control (38 ± 22 kPa) but reduces dynamic stiffness in PH (to 57 ± 18 kPa, paired t-test P=0.002). Similarly, basal oxygen consumption (before blebbistatin 0.21 ± 0.13 mM/hr and after 0.22 ± 0.13 mM/hr in control) was reduced by blebbistatin only in PH from 0.22 ± 0.07 mM/hr to 0.17 ± 0.06 mM/hr (paired t-test P=0.001).

We conclude that dynamic stiffness in experimental PH is increased and is partly due to cross-bridge cycling caused by incomplete relaxation, leading to increased basal oxygen consumption.

644 Hypotensive effect of quercetin is possibly mediated by down-regulation of immunotromprotease subunits in aorta of spontaneously hypertensive rats

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Background: Quercetin is flavonoid-based drug that shows antihypertensive and anti-inflammatory effects. Earlier we have shown its ability to inhibit proteasomal activity, however the molecular mechanisms are poorly studied.

It is unknown whether expression of genes encoding proteasomal subunits and proteasomal activities are altered in the aorta of spontaneously hypertensive rats (SHR). The goal of the present investigation is to elucidate if changes in proteasomal subunits gene expression and proteasomal activities are involved in antihypertensive effects of quercetin in SHR.

Methods: Utilizing real-time PCR analysis we have evaluated mRNA levels of proteasome and immunotromprotease subunits in aorta tissues of Wistar rats, SHR and quercetin-treated SHR. Quercetin (BCTP, Ukraine) was added to standard diet for 8 weeks in doses of 15 mg/kg. Proteolytic activities of proteasomes (peptidyl glutamine peptide hydrolase, trypsin-like and chymotrypsin-like activities) were measured using specific fluorogenic substrates. We also monitored hemodynamic parameters of animals (end-systolic pressure, end-diastolic pressure, stroke volume, ejection fraction, cardiac output) with "Millar Instruments" equipment. The structural changes in rats' aorta were determined by the morphometric analysis and electron microscopy in all experimental groups.

Results: The mRNA expression of immunotromprotease subunits (PSMB8 and PSMB9) were up-regulated comparing with constitutive ones (PSMB5 and PSMB1) in both Wistar and SHR. The mRNA level of genes encoding PSMB1, PSMB5, PSMB7, PSMB10 subunits of proteasome was significantly decreased in the tissue of SHR, comparing to Wistar rats. However, expression of genes encoding PSMB2, PSMB5 and PSMB14 was significantly up-regulated. The proteasome treatment provoked increase in mRNA levels of PSMB1 and decrease of PSMB2, PSMB8, PSMB9. PSMB1 in SHR aorta. Activities of proteasome did not differ between Wistar rats and SHR, but quercetin decreased trypsin-like and chymotrypsin-like activities of proteasomes in SHR group. Furthermore, cardiac output increased by 3.5 times, stroke volume by 1.7-fold, stroke rate by 2.3 times (P<0.001), ejection fraction in 2.5 times (P<0.001) in SHR. Quercetin application had normalized disturbed cardiodynamic parameters: ejection fraction increased in 1.7-fold (P<0.001), end-systolic pressure was decreased on 15.7% (P<0.001).

Conclusion: The inhibitory effect of quercetin on proteasomal process is observed in aorta and its antihypertensive effects is mediated not only by influence on catalytic activities of proteasome but also by effect on expression of genes encoding both proteasomal and immunotromprotease subunits.

645 Urocortin-2 improves right ventricular function and attenuates experimental pulmonary hypertension

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The contribution of mice lacking Urocortin-2 (UCN-2) to the pathogenesis of PH was determined. UCN-2−/− mice (UCN-2 knockout mice) were generated (GeneTargeting). Pulmonary hypertension was induced by chronic hypoxia (21% O2) for 9 weeks and the effect of UCN-2 on right ventricular hypertrophy was evaluated. UCN-2 deficiency caused a significant increase in RV systolic pressure and RV hypertrophy and impaired RV systolic function (ejection fraction: 32 ± 6 vs 40 ± 6, P < 0.01). The increased RV systolic pressure and RV hypertrophy were significantly attenuated in UCN-2−/− mice (ejection fraction: 41 ± 8 vs 53 ± 5, P < 0.01). The data suggest that UCN-2 may be a potential therapeutic target for the treatment of PH.

