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# POSTER SESSION 3

## Session held on 10 July 2016

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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. Underlying dystrophin defects cause 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 (CBR1 and CBR2), endogenous ligands (anandamide and 2-arachidonoylglycerol) 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 CBR1 antagonist AM251 prevented the formation of iPSC colonies ($p \leq 0.05$, 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 ($p \leq 0.0001$, 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.

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**An emerging role of T lymphocytes in cardiac regenerative processes in heart failure due to dilated cardiomyopathy**

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**Background:** Dilated cardiomyopathy (DCM) is a heart muscle disease of varied etiology that is treated with medical therapies, but cardiac function is reduced. DCM exhibit significant cardiomyocyte loss at the stage of heart failure (HF). After myocardial injury adult mammals do not sufficiently regenerate cardiomyocytes to compensate for lost cardiomyocytes. The recognition of endogenous cardiomyocytes and cardiomyocytes progenitors as a source for new cardiomyocytes raises the possibility of activating this process for myocardial repair. Recent studies have shown that formation of functional cardiomyocytes may come both of dedifferentiation and proliferation of pre-existing cardiomyocytes without complete reversion to a cardiac progenitor state or by cardiac differentiation of progenitor (embryological or dedifferentiated origin) cells. Dedifferentiated cardiomyocytes and progenitor cardiomyocytes express stem cell markers and early cardiac transcription factors. Oncostatin M a member of the IL-6 family of cytokines secreted by T lymphocytes regulates cardiomyocyte dedifferentiation in culture. We propose that T lymphocytes infiltrating heart in the disease play an important role in cardiomyocyte dedifferentiation after myocardial injury. Purpose: The aim of the study to assess the number of T lymphocytes, cardiomyocytes progenitors and dedifferentiated cardiomyocytes in endomyocardial biopsies from patients with DCM.
**Methods:** Endomyocardial biopsies were obtained from patients with DCM. Surface markers were analyzed by immunocytochemistry. The number of T lymphocytes, expressing CD4 and CD8 markers, cardiomyocytes progenitors and dedifferentiated cardiomyocytes, expressing c-kit, MDR1 and GATA-4, Nkx2.5 was detected in the same samples.
**Results:** In our study 14 endomyocardial biopsies from patients with DCM were analyzed. The number of CD4 +lymphocytes was 48 ± 8 cells per mm2, the number of CD8+lymphocytes was 26 ± 7 cells per mm2. The number of cardiomyocytes expressing stem cell markers c-kit, MDR1 and early cardiac transcription factors GATA-4, Nkx2.5 was 8 ± per mm2 in the same samples.
**Conclusions:** The results of this study demonstrate that there is a presence of increased numbers of T cells in endomycardial biopsies from patients with DCM where cardiomyocytes progenitors and dedifferentiated cardiomyocytes were detected too. Exact mechanisms of cardiomyocyte dedifferentiation are poorly understood but our results and evidence that cytokines secreted by T lymphocytes regulate cardiomyocyte dedifferentiation in culture show an emerging role of T lymphocytes in cardiac regenerative processes.

### 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 aging/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:** c-kitpos CD45neg eCSCs were isolated from the right atria appendage (~200mg) of different aged patients (32 to 85 years) by enzymatic digestion followed by MACS (Miltenyi). eCSCs were characterised for co-expression of ageing/senescence markers (p16INK4a, p53, p21, senescence-associated $\beta$-galactosidase) with known stemness/multipotency (Oct-4, Nanog, Bmi-1, TERT, Sox-2) and proliferation (Ki67) markers. Telomere length of eCSCs was determined using Q-FISH analysis. DNA damage was assessed using $\gamma$-H2AX. The growth (BrdU labelling), clonogenicity and differentiation potential of young and old eCSCs were also evaluated.
**Results:** The number of eCSCs isolated was similar regardless of age, gender and pathology (~45,000/gram of tissue). eCSCs isolated from young and old hearts showed age-correlated increased expression of ageing/senescence markers and decreased expression of stemness/multipotency and proliferation markers. Single cell expression analyses revealed heterogeneity within the eCSC population with eCSCs isolated from old hearts harboring a greater proportion of eCSCs with critically short telomeres and increased DNA damage. 'Aged-senescent' eCSCs showed limited cloning and growth capacity and impaired cardiac differentiation capacity. Moreover, 'aged-senescent' eCSCs expressed increased senescence-associated secretory phenotype (SASP) factors relative to their younger counterparts. Treatment with the canonical Wnt ligand, Wnt3a significantly increased the proliferation of 'aged-senescent' eCSCs to levels observed in younger eCSCs. Conversely a switch 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.

### 514

**Hippo signalling modulates survival of human induced pluripotent stem cell-derived cardiomyocytes**

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**Background/Introduction:** Hippo signalling is an evolutionarily conserved pathway that controls organ size by regulating apoptosis, cell proliferation and stem cell self-renewal. Recently, the pathway has been shown to exert powerful growth regulatory activity in cardiomyocytes. However, functional role of this stress- and cell death-related pathway in human cardiomyocytes is not known.

**Purpose:** Our aim was to investigate the role of transcriptional Hippo co-activators YAP and TAZ signalling in human induced pluripotent stem cell-derived cardiomyocytes (hiPSC-CM), and to test the effects of modulating the pathway on cardiomyocyte function and survival.
**Methods:** Human iPSC-CM were plated in different densities or treated with cardiotoxic anthracycline doxorubicin (3-15μM). Gene silencing of YAP and TAZ was achieved by RNAi. The mRNA levels of YAP and TAZ were assessed by real time PCR, and Cellomics ArrayScan was used to quantify YAP/TAZ nuclear translocation and cell death (with Topro3 necrosis marker and mitochondrial membrane potential marker TMRM). CellOPTIQ optical imaging platform was used to measure calcium transients in a high throughput manner.
**Results:** Our results showed that YAP and TAZ genes are abundantly expressed both in hIPSC-CM and adult ventricular cardiomyocytes. Nuclear translocation and mRNA levels of YAP and TAZ were increased with decreased cell density (for YAP: $p<0.001$, TAZ: $P<0.01$, n=3). Other extracellular stress signals such as doxorubicin increased YAP/TAZ translocation (p=0.0004, n=3) and cell loss in a dose-dependent manner in hiPSC-CM. We showed that transient paired downregulation of YAP and TAZ may increase Topro3-positive hiPSC-CM, marking a detrimental effect of their silencing (p=0.0078, n=4). Modulation of YAP/TAZ expression had no effect on hiPSC-CM calcium transient and contractility profiles.
**Conclusions:** Our results suggest that modulation of Hippo cascade may affect cell viability and necrosis of hIPSC-CM and thereby modulate the survival of human cardiomyocytes.

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### Biocompatibility of mesenchymal stem cells with a spider silk matrix and its potential use as scaffold for cardiac tissue regeneration

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One of the problems of using cell therapy for heart diseases is the hostile environment for cell retention/cell survival in the area were the cells are delivered. In order to minimize the impact the poor environment has on the implanted cells, we are working on using spider silk matrices as cell vehicles for the infarcted region.
Recombinant spider silk proteins (spidroins) are capable of self-assembling into fibres in aqueous solutions and can create very thin and resistant matrices with interesting mechanical properties. In this study, we improved both the cytocompatibility and the biological properties of these matrices functionalizing them with Fibronectin and Vitronectin; two extracellular matrix proteins associated to cell adhesion and retention. Thus, we cultured mesenchymal stem cells (MSC) for 8 days in these matrices: During the first 6 days, we compared the ability of the cells to grow in monolayers and their proliferation rates. Finally, we determined the impact of cell-matrix interactions in cell behavior by analyzing the rate of cell migration and changes in cell secretion during the last 48 hours of culture.
When comparing the MSC growing curve among the matrices - matrices without functionalization (A), matrices functionalized with fibronectin (B) and matrices functionalized with vitronectin (C) - we observed that the slopes of the regression lines that fitted into the growing curves of functionalized matrices were higher than the slope of the non-functionalized matrix. Moreover, Vitronectin functionalization was the most effective functionalization promoting cell growth: Am=6.37 < Bm=10.25 < Cm=12.22. Consistent with this, the analysis of the three cropped secretomes showed that Vitronectin functionalization also enhanced exocytosis (table1).
These results show that Vitronectin functionalized matrices are the best option to be tested as scaffold matrices for preclinical studies on small animal models.

5 main molecules in MSC culture media

| | fold change B/A-1 | C/A-1 |
|---|---|---|
| MCSF | 1.1 | 7 |
| SDF1 | 1 | 82 |
| HGF | 1.7 | 168 |
| MCP1 | 1.1 | 13 |
| VEGF | 2.7 | 30 |

Summary of the secretome analysis of MSC cultured on matrices functionalized with fibronectin or Vitronectin and compared to non-functionalized matrices (B/A-1 and C/A-1 respectively).

## 516

### A snapshot of genome-wide transcription in human induced pluripotent stem cell-derived hepatocyte-like cells (iPSC-HLCs)

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**Background/Introduction:** Cholesterol metabolism is causally involved in the pathogenesis of atherosclerosis and cell culture models for studying the pathophysiology of atherosclerosis are needed. However, primary human hepatocytes (PHH), the key cell type involved in cholesterol metabolism, are difficult to acquire and culture due to their tendency to dedifferentiate and lose their liver function in culture. Hepatocytes differentiated from human induced pluripotent stem cells (iPSCs) offer an alternative for PHH. The validation of the iPSC-derived hepatocytes and the detailed exploration of their transcriptomic profile is an essential step when developing functional hepatocyte cell models for e.g. exploring the lipid metabolism involved in the development of atherosclerosis.
**Purpose:** Our aim is to differentiate iPSC-derived hepatocyte-like cells (iPSC-HLCs), which recapitulate the functionality as well as the transcriptomic profile of primary human hepatocytes as closely as possible.
**Methods:** We produced iPSC lines from dermal fibroblasts of three different patients and characterized the iPSC lines in detail. We then differentiated iPSCs to hepatocyte-like cells (HLCs). To characterize the iPSC-HLCs produced we used a wide array of functionality tests and immunocytochemical (ICC) staining. In order to study the genome-wide gene expression, we performed Global Run-On sequencing (GRO-seq) of the iPSC-HLCs as well as of the iPSCs and PHHs. GRO-seq is a derivative of RNA-sequencing that aims to measure rates of transcription (instead of steady state RNA levels) by directly measuring nascent RNA production. In this method, active RNA polymerase is allowed to run on in the presence of Br-UTP and RNAs are hydrolyzed and purified using Brd-UTP antibody coated beads. The eluted RNA undergoes cap removal and end repair prior to reverse transcription to cDNA. Deep sequencing of the cDNA provides sequences of RNAs that are actively transcribed by RNA polymerase II.
**Results:** The iPSC-HLCs stain positive for alpha-fetoprotein (AFP), albumin (ALB), LDL receptor (LDLR) and Oil red O. Our principal component analyses of the GRO-seq data suggest that the gene expression profile of the iPSC-HLCs closely resembles the expression pattern of PHHs. For example, the iPSC-HLCs have low expression of pluripotency genes (NANOG, SOX2) and express many liver-specific genes like apolipoproteins (apoAI, apoAII, apoB), transthyretin (TTR, a prealbumin), as well as liver-enriched transcription factors such as HNF1a and HNF4a.
**Conclusion(s):** Our study presents a genome-wide transcription map of iPSC-HLCs, with low expression levels of pluripotency genes and higher expression of liver-specific genes, closely related to PHHs, thus demonstrating the potential of these cells as a laboratory model for human liver.

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### Can NOS/sGC/cGK1 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)/cGMP-dependent protein kinase I (cGKI) pathway in adult cardiac cells is extensively studied. Indeed, physiological levels of NO generated by NOS1 and NOS3 or pharmacological treatments with NO-donor drugs can modulate cardiac contractility and increase coronary blood flow throughout the Ser/Thr phosphorylation (sGC/cGKI pathway) and directly by S-nitrosylation of several proteins.
**Purpose:** Although the effects of NOS/sGC/cGKI pathway in adult cardiac cells are well known, the influence of this signaling as modulator of cardiac differentiation of embryonic stem cells (ESCs) is less defined. Therefore, we investigated NOS/sGC/cGKI in the early stage of cardiac differentiation of ESCs, studying i) enzyme expressions and activities during cardiac maturation and ii) the acute and chronic effect of pathway alteration.
**Methods:** Undifferentiated mouse ESCs were cardiac differentiated by Embryoid Bodies formation (EBs). Cardiac maturation was followed for 21 days. Beating EBs were monitored starting from 7th-10th day. At different stages of maturation, mNos3, mGuCy1b and mPrkgI expressions were detected by real-time PCR (Q-PCR); western blot analysis and enzymatic activity were used for protein evaluation.
**Results:** Q-PCR showed that during differentiation enzymes expression increased in a time-dependent mode and different time-courses. The peak of mNos3 expression was measured at d8 and then rapidly decreased. mGuCy1b and mPrkgI were detected starting from d5 and increased until d14, maintaining similar values until the end of observation period. Protein expressions were in line with genes expression, with detectable level of NOS3 only at the beginning of differentiation (d5-d8). Enzymatic activities were tested at d15: sGC activity was increased of 3 times with the NO-donor SNAP and inhibited by 50% with ODQ. Moreover, cyclic GMP was reduced when EBs were incubated for 30 min with L-NMA, suggesting an endogenous activity of NOS. The phosphorylation of Ser/Thr residues on target proteins, were increased by membrane permeable cyclic GMP, isosorbide-5-mononitrate and SNAP and reduced by KT5823, a selective cGKI inhibitor. Functionally, cell incubation with isosorbide-5-mononitrate from the beginning of differentiation slightly increased the percentage of beating EBs at early stages of cardiac maturation (d5-d8).
**Conclusions:** The time-dependent expression of NOS/sGC/cGKI 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.

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

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**Background:** HiPSC-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 hiPSC-CMs via co-culture with Ik1-expressing HEK293 cells.
**Methods:** HiPSC-CMs obtained commercially (Cor.4U - Axiogenesis, and Pluricyte - Pluriomics) were prepared according to manufacturers' protocols, and mixed with Ik1-expressing HEK293. The density of hiPSC-CMs was kept constant at 25k-40kcells/well (varying in different cell lines according to manufacturers' suggestions) to ensure a monolayer was formed. Ik1-expressing HEK293 were mixed with hiPSC-CMs at decreasing densities from 1:10 to 1:300, 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-40kcells/well hiPSC-CMs in the presence of increasing 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 Ik1-expressing HEK293 (1:10) lead to an increase in interval time between beats of approximately 60% on day 9 ($1972 \pm 592$ vs $1213 \pm 114$ms, n=8 $p<0.05$). Time for relaxation was also significantly prolonged in 1:10 and 1:30 compared with control on day 9, 283% and 128% ($875 \pm 265$ and $522 \pm 133$ms vs $229 \pm 26$ms, respectively, n=8 $p<0.01$), respectively. Earlier and later culture times showed no significant difference in spontaneous contractile activity up day 12. In contrast, 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-culturing with Ik1-expressing HEK293 may provide a method of adding Ik1 conductance to a network of hiPSC-CMs but different sources of hiPSC-CMs respond differently. With Cor.4U cells 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 Ik1 expressing HEK293 cells.

## 519
## Cell therapy of the heart studied using adult myocardial slices in vitro

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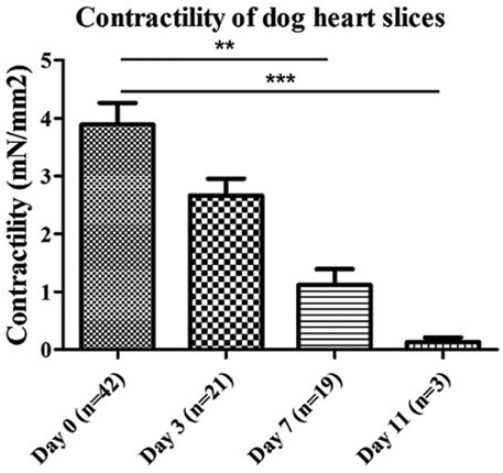
**Introduction:** Cardiac cell therapy is the introduction of stem cells in the heart to repair/replace damaged myocardium. In vivo studies have revealed that this therapy can induce arrhythmias, and the efficacy in improving myocardial function appears limited. A better understanding of the mechanisms involved during cell therapy is required but a suitable representative in vitro model is lacking. Organotypic heart slices are multicellular preparations with preserved structural, biochemical and electrophysiological properties.
**Purpose:** Here we use heart slices to study the mechanisms of functional integration, proliferation and direct/indirect effects on recipient myocardium of transplanted cells.
**Methods:** In this study human induced pluripotent stem cell-derived cardiomyocytes (iPSC-CMs) were cultured in vitro on 300μm thick vibratome-cut slices prepared from adult dog left ventricular tissue. Viability and functionality were assessed by force measurement, histology and immunohistochemistry. Calcium transients were recorded by optical mapping.
**Results:** iPSC-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 1Hz 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 iPSC-CMs, suggesting a lack of coupling with the recipient tissue. After 9 days in culture some iPSC-CM started to integrate 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.



Contractility of dog slices

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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 reservoir for the stem cells. Both preclinical and clinical data have shown that adipose derived stem cells (ASCs) 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 (assessed by immunofluorescence) and their ability to maintain multilineage differentiation. Alternatively, ASCs were also cultured as 3-D structure as spheroid bodies (SBs), by using the hanging drop technique. Luminex and ELISA assays were conducted to quantify key immunomodulators and angiogenic mediators to compare the two study groups. Western blots were used to study proteins involved in apoptosis.
**Results:** hASCs expressed similar surface markers as those documented for bone marrow MSCs including 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 TNFa stimulation, whereas cleaved caspase 3 was undetected in SB structures. Also, 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, therapeutic potential can be optimized. Our findings clearly showed that hASCs cultured as 3D 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 enhance the therapeutic potential of hASCs.

## 521
## 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 cell source for cardiac regenerative 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 provided by the beating heart. Therefore, we study the response to cyclic strain of undifferentiated and predifferentiated human CMPCs in a 2D environment, as well as how CMPCs respond to unidirectionally 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 simulated 'host' tissue.
**Methods:** To test mechanosensitivity of CMPCs in 2D and 3D environments, the response of L9TB CMPCs to uniaxial (cyclic) strain (10% with 0.5 Hz) was investigated. To represent the 3D environment, undifferentiated CMPCs were cultured in unidirectionally constrained and stress-free collagen/Matrigel hydrogels, where the constrainment provides a static strain to the cells. The cellular mechanoresponse 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 mimicked 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 mechanotransduction. Mechanotransduction is essential for optimal recruitment, migration, and mechanical integration 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 endogenous regeneration strategies.

## 522
## 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 (iPS-CM) provide unique alternative to model human cardiomyocyte differentiation, the function of the cardiac cells and the pathology of severe cardiac diseases. However, the differentiated iPS-CMs are classified to resemble embryonal or fetal like cardiomyocytes due to their gene expression pattern, size, shape and mononuclear nature. Moreover, the lack of t-tubule network has been suggested to be a reason for the slow excitation-contraction coupling and calcium handling. The culture platforms that orientate the cardiac cells could have a positive effect on the overall maturation state of the differentiated cardiomyocytes, e.g. patterned biomaterials could induce the orientation and furthermore the more mature phenotype of iPS-CMs.
**Methods:** We have utilized natural topography provided by the network formed by endothelial cells and fibroblasts in the maturation of iPS-CMs. The iPS-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 functionality and the level of the structural gene expression.
**Results:** Our results show that iPS-CMs were elongated and aligned with the vascular-like network and formed a synchronously beating cell construct. The electrical activity as well as calcium metabolism was shown to be normal. In addition, the cells responded to pharmaceutical agents as expected. Furthermore, the iPS-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 iPS-CMs in terms of cell structure and alignment as well as sarcomere orientation and function. Our results suggest that iPS-CMs cultured on these platforms present more mature phenotype compared to iPS-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

### 525
### Gene expression regulation in heart failure: from pathobiology to bioinformatics

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**Background:** Heart failure (HF) syndrome results from abnormalities in multiple biological processes that contribute to cardiac dysfunction. 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 human HF 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 and control subjects were analysed by RNA-Seq. Different informatic tests (edgeR and NOISeq BIO) were employed and compared. Several public tools were used to effect in silico analysis of the specific differentially expressed genes (DEGs).
**Results:** The statistical methods adopted, generated different lists of genes both for the number of DEGs and for gene symbols. Furthermore, 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 downregulated in both DCM and RCM. Finally, several genes also encoding for components of extracellular matrix, including ADAMTSL4 and ADAMTS8, belong to the extrapolated gene list of common DEGs.
**Conclusions:** Our data revealed a new map of gene expression changes in the DCM and RCM cardiac tissues. Several genes involved in crucial cellular mechanisms were not previously implicated in the molecular phenotype of HF. These new changes may be responsible for alterations found in cardiomyopathies. However, further studies are needed to lead to potential novel biomarkers and targets for therapeutic intervention in these pathologies.

