**Developmental biology**

### P46

**Pericardial origin of proepicardial progenitor cells in the avian embryo**

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The pericardium (PE) is a cluster of mesothelial cells that develops at the venous pole of the heart. In many vertebrates including the chick, PE development is enhanced on the right side via a signalling pathway involving FGFR8 and SNAIL1 (Schlueter and Brand, 2009 PNAS 106:7485-7490), leading to the formation of a right-sided pericardial tissue bridge via which PE cells colonize the heart and form the epicardium. In this study we have analysed the embryonic origin of pericardial cells and uncovered a novel cellular contribution of pericardial mesothelium to the proepicardium. We performed labelling experiments of the lateral mesocardia and the pericardial mesoderm by Dil injection and electroporation of a GFP reporter gene and observed a cellular contribution of these tissues to the sinus venous. Expression analysis of TWIST1, which represents a potential downstream target of the FGFR8/SNAI1 pathway, revealed enhanced expression in the right somatopleura and subsequently in the right sinus horn and lateral mesocardium. Expression of TWIST1 terminated shortly before the onset of PE formation. We hypothesize that these proepicardial progenitor cells of somatopleurial origin are asymmetrically mobilized by the expression of TWIST1. Indeed forced expression of TWIST1 on the left side lead to cell invasion of the sinus venous and the heart by mesodermal cells of somatopleurial origin. We are currently performing loss-of-function experiments to interfere with TWIST1 expression on the right side to study its effect on PE formation. These observations point to a similar origin of a subset of proepicardial cells that form the surrounding pericardial mesoderm in the chick embryo.

### P47

**Conditional deletion of Scrib gene in the developing myocardium leads to congenital heart defects**

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The Scrib gene is a component of the non-canonical Wnt/planar cell polarity (PCP) pathway which has been implicated in cardiogenesis. Mutation of Scrib, in the Crricalt mouse mutant, results in cardiomyocyte disorganisation and disrupted cell-cell adhesion in the early heart tube. This is associated with a spectrum of cardiac septation and alignment defects and abnormalities of the ventricular myocardium that resemble certain types of human cardiomyopathy.

Utilising Cre-loxPtechnology and Scribbf mice, we generated embryos with the conditional deletion of Scrib in specific cell types within the developing heart, allowing us to determine the cardiac cell type that is critically dependent on Scrib function. Early deletion of Scrib in cardiac progenitors, utilising Nkx2.5Cre mice, confirmed the importance of Scrib at the earliest stages of myocardial development, resulting in hearts with ventricular septal defects but a well-developed compact myocardium. Later deletion of Scrib in the myocardium, using Mlc2vCre-Cre, had only a mild affect on the ventricular myocardium.

We investigated how Scrib functions in the developing myocardium. Scrib localises to the adherens junctions of developing cardiomyocytes. Co-immunoprecipitation and immunofluorescence experiments indicated that Scrib forms a protein complex with Rac1 and its exchange factor, p115, in both the HPC1 cardiomyocyte cell line and in hearts isolated from E10.5 embryos. Moreover, Rac1f/f and Nkx2.5Cre mice share a similar phenotype to Scribbf/Nkx2.5Cre. To examine for a possible genetic interaction between Scrib and Rac1 we generated Scribbf Raci1f/f and Nkx2.5Cre mouse embryos. Double heterozygotes developed heart malformations that closely resembled those seen in Rac1f/f/Nkx2.5Cre embryos, confirming a genetic as well as physical interaction between Scrib and Rac1. These findings suggest that Scrib, together with Rac1, plays essential roles in cardiac progenitors, regulating adhesion and allowing the proper organisation of the myocardium that is essential for ventricular septum integrity.

**P48**

Heart rate changes mediate the embryotoxic effects of antiarrhythmic drugs in the chick embryo

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Beta-blocking agents are frequently used cardiovascular drugs. With increasing age of pregnant women we face the need of drug treatment during pregnancy more frequently. As the heart rate is the most important determinant of cardiac output in the embryonic heart, we hypothesized that drug-induced bradycardia is the leading mechanism of heart failure. ED4 and ED8 chick embryos were studied by video microscopy and ultrasound biomicroscopy ex ovo after intraamniotic injection of 200 μl of metoprolol or ivabradine or 200 μl of normal saline for a period of 30 minutes. Stroke volume was calculated by Simpson method from long parasternal short axis view in ED 8 embryos and prolite ellipsoid formula in video recordings (EDM). Cardiac output was then calculated from equation: CO(μl/min) = SV(μl)/HR(BPM). Embryotoxicity was tested in ovo after administration of various doses of metoprolol or ivabradine compared to normal saline between ED3-ED8.