646 A proclival evaluation of the anti-hypertensive properties of an aqueous extract of Agathosma (Buchu)

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Hypertension, currently a pandemic, is one of the main risk factors for cardiovascular disease, hypotrophic heart disease, renal disease, stroke and blindness. Ischaemic heart disease and stroke are currently the two causes of death worldwide. In view of the group interest in the utilization of herbal remedies as treatment options, either alone or in conjunction with pharmaceuticals, this study investigated the anti-hypertensive properties of an aqueous extract of Agathosma (Buchu).

Methods: Male Wistar rats received normal rat chow (C) or a high-fat (40% fat) diet (HFD) for 16 weeks. A group of both C and HFD rats received (i) Buchu extract as replacement for water for 1 day (day 17) or (ii) from week 12 (treatment) of the 16 weeks. Blood pressure (BP), food and water intake were not affected and no diuretic effect was observed. (iv) Buchu ingestion decreased body weight and visceral fat gain. Further, food and water intake were monitored throughout and urinary production measured. At termination, body weight and visceral fat were determined. Blood was collected and serum insulin, c-peptide, leptin, aldosterone (ELISA) and ACE activity (RGET) determined.

Results: (i) The HFD elevated body weight and visceral fat gain while the Buchu extract significantly decreased both (ii) BP rose steadily in HFD while this was completely prevented by the Buchu extract ingestion and normalised when used as treatment, having no effect on BP in C. (iii) Food and water intake were not affected and no diuretic effect was observed. (iv) Buchu ingestion decreased leptin levels and normalised the aldosterone levels that was increased by HFD. (v) No ACE inhibitor effect could be detected.

Conclusion: This aqueous extract of Buchu may serve as an alternative, cost effective natural ther-apy for the improvement of hypertension, also causing weight loss and an improved RAS.

647 The adiponectin level in hypertensive females with rheumatoid arthritis and its relationship with subclinical atherosclerosis

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Cardiovascular risk level in hypertensive (HT) pts with rheumatoid arthritis (RA) do not fully reflect- ing by SCORE or mSCORE (EULAR 2009). Thus an additional risk factors research is required. Ad- ponectin may be a biomarker of early atherosclerosis. Its little known about the association of adiponectin level and subclinical atherosclerosis in HT pts with RA.

We aimed to estimate adiponectin level in HT females with RA and its relationship with subclinical atherosclerosis. The study included 42 HT females with comorbid RA (mean age of 54 [50; 61.5] years) and 20 HT females (control group). The cardiovascular risk was calculated using mSCORE. RA disease activity was measured using DAS28 scale. Carotid ultrasound with stiffness indices detec-tion (ESC 2006) and endothelial-dependent flow mediated vasodilatation (EDVD) by D. Celemajer method were performed. The level of adiponectin was measured using ELISA kit test. Serum adiponectin level was significantly higher in the HT females with RA (13.12 [13.15; 14.8] ng/ ml) compared to control (p<0.05). HT females with RA and subclinical atherosclerosis were
characterized by significantly higher adiponectin level (p<0.05). Adiponectin level was correlated with the highest ratio r=0.34 (p<0.005). DASS2, r=0.36 (p<0.005), cardiovascular risk score mSCORE r=-0.33 (p<0.05), BMI r=-0.79 (p<0.005) and EDV/DVD r=-0.41 (p<0.005). AUROC index for adiponectin predictive role in subclinical atherosclerosis develop was 0.78 (95% CI 0.64-0.93; p<0.05).

Serum adiponectin level determining may be useful additional biomarker for early atherosclerosis develop in hypertensive females with rheumatoid arthritis.

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Markers for identification of renal dysfunction in the patients with chronic heart failure

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Purpose: To compare efficacy of various methods of evaluation of the renal function (RF) state in the patients with functional class (FC) I-V of chronic heart failure (CHF).

Methods: This study includes 60 patients with ischemic heart disease with FC I (n=23), FC II (n=19) and FC III-IV (n=18) of CHF. Control group included 20 healthy persons. All the patients were per-

Results: In the subgroup of patients with blood levels of TG ≥1,7 mmol/l levels of apelin were sig-

Results: Serum MMP-9, oxLDL levels (p<0.001) in the case group (MMP-9 0.39±0.155 ng/ml, oxLDL 1.41±0.099 mg/ml) were more than in the control group (MMP-9 0.22±0.087 ng/ml, oxLDL 1.33±0.163 µg/ml).