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### Human transcriptome in idiopathic dilated cardiomyopathy - a novel high throughput screening

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**Background and Aim:** Idiopathic dilated cardiomyopathy (DCM) is the leading cause of heart failure (HF), and the most common indication for cardiac transplantation. DCM is characterized by transcriptomic changes, which alter cellular processes, leading to failing phenotype. As the specific molecular mechanisms of DCM are largely unknown, the aim of this study was to develop a novel platform for screening of all human transcription factors (TFs) and to characterize the role of TFs in the molecular mechanisms of DCM.
**Methods:** Myocardial tissue samples from DCM and control human subjects were analyzed using novel screening platform, called Quantrrx, based on quantitative real-time polymerase chain reaction (qRT PCR). We screened all known and putative TFs (~2628) in the human genome.
**Results:** We identified 41 differentially expressed TFs. 18 genes were upregulated (fold change $>=$ 2, $p < 0.05$) while 22 genes were downregulated (fold change $>= 2$, $p < 0.05$) in dilated cardiomyopathy group. The analysis of the differentially expressed genes uncovered TFs affecting important signaling pathways in cardiac development and disease including MAPK and Wnt-signaling pathways and thus allowed the characterization of possible novel regulators that play role in HF.
**Conclusion:** Quantrrx is a new method to screen quickly and effectively all human TFs and provides a valuable resource for further investigation of molecular mechanisms of DCM as well as other diseases. Our data indicate that changes in the expression of 41 TFs affect important signaling pathways, which subsequently alter a number of biological processes in DCM patients and could serve as potential diagnostic or therapeutic targets.

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### A high-throghput approach unveils putative miRNA-mediated mitochondria-targeted cardioprotective circuits activated by T3 in the post ischemia reperfusion setting

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**Background:** Increasing experimental and clinical evidences indicate that a low T3 state (LT3S) in the post cardiac ischemia reperfusion (IR) setting favors long term adverse cardiac remodeling and worsens patients prognosis. We previously reported a cardioprotective role of T3 treatment and suggested the mitochondria as main effectors of this action. Although the regulation of cardiac miRNAs may be the presumable mechanism, a relation of cause and effects has never been demonstrated. A system biology approach may help investigating this important issue.
**Purpose:** The purpose of the study was to unveil putative mitochondria-targeted cardioprotective circuits activated by T3 in the early post IR setting and dependent on the regulation of micro RNAs.
**Methods:** To this aim, miRNA profiling and mitochondrial proteome were performed in a model of cardiac IR and the data were integrated through computational analysis. Breefly, 24h following IR, rats developing a low T3 state were treated with T3 (6μg/Kg die) or T3 vehicle for 48h.Thereafter 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 perinfarctual myocardium were purified from rats developing or not the lowT3S 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 cardio-protective pathways targeted to mitochondria.

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

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**Background:** The prevalence of uraemia is continuously increasing in developed countries. Uremic cardiomyopathy characterized by left ventricular hypertrophy and diastolic dysfunction is a common cardiovascular complication of uraemia; however, the underlying molecular mechanisms are not clear. The overexpression mR-212/132 has already been implicated in the development of left ventricular hypertrophy via modulation of the calcineurin pathway in TAC mice. Purpuse Therefore, here we investigated the effect of uraemia on the myocardial expression of miR-212/132 and the calcineurin pathway.
**Methods:** Uraemia was induced by 5/6 nephrectomy in male Wistar rats. Eight weeks later serum urea and creatinine levels were measured and transthoracic echocardiography was performed. Then RNA was isolated from left ventricles of nephrectomised and sham-operated rats and expression of miR-212/132 and atrogene-1 as well as MCIP1.4, components of the calcineurin pathway, was measured by qRT-PCR.
**Results:** In the nephrectomised group, serum urea and creatinine levels were significantly higher proving the development of uraemia. In the uremic group, left ventricular anterior and septal walls were significantly thicker, e' was significantly decreased and E/e' was significantly increased referring to left ventricular hypertrophy and diastolic dysfunction. In the uremic group, heart weight/body weight ratio was also significantly elevated as compared to the control group. In the uremic group, miR-212 was significantly overexpressed; however, miR-132 did not change significantly as compared to the control group. Moreover, atrogene-1 showed significant down-regulation and MCIP1.4 showed significant up-regulation in the uremic group.
**Conclusion:** Myocardial overexpression of miR-212 might play a role in the development of uremic cardiomyopathy by modulating the calcineurin pathway.

# 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-$\beta$ family. Under homeostatic 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

rupture. This detrimental effect on the healing process is most likely related to an exacerbated inflammatory response. In heart failure (HF) patients, GDF15 plasma levels are increased and high GDF15 levels are associated with a higher mortality. Despite this association, a causal role of GDF15 in adverse cardiac remodeling leading to HF is currently not very well established. We therefore aimed to study the role of GDF15 in a mouse model of non-ischemic HF development.

**Methods:** GDF15 knock-out (KO) mice and wild type (WT) mice underwent trans aortic constriction (TAC) with a 27 gauge needle. Correct placement of the TAC was confirmed by Doppler measurements of the carotid flow ratios. Cardiac function and geometry was assessed using echo at baseline, 7, 28 and 42 days after TAC. At day 42 immunohistochemistry was performed to study cardiomyocyte hypertrophy (WGA) and influx of different leukocyte subtypes. Flow cytometry was performed at 42 days after TAC on cardiac lymph nodes (LN), blood and spleen to study the effect of GDF15 deficiency on leukocyte subtypes .

**Results:** GDF15 KO mice have significantly increased end diastolic volume (EDV) and end systolic volume (ESV) after 6 weeks of TAC compared to WT mice (EDV: 93 μl versus 64 μl, ESV: 72 μl versus 38 μl, $p<0.05$). The increase of ESV is already present 7 days after TAC. GDF15 KO mice show increased heart weight/body weight ratios after 42 days of TAC compared to WT mice. (7.0 mg/g versus 9.2 mg/g, $p<0.01$) Though this observation implies increased cardiac hyperthropy in GDF15 KO mice, we did not observe a difference in cardiomyocyte hypertrophy. Flow cytometry did not show any alterations in the amount or activation status of leukocytes in the blood, LN or spleen after 42 days of TAC.

**Conclusions:** GDF15 deficiency aggravates adverse cardiac remodeling upon pressure-overload. Volume increase, both EDV and ESV is accelerated in GDF15 KO mice compared to WT mice. These changes are already present after 7 days of TAC. Increased heart weigth/body weight ratio in GFD15 KO mice is not related to an increased volume of individual cardiomyocytes. More in depth analyses are needed to provide a mechanistic explanation for the worsening of cardiac function in GDF15 KO mice.

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### Blocking heteromerization of platelet chemokines ccl5 and cxcl4 reduces inflammation and preserves heart function after myocardial infarction

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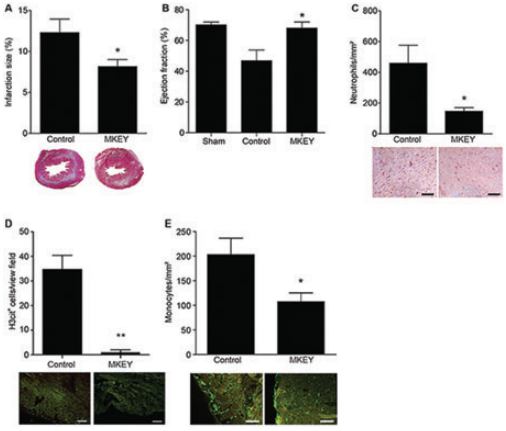
**Background:** Myocardial infarction (MI) is among the most common causes of death in developed countries and its incidence is still increasing. Finding new strategies to prevent and treat this threatening clinical event is thus of high priority. Inhibition of CCL5 was shown to have beneficial effects on the outcome of experimental MI in mice, yet might be accompanied by adverse immunologic side effects. In a previous study, we have demonstrated a pathophysiologic relevance for the heteromer formation of CCL5 and CXCL4 in the progression of atherosclerosis.

**Purpose:** To evaluate a specifically designed compound (MKEY) that blocks the CCL5-CXCR4 interaction in a mouse model of myocardial ischemia/reperfusion (I/R).

**Methods:** To examine the effect of MKEY in healing following I/R, 8 week-old male mice were intravenously treated with MKEY or scrambled control (sMKEY) from 1 day before, until up to 7 days after I/R. Myocardial function was evaluated using echocardiography and intraventricular pressure measurements and tissue viability, scar formation, leukocyte infiltration and the formation of neutrophil extracellular traps (NETs) was assessed by histology.

**Results:** MKEY treatment resulted in a significant decrease in infarction size and preserved heart function as compared to sMKEY-treated animals (Figure A, B). Moreover, MKEY treatment significantly reduced the inflammatory reaction following I/R, as revealed by specific staining for neutrophils, NETs and monocytes/macrophages (Figure C, D, E).

**Conclusion:** Disrupting chemokine heterodimers during myocardial I/R might have clinical benefits, highlighting the therapeutic benefit of blocking the interaction of platelet-derived chemokines, and in addition, reducing the inflammatory side effects while maintaining normal immune defense.




## 533

### Is there an association between low-dose aspirin use and clinical outcome in HFPEF? Implications of modulating monocyte function and inflammatory mediator release

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**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 those 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 28 HFPEF age- and sex-matched patients (14 aspirin, 14 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/without 10 $\mu$M 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 0.605, 95% confidence interval, 0.389- 0.961). Serum MMP2 and sCD163 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 invasion was reduced by 50% with low-dose aspirin ($p<0.01$). Inflammatory cytokines (IL1$\alpha$, IL1$\beta$, 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. Antiplatelet strategies to modulate monocytes may require further, prospective evaluation in HFPEF.

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### N-terminal truncated intracellular matrix metalloproteinase-2 expression in diabetic heart.

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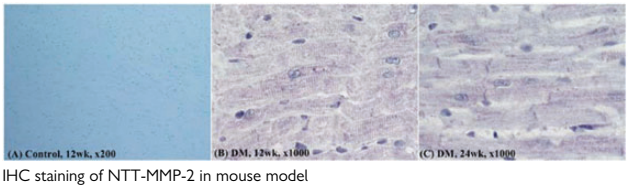
**Background:** Diabetic cardiomyopathy is a distinct form of cardiomyopathy and can be defined as cardiac damage and ventricular dysfunction which is independent of the concomitant coronary artery disease and/or hypertension. Matrix metalloproteinases (MMPs) are reported to account for increased myocardial collagen content in diabetic cardiomyopathy. Recently reported intracellular type of MMP-2, which is N-terminal truncated (NTT) type, is induced by oxidative stress and reported to account for cardiac dysfunction through activating innate immunity and apoptosis in various conditions.

**Purpose:** We hypothesized that NTT-MMP-2 is induced in diabetic cardiomyopathy. We aimed to evaluate the expression of NTT-MMP-2 in vitro and in vivo in connection with activated innate immunity and apoptosis.

**Methods:** H9c2 cells were cultured with intermediate and high glucose concentration (15, 30mM) for 2, 24, and 48 hours. Cells were analyzed with quantitative reverse transcription polymerase chain reaction (qRT-PCR) and gelatin zymography. AKT and NF-kB expression were also measured with western blot method. In vivo mouse model was induced with 40mg/kg of streptozotocin intraperitoneal injection for 5 days. After sacrificing mouse at 12 and 24weeks, pathological analysis including immunohistochemical (IHC) staining of NTT-MMP-2 were done.

**Results:** Quantitative RT-PCR showed that there was an expression of NTT-MMP-2 in H9c2 cell with glucose exposure compared to negative expression in control group, and it was dose and time-dependent. Also, there was a distinct expression of NTT-MMP-2 in IHC staining from diabetic mouse heart. There was no definite collagen accumulation and fibrosis from light microscopy (LM) evaluation, but there was a mitochondrial damage from electron microscopy (EM) evaluation.

**Conclusion:** NTT-MMP-2 expression was noted from both in vivo and in vitro model of diabetic cardiomyopathy. Further evaluation of its role in diabetic cardiomyopathy should be followed.




IHC staining of NTT-MMP-2 in mouse model

Expression of NTT-MMP-2 in H9C2 cell.

| NTT-MMP-2 qRT-PCR | Mean | SD |
|---|---|---|
| Control 2hr | 1.00 | 0.00 |
| High concentration 2hr | 1.19 | 0.09 |
| High concentration 24hr | 2.05 | 0.17 |
| High concentration 48hr | 1.00 | 0.39 |

qRT-PCR; quantitative real time polymerase chain reaction

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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. Median age was 63 (57;66) 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 naïve (N, CD45RA+CD62L+), central memory (CM, CD45RA-CD62L+), effector memory (EM, CD45RA-CD62L-) and terminally differentiated CD45RA-positive effector memory (TEMRA, CD45RA+CD62L-) CD45+CD3+CD4+ (Th) and CD45+CD3+CD8+ (Tcyt) cells were measured using multicolor flow cytometry.
It was found that relative number of Naïve Tcyt (p=0.034) was decreased and the relative number of TEMRA Tcyt (p=0.006) was increased in patients with calcification comparing with healthy donors. Meanwhile there were significant differences in number of Naïve Tcyt CD73+CD39- (p=0.042), TEMRA Tcyt CD73+CD39- (p=0.034), EM Th CD73+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 Tcyt (p=0.04), absolute count of EM Tcyt (p=0.01) and relative count of CD73-CD39- Tcells (p=0.04) in patients with BAV were significantly lower than those in patients with TAV. Taking together achieved results are proving the hypothesis of participation 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 purinergic signaling in the activation and functioning of these cells.

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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 still only partially explained. Various morphological and functional alterations have been associated with atrial fibrillation, including signs of inflammation in the atrial myocardium. Such inflammatory process is believed to contribute to structural remodelling and perpetuation of arrhythmia. Mast cells might be one of the inflammatory cell populations involved.
**Purpose:** Our aim was to characterize and quantify mast cells in the atrial myocardium of patients undergoing an open heart surgery with atrial fibrillation compared to those in sinus rhythm (SR).
**Methods:** Biopsies from the right and left atrium were obtained during elective open heart surgery. The samples were fixed with formaldehyde and embedded into paraffin. Sections were used to detect mast cells immunohistochemicaly, using anti-mast cell tryptase antibody. Systematic uniform random sampling was performed for collecting images that were subsequently used for quantification of mast cells. Frequency of cells was expressed as the number per square mm.
**Results:** Mast cells immunoreactive for mast cell tryptase were detected in samples from both patient groups and displayed their typical morphology. They presented usually as single cells in the endomysium or in clusters of several cells in the perimysial connective tissue of the atrial myocardium. Larger clusters were present in the epicardium, while in the endocardium only scattered single cells were found. The quantitative analysis of the frequency of mast cells in the atrial myocardium led to the following results: right appendage from patients with AF 4,73 $\pm$ 3,13 vs. 5,35 $\pm$ 4,32 with SR; left appendage from patients with AF 6,22 $\pm$ 3,12 vs. 5,17 $\pm$ 3,57 with SR, the left atrial free wall from patients with AF 6,58 $\pm$ 4,57 vs. 7,05 $\pm$ 4,43 in SR. When the both anatomical locations from the left atrium were combined there was 6,40 $\pm$ 3,92 in AF vs. 6,25 $\pm$ 3,90 in SR. In addition, cardiac mast cells were all positive for CD117 and no other CD117-positive cells were found.
**Conclusion:** The quantitative differences in mast cell frequency were not statistically significant when patients with AF and SR were compared. It is unlikely that these cells have any specific role in AF pathogenesis. CD117-positive cell population in the atrial myocardium corresponds to mast cells.

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### Characteristics of the inflammatory response in patients with coronary artery disease and arterial hypertension

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**Introduction:** The combination of coronary artery disease (CAD) and arterial hypertension (AH) usually accompanied by a significant morphological changes of coronary arteries. The study of the role of chronic inflammation in the development of cardiovascular complications in patients with CAD and AH still retains its relevance.
**Purpose:** To explore the characteristics of cellular and vascular inflammatory response in patients with CAD and AH.
**Methods:** A total of 324 patients with significant coronary artery stenosis >75% were examined. Group 1 included patients with CAD (n=44, mean age 54.5 $\pm$ 8.2 years); group 2 - patients with CAD and AH (n=280, mean age 55.9 $\pm$ 7.8 years). Groups differed in mass body index (MBI) (1 group — 27.2 $\pm$ 4.4 kg/m2; 2 group — 31.2 $\pm$ 4.9 kg/m2) and level of glucose (1 group — 5.34 $\pm$ 0.89 mmol/l; 2 group — 5.95 $\pm$ 1.54 mmol/l). Lipid profile parameters; cellular inflammatory markers (neutrophil-to-lymphocyte ratio, platelet-to-lymphocyte ratio); vascular inflammatory markers (uric acid, hs-CRP, TNF-alpha, homocysteine, interleukine 1$\beta$, 6, 8; sCD40, CD40L, MMP-9, TIMP-1); endothelial dysfunction markers (endothelin-1, nitrites) were measured.
**Results:** Value of MMP-9 and TIMP-1 was significantly higher than the norm in both groups, but a higher level of TIMP-1, IL-8 was registered in group 1; MMP-9 and uric acid were higher in group 2. Method of logistic regression identified the following factors associated with the presence of the combination of CAD and AH: history of myocardial infarction (OR 0.09 [CI 0.03; 0.27], p<0.001); uric acid (OR 1.01 [CI 1.00; 1.01], p<0.001); BMI (OR 1.31 [CI 1.18; 1.46], p=0.044); MMP-9 (OR 1.02 [CI 1.01; 1.03], p<0.001).
**Conclusion:** In patients with CAD and AH the imbalance of MMP-9/TIMP-1 prevailed possibly because of hemodynamic factors in AH and oxidative stress in obesity. It may be associated with the higher risk of cardiovascular events in this group.

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### Pro-inflammatory cytokines as cardiovascular events predictors in rheumatoid arthritis and asymptomatic atherosclerosis

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**Background:** Recently rheumatoid arthritis (RA) is considered as an important risk factor of cardiovascular diseases (CVD) and asymptomatic atherosclerosis (AA). In our previous studies we revealed close correlation between RA severity, pro-inflammatory cytokines levels and CVD manifestation. Purpose of this investigation was to assess the diagnostic and prognostic value of pro-inflammatory cytokines in the prediction of serious cardiovascular events.
**Methods:** 112 pts with RA (94 female and 18 male aged 37-74 years) were observed during 5 years. Baseline levels of pro-inflammatory cytokines (TNF-$\alpha$, IL-1$\beta$, IL-6) were determined. Outcomes included cardiovascular death, acute coronary syndrome (ACS) and stroke. We used ROC-analysis to evaluate the validity of determined cytokines in prediction of these CV events and Cox proportional hazard models to calculate the hazard ratios (HRs) for each outcome.
**Results:** Focal plaques and/or cIMT>0.90 were find out in 38 pts from 54 pts (70.4%) without history of IHD and stroke. The increased incidence of ACS and CV death was defined in RA pts with history of CVD or with AA. The risk of ACS and CV death was higher in these groups and was associated with elevated levels of IL-1$\beta$ (HR 1.15, 95% CI 1.06 to 1.19, HR 1.12, 95% CI 1.04 to 1.18, respectively; AUC=0.675, AUC=0.658 p<0.05, respectively) and IL-6 (HR 1.29, 95% CI 1.12 to 1.45, HR 1.32, 95% CI 1.18 to 1.44, respectively; AUC=0.646, AUC=0.628 p<0.05, respectively).
**Conclusions:** CV risk should be addressed with all pts affected by RA and AA. Elevated levels of IL1$\beta$ and IL-6 can be considered as reliable predictors of serious CV events such as ACS and CV death in RA and AA.