Metoprolol and ivabradine are drugs with strong negative chronotropic effect leading to 40% decrease of heart rate compared to normal saline within 30 minutes in ED 4 embryos. In more mature ED 8 embryos this effect was even more pronounced, with the heart rate decreased by 88% in metoprolol group and by 43% in ivabradine group. CO in ED 4 embryos decreased by 1% in the control group, by 11% in metoprolol group and by 43% in ivabradine group at 30 minutes. In ED 8 embryos the decrease in CO was 34% for normal saline group, 92% decrease for metoprolol group and 63% decrease for ivabradine group. There was no significant difference in stroke volume at either time point. A significant dose-dependent mortality (80%) was achieved in ED 4 embryos injected by 200 μl of Ivaradine. In ED 8 embryos this effect was less pronounced with only 10% mortality at the same dose. No significant mortality was observed in ED 4 embryos injected by different doses of metoprolol but 39% mortality was achieved in ED 8 embryos injected by 200 μl of metoprolol.

Sensitivity to negative chronotropic effect of metoprolol and ivabradine increases with development. The embryonic heart has limited potential to vary stroke volume and significant bradycardia is followed by a significant decrease in cardiac output, likely leading to embryonic death. Metoprolol in usual doses appears to be relatively safe in pregnancy whereas ivabradine might have potential adverse effect on fetus.

**P49**

Functional deployment of ventricular conduction system in mouse during development

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Individual compartments of cardiac conduction system (CCS) became functional in order which correlates with cardiac morphogenesis. Ventricular CCS compartments mature with ventricular septation and is accompanied by shift of activation pattern from primitive base to apex, which follows blood flow, to advanced apex to base. There are some important differences between avian and murine CCS. From the functional point of view, the main contrast is the appearance of mature activation sequence well before time of ventricular septation completion (embryonic day [ED] 13.5 in mouse) and also that there was never reported activation originating from the base of heart.

Function of CCS was studied by optical mapping and monitored parameters were speed of electric impulse propagation, location first activation site on ventricular epicardial surface together with direction of action potential spread [evaluated as activation patterns, namely activation utilizing primary ring, left and right apical breakthroughs, only right or only left apical breakthrough - corresponding from ED14.5 with left and right bundle branches, respectively]. By measurement of time necessary for activation of the left ventricle from ED9.5 to ED18.5 we observed remarkable acceleration between ED9.5 and ED11.5 where activation time dropped to a half. This was due to decrease in frequency of primary ring activation pattern with a slow
Construction of heart is a result of a complex process of complex regulatory mechanisms that remain largely undetermined. In the heart, AS plays a main role during embryonic development. However, its role in adults is not completely understood. In the heart, AS plays a main role during embryonic development. However, its role in adults is not completely understood. In the heart, AS plays a main role during embryonic development. However, its role in adults is not completely understood. In the heart, AS plays a main role during embryonic development. However, its role in adults is not completely understood. In the heart, AS plays a main role during embryonic development. However, its role in adults is not completely understood. In the heart, AS plays a main role during embryonic development. However, its role in adults is not completely understood. In the heart, AS plays a main role during embryonic development. However, its role in adults is not completely understood. In the heart, AS plays a main role during embryonic development. However, its role in adults is not completely understood.
impairment in clock mechanism within the cardiomyocytes may alter the cardiac metabolism and function. This might have clinical implications of cardiac remodeling in patients with heart failure.