The logistic analysis shows that MMP-9, oxLDL, CRP (MMP-9 OR=0.985, p<0.001; oxLDL OR=0.011, p<0.05; CRP OR=0.041, p<0.005) may play a role in the pathogenesis of the plaque rupture.

Conclusions: Serum MMP-9 enzyme level was directly correlated with Geniuni score (r=0.552, p<0.01), CIIS (r=0.340, p<0.01) and CRP (r=0.321, p<0.01) severely. Furthermore, serum MMP-9 enzyme increases with accordance of severity of the myocardial infarction with the statistical significance (p<0.01): the borderline abnormality group (CIIS<10, 0.227 ±0.099 ng/ml, possible injury (CIIS 10-15, 0.317 ± 0.132 ng/ml), probable injury (CIIS >15, 0.376 ± 0.132 ng/ml) groups. MMP-9 levels were significantly higher in the probable injury group patients (CIIS >15) compared to the possible injury group patients (CIIS 10-15) (p<0.001).

ROC Curve analysis shows that MMP-9 enzyme levels variance (area=0.87, p<0.001) are more than other biomarkers making it a dagnostically beneficial for the coronary atherosclerotic plaque rupture (CRP area=0.733, p<0.001, oxLDL area=0.635, p<0.05; picture 1).

Conclusion: Serum MMP-9, oxLDL and CRP are significantly involved in the pathogenesis of cor-

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cardio-hepatic syndromes in chronic heart failure: North Africa profile

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Purpose: Patients with chronic heart failure (CHF) have a variety of liver abnormalities, known as cardio-hepatic syndromes. The aim of the study was to evaluate the prevalence and importance of liver function tests (LFT) abnormalities in a group of patients with chronic heart failure.

Methods: The study included 1400 patients with chronic heart failure consecutively followed from 2010 until end of 2014 in care unit of CHF, departments of cardiology.

Results: The mean age of the patients was 50±13 years. The distribution by sex 930 (66,4%) men and 470 (33,6%) women. Liver function tests abnormalities were observed in patients with chronic heart failure: low albumin in 42% of the patients and CPR (C Reactive Protein) has a prognostic value in myocardial infarction.

Materials and Methods: The study included 30 patients (15 men and 15 women) with hypertension grades 2-3 at the age from 42 to 70. Patients with diabetes mellitus were not included into the study. The control group was consisted of 14 practically healthy people. The investigation complex included measuring blood levels of total cholesterol (TC), high density lipoprotein cholesterol (HDL-C), very low density lipoproteins cholesterol (VLDL-C), triglycerides (TG) and the calculation of low-density lipoprotein cholesterol (LDL-C).

Conclusions: Mild abnormalities of LFT are relatively frequent in patients with chronic heart failure, with a greater elevation of bilirubin than alaninotransferases. Patients with reduced ejection fraction had a higher prevalence of increased bilirubin. Total bilirubin was a predictor of adverse prognosis.

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To study other biomarkers that assess during myocardial infarction

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Background: When the coronary atherosclerotic plaque becomes vulnerable, a thrombus develops that causes acute coronary occlusion. The classic endothelial disruption occurs when an atheromatous plaque ruptures, exposing oxLDL, platelets and thus the underlying subendothelial matrix. This leads to inflammation and activation of the coagulation system.

Furthermore oxLDL (oxidized Low Density Lipoprotein) is involved in the coronary atherosclerotic plaque pathogenesis, MMP-9 (Matrix Metalloproteinase-9) enzymes play role during the plaque rupture and CPR (C Reactive Protein) has a proteics value in myocardial infarction.

Objective: To determine the involvement of oxLDL, MMP-9, CRP markers in the pathogenesis of myocardial infarction, to study their involvement in the injury of the myocardium and to evaluate the complications.

Methods: The study was conducted using case-control design. The main inclusion criteria of the 40 cases were that the patient should have a ruptured coronary atherosclerotic plaque, confirmed by clinical symptom, ECG, serum troponin I, and coronary angiography. Also 40 patients with coronary stenosis or chronic occlusion without ruptured plaque were included in the control group.

Serum MMP-9 enzyme and oxLDL titer were determined by ELISA according to the manufacturer’s recommended protocol. Additionally CRP was measured by full-automated analyzer. We used CIIS (cardiac infarction injury score) by ECG and Geniss score system (Coronary Angiographic Scoring System) for assessing the severity of coronary heart disease.