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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 classically 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 in vitro.
**Method:** Bone marrow cells obtained from femurs and tibiae of 8-10-week male FVB/N mice were cultured for seven days. The cells were divided into three groups: unstimulated, M1 and M2. The four-hour in vitro polarization into M1 phenotype was performed by using lipopolyssacharides and interferon-$\gamma$, and into M2 phenotype, interleukin-4 (n=4 animals; 107 cells per animal). RNA was extracted and after DNAse treatment, qPCR was used to analyse the differential gene expression of M1 markers interleukin-1$\beta$ (IL-1$\beta$), tumor necrosis factor-$\alpha$ (TNF-$\alpha$), 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 (Fizz1). Glyceraldehyde 3-phosphate dehydrogenase was used as an endogenous control.
**Results:** In M1, both TNF-$\alpha$ and IL-1$\beta$ gene expression increased compared to unstimulated (6.76 $\pm$ 0.48 and 6.26 $\pm$ 0.32 A.U., respectively). Moreover, both genes were more expressed in M1 compared to M2 (6.76 $\pm$ 0.48 vs -0.26 $\pm$ 0.56 A.U. and 6.26 $\pm$ 0.32 vs 0.14 $\pm$ 1.29 A.U., respectively). The Arg2 gene expression was also higher in M1 compared to unstimulated (9.98 $\pm$ 3,1 vs 2.27 $\pm$ 2.51 A.U.). For the CCL2 RNA, a trend towards higher expression in M1 was found, without reaching significance. Intringly, the amplification of IL-6 gene was absent by qPCR. In M2, MR gene was more expressed compared to M1 (4.41 $\pm$ 1.15 vs -12.37 $\pm$ 7.4 A.U.), while the amplification of Fizz1 by qPCR was inefficient.
**Conclusions:** The TNF-$\alpha$, IL-1$\beta$ and Arg2 RNA 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 Fizz1 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 dyslipidaemic syndrome in chest pain pathology anterior localized (TAP).
**Material and Method:** It watched metabolic clinical profile and antioxidant status to a number of 170 patients admitted in the Medical Clinic V, Hospital Clinic CF Iaşi and who have been diagnosed with various disorders with common symptoms: chest pain earlier. The results obtained 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), 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 redox status differs depending on the type of disease or age.
**Conclusions:** Patients considered as limit geriatric with cardiovascular 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

| Age | Patients under 65 years | Patients over 65 years |
|---|---|---|
| sex (m/f) | 57,40 | 69,8 |
| smoking | 1/1 | 3/1 |
| hta degree association | 44% Degree II, III | 9% Degree I, II |
| obesity | 74,4% | 11,6% |
| association | 86,20% | 100% |
| dyslipidaemia | 44,4% | 75% |
| vascular comorbidities | 75% | 88,8% |
| ischemic heart disease | 60,4% | 39,5% |
| HTA | 33,3% | 16,6% |
| peripheral arterial disease | 16,6% | 0,08% |
| Stroke | 25,0% | 11,1% |

Clinical features of patients with anterior chest pain (TAP)

## Signal transduction - Heart

### 545
### The association of heat shock protein 90 and TGFbeta receptor I is involved in collagen production during cardiac remodelling in aortic-banded mice

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**Background/Introduction:** Transforming growth factor $\beta$ (TGF$\beta$) plays crucial pathophysiological role in the maladaptive remodeling of the heart in response to pressure overload by triggering interstitial fibrosis and cardiomyocyte hypertrophic growth. The molecular chaperone heat shock protein 90 (Hsp90) has been shown to play a critical role in TGF$\beta$ signaling by stabilizing the TGF$\beta$ signaling cascade. Hsp90 is considered to be a protein stabilizer known to bind many receptors and thus protecting the corresponding signaling. TGF$\beta$ receptors (TGF$\beta$Rs) 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 TGF$\beta$RI 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 TGF$\beta$RI, Hsp90 and Col1 were determined by qPCR and Western blot. Cardiac fibroblasts were isolated from hearts of long term TAC-treated mice and functional refolding assay was used to determine Hsp90 activity levels in presence/absence of specific inhibitors (SB43152 and 17AAG). NIH-3T3 fibroblasts were assesed for co-localization of Hsp90 and TG$\beta$RI in the presence of TGF$\beta$ recombiant protein. In silico predictions of Hsp90-TG$\beta$RI protein-protein interactions were performed using FpClass data mining-based method, and ClusPro and ZDock algorithms. The degree of fibrosis was detected in mice heart sections stained with Masson's trichrome assay.
**Results:** TAC-treated mice showed Hsp90 higher expression ($p<0.05$*) compared to their Sham littermates at 3 h and 14 h after TAC (Fold: $3.0 \pm 1.2$; 3h; $1.8 \pm 0.3$; 14h), TG$\beta$RI (Fold: $1.9 \pm 0.3$; 3h; $2,0 \pm 0.6$; 14h) and Coll (Fold: $1.5 \pm 0.2$; 3h; $1.4 \pm 0.3$; 14h). Hsp90 folding activity increased in fibroblasts from 2 weeks TAC-treated mice (TAC $27139.5 \pm 504.1$ vs Sham $20789.9 \pm 146.0$, $p<0.005$**) but it did not change in the corresponding myocyte fraction. We detected Hsp90 in complex with TGF$\beta$RI with specific antibodies suggesting a potential physical interaction between the two. Hsp90 inhibition lessened the yield of collagen production (Fold: $0.5 \pm 0.02$, $p<0.005$**) as well as the phosphorylation of Smad2/3 ($p<0.005$**). These observations together with the significant increased activity of Hsp90 at the plasma membrane of TGF$\beta$-treated myocardial fibroblasts pointed to a functional cooperative partnership between Hsp90 and TGF$\beta$RI in the fibrotic process.
**Conclusion:** Our studies propose that the formation of Hsp90-TGF$\beta$RI 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 excitablitiy

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**Introduction:** The heart is controlled by the sympathetic and parasympathetic limbs of the autonomic nervous system with inhibitory signalling 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 and its potential role in arrhythmogenesis.
**Methods:** Mice with conditional deletion of inhibitory heterotrimeric G protein G$\alpha$O in the rostral ventrolateral medulla oblongata were generated to determine the effect of specific G$\alpha$O deletions on autonomic control and electrophysiological properties of the heart.
**Results:** G$\alpha$O deletion in the presympathetic area of the rostral ventral lateral medulla (RVLM) was not associated with changes in HR or the arterial blood pressure (BP) at 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 G$\alpha$O loss in the RVLM increases central sympathetic drive.
**Conclusions:** The data obtained suggests that G$\alpha$O-mediated signalling within the presympathetic circuits of the RVLM contributes to the autonomic control of the heart. G$\alpha$O deficiency in the RVLM is associated with exaggerated cardiovascular responses to stress, altered cardiovascular reflexes and electrical properties of the heart.

### 547
### Selenoprotein P regulates pressure overload-induced cardiac remodeling

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Selenoprotein P (SeP) is a liver-derived secretory protein that induces insulin resistance. Although clinical studies suggest insulin resistance is associated with congestive heart failure incidence independent of established risk factors, the role of SeP during cardiac remodeling is not well understood. In this study, we examine the role of SeP in regulating cardiac hypertrophy and function in response to pressure overload. Transverse aortic constriction (TAC) was applied to SeP knockout (KO) and wild-type (WT) mice. The mortality rate following TAC was significantly decreased in SeP KO mice compared to WT mice (32.5 % in KO mice (n=39) vs 51.3 % in WT mice (n=40) $p<0.05$). The ehocardiographically determined left ventricular (LV) ejection fraction and LV wall thickness at baseline were similar between SeP KO and WT mice. Interestingly, both LV septum and posterior wall thickness two weeks after TAC were significantly smaller in SeP KO than those in WT mice ($1.04 \pm 0.04$ vs$1.27 \pm 0.03$ mm, $p<0.05$; $0.92 \pm 0.04$ vs $1.12 \pm 0.04$ mm, $p<0.05$). LV weight/body weight (BW) and lung weight/BW were significantly smaller in SeP KO than those in WT mice ($4.41 \pm 0.18$ vs $5.39 \pm 0.22$, $p<0.05$; $6.88 \pm 0.47$ vs $10.57 \pm 0.69$, $p<0.05$). mRNA expression of ANF and BNP was significantly reduced in SeP KO compared to WT mice ($p<0.05$). Furthermore, mRNA expression of collagen 1a1, marker of fibrosis, was significantly decreased in SeP KO compared to WT. These results suggest that the absence of endogenous SeP attenuates cardiac hypertrophy and fibrosis by pressure overload. In conclusion, SeP is a regulator of cardiac hypertrophy and possibly plays a maladaptive role against progression of congestive heart failure.

### 548
### Study of adenylyl cyclase activity in erythrocyte membranes in patients with chronic heart failure

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**Purpose:** study of activity adenylyl cyclase (AC) in erythrocyte membranes in patients with chronic heart failure (CHF).
**Methods.**The study included 56 post-MI male patients aged from 45 to 55 (mean age $51.2 \pm 4.6$ years) years with CHF (NYHA FC II-III). All the patients were divided into two groups according to the New York Heart Classification (NYHA) functional class (FC). Group 1 consisted of 30 patients with CHF FC-II and Group 2 consisted of 26 patients with CHF-III. The AC activity in red blood cells homogenate was determined according to the method of Y. Salomon.
**Results:** Basal AC activity was less by 31.9% in patients of Group 1 compared to the control group ($4.15 \pm 0.14$ vs $6.1 \pm 0.19$ pmol/mg/min); in patients of Group 2 it was less by 41.6% compared to the control group ($3.56 \pm 0.13$ vs $6.1 \pm 0.19$ pmol/mg/min) and by 14.2% compared to Group 1 patients. In the control group, we found an increase in the epinephrine-stimulated AC activity of about 2 times in comparison with basal level ($11.3 \pm 0.5$ vs $6.1 \pm 0.19$ pmol/mg/min, $P<0.01$). In Group 1 patients, the epinephrine-stimulated AC activity was lower about 2 times compared to the control group ($5.5 \pm 0.19$ pmol/mg/min vs. $11.3 \pm 0.5$pmol/mg /min). In Group 2 patients, this parameter was reduced to $3.85 \pm 0.19$ pmol/mg/min and was 65.9% ($P<0.05$) lower than in the control group and 28.7% lower than in Group1 patients. A significant increase in the epinephrine-stimulated AC activity of erythrocyte membranes in healthy controls by 85% reflects an adequate response of the

membrane AC to stimulation. Revealed disturbances in ESAC activity in patients with CHF reflect the desensitization of the erythrocyte AC system, which is exacerbated by severity of the CHF.
Conclusion, in patients with CHF FC II-III observed desensitization of the adenylate cyclase system, which is more pronounced in patients of Group 2.

### 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 contractile 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 ventricle dilation and systolic dysfunction. The vitamin K antagonist, Warfarin, had no effect on both atrial and ventricle remodeling. The increase in hypertrophic markers such as brain natriuretic peptide and $\beta$-myosin heavy 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, 10 nM thrombin upregulated hypertrophic markers and plasminogen 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 / FVIIa 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 tissue factor (TF) / FVIIa-complex formation. This occurs in several cell types including monocytes and aortic smooth muscle cells. Caveolae are well characterized cell membrane signaling compartments, but their role in TF signaling is poorly understood.
**Purpose:** To clarify the mechanism behind the TF-induced phosphorylation of the IGF-1R, we utilized 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, PC3 prostate or MDA-MB-231 breast cancer cells were treated 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 Duolink In Situ proximity ligation assay (PLA) and western blot (WB), and the nuclear localization of the IGF-1R was assessed by PLA or by WB on fractionated cell lysates.
**Results:** FVIIa treatment (10 and 100 nM) increased the phosphorylation of the IGF-1R after 30 minutes 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 transcription. The IGF-1R was similarly activated by Cav1 siRNA knockdown. Additional experiments showed that pretreatment with the Cav1 scaffolding domain peptide completely abolished the effects of FVIIa regarding IGF-1R phosphorylation and nuclear translocation. The formation of the TF/FVIIa-complex did not alter Cav1 protein levels but induced a Src-dependent phosphorylation of tyrosine 14 on Cav1 after 10 minutes. Inhibition of 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 emphasizes 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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**Purpose:** Thoracic aortic aneurysm develops as a result of complex series of events that alter the cellular structure of the aortic wall. It has been shown in our and other previous studies, that patients with defects of left ventricular outflow tract may have mutations in NOTCH1 gene. Notch signaling between endothelial and smooth muscle cells plays an important role for smooth muscle differentiation, which is altered in patients with ascending aortic aneurysm (AoA). The aim of this study was to assess the expression level of Notch signaling components in endothelial and smooth muscle cells derived from aneurysms in patients with bicuspid aortic valve (BAV) and tricuspid aortic valve (TAV).
**Methods:** Human aortic endothelial cells (HAECs) and smooth muscle cells (SMC) were isolated from tissue fragments of BAV- and TAV-associated thoracic aortic aneurysm patients and from healthy donors used as controls. The baseline level of Notch receptors, ligands and target genes was estimated by qPCR.
**Results:** Endothelial cells of AoA patients had significantly lower mRNA levels of NOTCH1, NOTCH2, NOTCH4 and DLL4 comparing to controls. However the mRNA level of direct Notch target HEY1 was higher in HAEC of AoA patients. On the contrary, SMC of the patients had significantly higher mRNA levels of Notch receptors: NOTCH1, NOTCH2, NOTCH3 comparing to controls, while levels of direct Notch target genes, such as HEY1, HES1, was not changed in SMC of the patients.
**Conclusions:** Expression level of Notch receptors, ligands and effectors is altered in HAEC of AoA patients. In contrast, in SMC of the patients the level of Notch receptors is changed comparing to controls, but not the level of Notch effector genes such as HEY1 and HES1. Our results show that Notch signaling is differently altered in endothelial and smooth muscle cells of AoA patients. This corresponds 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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Angiogenesis is the process in which endothelial cells and perivascular cells create new vessels under the influence of a broad spectrum of stimuli. This process is tightly regulated and imbalances in signalling can be a causative or a progressive factor in many vascular defect associated diseases. Emerging evidence suggests an important role for Frizzled/Wnt signalling 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.
In vitro endothelial tubule formation was studied in a 3D coculture system, in which human umbilical vein endothelial cells (HUVECs) and pericytes were cocultured in a collagen matrix. Short interference 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 proliferation and migration, two essential components of angiogenesis, were severely inhibited after knockdown of Fzd5. In order to define the molecular cause for the inhibition of angiogenesis after knockdown of Fzd5, known downstream Fzd/Wnt transduction cascades were studied. No alterations were observed in various parameters of the canonical Wnt/$\beta$-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 Flt1 and Angpt2, two important factors in vascular regression. The up regulation of Angpt2 could be suppressed by a combined knockdown of Fzd5 and the transcription factor Ets-1.
These data indicate that the degenerative effect of Fzd5 silencing on vascular structure formation in vitro is associated with an inhibitory effect on endothelial proliferation and migration. Wnt/$\beta$-catenin signalling and Wnt/Ca2+ signalling are not affected by knockdown of Fzd5 in HUVECs. Instead, there could be a role for the transcription factor Ets-1 in Fzd5 signal transduction, as it is involved in the up regulation of Angpt2 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 cardiovascular 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 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 it is not known.
**Purpose:** The present study was purported to assess the effect of three benzopyran analogues on the vascular reactivity and H2O2 production in aortic rings isolated from rats with streptozotocin-induced diabetes mellitus (DM) and mammary arteries harvested from coronary artery disease patients with and without DM subjected to by-pass grafting.
**Methods:** The effect of KL-1487, KL-1492, KL-1507 (10μmol/L) on endothelium-dependent relaxation (EDR, assessed in the organ bath-system) and H2O2 production (determined by ferrous oxidation xylenol orange assay) have been studied in diabetic vs. non-diabetic murine and human vascular fragments. Results: We found an important decrease in EDR in diabetic vessels whereas H2O2 generation was significantly increased in both humans and rats. Incubation of vascular segments with all investigated compounds attenuated H2O2 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

### 556
### Cardiac fibroblasts as inflammatory supporter cells trigger cardiac inflammation in heart failure

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**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 chemoactive 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 fibroblasts 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 demonstrates 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 fibroblast 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 osteogenesis in atherosclerosis.
**Purpose:** to study whether Gal-3 mediates valve calcification in AS.
**Methods:** Blood samples and 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 new therapeutic approach to delay the progression of AV calcification in AS.

## 558
## Omega-3 polyunsaturated fatty acids- can they decrease risk for ventricular fibrillation?

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**Background:** Reports, including ours, indicate that lower omega-3 ($\omega$-3) index accompanied by cardiac extracellular and myocardial connexin-43 remodelling and enhanced autoantibody production to the adrenergic beta-1 receptors (b1-AAB) are implicated in development of heart failure and increased incidence of lethal arrhythmias. Based on these results we aimed to explore the effect of $\omega$-3FA suplementation on $\omega$-3 index, b1-AAB, matrix metalloproteinases (MMP), connexin 43 (Cx43) and susceptibility to arrhythmias in aged male (♂) and female (♀) spontaneously hypertensive rats (SHR).
**Methods:** 1 year-old SHR and age-matched healthy Wistar rats (WR) fed with $\omega$-3FA (Vesteralens, Norway, EPA+DHA 200mg/day/2month) were compared with untreated rats. Gas chromatography was used for determination of red blood cells $\omega$-3FA and $\omega$-6FA composition. Blood serum was used for the detection of b1-AAB. Left ventricular tissue was taken for examination of MMP-2 activity using zymography; Cx43 expression and its cellular distribution using Western blot and immunohistochemistry. Susceptibility to electrically-induced ventricular fibrillation (VF) was tested using Langendorff-perfused heart.
**Results:** Compared to healthy WR $\omega$-3 index was lower in both ♂ and ♀ SHR. This parameter was significantly increased due to $\omega$-3 FA intake in both sexes of SHR. ♂ and ♀ SHR also exhibited a significant increase of serum levels of b1-AAB, activity of MMP2, down-regulation and miss-localisation of Cx43. It was associated with higher incidence of VF. $\omega$-3FA intake resulted in significant decrease of b1-AAB levels and MMP2 activity, upregulation of Cx43 and partial elimination of Cx43, its miss-localisation in both ♂ and ♀ SHR. Moreover, $\omega$-3FA decreased incidence of electrically-induced VF.
**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 derived from this degradation are present in the circulation and are a stimulus for production of anti-elastin antibodies. They can form circulating immune complexes with corresponding antigens. It is well known that elastin metabolism is pathologic in patients with coronary artery disease (CAD). The aim of our study was to: Measure levels of (1) elastin derived peptides (EDP) and (2) elastin-antielastin circulating immune complexes (EA CIC) in sera of patients with CAD and to compare them with these in healthy controls.
**Material and Methods:** The levels of EDP and EA CIC were studied in sera of 63 patients (mean age-62.5 $\pm$ 12.4 years, CAD duration 9.88 $\pm$ 3.12 years). Forty-two healthy persons were used as controls (mean age-58.9 $\pm$ 7.56.). An elastin-specific ELISA for detection of EDP was used. EA CIC were investigated by a method for immune complexes detection by means of ELISA-type techniques.
**Results:** Patients with CAD showed statistically significantly higher levels of EDP (0.196 $\pm$ 0.073) than healthy controls (0.085 $\pm$ 0.033) $p<0.001$. Patients with CAD showed statistically significantly higher levels of EA CIC (0.063 $\pm$ 0.027) than healthy controls (0.014 $\pm$ 0.004) $p<0.05$.
**Conclusion:** These findings suggest that elevated levels of EDP and EA CIC are associated with the development of coronary artery disease.

## 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 dysfunction 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:** We have tested this hypothesis in a chick model of HLHS induced by left atrial ligation (LAL) at embryonic day (ED) 4.
**Methods:** At ED8 and 12, modifications of myocardial architecture and fibrosis were studied by histology and imnmunoconfocal microscopy, and the amount of collagen was quantified by image analysis and mass spectrometry (MS).
**Results:** Histologey 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. Hypoxyprobe 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.
**Conclusions:** We conclude that abnormal hemodynamic loading leads to myocardial hypoxia, 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 60.47 $\pm$ 7.4) was enrolled. Blood samples were drawn on the 1st (T1), 3d (T2), 7th (T3), 14th (T4) days of STEMI and 6 month after STEMI (T5). The serum levels of MMP-2, 3, 9 were determinated by the immunoassay (ng/ml).
**Results:** All pts underwent reperfusion therapy, 6 pts by the primary PCI, 15 pts - PCI after successful fibrinolysis (67%). Average reperfusion time was 5.01 $\pm$ 3.5 h. The levels of MMP-2 were decreased in 30% pts and 70% pts had normal levels. Than MMP2 levels was increased or normalized to T4. Although there were no significant dynamics according to average value: 224.95 $\pm$ 84.14 (T1) $\rightarrow$ 190.56 $\pm$ 63.85 (T2) $\rightarrow$ 224.5 $\pm$ 90.11 (T3) $\rightarrow$ 233.3 $\pm$ 70.23 (T4) $\rightarrow$ 262.86 $\pm$ 96.33 (T5). The nonlinear dynamics of MMP-3 was revealed. Levels of MMP3 were increased to T3 ($p<0.05$), it was no changed to T4. Then it was continued to rise and increased in 2.5 times from T1 to T5 - 23.27 $\pm$ 8.71. MMP-9 had also the nonlinear dynamics. It was above than normal range - 905.92 $\pm$ 772.41 at T1. Further we revealed decreasing of it to T3 — 538.0 $\pm$ 520.6 ($p<0.05$), from T3 to T4 it was unchanged, but to T5 it decreased too - 365.54 $\pm$ 449.4 ($p<0.05$).
**Conclusion:** Thus, the dynamic of studied metalloproteinases was different. The levels of MMP-9 were nonlinear decreasing from T1 to T5, whereas, the levels of MMP-3 were nonlinear increasing. Serum levels of MMP-2 were normalizing (if its were reduced) or increasing to 14th day.