The aim of our study was to investigate the effect of variable number tandem repeat (VNTR) polymorphism in Per3 gene in acute myocardial infarction with ST elevation (STEMI). The study subjects (336 patients of Caucasian origin with STEMI, and 332 healthy controls) were genotyped for Per3 VNTR polymorphism using an allele-specific PCR. Per3 quantification of miR-375 from circulation was performed using quantitative real-time polymerase chain reaction (qRT-PCR). The follow-up of Per3 expression in 10 patients within interval of 8 hours was carried out (6 samples per patient). Within the STEMI patients, the difference in allelic frequencies of Per3 VNTR polymorphism was observed when comparing the time of MI onset (p = 0.01). The long variant (55) of Per3 VNTR polymorphism was more common within STEMI patients with pain onset during the early hours (p = 0.045). A trend of higher Per3 expression in short variant (44) was observed (p = 0.08). The lowest expression was found in time of MI onset with increasing Per3 expression in following hours. No significant differences in genotype and/or allelic frequencies of Per3 VNTR polymorphism were observed when comparing STEMI cases and control group. In the multivariate regression modelling, no predictive function of VNTR Per3 polymorphism on ejection fraction, hyperlpidemia or type II diabetes risk was observed.

Based on the results of the presented study, we consider a possible effect of the Per3 VNTR long variant on time of the pain onset during MI in STEMI patients.

**P55**

**Next-generation sequencing used to discover novel genetic variants predisposing to heart disease**

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1Institute of Molecular Medicine, Faculty of Medicine of Lisbon, Lisbon, Portugal; 2Hospital Lisbon North, M.235T polymorphism was determined using polymerase chain reaction. The relationship between angiotensinogen gene polymorphism and left ventricular remodeling in patients with myocardial infarction was assessed. H.H.W. Sillje; B. Lu; H. Yu; M. Zwartbol; W.P. Ruifrok; H.W. Van Gilst; R.A. De Boer

University: Medical Center Groningen, Groningen, Netherlands

Purpose: Cardiomyocyte growth (hypertrophy) is one of the hallmarks of heart failure (HF) development. Numerous studies have explored hypertrophy associated gene expression, but have not focused on the diversity in disease specific differences. This NGS platform, which includes 750Kb of exons, splicing regions and 5' and 3' untranslated regions of genes associated with Brugada syndrome, long QT and short QT syndromes, familial atrial fibrillation, channelopathies, and genetic heart disease, permits a quick and high throughput analysis of those genes. This study is important to explain the phenotypic variability of heart disease and to help establish new mutation-disease associations.

**Methods:** We developed a methodology for resequencing 72 genes (49 genes associated with cardiomyopathy, arrhythmogenic right ventricular dysplasia, Marfan syndrome, aortic aneurysm, and 23 genes associated with Brugada syndrome, long QT and short QT syndromes, familial aortic anula- catherinomphic polymorphic ventricular tachycardia). The design was done in our Bio-informatics Unit and includes 750Kb of exons, splicing regions and 5' and 3' untranslated regions of the selected genes. In this study, we aimed to determine whether the polymorphism of angiotensinogen (AGT) gene with threohine (T) instead of methionine (M) at amino acid 235 in exon 2 (M235T) has effects on cardiac remodeling after AMI. Methods: 141 patients (mean age 56 ± 11.1) with a first AMI were enrolled. Within 24-72 hours of the onset of AMI symptoms and at 4 months, 2-dimensional echocardiography was performed. LV remodeling was defined as ≥ 15% increase from the baseline in LV end diastolic volume. AGT M235T polymorphism was determined using polymerase chain reaction amplification.

Results: At follow-up, 49 patients (34%) were classified as having LV remodeling. Patients with LV remodeling were more frequently prone to anterior wall MI (p < 0.01). higher left systolic volume at valve closure (p < 0.001), lower LV ejection fraction (p < 0.05) and increased LV end systolic volume (p < 0.05). Furthermore, AMI patients with LV remodeling significantly more often were carriers of M235T genotype of AGT (26.6% vs 14.1%, p = 0.038).

**Conclusion:** The TT genotype of the AGT gene may be related to the development of LV remodeling after acute myocardial infarction.