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Interconnections of apelin levels with parameters of lipid metabolism in hypertension patients

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Objective: Endogenous peptide apelin is an important cardiovascular biomarker that is involved in the regulation of blood pressure and cardiac function, glucose metabolism and exhibits antiatherosclerotic properties. The aim of this study was to evaluate the interconnections of apelin blood levels with parameters of lipid metabolism in hypertension patients.

Materials and Methods: The study included 30 patients (15 men and 15 women) with hypertension grades 2-3 at the age from 42 to 70. Patients with diabetes mellitus were not included into the study. The control group was consisted of 14 practically healthy people. The investigation complex included measuring blood levels of total cholesterol (TC), high density lipoprotein cholesterol (HDL-C), very low density lipoproteins cholesterol (VLDL-C), triglycerides (TG) and the calculation of low-density lipoprotein cholesterol (LDL-C) and atherogenic coefficient (AC). The level of apelin was determined by Enzyme-linked immunosorbent assay.

Results: In the subgroup of patients with blood levels of TG≥1,7 mmol/l levels of apelin were sig-

Results: The obtained data can be confirmed the antiatherogenic properties of apelin.

Conclusions: It has been determined that hypertriglyceridemia in hypertension patients associated with decreased blood levels of apelin and this factor inversely correlates with pro-atherogenic lipids and positively correlates with antiatherogenic lipids. The obtained data can be confirmed the anti-

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Plasma proteomics in hypertension: prediction and follow-up of albuminuria during chronic renin-angiotensin system suppression

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Background/Introduction: Albuminuria is a risk factor strongly associated with cardiovascular disease, the first cause of death in the general population. The search for potential biomarkers identifying patients with sustained and de novo development of albuminuria under renin-angiotensin system (RAS) suppression may represent an effective strategy for adequate intervention. The findings obtained could contribute to a better understanding of the mechanisms involved in the pathogenesis.

Purpose: The application of different proteomic strategies could elucidate specific molecular pathways involved in the pathogenesis and may provide predictors and chronic organ damage indicators.

Methods: In this work, 24 plasma samples of patients with different degrees of renal impairment (normoalbuminuria, de novo albuminuria and sustained albuminuria) were analyzed using a "multi-omic" approach: two-dimensional difference in gel electrophoresis (2D-DIGE) and isobaric tags for relative and absolute quantitation (iTRAQ) labeling followed by liquid chromatography-tandem mass spectrometry (LC-MS/MS). Significant variations were validated in an independent cohort of 105 subjects using two different methodologies: turbidimetry, an assay focused on clinical diagnostic and selected reaction monitoring (SRM), a proteomic approach with great clinical potential.

Results: Proteomic analysis of plasma has allowed identifying two protein profiles with an important value from a clinical point of view: 1) proteins with predictive value of de novo albuminuria that are related to immune system response and 2) sustained albuminuria indicator proteins related with chronic renal damage.

Conclusions: The study carried out showed two different protein profiles which may be very useful for predicting the development of de novo albuminuria as well as to monitor renal damage. These results highlighted alterations in specific molecular pathways related with immune response and the pathogenesis of organ damage. The possibility of a future strategy based on anti-immune therapy to treat hypertension which could help to prevent the development of albuminuria and hence, the progression of kidney damage.

Soluble RAGE levels in plasma of patients with cerebrovascular events
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Background: There is growing evidence implicating the participation of RAGE-ligand interaction in the development and progression of various immune-mediated disorders, including vascular disease.

Purpose: The aim of the present study was to evaluate the sRAGE plasma levels in patients with ischemic stroke or transient ischemic attack in order to identify a biomarker of differentiation in the genesis of these diseases.

Methods: This study included 87 Caucasian subjects (50 males and 37 females) with cerebrovascular event. Plasma levels of sRAGE were determined using a kit for the immunoadsorption enzyme, commercially available.

Results: Our study showed that the plasma concentration of sRAGE is significantly lower in patients with ischemic stroke compared to patients with transient ischemic attack and to controls.

Conclusions: This feedback appears to confirm that transient ischemic attack, in absence of documented organic pathology, does not seem to recognize the atheromasic origin as its primary cause. This analysis contributes information about the pathophysiology of vascular cerebral disease and, in particular, these results reaffirm strong prothrombotic and inflammatory components to the pathophysiology of stroke.