### 564
**Deletion of the alpha-7 nicotinic acetylcholine receptor changes the vascular remodeling induced by transverse aortic constriction in mice.**

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**Background/Introduction:** The vascular remodeling is a response to hemodynamic forces. The mechanical stress causes modifications in the vascular wall, such as reorganization of cellular composition, vascular matrix and vascular inflammatory responses. The $\alpha$7 nicotinic acetylcholine ($\alpha$7nAChR) receptor is found in many cells types, including the cells of the immune system. In several inflammated tissues, the activation of the $\alpha$7nAChR by acetylcholine inhibits the production of inflammatory cytokines and chemokines through the vagal reflex, thus producing an anti-inflammatory effect. It is not yet the contribution of the $\alpha$7nAChR for the process of vascular remodeling.
**Purpose:** The aim of this study was to evaluate the effect of the deletion of $\alpha$7nAChR in the vascular remodeling developed in response to transverse aortic constriction (TAC).
**Methods:** We used wild type mice (WT) and knockout mice with deletion of $\alpha$7nAChR ($\alpha$7-KO) at 10 weeks of age. Mice were divided into the following groups: WT SHAM, WT TAC, $\alpha$7 SHAM and $\alpha$7 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 ($0.32 \pm 0.001mm^2$) and $\alpha$7 TAC ($0.31 \pm 0.02mm^2$) groups when compared to their respective controls WT SHAM ($0.26 \pm 0.02mm^2$) and $\alpha$7 SHAM ($0.28 \pm 0.012mm^2$). A similar pattern was also observed for the area of the lumen, in which the values of WT TAC ($43 \pm 5.66\%$) and $\alpha$7 TAC ($38.40 \pm 2.41\%$) groups were larger when compared to their controls WT SHAM ($28 \pm 0.71\%$) and $\alpha$7 SHAM ($29 \pm 3.61\%$). While the WT TAC group had a significant increase in the deposition of collagen type I ($7.35 \pm 0.88\mu m^2$) and III ($2.97 \pm 0.75\mu m^2$) when compared to SHAM, the deletion of $\alpha$7nAChR inhibits this process maintaining the level of both types of vascular collagen as in SHAM and $\alpha$7 SHAM operated groups. Regarding the density of cells, the $\alpha$7 TAC group had the highest values.
**Conclusion:** The results demonstrate that the TAC promotes a positive vascular remodeling in the proximal aorta of both WT and $\alpha$7nAChR knockout mice. In response to TAC, the vascular deposition of collagen and the density of cells are 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**

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**Background:** 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 proteins were enriched from venous 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 laminins. Chymase and tryptase, two serine proteases commonly attributed to mast cells, were among the upregulated proteins. Using immunohistochemistry, however, chymase expression was localised to smooth muscle cells in varicose veins. The effect of chymase and tryptase on the venous ECM was explored by incubating normal saphenous veins with recombinant enzymes. Proteomics analysis revealed extensive ECM degradation after digestion with tryptase. 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-ß), tumor necrosis factor-$\alpha$ (TNF-$\alpha$) or angiotensin II (Ang II), a number of ECM genes differentially expressed in varicose veins, including mimecan, changed in response to TGF-ß and TNF-$\alpha$ 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 vascular ECM in varicosis.

## Ion channels, ion exchangers and cellular electrophysiology - Heart

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

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**Introduction:** Microtubule-associated protein RP/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 is located in adult cardiomyocytes at the intercalated 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 localisation of sodium channels in neurons. We here investigated the effects of EB1 on cardiac sodium channel function and its modulatory 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 (Scn5a1798insD+/-) 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. Overexpression of MAPRE1/EB1 in HEK293 cells together with SCN5A/Nav1.5 led to an increase in sodium current density without affecting kinetic properties, indicating an increased membrane trafficking of the Nav1.5 protein.
**Conclusion:** In this study we demonstrate a functional role for EB1 in cardiac conduction and we highlight its direct regulation of the cardiac sodium channel Nav1.5. We recently produced lentiviruses in order to knock-dow and overexpress EB1 in order to characterize its modulatory effect on ion currents and action potential parameters in hiPS-CM using the dynamic clamp technique.

### 569
**Investigation of electrophysiological abnormalities in a rabbit athlete's heart model**

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**Introduction:** Most sudden cardiac death events in athletes are associated with cardiac muscle structural disorders. However, the underlying cause remains unclear in 3-6% of such death events. Apart from the structural disorders, functional remodeling (e.g. reduced repolarization reserve) might also lead to life-threatening ventricular tachyarrhythmias. In a new study, the effect of the long-term exercise training was tested on the electrical activity of the myocardium in a new rabbit athlete's heart model.
**Methods:** New-Zealand white rabbits were randomized into a 'Sedentary' and to an 'Exercised' ('Ex') group (n=7). Animals of the 'Ex' group were trained during a 12-week long treadmill-running protocol. Echocardiography and resting ECG recording were performed under ketamine anaesthesia. At the end of training protocol, proarrhythmic sensitivity were tested with dofetilide (50 nM) in 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-ß, fibronectin-1, collagen-I,-III, MMP-2 and TIMP-1 were quantified with RT-qPCR to determine the collagen metabolism.
**Results:** Echocardiography on the 12th week showed significant increase in the internal end-diastolic diameter of the left ventricle (LVIDd) in the 'Ex' group ($17.4 \pm 0.3$ vs. $14.7 \pm 0.8$ mm, $p<0.05$) compared to the 'Sedentary' group. Resting heart rate was significantly lower ($198 \pm 4$ vs. $253 \pm 8$, $p<0.05$), PQ, QT, RR, Tpeak-Tend intervals 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 in vitro, 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 LVIDd 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 variability 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.
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### 570
**Upregulation of expression of multiple genes in the atrioventricular node of streptozotocin-induced diabetic rat**

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**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 correlate 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 healthy controls. Previous in vivo and in vitro studies have demonstrated reduced heart rate in the streptozotocin (STZ) — induced diabetic rat. Diabetes can increase the duration of the sinoatrial node (SAN) action potential and prolong sino-atrial conduction time. 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 rat. Recent studies have demonstrated a reduction in peak L-type Ca2+ current, faster time-dependent inactivation, a negative shift in the voltage dependence of inactivation, and a slowing of restitution parameters in AVN cells from STZ rat. Modification of ion channel properties either by altered trafficking and expression, or post-translational modification of channel gating properties, may have a significant impact on AVN function, and result in clinical AVN abnormalities.
**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.

**Methods:** Diabetes was induced in male rats with STZ (60 mg/kg bodyweight, i.p.) and age-matched Control 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 compared to Control rats. Genes encoding expression of multiple ion channel proteins were upregulated (2-9 fold) in STZ compared to Control-AVN as follows: ATP1B, ATP2B, NCX1, TRPC1, CAV$\beta$2, CLCN3, SCN3A, HCN3, HERG1, KIR6.2, KIR3.1, KIR3.4, K2P3.1, SK2. In addition various other genes that encode proteins that regulate ion transport were also upregulated in STZ compared to Control-AVN as follows: CASQ2, RYR2, RYR3, ANP.
**Conclusions:** These changes in gene expression are likely to contribute to the electrophysiological disturbances seen in the diabetic AVN.

### 571
### miR-1 as a regulator of sinoatrial rhythm in endurance training adaptation

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Endurance exercise induces a physiologic remodelling of the heart in order to compensate for the increased metabolic need. One of the most common adaptations in endurance athletes is bradycardia. Recently, it was demonstrated that trained mice display bradycardia associated with a down-regulation of the sinoatrial If current.
MicroRNAs (miRNAs), small non-coding RNAs able to block translation of target mRNA, have been recognized as novel modulators of (patho)physiologic cardiac remodelling, and their expression can be altered by training. miR-1 is for example upregulated in the sinoatrial node of trained mice. Luciferase reporter essay shows that miR-1 can directly target HCN4 mRNA, a channel important in setting the heart rhythm. In order to understand the role of miR-1 in cardiac pacemaking we first created both a line of mouse embryonic stem cells containing a construct for the over-expression of miR-1 (mESC-miR-1) and a control line containing the empty construct (mESC-empty). We differentiated cells into spontaneously beating cardiomyocytes following a standard protocol and selected sinoatrial-like cells by CD166 expression. Undifferentiated mESC-miR-1 showed 200-fold overexpression of miR-1 compared to mESC-empty but this did not affect either pluripotency or their capacity to differentiate into cardiomyocytes. Preliminary results show that in mESC-miR-1 the proportion of CD166+ SAN-like cells is higher than that in control mESC-empty (14.0 $\pm$ 2.7% Vs. 7.4 $\pm$ 2.6%). Electrophysiological analysis confirmed a decrease in the action potential rate due to miR-1 over-expression. This evidence is consistent with the idea that miR-1 could play a leading role in regulating cardiac excitability, probably participating in the establishment of bradycardia in endurance athletes.

### 572
### Selective sodium-calcium exchanger inhibition reduces myocardial dysfunction associated with hypokalaemia and ventricular fibrillation

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**Introduction:** Hypokalaemia (HK) increases the risk of arrhythmias such as ventricular fibrillation (VF). Both HK and VF are thought to elevate the cellular Ca through activation of Ca-influx via the Na/Ca-exchanger (NCX), which may cause arrhythmias, myocardial dysfunction and contracture. This study investigated the possible beneficial effect of selective NCX inhibition on alterations of left ventricular mechanics occurring during HK and VF.
**Methods:** Experiments were performed on isolated guinea pig and rat hearts. ECG and left ventricular pressure (LVP) were recorded throughout the experiments and analysed off-line. NCX inhibition was achieved with ORM10962 (ORM, 1$\mu$M).
**Results:** HK solution increased LVP indicating net cellular Ca gain. Administration of ORM significantly diminished the HK-induced increase in LVP. ORM did not affect the ECG parameters. In separate experiments, VF was induced by rapid pacing in isolated rat hearts. Onset of VF resulted in left ventricular contracture, the fast component of which occurred within 5 seconds and was significantly attenuated by ORM. Similarly, the slowly developing component, evaluated after 3 min of fibrillation, was also decreased in the ORM-treated hearts. After defibrillation, LVP amplitude showed a mild increase in the ORM-treated hearts compared to the control group, but this effect did not reach statistical significance.
**Conclusions:** The increased LVP during HK and the myocardial contracture during VF observed in this study support the literature arguing development of elevated Ca load in these conditions. As reflected by the diminished LVP and contracture, the results suggest that NCX inhibition may protect the myocardium against the excess Ca load, both in HK and during VF. However, further studies are required to investigate whether these effects can result in decreased arrhythmia propensity in HK and/or reduced defibrillation after successful defibrillation.

### 573
### Effect of racemic and levo-methadone on action potential of human ventricular cardiomyocytes

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**Background:** Racemic and levo-methadone treatment has been implicated in cardiac QT-prolongation and development of Torsade de Points. The effect is attributed to the blockage of the repolarizing rapid delayed rectifier potassium current IKr/hERG, that has been characterized in re-expressed channels. Recently, methadone was demonstrated to block the depolarizing cardiac sodium current INa/Nav1.5, thus exerting an effect opposite to that due to IKr/hERG channel blockade.
**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:** Racemic methadone (0.1-10$\mu$M) reduced AP duration (APD) in a concentration-dependent manner. The effect was not prevented by naloxone (1 $\mu$M), 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 $\mu$M) reduced APD of human ventricular cardiomyocytes, showing properties comparable to those of the racemic form. Other AP parameters, including amplitude and maximal diastolic potential were not modified either by racemic 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 hERG, other channels, such as INa/Nav1.5, are likely to be affected by methadone. Latter effect probably counterbalances and overcomes the reduction of repolarization due to IKr/hERG channel blockade.

### 574
### Acute temperature effects 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 ovo.
**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 ovo for comparison and validation of in vitro measurements. Artificial stimulation experiments were performed in vitro and in ovo to uncover conduction limits of heart segments.
**Results:** Decrease in temperature from 37 °C to 34 °C led in vitro to 22% drop of the heart rate and unchanged amplitude of Ca2+ transients, compared to 25% acceleration in ovo. Increase in temperature from 37 °C to 40 °C led in vitro to 20% and in ovo to 23% increase of the heart rate, and a significant decrease in amplitude of Ca2+ transients (atrium -35%, ventricle -38%). We observed in vitro wide spectrum of arrhythmias, of which the most common was atrioventricular block (57%). We found variability of atrioventricular block locations. Pacing experiments in vitro and in ovo suggested that the atrioventricular blocks were likely caused by relative tissue hypoxia and not by the tachycardia itself.
**Conclusion:** 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.

## Vasculogenesis, angiogenesis and arteriogenesis

### 577
### 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 allogenic 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 used 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 (LDPI) prior, as well as 7, 14 and 21 days post ligation. We calculated a perfusion index (ligated/unligated leg, 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 evaluation of hind limb musculature was done to examine perivascular cell infiltration, density of vessels and cell-cell-interactions.
PI decreased from preoperatively 0.97 $\pm$ 0.08 to 0.19 $\pm$ 0.05 in all groups post ligation. Already one week after ligature PI increased in mice who had received human monocytes (HuMo 0.39 $\pm$ 0.12) as well as in the BalbC-control group (BalbCCo 0.35 $\pm$ 0.17). At day 14 PI in HuMo was 0.57 $\pm$ 0.07 vs. BalbCCo 0.50 $\pm$ 0.15 (n.s.). A difference in perfusion became significant at day 21: HuMo 0.65 $\pm$ 0.12 vs. BalbCCo 0.50 $\pm$ 0.14 (n= 9; $p<0.05$).
Histological evaluation of the thigh showed significant more collateral arteries in the adductory muscles within the ligated site after HuMo transplantation (8.3 $\pm$ 1.51) in comparison to the BalbCCo (6.3 $\pm$ 1.5; each n= 9, $p<0.05$). Muscle tissue in the lower leg was histological examined for capillary density and hypoxia (HIF1$\alpha$) as a measure for angiogenesis. The gastrocnemius muscle of mice who did not receive human monocytes showed an increase in capillary density as marker for ischemia: BalbCCo 1001 $\pm$ 305 vs. 629 $\pm$ 154 Cap./mm$^2$ HuMo (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 HuMo from initially postoperative 6 up to 2.3 vs. 3.7 BalbCCo at day 7. At day 14 the condition of mice improved even more: HuMo 1.17 vs. 4 BalbCCo. The most importand clinical difference between the two groups was found after 21 days: HuMo 0.17 vs. 3.3 BalbCCo

(n=9). Conclusion: transplantation of xenogenic monocytes can improve collateral vessel growth and increase clinical outcome of mice. These results verify our hypothesis that controlled triggering the inflammatory part in collateral vessel growth by a local host vs. graft reaction in ischemic mouse hind limb could be a usefully new strategy in promotion of collateral vessel growth.

### 578

### 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 ischaemia. The exact mechanism is as yet unclear. By better understanding of the physiology, one may attempt to replicate these adaptive mechanisms in non-OSA ischaemic heart disease (IHD) patients to better augment CCC.
**Purpose:** The study objective was to assess whether Hypoxia Inducible Factor-1$\alpha$ (HIF-1$\alpha$) and Vascular Endothelial Growth Factor (VEGF) play a role in the development of CCC in patients with OSA.
**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-1$\alpha$ (HIF-1$\alpha$ ELISA Kit, Antibodies-online Inc, Atlanta, GA, USA) and VEGF (Human VEGF Elisa Kit, KHG0111, Invitrogen Corporation, Camarillo, 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-1$\alpha$ increased with increasing Rentrop Score (p=0.04), in all patients. VEGF levels were however not significantly higher [p=0.31]. HIF-1$\alpha$ 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 differ significantly 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-1$\alpha$ (p=1.00) 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-1$\alpha$ (p=0.825) and VEGF (p=0.454)
**Conclusion:** This is the first study to date that links OSA, CCC, and plasma HIF-1$\alpha$ and VEGF levels. Augmented HIF-1$\alpha$ in moderate/severe OSA patients might be an important mediator in the development of CCC, but not in patients with no/mild OSA.

### 579

### Initiating cardiac repair with a trans-coronary sinus catheter intervention in an ischemia/reperfusion porcine animal 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 (PICSO) 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 (control-group, n=8), 4 hours PICSO in the intact heart (PICSO-A, n=10); PICSO (started 15 min. after ischemia (PICSO-B, n=11). Specimen 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, p53 positivity was measured as percentage of total amount of pixels and Ki67 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 PICSO groups as compared to control ($p<0.05$). Significant upregulation could further be found in veins of PICSO groups as compared to control and sham-operated animals ($p<0.05$). p53 was significantly downregulated in myocardial tissue of pigs from PICSO A group in comparison with control pigs ($p<0.05$). Ki67 was significantly upregulated in PICSO A in comparison with controls ($p<0.05$).
**Conclusion:** Significant upregulation of angiogenetic proteins stimulates a creating of new coronary vasculature as a result to temporarily blocking venous drainage, thus activating endothelium. Furthermore the downregulation of p53 is construed as shortage of myocardial damage, usually leading to apoptosis. Whereas upregulation of the proliferating marker Ki67 indicates that a trans-coronary sinus catheter intervention enables cell cycle reentry.
In conclusion, this study substantiates the concept that the PICSO catheter displays beneficial effects on pathologically affected myocardium by exciting neoangiogenesis and cardioprotection leading to structural repair of the damaged heart.

### 580

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

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**Introduction:** After arteriolar occlusion, outward remodeling of pre-existing arteriolar collaterals occurs chronically and increased shear 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 E14, a 4-vessel-segment 'collateral unit' was chosen at an arteriolar or venular anastomosis, a unit comprising the two consecutive vessel segments from each side of the 'anastomosis 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 24 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 h PO; venules: 2 h PO). In parallel, WSR showed an initial increase (arterioles: for 5 h PO; venules for 1 h 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 segment and smallest venular collateral segment before occlusion.
**Conclusions:** In the CAM, collateral enlargement occurs 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.

## Endothelium

### 583

### EDH-type responses to the activator of potassium KCa2.3 and KCa3.1 channels SKA-31 in the small mesenteric artery from spontaneously hypertensive rats

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**Background:** Endothelium-derived hyperpolarization (EDH) type relaxation is NO- and prostacyclin I2 (PGI2)-independent hyperpolarization, with activation of small (KCa2.3) 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 activator SKA-31 increased coronary flow in diabetic rats, reduced systemic blood pressure in hypertensive mice, normotensive dogs and pigs.
**Purpose:** Influence of SKA-31 on EDH-KCa2.3/KCa3.1 type responses in spontaneously hypertensive rats (SHR) in endothelium-intact small mesenteric arteries.
**Methods:** Vasorelaxation examined in the wire myography are expressed as the percentage of phenylephrine pre-contracted tone. Expression of KCa3.1 and KCa2.3 mRNA was confirmed by RT-PCR.
**Results:** Concentration-dependent relaxation induced by SKA-31 (0.01-10 $\mu$M) was reduced in SHR ($pD2=5.5\pm0.1$, $Emax=75.8\pm6.1\%$, n=13) compared with normotensive rats (WKY) ($pD2=6.0\pm0.1$, $Emax=92.8\pm2.3\%$, n=12 $p<0.01$, $p<0.05$, respectively). Endothelium denudation attenuated maximal effect of SKA-31-induced relaxation in both groups (WKY: $Emax=33.2\pm2.1\%$, n=5, $p<0.001$; SHR: $Emax=19.6\pm7.1\%$, n=4, $p<0.001$) in comparison with the respective control. EDH-KCa2.3/KCa3.1-type response was investigated as SKA-31-evoked vasorelaxation in the presence of inhibitors of NO synthase (L-NAME; 100 $\mu$M) and cyclooxygenase (indomethacin; 10 $\mu$M) and it was reduced in WKY ($pD2=4.8\pm0.1$, $Emax=64.9\pm7.3\%$, n=12 $p<0.001$, $p<0.01$, respectively) and in SHR ($pD2=5.0\pm0.1$, $Emax=63.0\pm6.6\%$, n=15, $p<0.01$). KCa blockers of KCa2.3 (UCL1684; 0.1 $\mu$M), KCa3.1 (TRAM-34; 10 $\mu$M) and large conductance KCa1.1 (iberiotoxin; 0.1 $\mu$M) attenuated vasorelaxation in WKY ($Emax=43.4\pm4.2\%$, n=9, $p<0.05$; $Emax=36.4\pm5.8\%$, n=6, $p<0.05$; $Emax=28.8\pm5.5\%$, n=5, $p<0.01$; respectively) and also in SHR ($Emax=46.5\pm4.8\%$, n=8, $p<0.05$; $32.2\pm6.0\%$, n=6, $p<0.01$; $21.9\pm2.6\%$, n=6, $p<0.01$; respectively) compared to the respective control. In SHR we observed significantly reduced expression of KCa3.1 and KCa2.3.
**Conclusions:** The SKA-31-induced relaxation was attenuated in SHR compared with WKY, in which NO or PGI2 seem to play the major role. EDH-KCa2.3/KCa3.1 type responses to SKA-31 did not differ between WKY and SHR although expression of these channels in SHR is decreased. In the next step we will investigate influence of SKA-31 on the acetylcholine-induced relaxation and KCa2.3/KCa3.1-activated downstream hyperpolarizing pathways in mesenteric arteries from SHR.