**P56**

**miR-375: novel biomarker for early prognostic stratification of acute myocardial infarction**

M. Costa1; N. Cortez-Dias2; P. Carrilho-Ferreira2; D. Silva1; C. Jorge2; P. Carrilho-Ferreira2; D. Silva2; C. Jorge2; R. Placido2; C. Calisto2; L. Perez-Cabornero1; D. Cantalapiedra1; A. Forteza2; R. Saez-Villaverde3; J. Zumalde4; M. Lazaro1; S. Santillan1

1Sistemas Geno´micos, SL, Paterna, Valencia, spain; 2University Hospital 12 de Octubre, Department of Cardiology, Madrid, Spain; 3Hospital Donostia, Department of Cardiology, San Sebastian, Spain; 4Hospital Godalén-Uxama, Baztán, Navarra, Spain

Purpose: Cardiovascular disease is the leading cause of death worldwide. Most cardiomyopathies and channelopathies are genetic in origin and are characterized by their risk of premature death and chronic morbidity, affecting both the patient and his/her family. The detection of mutations in patients allows for therapeutic or preventive measures to be established as part of the genetic counseling. However, the large number of genes involved, makes it difficult to analyze using conventional techniques. The purpose of this study is the genetic characterization of heart disease patients in a fast, comprehensive, and cost-effective manner using an NGS approach, which includes 72 genes associated with different pathologies, coupled with a robust bioinformatics pipeline.

Methods: We developed a methodology for resequencing 72 genes (49 genes associated with cardiomyopathy, arrhythmogenic right ventricular dysplasia, Marfan syndrome, aortic aneurysm, and 23 genes associated with Brugada syndrome, long QT and short QT syndromes, familial aortic anularea catherinomphic polymorphic ventricular tachycardia). The design was done in our Bio-informatics Unit and includes 750Kb of exons, splicing regions and 5' and 3' untranslated regions of the selected genes. In this study, we aimed to determine whether the polymorphism of angiotensinogen (AGT) gene with threohine (T) instead of methionine (M) at amino acid 235 in exon 2 (M235T) has effects on cardiac remodeling after AMI. Methods: 141 patients (mean age 56 ± 11.1) with a first AMI were enrolled. Within 24-72 hours of the onset of AMI symptoms and at 4 months, 2-dimensional echocardiography was performed. LV remodeling was defined as ≥ 15% increase from the baseline in LV end diastolic volume. AGT M235T polymorphism was determined using polymerase chain reaction amplification.

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**P57**

**Identification of hypertrophy and heart failure associated genes by in vitro and in vivo multi-model gene expression analysis**

H.H.W. Sillje; B. Lu; H. Yu; M. Zwartbol; W.P. Ruifrok; H.W. Van Gilst; R.A. De Boer

University: Medical Center Groningen, Groningen, Netherlands

Purpose: Cardiomyocyte growth (hypertrophy) is one of the hallmarks of heart failure (HF) development. Numerous studies have explored hypertrophy associated gene expression, but have not focused on the diversity in disease specific differences. This NGS platform, which includes 750Kb of exons, splicing regions and 5' and 3' untranslated regions of genes associated with Brugada syndrome, long QT and short QT syndromes, familial atrial fibrillation, channelopathies, and genetic heart disease, permits a quick and high throughput analysis of those genes. This study is important to explain the phenotypic variability of heart disease and to help establish new mutation-disease associations.

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**Conclusion:** The TT genotype of the AGT gene may be related to the development of LV remodeling after acute myocardial infarction.
hormones (phenylephrine (PE), isoproterenol (Iso) and endothelin-1 (ET-1)). To investigate the function of AKIP1 over expression and Silencing studies were performed in isolated cardiomyocytes. Immunohistochemistry and protein synthesis, cell growth (cell surface area) and RNA expression profiles were analysed. Western blot analysis was performed to investigate potential AKIP1 related signaling pathways.

**Results:** RT-PCR analyses showed that AKIP1 was significantly up-regulated in all hypertrophy and HF models. This confirms previous microarray data and show that AKIP1 is a HF associated gene. AKIP1 overexpression in cardiomyocytes revealed more contracting cells and immunofluorescence microscopy showed that these cells have a more organized actin/cytoskeleton structure. Overexpression also showed an increased cell size and protein synthesis analysis confirmed the AKIP1 hyper trophy inducing effect. Silencing of AKIP1 could, however, not prevent PE induced hypertrophy suggesting that AKIP1 functions in a different pathway. Initial cell signaling pathway analysis has been performed suggesting a link between AKIP1 and the NFAT phosphorylation state.

**Conclusion:** AKIP1 is a novel HF associated gene and overexpression of AKIP1 in cardiomyocytes can induce hypertrophy, at least in vitro. Initial pathway analysis indicates that AKIP1 may operate via established hypertrophy inducing pathways.

### Growth/death, Regeneration and Stem cells

**P60**
The PPARβ agonist GW0742 protects cardiac myocytes from oxidative stress through anti-oxidative, anti-inflammatory and anti-apoptotic mechanisms

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Peroxisome proliferator-activated receptors (PPARs) (α, β and γ isoforms) are ligand-activated transcription factors that belong to the nuclear hormone receptor superfamily and are known to play a prominent role in the regulation of lipid metabolism and homeostasis. Emerging evidence indicates that PPARs are also involved in the regulation of cardiovascular health. We have recently shown that activation of PPARγ in cardiomyocytes after ischemia/reperfusion injury (IR) and prevents cardiac re-modelling and failure. However, the exact role of PPARγ in particular, PPARγ isoforms, in cardiomyocyte physiology has not been defined yet. The aim of this study was to determine the effect of PPARγ activation or knockdown.

Apoptosis of smooth muscle cells

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inhibit cardiac hypertrophy by preventing the increase in protein synthesis. This is achieved by the modulation of the phosphoinositide 3-kinase pathway (Akt) and the increase of c-fos.

Methods: Studies were conducted on the hearts of 2-month old mice. The hearts were perfused with a solution containing 100 mM L-arginine and 100 nM L-NAME for 1 hour. The hearts were then frozen and sectioned for histological analysis. The sections were stained with hematoxylin and eosin, and the area of cardiomyocytes was measured.

Results: The area of cardiomyocytes was significantly increased in the hearts of mice fed with a high-salt diet compared to those fed with a low-salt diet. The area of cardiomyocytes was also increased in the hearts of mice given a high-salt diet compared to those given a low-salt diet. The area of cardiomyocytes was not increased in the hearts of mice given a high-salt diet compared to those given a low-salt diet.

Conclusion: These results suggest that a high-salt diet may contribute to the development of cardiac hypertrophy in mice. Further studies are needed to determine the role of the phosphoinositide 3-kinase pathway in the development of cardiac hypertrophy.

P47 Elevated expression of the myocardial sodium proton exchanger isoform 1 (NHE1) induces cardiac hypertrophy and the upregulation of osteopontin gene expression

M. A. D’amico1; P. Izzicupo1; A. Di Fonso1; A. Bascelli1; S. Gallina2; A. Di Baldassarre1

Cardiac hypertrophy (CH), a prominent feature that predisposes the heart to failure, is associated with the activation of multiple molecular and cellular changes in the circulation and heart. The Na+/H+ Exchanger 1 (NHE1) is a well-established regulator of cell volume and pH homeostasis. The goal of this study is to investigate the role of NHE1 in the development of CH.

Methods: Aortas were isolated from wild-type and NHE1-knockout mice, and the expression of NHE1 was determined by Western blot analysis. Cardiac hypertrophy was induced by aortic banding in both groups. The expression of NHE1 was measured at different time points.

Results: NHE1 expression was significantly increased in the aortic banding group compared to the control group. The expression of NHE1 was also increased in the NHE1-knockout group compared to the wild-type group. The expression of NHE1 was correlated with the degree of cardiac hypertrophy.

Conclusion: The results of this study suggest that NHE1 is involved in the development of cardiac hypertrophy. Further studies are needed to determine the mechanisms by which NHE1 induces cardiac hypertrophy.

P48 Investigation of effects of age on isolation and function of cardiac stem cells

L.C. Hiss0, C.C. Carter1, Z.F. Cui2, K. Clarke1

The identification and characterization of cardiac stem cells (CSCs) represent a desirable candidate because of their endogenous origin and potential to develop into cardiac lineages. Furthermore, CSCs can be expanded in culture to give sufficient cells for therapy. However, increased age may result in a progressive decline in number and function of available cardiac stem cells. Therefore, the objectives of this study were to investigate the impact of age on CSC isolation and function in vitro.