### 584

### The peculiarities of endothelial dysfunction in patients with chronic renocardial syndrome

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Endothelial dysfunction is a marker of vascular disease, as well as the development and progression of hypertension in chronic kidney disease is called chronic renocardial syndrome.
**Objective:** To study the characteristics of endothelial dysfunction in chronic renocardial syndrome.
**Results:** The study included patients with chronic renocardial syndrome, hypertension in chronic glomerulonephritis (n = 105, 67 men and 64 women). Vasomotor function analysis showed that the vasodilatory response in less then decompression was expressed in patients with night-peaker - 5,47 (3,04; 11,72)% ($p<0.00014$, compared with the other groups, which is in the group of patients

with non-dipper was 11,63 (7,76; 18,92)%, dipper 8,94 (7,04; 15,46)%, and over-dipper 7,24 (5,82; 13,32)%. inverse correlation in the form of reduced vasodilatory effect on the diagnostic tests available between concentric hypertrophy r=-32 (p=0.001), stroke volume, r=-0,32 (p=0.02), the type of the daily blood pressure non-dipper r=-0.27 (p=0.009), the degree of nocturnal diastolic blood pressure r=-0,25 (p=0,014), and stroke volume index r=-0,25 (p=0.016) rates dilation of the brachial artery in the sample with nitroglycerin r=-0.24 (p=0.017) and normal geometry r=-0,22 (p=0.026), the type of change in blood pressure dipper r=-0,22 (p=0.032), the degree of nocturnal systolic BP r=-0,22 (p=0.030) and the ratio of the velocity of early diastolic filling and atrial r=-0,21 (p=0.037). direct correlation occurred between endothelial dysfunction and the degree of diastolic dysfunction r=0,37 (p=0, 00,038), the relative thickness of LV, r=0,28 (p=0.008), concentric remodeling, eccentric hypertrophy (r=0,25 and 0.23, respectively, p=0.015 and p=0.020), diastolic dysfunction rigid type r = 0.25 (p=0.026), normal LV geometry r = 0,21 (p=0,036), night-peaker diurnal changes in blood pressure r=0,23 (p=0.026), systolic myocardial dysfunction r=0,21 (p=0.036).
At correlation analysis between lipid indicators vasotonics negative correlation dependence between 6-ketoPGF1 a and holesterol (r=-0,28 and r=-0,31, accordingly, $p<0,05$), and LPLD (r=-0,29 and r=-0,32, accordingly, $p<0,05$) has been revealed. Given results testify to negative influence of DL on level of vasodilatators and antiagregants. It can be connected with oppression prostatsiklinsinthetase activity and changes of accumulation and sensitivity of cells to NO. Correlation dependence between tromboxane B2 and triglyceride level has been especially expressed in group of patients with DL IV type (r=0,43, $p<0,01$). Level of another vasoconstrictor - endothelin-1 correlated with concentration of holesterol and LPLD (r=0,36 and r=0,38, $p<0,05$).
Output. The presence of endothelial dysfunction is the factor most often combined with left ventricular remodeling in concentric type. Dysbalance of endothelial and cellular factors that finally leads to vasoconstriction.

## 585

### Endothelial dysfunction, atherosclerosis of the carotid arteries and level of leptin in patient with coronary heart disease in combination with hepatic steatosis depend from body mass index.

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**Objective:** To compare the relationship endothelial dysfunction, atherosclerosis of the carotid arteries and level of leptin in patient with coronary heart disease in combination with hepatic steatosis depend from body mass index (BMI).
**Methods:** Examined 31 men (mean age 56,7 $\pm$ 5,6 years) with coronary heart disease and hepatic steatosis. Allocated 3 groups according to BMI: group 1 consisted of 9 (29%) people (BMI 25 to 29,9 kg/m2), group 2 - 13 (42%) (BMI 30 to 34,9 kg/m2), group 3 - 9 (29%) (BMI 35 to 39,9 kg/m2). We studied the levels of leptin. The reactive hyperemia test for assessment of endothelial dysfunction was consecutively performed in all patients.
**Results:** According to the reactive hyperemia test data, endothelial dysfunction was found in 7 patients (77%) in a group 1, and in all patients in a group 2 and 3. Reactive hyperemia index (RHI) was lower (4,1 $\pm$ 5,3%,) in the group 2 and in group 3 (4,5 $\pm$ 1,7%) than in group 1 (7,9 $\pm$ 5,4%) ($p<0,05$). Frequency of exposure of atherosclerotic carotid plaques in a group 1 — 100%, was higher than in a group 2 — 76% and in a group 3 - 88%; point plaque in a group 1 — 33%, was higher than in a group 2 — 7% and in a group 3 - 22%. The mean level of leptin in a group 3 was higher (43,6 $\pm$ 20,2 ng/ml) than in group 2 (24,4 $\pm$ 14,6 ng/ml) and 1 (16,1 $\pm$ 9,8 ng/ml). There was correlation between the endothelial dysfunction and degree of stenosis of carotid vessels of head (r=0,58, $p<0,05$) and level of leptin (r=0,57, $p<0,05$) in a group 2 however in a group 1 and 3 such connection was not observed.
**Conclusion:** Thus, in patients with coronary heart disease in combination with in combination with hepatic steatosis RHI was lower in the group 2 (BMI 30 to 34,9 kg/m2) and in group 3 (BMI 35 to 39,9 kg/m2) than in group 1 BMI 25 to 29,9 kg/m2). There was correlation between the endothelial dysfunction, degree of stenosis of carotid vessels of head and level of leptin in a group 2.

## 586

### Role of non-coding RNAs in thoracic aortic aneurysm associated with bicuspid aortic valve

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**Background/Introduction:** An individual with a bicuspid aortic valve (BAV) runs 50-70% higher risk of developing thoracic aortic aneurysm (TAA) compared to the normal population with tricuspid aortic valve (TAV). BAV is the most common congenital heart defect present in 0.5-2% of the population. It has been proposed that inherent changes, as a consequence of genetic mutation or altered flow pattern, cause the increased aneurysm susceptibility. The role of non-coding RNA (ncRNA) has not been studied previously in the context of BAV associated TAA. Here, we investigated the functional role of ncRNAs in the pathogenesis of TAA.
**Purpose:** Recently it has been reported that ncRNAs can regulate other ncRNAs thus establishing a hierarchical order of regulation. We hypothesised that a hierarchical regulatory mechanism imposed by ncRNAs/ncRNA families may play a role in vasculopathy of thoracic aortic aneurysm (TAA) in a tissue specific manner in BAV patients. Here, we aimed to discover "master-ncRNAs" important in the BAV associated TAA.
**Methods & Results:** With the aid of a systems biology approach using data obtained from proteomic analysis of the human non-aneurysmal aortic samples, we constructed a gene-interaction network in BAV and TAV patients and ranked microRNAs (miRNAs) in order of their likely influence on the disease development. Our result based on in-silico network analysis followed by qRT-PCR and in situ hybridization revealed that microRNA families are differentially expressed in human aortic samples in BAV.
As our goal was to establish a hierarchical order of regulation, we also investigated long non-coding RNA (lncRNAs), which can act as a sponge to regulate miRNAs. Due to the exposure of BAV ascending aorta to the non-physiological hemodynamic, we also studied the differential expression of the lncRNAs in endothelial cells, obtained from patients, to laminar vs. turbulent shear stress.
**Conclusion:** In summary, using systems biology approach we have established a hierarchical order of signaling influenced by ncRNAs and miRNAs, which may explain the higher propensity of BAV to develop TAA.

## 587

### Cigarette smoke extract abrogates 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 main mediator of cellular adaption to oxidative stress NRF2 and its target genes heme oxygenase 1 and NAD(P)H dehydrogenase (quinone 1) were strongly increased by CSEaq in a dose-dependent manner. High laminar flow induced elongation of endothelial cells in the direction of flow, activated the PKB/AKT pathway, followed by increased eNOS expression and subsequent NO release. This increase 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 molecules 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.

## 588

### 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 modeling 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-hyaluronat antibodies (HR=4.14 95% 1.54-14.02). According to ROC-analysis 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.

## 589

### Novel potential properties of bioactive peptides from spanish dry-cured ham on the endothelium.

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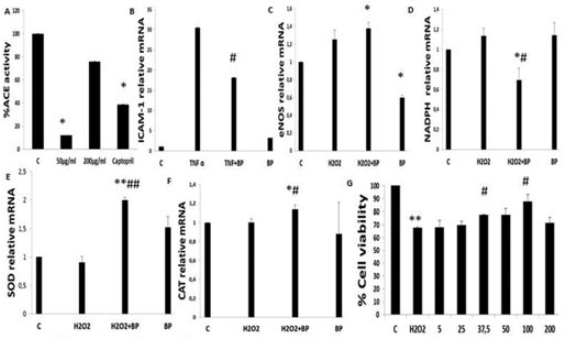
**Background:** Bioactive peptides (BP) showing angiotensin I converting enzyme (ACE) inhibitory capacity have been widely pursued for the management of hypertension and are believed to exert beneficial physiological effects on the endothelium.
**Purpose:** To test ACE inhibitory capacity of a protein 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 (Myrus®). Transfected cells were treated with cured ham hydrolysate (5-500 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/μl or H2O2 300 μM and/or BP.

**Results:** BP do not affect cell proliferation but significant ACE inhibition was observed at 50μg/mL. BP prevented the TNFα-increased ICAM-1 mRNA expression. H2O2 decreases cell viability and BP significantly reversed the H2O2-damage at 37.5 and 100 μg/mL. In the presence of H2O2 they also increased expression of eNOS 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.




**A: ACE ihibitory activity after peptides treatment. B,C,D,E, and F: Quantitative RT-PCRs show the changes of mRNA relative levels after 100 ng/ml TNFα or/and 50 μg/ml peptides treatment in B, and after 300 μM $H_2O_2$ or/and 50 μg/ml peptides treatment in C, D, E, F. G:Bioactive peptides dose-dependently reversed the $H_2O_2$-damaged cellular viability. *, ** or *** indicates P < 0.05, 0.01 or 0.001 for comparison between control, #, ## or ### indicates P < 0.05, 0.01 or 0.001 for comparison between $H_2O_2$ alone and $H_2O_2$+BIO or TNF alone and TNF +BIO.**

Results

# 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 densitiy 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-; CM), intermediate monocytes (CD14++ CD16+; IM) and non-classical monocytes (CD14+CD16++; NCM) by flow cytometry. Lipoprotein subfractions 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 densitiy lipoprotein (LDL) were not associated with monocyte subtypes. IDL was not associated with CM (r=-0.18; p=0.09) and NCM (r=0.16; p=0.13) but correlated significant 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 IQR 4.3-8.3% 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 subtypes 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 "Statistica 10"software.

**Results:** Dyslipidemia was revealed in patients with early RA in 68% (RA in 65%). In addition, the increase in total blood cholesterol (TBC) in early RA - 61% (in RA-55%) 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 (5.3) 7,2mmol\L) (p<0.05). Increased level of triglycerides (TG) in early RA was 1.5 times more frequent 51%, (in RA-33%), the mean value was 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 was 3.2 mmol\L in early RA, (2.6 mmol\L- in RA), decreased 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—100%, (80%-in RA), C- reactive protein (CRP) showed 10% difference -71% 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 23mg\L (19mg\L—in RA). The correlations between total cholesterol and CRP (r=0.23, p<0.05), LDL and CRP (r=0.21, p<0.05) were revealed.

**Conclusion:** 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).

# Atherosclerosis

### 596

### Macrophages differentiated in vitro are heterogeneous: morphological and functional profile in patients with coronary artery disease

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**Introduction:** Monocytes and tissue macrophages, cells involved in the inflammatory process, play a crucial role at any stage of coronary artery disease (CAD). Macrophages are hallmarked by morpho/phenotypic heterogeneity described also in atherosclerotic plaque where the presence of a particular macrophage phenotype may have harmful or beneficial functions on CAD development. Tissue macrophages are not easily obtained and monocyte-derived macrophages (MDMs) are accepted as a good surrogate. We previously reported that in healthy subjects MDMs spontaneously differentiated in vitro show two dominant morphotypes, spindle and round, with pro- and anti-inflammatory proprieties respectively. In particular, round MDMs show high efferocytic capacity respect to spindle.

**Purpose:** This study is conceived to delineate the morphological and functional profile of MDMs obtained from CAD patients compared to those of healthy subjects.

**Methods:** Monocytes were isolated from venous blood of 25 healthy subjects (50 ± 15 years) and from 50 CAD patients (61 ± 11 years) and differentiated for 7 days in medium supplemented with 10% autologous serum. The uptake of apoptotic Jurkat T cells, for efferocytosis assay, was detected by flow cytometry. Transglutaminase 2 (TG2) and tissue factor (TF) were determined by immunofluorescence and western blotting. Thrombin generation was evaluate using a thrombinoscope.

**Results:** Morphologically, MDMs of CAD patients show a prevalence of round morphotype respect to spindle. Nevertheless, these MDMs displayed less efferocytic capacity compared to control. Impaired efferocytosis may be due to the reduces levels of TG2, protein involved in phagosome formation. Moreover, CAD MDMs present higher TF levels that are associated with a quickly thrombin generation.

**Conclusions:** MDMs of CAD patients show a pro-inflammatory and a pro-thrombotic profile characterized by reduced efferocytic capacity and increased of TF levels. MDMs of CAD patients can contribute to plaque progression and activation besides that to thrombus formation. Drug handling of different macrophage phenotypes may provide a basis for new therapeutic strategies able to limit the progression of atherosclerosis.

### 597

### Palmitoylethanolamide promotes anti-inflammatory phenotype of macrophages and attenuates plaque formation in ApoE-/- mice

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**Introduction:** The endogenous fatty acid amide palmitoylethanolamide (PEA) is a lipid-derived mediator, which does not bind to the cannabinoid receptors CB1 or CB2, but exerts potent anti-inflammatory effects by ligating type-α peroxisome proliferator-activated receptors (PPAR-α). PEA has shown to possess therapeutic potential in inflammatory disease models, but the role of PEA and its promise as a therapeutic agent in atherosclerosis remain unexplored.

**Purpose:** We aimed to evaluate the therapeutic potential of chronic PEA treatment in atherosclerotic mice.

**Methods:** The anti-inflammatory efficacy and mechanism of PEA were first investigated in primary bone marrow-derived macrophages (BMDM) under stimulation with lipopolysaccharides (LPS). As an in vivo approach, 6-8 week-old female apoliporotein E deficient (ApoE-/-) mice on a high fat diet were treated with either vehicle or PEA (3 mg/kg/day) for 4 weeks. Lesion size and macrophage content of plaques were determined in aortic root sections. Furthermore, leukocyte subpopulations and cytokine expression levels at the tissue level were studied by flow cytometry and quantitative PCR, respectively.

**Results:** In LPS-stimulated BMDMs, PEA reduced the expression pro-inflammatory cytokines in a dose-dependent manner and through the activation of PPAR-α. Without affecting body weight or plasma cholesterol level, chronic in vivo administration of PEA was effective in attenuating atherosclerotic lesion size in ApoE-/- mice. Absolute macrophage-positive area of the lesions was also reduced in PEA-treated mice, but when normalized to total plaque area, macrophage content was comparable between the treatment groups. PEA treatment downregulated the expression of M1-type macrophage markers while enhancing M2 marker expression particularly in the spleen. Unexpectedly, PEA-treated mice had increased levels of classical monocytes in the circulation and aorta, an effect that occurred through a yet unknown mechanism.

**Conclusions:** Our data show that PEA evokes potent anti-inflammatory effects in cultured primary macrophages, which translates into an atheroprotective effect in a model of early atherosclerosis. Future studies will be instrumental to clarify the underlying mechanisms and to evaluate whether this treatment strategy has efficacy also in pre-established and more advanced atherosclerosis.

## 598

### Amiodarone versus esmolol in the perioperative period: an in vitro study of coronary artery bypass grafts

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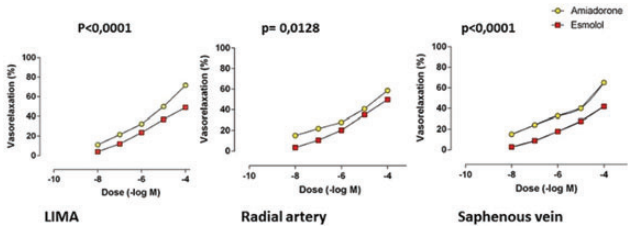
**Background:** Arrhythmias, particularly atrial fibrillation(AF) is a major concern after coronary artery bypass grafting(CABG) surgery. Beta blockers and amiodarone are indicated in both prophylaxis and treatment of AF in the perioperative period.
**Purpose:** This study is conducted to define and compare the vasoactive effects of esmolol and amiodarone, the two most commonly used agents, on left internal mammarian artery (LIMA), radial artery(RA) and Saphenous vein(SV) grafts in in vitro tissue bath system.
**Methods:** Ninety six vascular rings (32 IMA, 32 RA and 32 SV graft samples) obtained from 40 CABG patients (24 male, 16 female; mean age 54,25 ± 7,5 years) were evaluated after testing for vascular functionality of smooth muscle and endothelium. Vascular contractility of graft samples was assessed with phenylephrine; and the presence of a functional endothelium was tested with carbachol. Of 96 functional grafts, half was treated with esmolol and half was treated with amiodarone. The amount of relaxation of phenylephrine-precontracted grafts were assessed in terms of vasodilatation response to increasing doses of drug(amiodarone or esmolol) added to the tissue bath system. The concentration-response curves were constructed after transfer of data via Transducer Acquisition System (MAY IOBS 99, FDT 05 Ankara-Turkey) and MAY-MASTER MP36 analysis software.Patients with Diabetes Mellitus, Chronic Renal Failure and Peripheral arterial disease were excluded.
**Results:** When the samples from IMA, RA and SV grafts were compared in terms of amount of vasodilatation response to esmolol and amiodarone, all graft samples exhibited significantly higher vasodilatation with amiodarone($p<0,0001$, p=0,0128 and $p<0,0001$ respectively) when compared to esmolol. The percentage vasodilatation responses with esmolol were 48,99 ± 2,28% for IMA, 49,77 ± 3,03% for RA and 41,90 ± 4,05% for SV. The logarithmic 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, percentage vasodilatation responses with amiodarone were 71,65 ± 5,18% for IMA, 58,61 ± 5,87% for RA and 65,07 ± 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 perioperative period. Although amiodarone has a class IIa 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.