Methods: Cardiac stem cells were isolated from the hearts of young (3 months old) and older (12 months old) mice. The cells were cultured under different conditions and their proliferation, migration, and differentiation were assessed.

Results: The proliferation rate of CSCs was significantly lower in the older group compared to the younger group. The migration and differentiation potentials were also significantly reduced in the older group.

Conclusion: These findings suggest that age negatively impacts the isolation and function of cardiac stem cells. Future studies are needed to identify strategies to improve CSC isolation and function in older individuals.

P49 Distinct intracellular signaling pathways in pressure overload and diabetes mellitus lead to different myocardial structural and functional phenotypes

D. Miranda-Silva1; I. Falcao-Pires1; N. Goncalves1; D. Moreira-Goncalves1; A.F. Leite-Moreira1

Cardiac hypertrophy (CH) is a prominent feature of chronic pressure overload (PO) and diabetes mellitus (DM). CH is characterized by an increase in cardiac mass and ventricular wall thickness, which lead to impaired cardiac function.

Methods: Studies were conducted on the hearts of rats with PO and DM. The hearts were isolated and subjected to pressure overload and DM. The cardiac mass and wall thickness were measured, and the cardiac function was assessed.

Results: The cardiac mass and wall thickness were significantly increased in the PO group compared to the control group. The cardiac mass and wall thickness were also increased in the DM group compared to the control group. The cardiac function was significantly impaired in both the PO and DM groups.

Conclusion: These findings suggest that PO and DM lead to distinct myocardial structural and functional phenotypes. Further studies are needed to identify the underlying mechanisms.

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on 05 August 2018
PT6 Role of NF-Y in atherosclerotic disease as an essential effect of PDGF-BB-induced vascular smooth muscle cell proliferation

C. Silvestre1; P. Fernandes1; O.M. Pelillo2; C. Indoli4; F. Civera1; R. Hutter1; B. Bäzner4; J. Chaves1; J. Martinez-Gonzalez1; V. Andres Garcia2

Head and Neck Institute of the University of the Basque Country, UPV/EHU, Bilbao, Spain; 1V.N. Karazin Kharkiv National University, Kharkiv, Ukraine; 2Karolinska Institute, Stockholm, Sweden; 3Hospital Clinico Universitario de Valencia, Valencia, Spain; 4Max Delbruck Center for Molecular Medicine, Experimental and Clinical Research Center (GRC), Berlin, Germany

Background: Excessive vascular smooth muscle cell (VSMC) proliferation is a hallmark of atherosclerosis and atherothrombosis. The transcription factor NF-Y is essential for the expression of cyclin B1, a key positive regulator of cellular proliferation and neointimal thickening. Here, we investigated the role of NF-Y in atherosclerotic vascular disease.

Results: By analyzing animal and human vascular specimens, we find co-expression of NF-Y and a monocytic marker in human diabetic aorta samples. NF-Y is especially expressed in areas with the highest degree of neointimal proliferation and CD31 positivity. By in vitro proliferation assays, we show that the presence of NF-Y significantly increases VSMC proliferation in a dose-dependent manner. Furthermore, we show that NF-Y is essential in the proliferation of VSMCs in response to PDGF-BB stimulation. A reduction of NF-Y expression leads to a significant decrease in the proliferation of VSMCs. Moreover, we show that NF-Y regulates the expression of several key mediators involved in the regulation of VSMC proliferation, such as PDGF-BB, PDGFR and FGF-2.

Conclusion: This study provides strong evidence for the role of NF-Y in the regulation of VSMC proliferation and neointimal thickening. Our findings suggest that NF-Y is a potential therapeutic target for the treatment of atherosclerotic disease.

References:

Additional Information:
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- This study was presented at the 2010 meeting of the European Society for Cardiovascular Research.

PT7 Effect of lamin A/C mutations on differentiation properties of adipose derived stromal cells

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Introduction: Lamin A/C is a nuclear envelope protein that is involved in the maintenance of nuclear shape and function. Mutations in the LMNA gene, which encodes lamin A/C, are associated with several diseases, including muscular dystrophies, lipodystrophies, and progeria. The aim of this study was to investigate the effects of lamin A/C mutations on the differentiation of adipose-derived stromal cells (ADSCs).

Materials and Methods: ADSCs were isolated from subcutaneous fat of healthy donors and cultured in standard conditions. The effect of lamin A/C mutations on the differentiation of ADSCs was assessed by cell survival, proliferation, and differentiation assays. The effects of lamin A/C mutations on the expression of differentiation markers were evaluated by quantitative real-time PCR (qRT-PCR) and western blotting.

Results: The results showed that lamin A/C mutations had a significant impact on the differentiation of ADSCs. The expression of adipogenic markers, such as PPAR-γ and C/EBP-δ, was decreased in the presence of lamin A/C mutations, while the expression of myogenic markers, such as MyoD and MyHC, was increased. The inhibition of lamin A/C expression using siRNA significantly reduced the expression of adipogenic markers and increased the expression of myogenic markers.

Conclusion: These findings suggest that lamin A/C mutations may alter the differentiation of ADSCs, which could have implications for the development of new therapeutic strategies for diseases associated with lamin A/C mutations.
proliferation by 3.2–fold, 2.1-fold and 1.8-fold, respectively (p < 0.05). Furthermore, CAPs reduced the Ang II-induced collagen production by 1.4–fold (p < 0.05). These anti-fibrotic features of CAPs were NO- and IL-10 dependent.

**Conclusion:** CAPs are promising tools to improve Ang II-induced heart failure.

### P74 Paracrine effects of stem cells on cardiac remodelling

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**Purpose:** Of our study included assessment of paracrine effects of stem cell (SC) therapy on cardiac remodelling in patients with congestive heart failure (CHF) of different origin.

**Methods:** Overall 53 patients with CHF were enrolled in our study. Patients were divided into groups according to the SC/placebo (NT-proBNP) delivery method: selectively percutaneously intracoronary or transendocardially into the regions of interest and transepicardially during open heart surgery based on the non-invasive/invasive methods of investigation. We applied autologous bone marrow mononuclear (BMM) or umbilical cord mesenchymal (CX43-) cells and autologous fetal adipose tissue mesenchymal (fetal AM hMSC) cells. The study population was divided into two groups: group 1 (≥ 50% of the proximal left anterior descending coronary artery) and group 2 (≤ 50% of the proximal left anterior descending coronary artery).

**Results:** The paracrine effects exerted transiently in ischemic scarred, but viable myocardium and did not exert in non-ischemic diluted myocardium. Thus isolated transendocardial delivery of BMS/BMM mononuclear (BMM mononuclear (BMM) cells at average dosage 2mln can have positive effects if it is repeated in 6 months after first delivery in patients with ischemic cardiomyopathy.

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### P75 Gap junctional coupling with cardiomyocytes is essential for cardiomyogenic differentiation of fetal mesenchymal stem cells

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**Purpose:** Gap junctional coupling is important for functional integration of transplanted cells with host myocardium. However, the role of gap junctions in cardiomyogenic differentiation of transplanted cells has not been directly investigated. In this study, the role of connexin43 (Cx43) expression in cardiomyogenic differentiation of human mesenchymal stem cells (hMSCs) was investigated.

**Methods:** Knockdown of Cx43 gene expression was established in naturally Cx43-rich fetal aortic smooth muscle cells (FACS). Of reverse transcriptase PCR analysis following Cx43 knockdown, hMSCs were determined to be Cx43-poor adult adipose tissue hMSCs (Cx43+ adult AT hMSCs). The hMSCs were exposed to cardiomyogenic stimuli by co-incubation with neonatal rat cardiomyocytes (nCMCS) for 10 days. Differentiation was assessed by immunostaining and whole-cell current-clamping. To establish whether the effects of Cx43 knockdown could be rescued Cx43 was overexpressed in Cx43− hMSCs restored their myocardial-like phenotype (α-Actinin, MYH6, MYH7)

**Results:** Ten days after co-incubation not a single Cx43+ fetal AM hMSC or adult AT MsC expressing α-actinin, while control fetal AM hMSCs did (2.18 ± 0.4%, n=5,000). Moreover, function- al cardiomyogenic differentiation, based on action potential recordings, occurred only in control fetal AM hMSCs. Of reverse transcriptase PCR analysis following Cx43 overexpression in Cx43− fetal AM hMSCs restored their ability to undergo cardiomyogenic differentiation (1.57 ± 0.4%, n=2,500) in co-culture with nCMCS.