Amiodarone versus Esmolol

## 599

### BMPRII signaling of fibrocytes, a mesenchymal progenitor cell population, is increased in STEMI and dyslipidemia

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**Introduction:** Inflammation is a hallmark feature of ST-elevation myocardial infarction (STEMI). Fibrocytes, a Collagen-I+CD34+CD45+ mesenchymal progenitor cell population accumulate in cardiac tissue of a murine ischemia/ reperfusion model. In ACS patients, decreased levels of circulating fibrocytes were found, compared to healthy controls. Bone morphogenic protein receptor II (BMPRII) is involved in the vascular remodeling of lung and heart. Therefore, we studied BMPRII expression in fibrocytes at the culprit lesion site (CLS).
**Methods:** We sampled blood from the CLS and a femoral site in the course of primary percutaneous coronary intervention (pPCI) from STEMI patients (n=50, male=78%, mean age=61 ± 13y). Another sample was acquired 72h after pPCI (n=21). A cohort of healthy controls (n=20, male=48%, mean age=51 ± 8y) served as controls. Flow cytometry was employed to characterize fibrocytes.
**Results:** Fibrocytes were increased at the CLS compared to femoral blood (722 [276-1298] vs. 324 [180-589], p=0.0001). 72h after STEMI, peripheral fibrocytes were decreased (246 [151-468] vs. 153 [102-252], p=0.006). Peripheral fibrocyte counts during pPCI were similar to those of controls.
No differences were found in BMPRII expression between coronary and femoral blood of STEMI patients; however, BMPRII expression was higher in patients than controls (MFI 22106 [13142-34125] vs. 13099 [8944-20231], p=0.014). In patients suffering from dyslipidemia, BMPRII on fibrocytes was substantially increased (MFI 26056 [13195-54807] vs. 19913 [13635-22965], p=0.009). 72h after pPCI, BMPRII was significantly upregulated on fibrocytes (MFI 22294 [17973-34125] vs. 31149 [27722-45724], p=0.044).
**Conclusions:** The more than two-fold increase of fibrocytes at the CLS and subsequent decrease 72h after pPCI in peripheral blood supports the concept of an active process. BMPRII expression is increased in STEMI patients, particularly in patients with dyslipidemia, suggesting lipid-induced inflammation, and the activation of fibrotic vascular remodeling.

## 600

### The characteristics of atherogenesis and systemic inflammation in rheumatoid arthritis

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**Introduction:** Atherosclerosis is a great contributor to the development of cardiovascular complications in rheumatoid arthritis (RA). Chronic inflammation causes dysproteinemia associated with high atherogenicity.
Purpose is to assess the association between immune-inflammatory markers and atherogenesis in women with rheumatoid arthritis (RA).
**Materials and Methods:** The study comprised 204 women with RA, including 65 (32%) with early RA lasting less than 1 year. The RA was diagnosed by the criteria ACR\EULAR 2010. The patients' average age in early RA was 55.7 ± 8.9, in RA — 54.9 ± 8.4 years. The risk factors of cardiovascular disease, the markers of inflammatory activity, the activity of RA (Visual Analogue Scale (VAS), DAS28) were analyzed, blood lipid profile was studied. Statistical analysis of the results was performed with "Statistica 10" software.
The results Increased total cholesterol level was in75% of cases with early RA (in 57% with RA). The mean value of total cholesterol was 5.4 ± 3.9 in early RA, (6.0 ± 2.9 in RA) mmol\L ($p<0.05$). The increase of low-density lipoproteins in early RA was 63%, (in RA - 36%), the mean value was 2.6 ± 0.3 in early RA, (3.1 ± 0.6 in RA) mmol\L. The increased triglyceride level was 51% in early RA, (34% in RA), the mean value was 1.5 ± 0.6 in early RA, (1.8 ± 0.7 in RA) mmol\L. Reduced levels of high-density lipoproteins (HDL) was 55% in early RA, (32% in RA) ($p<0.05$). The conditions associated with atherosclerosis in RA are more common in RA than in early RA: ischemic heart disease was 3 times more frequent in RA ($\chi^2 = 8.6$, $p<0.001$), ankle-brachial index <0.9 - in 1.8 times ($\chi^2 = 8.5$, $p<0.001$), transient ischemic attack occurred only in RA. The correlation between total cholesterol levels and DAS28 (r=0.8, $p<0.002$), VAS (r=0.9, $p<0.008$), C- reactive protein (CRP) (r=0.4, $p<0.001$), erythrocyte sedimentation rate (ESR) (r=0.6, $p<0.003$), rheumatoid factor (RF) (r=0.9, $p<0.001$) was revealed . There is correlation between CRP level and blood lipid profile parameters: triglycerides (r = -0.32, $p<0.002$), HDL (r = -0.22, $p<0.005$).
**Conclusions:** Thus, atherosclerosis in early RA has the following characteristics: increased blood atherogenicity in early stage of RA occurring in 1.2 time more common, it is associated with immune-inflammatory markers (RF, CRP, ESR), $p<0.005$ and the activity of the disease (VAS, DAS28), $p<0.005$.

## 601

### Role of adenosine-to-inosine RNA editing in human atherosclerosis

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**Background:** Adenosine to inosine (A-to-I) RNA editing is catalysed by ADARs (adenosine deaminases acting on RNA) and is an important posttranscriptional regulator of RNA metabolism. Although RNA editing is essential for life, its role in cardiovascular disease is unknown.
**Methods and Results:** RNA-sequencing (RNA-seq) of human endothelial cells revealed that ADAR1 is the main RNA editor in HUVECs. The vast majority of editing events are detected in Alu regions due to their ability to form double-stranded structures, a prerequisite for RNA editing. A-to-I RNA editing of the primate-specific Alu elements is observed in 25% of poly(A)+ transcripts and in 44% of ribosomal RNA-depleted transcripts. Cathepsin S (CTSS), an extracellular matrix degrading enzyme, has 3 Alu elements in its 3'-untranslated region and is among the most extensively edited mRNAs. Interestingly, ADAR1 silencing downregulates CTSS mRNA expression, whereas ADAR1 overexpression results in an increase of both CTSS RNA editing rate and CTSS mRNA expression. Mechanistically, RNA immunoprecipitation (RIP), luciferase reporter and iCLIP assays showed that A-to-I RNA editing of the Alu elements in the 3'UTR of CTSS mRNA regulates the recruitment of the stabilizing RNA-binding protein HuR to CTSS 3'UTR and, thus, controls CTSS mRNA stability and expression. CTSS Alu RNA editing is increased under hypoxic or pro-inflammatory conditions in vitro, as well as in patients with coronary or carotid atherosclerotic vascular disease. Further, the extent of CTSS Alu RNA editing rate correlated with various markers of subclinical atherosclerosis including intima-media thickness and number of atherosclerotic plaques. Importantly, ADAR1 and the extent of CTSS RNA editing correlated with CTSS expression levels in patients' samples from 6 non-overlapping cohorts of patients with inflammatory vascular diseases (total n=366), including peripheral blood mononuclear cells, carotid atherosclerotic plaques and thoracic aortic aneurysms.
**Conclusion:** This study shows for the first time that Alu A-to-I RNA editing is a critical modulator of inflammatory gene expression in all stages of atherosclerotic vascular disease development.

## 602

### Presence of bacterial DNA in thrombus aspirates of patients with myocardial infarction

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**Introduction:** Infectious agents, especially periodontal bacteria, has been suggested to be involved in inflammation contributing to plaque instability 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, Tannarella forsythia, Treponema denticola, Helycobacter pylori, Mycoplasma pneumoniae, Staphylococus aureus, Prevotella intermedia, and Streptococcus mutans.
**Results:** Four different species have been detected in ten thrombi. The most frequent bacterial DNA found was from viridans group streptococci (6 patients, 5,5%), followed by DNA from Staphylococus aureus (2 patients, 1,8%); one patient presented DNA from Porphyromonas gingivalis (0,9%) 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 thrombi 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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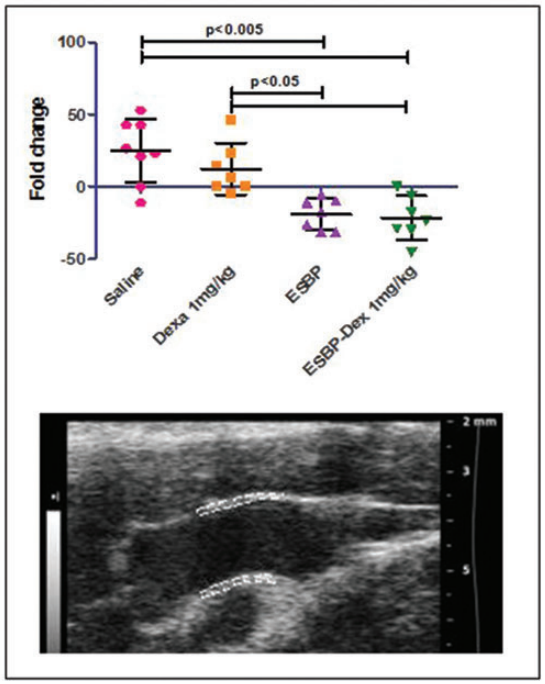
**Background:** Atherosclerosis is characterized by acute and chronic vascular inflammation and leukocyte infiltration that result in plaque formation, instability, and rupture. E-selectin is the adhesion molecule expressed on activated endothelium that recruits leucocytes to the inflammation site, making 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, with 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 4 treatment groups: ESBP-Dex, ESBP, free dexamethasone or saline, 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).
**Conclusion:** 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 (NCM, CD14+CD16++), and an intermediate subset (IM, CD14++CD16 +) whereby CM are mainly phagocytes, NCM patrol along the endothelium and IM exhibit 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 matrixmetalloproteinases 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 analyse the expression of 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 with 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 Ly6-C 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 NCM ($p<0.005$). 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 NCM ($p<0.05$). 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 . Ly6-C high expressing mouse monocytes are also able to bind higher amounts of plasminogen in comparison to Ly6-C 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 atherosclerosis.

## 605

### Apelin-13 treatment enhances the stability of atherosclerotic plaques

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*[1]Swiss Federal Institute of Technology of Lausanne, Lausanne, Switzerland; [2]University Federal of Minas Gerais, Physiology and Biophysics, Belo Horizonte, Brazil; [3]Geneva University Hospitals, Geneva, Switzerland*

**Background:** Atherosclerosis remains one of the main causes of worldwide death and morbidity, Fpr this reason, substantial efforts have been made to identify novel approaches to improve the management of this disorder. Apelin is an endogenous peptidergic 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 atherosclerosis.
**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 composition. 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.

### 607
### Association of neutrophil to lymphocyte ratio with presence of isolated coronary artery ectasia

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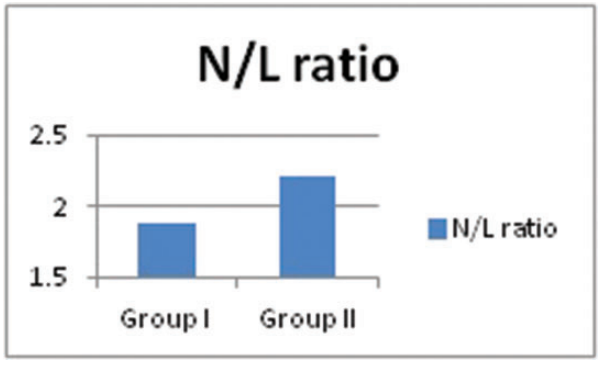
**Objectives:** Coronary artery ectasia (CAE) has been defined as a dilated artery luminal diameter that is at least 50% greater than the diameter of the normal portion of the artery. Isolated CAE is defined as CAE without significant coronary artery stenosis and isolated CAE has more pronounced inflammatory 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, diabetes, 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.



NLR

Table (1): Baseline characteristics of angiographicly normaly and ectatic coronary vessels

| P | Normal ( n = 30 ) Mean ± SD | n (%) | Ectasia ( n = 83 ) Mean ± SD | n (%) | |
|---|---|---|---|---|---|
| 0.564 | 50.35 ± 6.74 | | 51.43 ± 8.63 | | Age ( years ) |
| 0.078 | | 14 (46.7) | | 54 (65.1) | Sex ( male ) |
| 0.008 | 1.98 ± 0.56 | | 2.79 ± 1.70 | | N/L ratio |
| | | | | | Distribution of ectasia |
| | | | | 56 (67.5) | LAD |
| | | | | 28 (33.7) | LCX |
| | | | | 57 (68.7) | RCA |
| | | | | | Markis classification |
| | | | | 45 (54.2) | Type 1 |
| | | | | 38 (45.8) | Type 3 |

## Calcium fluxes and excitation-contraction coupling

### 610
### The coxsackie- and adenovirus receptor (CAR) regulates calcium homeostasis in the developing heart

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The coxsackie- and adenovirus receptor (CAR) is a cell adhesion protein of the Ig superfamily which serves as receptor for different coxsackie- and adenoviruses. CAR is strongly expressed throughout the developing heart and in contrast, during adulthood CAR is concentrated found at the intercalated discs. CAR knockout (KO) mouse models show malformation of the heart which leads to death between embryonic days E11.5 and E12.5 indicating an important function of CAR during heart development. Conditional CAR KO mouse models revealed impairment during excitation conduction in the mature heart. The aim of this study was to investigate the physiological function of CAR during early heart beats with regard to intercellular communication and Ca2+ cycling in embryonic cardiomyocytes. By using a global CAR KO mouse model, the investigation of cultivated E10.5 CAR KO cardiomyocytes and E11 CAR KO hearts revealed a significant higher beating frequency. Calcium imaging recordings of spontaneous Ca2+ transients in CAR KO cardiomyocytes showed a significant faster systolic Ca2+ decline compared to wildtype. The analysis of the cardiac Ca2+ extrusion mechanisms revealed a higher activity for NCX and SERCA2 in CAR KO cardiomyocytes. Gene expression and protein level of both NCX and SERCA2 was not changed in CAR KO hearts. Dye spreading studies with lucifer yellow indicated increased cell coupling of cultivated E10.5 CAR KO cardiomyocytes. Cx45 expression was downregulated in CAR KO hearts, however the observed increased cell coupling suggested in CAR KO hearts a remodelling of gap junctions that increases intercellular communication and excitation conduction resulting in the increased embryonic heart beat as recorded for CAR KO embryos. Due to the strong coexpression with Cx43 and Cx45 it can be suggested that CAR is localised in a larger protein complex involved with ZO-1 at the junctional sites. There, CAR may promote correct localisation of Cx45 and ZO-1 and cell-to-cell coupling. Taken together, CAR regulates intercellular communication between embryonic cardiomyocytes, is able to influence spontaneous Ca2+ cycling and is therefore an important regulator for embryonic heart beating.

### 611
### 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 37°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. Ionic Ca2+ currents were evaluated during whole cell patch-clamp in the presence or absence of HMW-AGEs (200 μg/ml). Perfusion of BSA 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 glycated molecules. After 4 minutes HMW-AGEs application, peak ICaL measured at +10 mV significantly decreased (-5.12 ± 0.59 pA/pF vs -7.72 ± 1.01 pA/pF at baseline, ncells =11, $p<0.05$). In comparison, 200 mg/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.

### 612
### Measuring electrical conductibility of cardiac T-tubular systems

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**Introduction:** The transverse axial tubular system (TATS) propagates the action potential to the cardiomyocyte core, ensuring a synchronous and uniform Ca2+ release throughout the cell. Cardiac pathological settings are commonly associated with structural alterations of the TATS (Ferrantini et al., Cell. Mol. Life Sci. 2013). We recently observed that a number of TATS elements are unable to conduct the electrical activity in pathological myocardium (Sacconi et al., PNAS 2012). We also found that these local electrical defects lead to non-homogeneous Ca2+ release and delayed myofilament activation that significantly contribute to mechanical dysfunction (Crocini et al., PNAS 2014; Crocini et al., J Mol Cell Cardiol. 2016).
**Purpose:** Reduction of TATS conductibility can be related to observed electrical defects. Here, we aim at studying whether structural changes can represent the source of increased electrical resistance in TATS.
**Methods:** We employ fluorescence recovery after photo-bleaching (FRAP) microscopy to probe the diffusion properties of TATS lumen in isolated cardiomyocytes. T-tubules of cardiomyocytes from rodent models of cardiac diseases are labelled using fluorescent dextran that freely diffuses from extracellular space to TATS lumen. The fluorescent dextran present inside TATS lumen is photo-bleached and the diffusion of unbleached dextran from extracellular space to TATS is monitored using confocal imaging.
**Results and Conclusion:** We designed a mathematical model that correlates the apparent diffusion of dextran inside TATS with the geometrical properties of the network. Then, exploiting the analogy between diffusion and electrical conductivity we link the diffusional properties of TATS with its electrical resistance. The method was first validated in control and acute detubuled cells and then applied to probe the electrical conductibility of two different pathological models in which we previously observed TATS electrical defects. In line with our hypothesis, we found an increased resistance in both diseases, highlighting the relationship between electrical conductibility and functional defects.

### 613
**Postnatal development of cardiac excitation-contraction coupling in rats**

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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 sarcolemma and thus the excitation signal throughout the cell volume. This morphological feature develops postnatally at early phase, when myocytes undergo of rapid growth and structural remodelling.
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 depolarization pulses 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.
Initial formation of sarcolemmal tubules 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 network was developed. Throughout the observed age interval the cell size, area and membrane capacitance were increasing and around D28 reached values typical for smaller myocytes of adult rat hearts. The development of calcium currents showed increase in current density that saturated around D11. Similarly, the rate of Ca-current decay increased with age, reaching values typical for adult myocytes after D10. These data suggest that functional local calcium signalling is present already at D11, when the t-tubules only start to form. This conclusion is supported especially by the dynamics of calcium current decay, which at D11 becomes as fast as in adult cardiac myocytes.

### 614
**Role of altered Ca2+ homeostasis during adverse cardiac remodeling after ischemia/reperfusion**

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Acute myocardial infarction (MI) due to coronary artery occlusion represents a major cause of morbidity and mortality in humans. Adverse ventricular remodeling by fibrous tissue occurs at the site of MI and remote to it. An unfavorable consequence of myocardial remodeling is Heart failure (HF). HF is characterized by systolic and diastolic dysfunction and abnormalities of intracellular Ca2+ handling with altered disturbed excitation-contraction coupling (EC-coupling). In each heartbeat, membrane depolarization during an action potential activates L-type Ca2+ channels. The subsequent Ca2+ entry activates the intracellular Ca2+ release channels, named ryanodine receptors (RyRs), which are located in the membrane of the sarcoplasmic reticulum (SR). This mechanism of Ca2+ -induced Ca2+ release is the key in the excitation-contraction (EC) coupling. However, intracellular Ca2+ is also involved in activation of Ca2+ -dependent transcription factors in adverse cardiac remodeling. Here we analyzed the [Ca2+] in a model of I/R at 1 week in which we ligated the left anterior descending coronary artery (LAD) for 40 min. We show a comparison between remote and infarted zone in Ca2+ recordings in cardiomyocytes.
**Methods:** To induce the stenosis a 6/0 ProleneTM (EthiconTM) nylon suture was placed around the left anterior coronary artery (LAD) reducing the vascular light with the help of a small piece PE-10 tube. Ventricular myocytes were isolated from Sham and I/R rats by enzymatic dissociation (collagenase type II, Worthington) using Langendorff perfusion. [Ca2 +]i transients and Ca2+ sparks were recorded in intact myocytes loaded with fluorescent Ca2 + dye (Fluo-3 AM) while prefused with normal Tyrode solution. Images were obtained with confocal microscopy (Leica TCS SP5).
**Results:** IR treatment produces a decreased cytosolic ([Ca2 +]cyto) and intranuclear ([Ca2 +]nuc) transients in adult cardiomyocytes, not only in the risk zone but also in the remote zone. Moreover, IR treatment induces a reduction in SR Ca2+ content that was similar on both zones, but no significant differences between two zones.
**Conclusion:** 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+-dependent regulation of gene expression.

### 615
**Experimental study of sarcoplasmic reticulum dysfunction and energetic metabolism in failing myocardium associated with diabetes mellitus**

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**Background:** Contractile dysfunction in heart failure (HF) and diabetes mellitus (DM) is tightly associated with the abnormal excitation-contraction coupling and sarcoplasmic reticulum (SR) dysfunction. The role of changes in the intracellular calcium handling in the pathogenesis of heart failure associated with diabetes remains poorly understood.
**Purpose:** We studied the sarcoplasmic reticulum (SR) function and energy production in failing myocardium associated with diabetes mellitus in the experimental study.
**Methods:** Study was performed on adult Wistar rats with postinfarction cardiosclerosis and diabetes mellitus alone and combined. The postinfarction cardiosclerosis (PICS) developed after 6 weeks of coronary occlusion and diabetes mellitus was induced by single injection streptosotozine (60 mg/kg i.p.). Inotropic reaction on rest period were studied on papillary muscles, dissected on 6 mm length and less than 1 mm thick and bathed at 37 C in oxygenated Krebs-Henzelait solution. Muscles were stimulated at a frequency of 0.5 Hz. Post-rest reaction of myocardium assessed for analyze the sarcoplasmic reticulum function (4 - 60 sec). The protein levels of SERCA2a and RyR2 in myocardium were determined by Western blotting. Oxygen consumption by mitochondria was measured using a Clark-type oxygen electrode. The activity of lactate dehydrogenase and succinate dehydrogenase were determined by histochemical method.
**Results:** Experimental animal models showed that development of heart failure (HF) alone led to post-rest contraction depression. The post-rest twitches were not depressed in case of the combination of diabetes Mellitus (DM) and chronic ischemic myocardial damage. The extent of changes in RyR2 levels was smaller than the degree of changes in SERCA2a levels when pathology developed. The development of monopathologies resulted in a significant decrease in the intensity of energy production. The degree of the energy production activity in diabetic rats was more significant decrease in compare with postinfarction rats, despite the fact that contractile disturbance of postinfarction rats was more severe. However, the development of the combined pathology resulted in the preservation of the energy production near the control level.
**Conclusion:** Induction of hyperglycemia at early stages of HF resulted in smaller changes in the expression of Ca2+-ATPase and ryanodine receptors and lesser disturbance of the energy metabolism related to glycolysis, Krebs cycle, and oxidative phosphorylation in comparison with monopathologies.

## Hibernation, stunning and preconditioning

### 618
**Volatile anesthetic preconditioning attenuates ischemic-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. In experimental studies, volatile anesthetic drugs are proven to mimic the IP. However, clinical data is still lacking with a strong evidence.
**Purpose:** The study was conducted to evaluate the cardioprotective effect of volatile anesthetic preconditioning (AP) in the diabetic and non-diabetics patients undergoing heart surgery. We applied sevoflurane as AP agent and studied its cardioprotective effect and postoperative outcome.
**Methods:** Total 60 patients were recruited. 30 Diabetic Mellitus (DM) patients were randomly selected into two 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 with AP. Patients of the AP group received 1% MAC sevoflurane for 5min, 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 trabeculae harvested for Western Blot analysis of total and phosphorylation of PKC, PTEN, STAT3, 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 $\pm$ 1.03 ng/ml vs. 1.65 $\pm$ 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 $\pm$ 0.62 ng/ml vs. 1.2 $\pm$ 2.45ng/ml. Total amount of CK-MB released was higher in the group without AP than groups with AP (39.2 $\pm$ 3 vs 36.3 $\pm$ 4 in DM groups and 28.5 $\pm$ 3.4 vs 21.4 $\pm$ 5.6 in non-DM groups respectively, $p<0.02$). In the Western Blot, only phosphorylation of PKC and total-STAT3 showed some difference. Lengths of postoperative intubation, 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 the isolated hearts of healthy and 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 STZ (65 mg/kg, i.p.). Diabetic and healthy rats were subjected to RIP induced by 3 cycles of 5-min ischemia / 5-min reperfusion of cuff occlusion of the right hind limb. Delayed phase was investigated 24-h 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 2-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 IS/AR to 11,7 $\pm$ 3,2% and delayed phase to 17,8 $\pm$ 1,8% vs. IS/AR 33 $\pm$ 3% in controls ($p<0,05$). In addition, LVDP recovery was increased to 70 $\pm$ 11,3% and to 83,4 $\pm$ 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

protective effect of RIP may share similar pathways with the mechanisms of increased resistance to ischemia in the acute phase of DM.

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### Post-conditioning with 1668-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 TLR9 ligand 1668-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/6 mice. Mice where treated with CpG i.p. 5 min. before reperfusion. Control group received PBS; sham group did not undergo ischemia. After 3, 7 and 28 days M-mode echocardiography and Millar® left ventricular (LV) pressure volume catheter measurements were performed. Hearts where excised and harvested for immunohistochemical analysis. Gene expression (Taqman® 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 action. Total LV collagen area using picrosirius red planimetry 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. TLR1 mRNA was induced in CpG PCon hearts, while TLR4 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 involve 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 has been shown in elder human and animal hearts. However, the onset of unfavourable 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 (1.5 months), younger adult (3 months) and mature adult (6 months) male Wistar rats, in Langendorff-perfused hearts exposed to 30-min I/120-min R without or with prior PC. 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 anesthesed rats (pressure cuff inflation (200 mmHg)/deflation). We measured infarct size (IS, TTC staining), susceptibility to ventricular arrhythmias and recovery of contractile function (left ventricular developed pressure, LVDP).
**Results:** Maturation did not affect heart function, however, it impaired cardiac response to lethal IR injury (IS increased by 40% and 65%, respectively, vs. juvenile group) and promoted arrhythmogenesis. IPC reduced occurrence of arrhythmias, IS and improved LVDP recovery in younger animals, while its efficacy was attenuated 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.
**Methods:** Male Wistar rats were exposed to hypoxic preconditioning (5600 m, 3 hours) or periodic hypobaric hypoxia (PHH, "lifting" at 5600 m in barochamber, 6 séances of 1 hour in every 72 hours). 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 Chance, mRNA and protein expression was assayed by RT PCR and Western blotting.
**Results:** 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, V3/V4, ATP/O 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, ATP/O 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-1$\alpha$ and HIF-3$\alpha$ mRNA. In the hypomethabolic phase, the antioxidant protection was increased due to induction of MnSOD protein, and membrane-associated mechanisms of cardioprotection were raised through the induction of caveolin-3. In the hypermetabolic 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, hypermetabolic and adaptive has been established. The hypometabolic and hypermetabolic phases are associated with various mechanisms of myocardial protection 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 L345P, (iv) Des A357P, (v) Des L370P, (vi) Des D399Y. Obtained data were normalized to the basal OCR level due to the obstacle of protein normalization. One-way ANOVA was used to evaluate statistical significance, with $p<0.05$ considered significant. Tukey's post-hoc analysis was performed to compare groups.
**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 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 $\pm$ 0.09 for non-transduced cells, 2.36 $\pm$ 0.09 for Des WT, 1.85 $\pm$ 0.12 for Des L345P, 1.81 $\pm$ 0.11 for Des A357P, 1.95 $\pm$ 0.11 for Des L370P and 1.87 $\pm$ 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 showed 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

### Methylene 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 purported to characterize the effects of MB (0.1 $\mu$M) on mitochondrial respiration, calcium sensitivity, and MAOs-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 spectrofluorimetrically.
**Results:** In RHM respiring on both complex I and II substrates (glutamate/malate and succinate + rotenone, respectively) a significant increase in all bioenergetic parameters was found in treated vs. non-treated mitochondria. No changes in sensitivity to Ca2+-induced opening of the mitochondrial permeability transition pore was 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 an opposite effect in mitochondria energized with succinate (+ rotenone). Incubation of RHM with MB in the presence of MAO-A and B inhibitors (clorgyline or selegyline, 10 μM) significantly reduced H2O2 release in mitochondria respiring on glutamate & malate and had no effect in the presence of the CII substrate.
**Conclusions:** In diabetic rat hearts, methylene blue improved mitochondrial respiratory function regardless the substrates used and elicited a dichotomic, 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
### Doxorubicin 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 cardiac 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 30μM. The OCR values are corrected for total protein concentration and normalised to baseline measurements. Acute injection of DOX resulted in a significant concentration-dependent increase in the real-time OCR of ARVC (1μM DOX 0.974 ± 0.009 vs. control 0.952 ± 0.015; 3μM DOX 1.18 ± 0.033 vs. control 0.954 ± 0.038; 10μM DOX 1.64 ± 0.136 vs. control 0.964 ± 0.057: $P<0.001$; 30μM 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 (CM) health. Atrogin1 and MuRF1 are muscle specific ubiquitin-ligases, and we recently demonstrated that loss of Atrogin1 impairs the turnover of the ESCRTIII protein CHMP2B, whose accumulation leads to block of autophagy, resulting, during ageing, in hypertrophic cardiomyopathy (1). To address the individual roles and interplay of these ubiquitin ligases in the control of CM proteostasis, we compared the molecular phenotype of MuRF1 KO and Atrogin1/MuRF1 double KO (DKO) mice, and that of mice treated with the UPS inhibitor Bortezomib.
**Methods:** Heart function and structure were assessed by ECHO, histology, IF and TUNEL assay. RTqPCR and WB assessed markers of UPS, ER/SR stress, ALS and extracellular matrix. Bortezomib treatment was performed both in vitro and in vivo.
**Results:** The cardiac phenotype of MuRF1 KO mice was analyzed at various ages from 3 to 24 mo. Loss of MuRF1 leads to CM hypertrophy, and age dependent systolic dysfunction. Ablation of MuRF1 increased collagen I and VI deposition, activation of MMP9, MMP12 and TGF-β signaling. Such fibrotic remodeling was not caused by CM death, nor myofibroblast activation, thus suggesting that MuRF1 has a role in CM dependent regulation of the extracellular matrix dynamics. The prevailing features of DKO hearts were similar to those observed in Atrogin1 KO mice, however with accelerated and worsened age-related cardiac dysfunction. In fact, DKO hearts had hypertrophied CM (LV CM size, DKO: 449.94 ± 6.99 vs WT: 225.77 ± 5.01, in $\mu m^2$), fetal gene reactivation, increased CM apoptosis, replacement fibrosis and impaired relaxation, showing, already at 9 mo., ALS impairment and intracellular protein aggregates, all features present in aged (16 mo.) Atrogin1 KO mice. These data support that accumulation of misfolded proteins, caused by simultaneous Atrogin1 and MuRF1 ablation, overloads the ALS, which is, however, dysfunctional due to the impaired Atrogin1/CHMP2B axis. In further support of this, CM proteotoxic damage, CHMP2B accumulation and ALS impairment resulted, both in cultured CM and in normal hearts, from non specific inhibition of the UPS with Bortezomib.
**Conclusions:** These data improve our understanding on the role of the muscle specific ubiquitin ligases in the control of CM proteostasis, and are of immediate clinical relevance, given the increasing use of proteasome inhibitors as anti-tumor treatment.

### 631
### Suppression of Wnt signalling in a desmoglein-2 transgenic mouse model for arrhythmogenic cardiomyopathy

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**Background:** Mutations in the desmoglein-2 (DSG2) gene have been identified in patients affected with arrhythmogenic cardiomyopathy (ACM), a progressive heart muscle disease and a leading cause of sudden cardiac death in the young and athletes. The mechanisms underlying DSG2 dysfunction involved in the pathogenesis of ACM remain poorly understood.
**Purpose:** The aim of the study was to investigate the molecular pathogenesis of ACM due to DSG2 mutations in transgenic animal models.
**Methods:** Human full−length wild−type and mutant (c.1672C>T, p.Q558*) cDNA sequences for human DSG2 were cloned into a vector containing alpha−MyHC promoter, and tagged at the C-terminus with the Flag sequence. Transgenic mouse lines with cardiac-restricted overexpression of the wild-type DSG2 (Tg-hWT) and mutant DSG2 lacking transmembrane and intracellular protein domains (Tg-hQ) were developed. Phenotypic characterization was performed by electrophysiological, histological and electron microscope analyses. The disease molecular pathogenesis was investigated in transgenic and wild-type heart samples by immune-histochemical and western blot examinations.
**Results:** A proper localization of Flag-tagged human DSG2 to the intercalated discs was demonstrated for Tg-hWT mouse cardiomyocytes, but not for both Tg-hQ10 and Tg-hQ13 cardiomyocytes which showed a cytoplasmic localization of the truncated form of the protein. Whereas Tg-hWT mice had no detectable histologic, morphological, or functional cardiac changes, the Tg-hQ mouse hearts displayed cardiomyocytes loss and biventricular fibrous tissue replacement, similar to those of ACM patients. Ultra-structural analysis revealed reduction of desmosome number, density, and length at the cardiac intercalated discs, together with swollen cisternae of endoplasmic reticulum, consistent with a 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-hQ mice at 3-, 6- and 12-months of age displayed reduction of active beta-catenin, together with decrease expression of c-Myc, cFos and Ciclin-D1 down-stream targets, and reduction of glycogen synthase kinase 3-beta levels.
**Conclusions:** These findings suggest that inhibition of Wnt/beta-catenin signalling 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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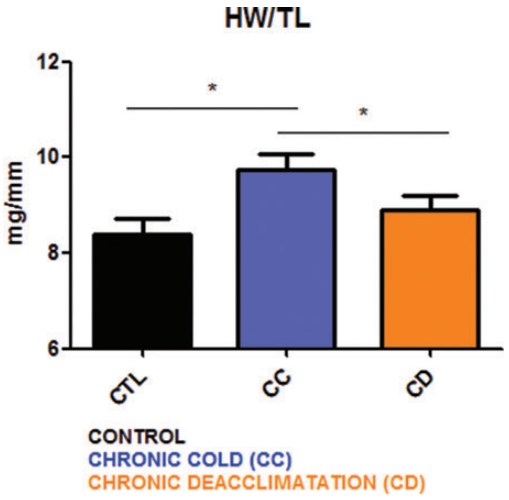
**Introduction:** Of the four seasons, winter has the highest mortality and morbidity from cardiovascular complications. Chronic exposure to cold is known to cause hypertension and cardiac hypertrophy, although cold-induced cardiac hypertrophy is independent of elevations in blood pressure. Therefore we aim to study how cold temperatures affect cardiac hypertrophy and whether this phenomena is reversible after return to a thermo-neutral temperature.
**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 the morphologic level, we found that chronic cold exposure induced a significant increase in the heart weight/tibia length (HW/TL) ratio. Moreover, the area of the cardiomyocytes was analyzed and we observed that the cell size was increased after cold exposure indicating that after 3 weeks of cold the mice develop cardiac hypertrophy. At the gene expression level, the hypertrophic marker genes atrial natriuretic factor (Anf), α-Actinin and the ratio α/β-Myosin heavy chain (Mhc) were up-regulated in these conditions whereas the genes involved in fatty acid oxidation like pyruvate dehydrogenase kinase (Pdk)-4 and medium-chain acyl-CoA dehydrogenase (Mcad) were significantly down-regulated. Moreover, the glucose transporter Glut1 was induced after cold exposure suggesting a switch in the energy substrate used by the cardiac tissue under this hypertrophic condition. By contrast, one week thermo-neutral acclimatization led to a significant decrease in the HW/TL ratio and in the cardiomyocyte area compared to animals exposed to chronic cold. Furthermore, the hypertrophic marker genes α-Actinin and the ratio α/β-Mhc were significantly reduced after thermo-neutral conditions and the genes involved in fatty acid oxidation and the glucose transporter Glut1 were completely restored when compared to cold exposed hearts.
**Conclusions:** Our data indicate that mice subjected to three weeks of cold develop a marked cardiac hypertrophy accompanied by indications of a switch from fatty acids to glucose metabolism. Moreover, one week of thermo-neutrality led to a complete reversion of the cardiac hypertrophy and the metabolic expression changes induced by cold exposure.



Hearth Weight/Tibia Length Ratio

### 633
### CD45 is a sensitive marker to diagnose lymphocytic myocarditis in endomyocardial biopsies of living patients and in autopsies

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**Introduction:** To diagnose lymphocytic myocarditis (LM) immuno-histopathological examination of endomyocardial biopsies (EMB) is used with a cut-off value of ≥14 leukocytes/mm$^2$, composed of CD3 and CD68 positive cells. We hypothesized that a more common leukocyte marker, CD45, instead of CD3 could increase the diagnostic sensitivity. Moreover, we also compared CD45 positivity in the EMB area of the left ventricular posterior wall (LVPW) to relation to the rest of the myocardium.
**Methods:** In hearts of mice with acute viral myocarditis (n=9), controls (n=7) and in the EMB area of the LVPW obtained from autopsy hearts of patients diagnosed with LM (n=18) and controls (n=6) were stained with anti-CD68, -CD3 and -CD45. Subsequently, cells were quantified per mm2. Anti-CD45 cells were also quantified in the remaining LVPW.
**Results:** In mice with myocarditis the number of CD45+CD68 cells/mm2 was significant increased compared to the number of CD3+CD68 cells/mm2. When applying the threshold of ≥14 leukocytes/mm$^2$, 44% of the mice would be diagnosed for LM with the use of CD3+CD68, however 100% of the mice would be diagnosed for LM with the use of CD45+CD68. In the EMB area of autopsied hearts, using the cut-off value of ≥14 leukocytes/mm$^2$, CD3+CD68 could only confirm 39% of the diagnosis of LM, while the CD45+CD68 could confirm 56% of the LM cases. Interestingly, a significant increase of CD45 positive leukocytes/mm2 was observed in the EMB area when compared to the remaining LVPW in LM patients.
**Conclusions:** The use of the common leukocyte marker CD45 increases the sensitivity of the diagnosis of LM. Furthermore, the inflammatory infiltrate in the EMB area is significant increased compared to the remaining LVPW, indicating that the sampling area constitutes the highest chance for the histological diagnosis of LM.

### 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 question of the cellular origin of atrial AT.
**Methods:** Human right atrial specimen obtained during cardiac surgery were used for histological, biochemical studies (n=60) and to harvest epicardial progenitors (n=20). Epicardial progenitor-derived cells (EDPCs) were maintained in culture conditions and characterized using flow cytometry, proteomic and genic expression assays. To study the ability to hEDPC to differentiate into adipocyte, progenitors were treated with adipogenic medium from 3 weeks. In order to determine the cellular origin of atrial adipose tissue, we studied a lineage tracing Wt-1-Cre-Rosa-tdT+/+ mice model (n=7). Mice atrial tissue sections were studied by histological and immunostaining assays. Similar to human EPDCs, cells were harvested from Wt1-Cre-Rosa-tdT+/+ atrial tissue and studied in cell culture.
**Results:** In the sub- and epicardial layer of atrial section, cells were positives for epicardic progenitor marker Wilm's tumor-1 (Wt1) and pre-adipocyte marker pre-adipocyte factor 1 (Pref-1) suggesting that EPDCs could engaged in the adipogenic fate. This hypothesis was tested in vitro, using human and mice EPDCs harvested from atrial samples; atrial EPDC underwent an epithelio-to-mesenchymal transition (EMT) and acquired mesenchymal phenotypes and could differentiate into osteocyte or chondrocyte. When cultured using an adipogenic medium, around 40% of EPDCs cells showed lipid droplet stained with oil red and expressed mature adipogenic markers perilipin, PPAR? and C/EBP$\alpha$. These results were supported by the formation of lipid droplet-tomato+ observed in mEPDCs induced by adipogenic medium. To follow the fate of Wt1 we developed a lineage tracing Wt-1-Cre-Rosa-tdT+/+ mice model. We found that a number of adipocytes that compose the atrial EAT could derive from aEPDC through an EMT process.
**Conclusion:** Atria 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 spam. IF (Intemittent 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 kinase 2 (GRK2), which contributes to dysfunctional 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 ($p<0.01$) 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 IF HF 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 GRK2 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.

### 636
### High fat diet improves cardiac remodelling and function after extensive myocardial infarction in mice

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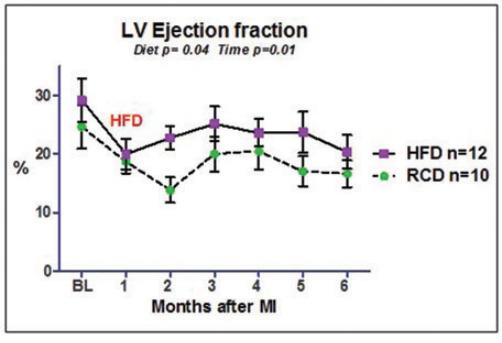
**Background:** Although obesity is considered a major risk factor for cardiovascular diseases, it is associated with lower mortality and a better outcome in patients with chronic heart disease ("the obesity paradox").
**Purpose:** We aimed to determine whether a high-fat diet (HFD) can protect the failing heart after extensive myocardial infarction (MI).
**Methods:** We induced MI in 12-week old balb/c female mice. Twenty-four hours later, a first echocardiography (echo) was performed to confirm significant left ventricular (LV) dysfunction. One month post-MI, a second echo was done and mice were randomized into 2 groups: HFD, (n=20) and regular chow diet (RCD), (n=20). Serial metabolic and echo studies were performed once a month following randomization. During 6 months of follow-up, HFD-fed mice gained significantly more weight (28gr ± 1.1 vs. 23gr ± 0.4, p=0.001, [mean ± SEM]), and had higher plasma levels of cholesterol, LDL, HDL and glucose, compared with RCD. Survival was similar between the groups. Significantly, compared with RCD, HFD attenuated LV dysfunction (Figure, p=0.04), and reduced LV diastolic dilatation (12.2 ± 2.2% vs. 2.6 ± 2.4%, p=0.003), at 6 months after MI.
**Conclusions:** High-fat diet attenuates adverse cardiac remodelling and dysfunction. Our study challenges the traditional dogma and suggest new pathway that could be targeted to reserve or halt the progression of heart failure.



High fat diet attenuated LV dysfunction.

### 637
### Epigenetic therapy reduces cardiac hypertrophy in murine models of heart failure

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**Background:** Heart failure with preserved ejection fraction (HFpEF) is one of the leading causes of global morbidity and mortality. HFpEF is driven by pathological remodelling 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 HFpEF.
This study investigated the therapeutic potential of the DNA methyltransferase inhibitor, 5-azacytidine (5aza) to inhibit pathological hypertrophy in the heart using preclinical models of HFpEF, the transaortic constriction (TAC) model and the Angiotensin-II (AngII) infusion model.
**Methods:** Wild type C57Bl6/J mice underwent surgical constriction 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 control group. TAC mice were treated four weeks post-surgery with intraperitoneal administration of either placebo or 5aza. AngII mice began 5aza treatment every four days after pump implantation. Cardiac structure and function was examined in vivo using non-invasive echocardiography.
**Results:** Echocardiographic analysis revealed that TAC and AngII mice treated with 5aza displayed a significant reduction in the interventricular septal wall and left ventricular posterior wall thickness compared to mice which received placebo treatment. Reduction in left ventricular mass was also evident in both models, even when 5aza treatment was initiated in the TAC model after cardiac hypertrophy was established.
**Conclusion:** Therapeutic options for HFpEF patients are limited. Inhibition of DNA methylation using 5aza 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+ Epicardial Progenitors results in coronary vascular defects, fibrosis and cardiac hypertrophy

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**Background:** Epicardial progenitors (EPs) of the mammalian heart express Wilms' tumor 1 (Wt1) and contribute to coronary vasculature, 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 (EPDCs).
**Methods:** We have generated several conditional hypoxia gain and loss of function models (GOF/LOF) in Wt1+EPs. We have performed electrocardiography, nuclear magnetic resonance (NMR), histological analysis and transmission electron microscopy (TEM).
**Results:** Echocardiography analysis shows increased width of Left Ventricular Posterior Wall (LVPW) and Inter-Ventricular Septum (IVS) and hypertrophy of the papillary muscles in mutant hearts. Histological characterization reveals diffused fibrosis of the IVS, LV, atrial wall and subepicardial region, consistent with the observed enlarged QRS segment. Interestingly main coronary vessels are evidently dilated as confirmed by NMR and present intracoronary fibrin clots by hematoxylin & eosin staining. Furthermore, GOF hearts display myocardial inflammatory infiltration as well as interstitial and pericardial hemorrhages, dying suddenly presumably due to coronary rupture. Von Hippel Lindau (VHL) _a negative regulator of Hypoxia Inducible Factors (HIFs)_ regulates fibronectin assembly, thus we investigated whether extracellular matrix components of the coronary vasculature might be affected in this model and potentially contribute to vascular permeability and coronary instability. We have found that embryonic mutant vessels present abnormal fibronectin levels, together with defective elastic fibers, pointing to elastin as a potential novel target of VHL. In addition we have generated HIF1 and HIF2 LOF models in the epicardium, finding that while epicardial lack of HIF1 seems to be dispensable, HIF2 deletion in the Wt1 lineage leads to increased LV volume and decreased LVPW width together with decreased cardiac function, suggestive of cardiac dilation. We are currently determining the molecular mechanisms behind these phenotypes.
**Conclusions:** Our data indicate that altered balance in the VHL/HIF signaling in the epicardium leads 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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**Introduction:** Cardiac adaptation to ischemic or toxic lesions may result in diastolic or systolic dysfunction, or both. Conditions of their development are not quietly understood.
**Purpose:** The aim of the study is to distinguish conditions determining the form of cardiac dysfunction in rats at ischemic or toxic models of the heart failure.
**Methods:** The study followed the European Convention for the Protection of Vertebrate Animals Used for Experimental and other Scientific Purposes (No. 123 of 18 March 1986). The ischemic or toxic models of the heart failure in Wistar rats were created by injections of isoproterenol (ISO) or doxorubicin (Dox), respectively. ISO was injected twice (120 or 180 mg/kg) and doxorubicin (2 mg/kg) once each week. Rats narcotized by ketamine (100 mg/kg) were subjected to catheterization of the aorta and left ventricle (LV) by Millar micromanometers and to echocardiography. Isolated cardiomyocytes were loaded by Fluo-4 and Ca++ transients were detected with Zeiss microscopy.
**Results:** At four weeks after lower ISO dose, the hearts exhibited 2-fold increased LV enddiastolic pressure and twice reduced relaxation constant (RC, 64+9 vs. 129+10 s-1 in control) while LV systolic pressure, +dP/dt and contractility index (CI, +dP/dt/P) were only slightly lower. The echocardiographic study of these hearts revealed only slightly increased LV diastolic and systolic volumes and slightly decreased ejection fraction (EF). Rats received higher ISO dose had 2.5 times higher LV diastolic volume and decreased EF by 32%, augmented LV enddiastolic pressure, and decreased CI and RC by 20 and 35%, respectively. Calcium transients showed greatly slowed signal decrement with plateau formation. The comparison of the data at 4 and 10 weeks after start of Dox injections revealed a clear worsening of all indices, namely, EF decreased by 7 and 22%, CI by 13 and 41%, RC by 22 and 55%, respectively, ($p<0.05$ for all). Calcium transients had lower peak, slowed signal decrement and elevated diastolic Ca++ level.
**Conclusion:** Diastolic dysfunction with slowed relaxation and increased myocardial stiffness occurs at lower degree of cardiac lesions induced by ISO or Dox while systolic dysfunction with decreased EF and profound fall in CI and RC develops later. Thus, diastolic dysfunction may be considered as the first step of cardiac adaptation to developing cardiomyopathy helping to maintain myocardial contractility and pump function of the heart.

### 640
### Colchicine aggravates coxsackievirus 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 disrupts microtubule assembly. In several clinical trials, colchicine was found to be a safe and effective treatment option for pericarditis patients, despite the fact that pericarditis 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 coxsackievirus B3(CVB3)-induced myocarditis.
**Methods:** Four groups of C3H/HeJ mice were included: Control mice (n=8), mice infected with CVB3 (100.000 PFU, n=10), mice with colchicine administration (2 mg/kg i.p, 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 (immuno)histochemical 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 in CVB3-infected mice ($p<0.01$) 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 induced massive apoptosis in the white pulp and significantly inhibited the virus-induced increase of megakaryocytes in the spleen ($p<0.001$). Finally, we observed that colchicine significantly increased CVB3 levels in both the pancreas and the heart.
**Conclusions:** Colchicine treatment in CVB3-induced 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.
**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:** 131 patients with known CHD combined with moderate to severe COPD and in regular chronic treatment according to international guidelines 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 $63.35 \pm 8.3$) 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 mean pulmonary artery pressure (mPAP) ($r = 0.25$, $P < 0.001$) 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).

**Conclusion(s):** These findings indicate that OPN may be 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 s.c./kg), 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 H1190-7, 2010). Static passive stiffness is determined from the force-length relation. A sinusoidal length change is imposed to determine dynamic passive stiffness (amplitude $\pm$ 0.075 Lo at 5 cycles/s, where Lo is the muscle length giving maximum active force). Dynamic stiffness is determined at 0.925 Lo from $\Delta$force/$\Delta$length during stretch without stimulation. Stiffness is normalized by muscle cross-sectional area/0.925 Lo to allow comparison between muscles. The contribution of cross-bridge cycling to stiffness is estimated using 10$\mu$M blebbistatin. Basal oxygen consumption is measured during length changes without stimulation.

Static stiffness is 67 $\pm$ 17 kPa (mean $\pm$ SD) in control and 102 $\pm$ 32 kPa in PH (P=0.006). Dynamic stiffness is smaller than static stiffness: 40 $\pm$ 19 kPa and 74 $\pm$ 37 kPa, respectively (P<0.001). Blebbistatin has no effect on dynamic stiffness in control (38 $\pm$ 22 kPa) but reduces dynamic stiffness in PH (to 57 $\pm$ 18 kPa, paired t-test P=0.002). Similarly, basal oxygen consumption (before blebbistatin 0.21 $\pm$ 0.13 mM/s and after 0.22 $\pm$ 0.13 mM/s in control) was reduced by blebbistatin only in PH from 0.22 $\pm$ 0.07 mM/s to 0.17 $\pm$ 0.06 mM/s (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 immunotroteasome 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 changed in the aorta of spontaneously hypertensive rats (SHR). The goal of the present investigation is to elucidate if changes in proteasomal subunits genes 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 immunoproteasome subunits in aorta tissues of Wistar rats, SHR and quercetin-treated SHR. Quercitin (BCPP, Ukraine) was added to standard diet for 8 weeks in dose of 15 mg/kg. Proteolytic activities of proteasome (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 rat's aorta were determined by the morphometric analysis and electron microscopy in all experimental groups.

**Results:** The mRNA expression of immunoproteasome 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, PSMC1, PSMB5, PSMB10 subunits of proteasome was significantly decreased in aorta tissue of SHR, comparing to Wistar rats. However, expression of genes encoding PSMB2, PSMB8, PSMB9 and PSME1 was significantly up-regulated. The quercetin treatment provoked increase in mRNA levels of PSMB1 and decrease of PSMB2, PSMB8, PSMB9, PSME1 in SHR aorta. Activities of proteasome did not differ between Wistar rats and SHR, but quercetin decreased trypsin-like and chymotrypsin-like activities of proteasome in SHR group. Furthermore, cardiac output was decreased in 3,5 times (p<0.0001), stroke volume in 3 times (p<0.0001), ejection fraction in 2,5 times (p<0.0001) in SHR. Quercitin application had normalized disturbed cardiodynamic parameters: ejection fraction was increased in 1,7 fold (p<0.0001), end-systolic pressure was decreased on 15,7 % (p<0.0001).

**Conclusions:** The inhibitory effect of quercetin on proteasomal proteolysis 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 immunoproteasomal subunits.

### 645

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

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Urocortin(UCN)-2 is highly expressed in the cardiovascular system and has shown promising therapeutic effects in several studies in human and experimental heart failure. This study analysed the levels of UCN-2 in human and experimental pulmonary arterial hypertension (PAH), and the effects of UCN-2 treatment in an animal model of RV failure, secondary to PAH.

Rats were submitted to monocrotaline (MCT,n=35)/vehicle (CTRL,n=27) administration, or pulmonary artery banding (PAB,n=13)/Sham (n=5). After 14 days, animals were randomly assigned to receive either UCN-2(5$\mu$g/Kg/day) or vehicle. Functional measurements were performed 23-25days after MCT or PAB, and after euthanasia tissue was collected. Moreover, RV, lung and blood samples were collected from PAH patients(n=7) and non-PAH controls(n=6). Only significant data (mean $\pm$SEM, p<0.05) are given.

Functional studies revealed that MCT group developed PAH, as shown by increased RV end-systolic pressure (MCT vs CTRL: 60 $\pm$ 3 vs 22 $\pm$ 1mmHg), end-diastolic pressure (6.0 $\pm$ 0.7 vs 3.7 $\pm$ 0.3mmHg), and decreased ejection fraction (32 $\pm$ 4 vs 75 $\pm$ 3%) and pulmonary artery acceleration time (13.6 $\pm$ 0.57 vs 25.7 $\pm$ 2.61ms). UCN-2 treatment attenuated these changes (48 $\pm$ 4; 4.3 $\pm$ 0.3mmHg; 60 $\pm$ 3% and 20.0 $\pm$ 1.11 ms, respectively). Also, UCN-2 treated rats had higher survival rate (76 vs 44%) and exercise capacity (MCT vs CTRL vs MCT+UCN-2: 219 $\pm$ 88 vs 749 $\pm$ 71 vs 573 $\pm$ 88m). PAH rats presented RV hypertrophy as shown by the morpho-histological analysis (RV weight/tibia length ratio, MCT vs CTRL: 0.08 $\pm$ 0.00 vs 0.04 $\pm$ 0.00 g/cm; cardiomyocyte cross-sectional area: 353 $\pm$ 25 vs 234 $\pm$ 25$\mu$m2). UCN-2 therapy attenuated RV remodelling (0.06 $\pm$ 0.00g/cm and 283 $\pm$ 22mm2, respectively). MCT-group isolated cardiomyocytes developed higher passive force compared to CTRL-group at the sarcomere lengths of 2.2 (MCT vs CTRL: 5.09 $\pm$ 1.14 vs 2.48 $\pm$ 0.56N/m2) and 2.3um (8.10 $\pm$ 1.77 vs 4.02 $\pm$ 0.82N/m2). UCN-2 restored passive force development (3.44 $\pm$ 0.70 and 5.36 $\pm$ 0.97N/m2, respectively). Plasmatic levels of UCN-2 were increased in MCT rats with decompensated RV function, compared to compensated RV (p=0.0338). PAB rats treated with UCN-2, showed RV-specific decrease in cardiomyocyte hypertrophy (PAB vs SHAM vs PAB+UCN-2: 534 $\pm$ 46 vs 280 $\pm$ 29 vs 387 $\pm$ 31$\mu$m2) and fibrosis (11.96 $\pm$ 1.2 vs 2.23 $\pm$ 0.3 vs 2.66 $\pm$ 0.2%). Moreover, UCN-2 levels in the buffy coat from blood of human PAH patients were higher than in controls (p=0.035), while a trend toward an up regulation was seen in the RV and Lung of PAH patients (p=0.0663 and p=0.0734,respectively).

UCN-2 levels are altered in human and experimental PAH. UCN-2 treatment attenuates PAH and RV dysfunction and increases survival in MCT-induced PAH, and has direct anti-remodelling effects on the pressure-overloaded RV. UCN-2 has a relevant role in the pathophysiology of PAH, and might be a new treatment option in this condition.

### 646

### A preclinical 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, hypertrophic heart disease, renal disease, stroke and blindness. Ischaemic heart disease and stroke are currently the top 2 causes of death worldwide. In view of the growing 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 from day 1 (prevention) or (ii) from week 12 (treatment) of the 16 weeks. Blood pressure (BP), 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 (FRET) 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 rise 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 therapy for the improvement of hypertension, also causing weight loss and an improved RAS.

## Biomarkers

### 648

### 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 reflecting by SCORE and mSCORE (EULAR 2010). Thus an additional risk factors research is required. Adiponectin 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 [50,3; 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 detection (ESC 2006) and endothelial-dependent flow mediated vasodilatation (EDVD) by D. Celermajer method were performed. The level of adiponectin was measured using ELISA kit test.

Serum adiponectin level was significantly higer in the HT females with RA group (13.4 [12.5; 14.8] mg/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 hip\waist ratio r = 0,34 ($p<0.05$), DAS28 - r=0,36 ($p<0.05$), cardiovascular risk mSCORE - r=0,33 ($p<0.05$), BMI - r=0,79 ($p<0.05$) and EDVD - r=-0,41 ($p<0.05$). 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.

### 649

### 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-IV 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 performed glomerular filtration velocity (GFV) by formulae MDRD, microalbuminuria (MAU), urine enzymes concentrations: alaninaminotransferase (ALT), aspartataminotransferase (AST), alkaline phosphatase (AP).
Results of investigations showed that in the patients with CHF FC I, II, and III-IV parameters of Cr were $90,33 \pm 12,15$, $99,1 \pm 5,98$, $111,75 \pm 5,5$ respectively. GFV in the patients with FC I and II was $90,1 \pm 7,1$ and $80,6 \pm 1,3$ ml/min/1,73m2 with reliable reduction in the patients with CHF FC III-IV $71,6 \pm 6,8$ ml/min/1,73m2. The patients with CHF FC I had no GFV<80 ml/min, and in the patients with CHF FC II it was observed in 16% of patients and in 50% of patients with CHF FC III. Analysis MAU showed that in 30% of patients: 3 with CHF FC I, in 5 — CHF FC II and 10 — with CHF FC III (55,6%) there was noted presence of MAU. In the patients with FC I and II CHF there was noted reliable increase in fermenturia level in comparison with control group: ALT-by 30% and 50%, AST — by 25% and 39%, AP — by 39% and 79% respectively ($p<0,05$). In the patients with FC III-IV of CHF there was revealed reliable increase in ALT, AST, AP by 79%, 50%, 111% respectively ($p<0,01$), that indicated about damage of congruentness of cytoplasmatic membranes of tubular epithelium of the kidney tubules. There was revealed direct correlation, which level of MAU directly correlated with of FC CHF, fermenturia and Cr level in the blood serum ($p<0,05$) and there was noted inverse corelational relation with GFV.
**Conclusion:** Thus, measurement MAU, fermenturia may be considered as reliable glomerotubular markers for evaluation of the RF state in the patients with CHF.

### 650

### 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 $50 \pm 13$ 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, increased total bilirubin in 17%, elevated alkaline phosphatase in 13%, elevated aspartate aminotransferase in 24%, alanine aminotransferase elevation in 18% of patients. The proportion of patients with reduced ejection fraction ($\leq$50%) who had elevations in total bilirubin was 23%. We note baseline abnormalities in bilirubin, alkaline phosphatase and albumin were more common in patients who died.
**Conclusions:** Mild abnormalities of LFT are relatively frequent in patients with chronic heart failure, with a greater elevation of bilirubin than aminotransferases. Patients with reduced ejection fraction had a higher prevalence of increased bilirubin. Total bilirubin was a predictor of adverse prognosis.

### 651

### To study other biomarkers that assess during myocardial infarction

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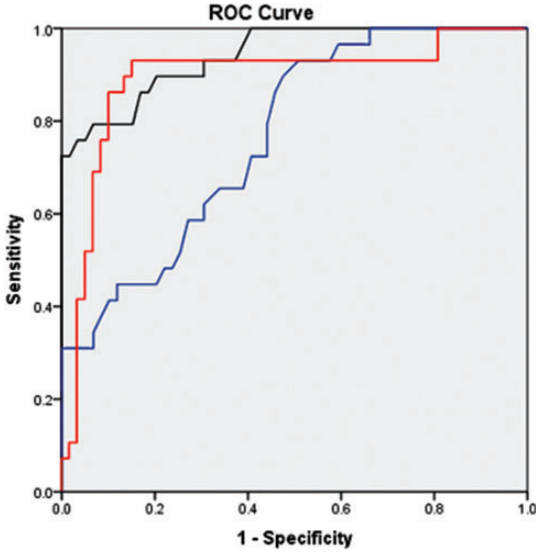
**Background:** When the coronary atherosclerotic plaque becomes vulnerable, a thrombus develops on that ruptured plaque and then occludes the coronary artery, which causes an acute blood deficiency in the downstream myocardium.
Furthermore oxLDL (oxidized Low Density Lipoprotein) is involved in the coronary atherosclerotic plaque pathogenesis, MMP-9 (Matrix Metalloproteinase-9) enzymes plays role during the plaque rupture and CPR (C Reactive Protein) has a prognostic 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 case group are 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 plague were included in the control group.
Serum MMP-9 enzyme and oxLDL titers were determined by ELISA according to the manufacturer's recommended protocol. Additionally CRP was measured by full-automated analyzater. We used CIIS (cardiac infarction injury score) by ECG and Gensini score system (Coronary Angiographic Scoring System) for assessing the severity of coronary heart disease.
**Results:** Serum MMP-9, oxLDL levels ($p<0.001$) in the case group (MMP-9 $0.396 \pm 0.155$ ng/ml; oxLDL $1.411 \pm 0.099$ μg/ml) were more than in the control group (MMP-9 $0.223 \pm 0.087$ ng/ml; oxLDL $1.332 \pm 0.163$ 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.
Serum MMP-9 enzyme level was directly correlated with Gensini score (r=0.552, $p<0.01$), CIIS (r=0.340, $p<0.01$) and CRP (r=0.321, $p<0.01$) titers.
Furthermore, serum MMP-9 enzyme increases with accordance of severity of the myocardium injury with the statistical significance ($p<0.01$): the borderline abnormality group (CIIS<10, $0.227 \pm 0.099$ ng/ml), possible injury (CIIS 10-15, $0.317 \pm 0.132$ ng/ml), probable injury (CIIS >15, $0.376 \pm 0.132$ 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 diagnostically 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 coronary atherosclerotic plaque rupture in the myocardial infarction.




Roc Curve

### 652

### 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 antiatherogenic 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$\geq$1,7 mmol/l levels of apelin were significantly lower than in healthy volunteers (0,851 (0,841; 0,877) ng/ml versus 1,087 (0,861; 1,318) ng/ml, $p<0,05$) and significantly lower than in the subgroup of patients with the levels of blood TG<1,7 mmol/l (0,851 (0,841; 0,877) ng/ml versus 0,919 (0,861; 1,412) ng/ml, $p<0,05$). Correlation analysis in the whole group of patients with hypertension showed significant negative correlation levels of apelin with blood levels of TG (r=-0,56, $p<0,01$), VLDL-C (r=-0,56, $p<0,01$), AC (r=-0,56, $p<0,001$) and a positive correlation with HDL-C ($r = +0,44$, $p<0,05$).
**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 antiatherogenic properties of apelin.

### 653

### 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.

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## 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.