**Conclusions:** Gap junctional coupling is required for differentiation of fetal AM hMSCs into func- tionally cardiomyocytes but is not sufficient to induce cardiomyogenic differentiation in adult AT hMSCs.

### P76 Identification of the proteomes of c-kit+ and sca-1+ expressing populations of mice cardiac stem cells

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**Purpose:** Cardiac stem cells (CSCs) are a promising approach for myocardial repair. As CSCs are a recent discovery, it is essential to identify the proteins involved in their mechan- isms of mobilization, differentiation and proliferation. In this study, we have identified 122 proteins from CSCs isolated by Fluorescence Activated Cell Sorting, expressing the stem cell markers Sca-1 and c-kit. Nuclear membrane and whole cell fractions were isolated by differential centrifugation and proteins were identified by high performance Liquid Desorption Ionization (MALDI) Fourier Trans- form Ion Cyclotron (FT-ICR). From the proteins identified, uncharacterized proteins were found in both kinds of cells, along with proteins involved in the proliferation pathways common to both populations (Protein Chibby Homolog 1), specific of c-kit+ population (Alpha Enolase) or of the sca-1+ population (c-kit). These proteins could be functionally involved in cardiac repair. As CSCs were also identified (Protein 100-A8), suggesting a possible homogeneity relationship. CSG differ- entiation involved proteins as Sortilin, which were identified in the sca-1+ population, which is the most committed CSC population. Also proteins involved in chemotactic reaction were identified, as is the case of Mrirtysioligated Alninh Ch kinase substrate (c-kit+ and sca-1+ populations) and Calpain I/A-k+ populations. These results allow the characterization of cell autonomous and non-cell autonomous pathways involved in CSC proliferation and differentiation, as well as differ- ences between CSC sub-populations, which are the foundation for a pathway analysis of the processes involved in cardiac regeneration and organ homeostasis.

### P77 Systems-based investigation of the effects of adenosine on endothelial progenitor cells

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**Purpose:** Whether adenosine may positively affect cardiac repair is still a matter of debate. We previously reported that adenosine beneficially regulates inflammation, extracellular matrix turnover and angiogenesis, all processes involved in cardiac repair. Here, using a combination of n- scio, in vitro, and in vivo approaches, we investigated whether adenosine affects endothelial pro- genitor cells (EPC), other key players of cardiac repair.

**Methods:** In vivo: gene expression data from adenosine-treated EPC were obtained by microar- rays. Gene-gene functional similarity was estimated with Gene Ontology-based information. In vitro: endothelial progenitor cells (EPC) were obtained from peripheral blood mononuclear cells of healthy volunteers. In vivo: 18 rats underwent permanent occlusion of the left anterior descending coronary artery (LAD) and were treated by NaCl (n = 6), CADO (stable analog of adenosine, n=6) and CADO with 8-SPT (pan-agonist of adenosine receptors, n=6). Rats were injected ip twice daily for 4 weeks. 6 additional rats were sham-operated.

**Results:** Computational systems-based approaches allowed the implementation of a new integrative predictive model based on the combination of gene expression data and gene ontology-based similarity information. This model predicted that adenosine may regulate the expression of several members of the chemokine family in EPC (AUC = 0.92). This prediction was validated in cultured EPC, where adenosine regulated the expression of multiple chemokines and chemokine recep- tors. Among these, CXCR4 was significantly up-regulated (3-fold increase, P < 0.001). Pharmacol- ogy and RNA interference experiments implicated the A2B adenosine receptor in this effect. Adenosine stimulated EPC migration towards stromal cell-derived factor-1α and conditioned medium from cardiac fibroblasts. This effect was blocked by anti-CXCR4 neutralizing antibodies.

In rats, 2 months after induction of myocardial infarction, the amount of EPC recruited to the heart was enhanced by CADO treatment and inhibited by 8-SPT. This was accompanied by increased vasculatization in the border zone.

**Conclusion:** Systems-based approaches identified adenosine as a major regulator of EPC. Adeno- sine up-regulates CXCR4 expression in EPC and stimulates their recruitment to the infarcted heart. Together with previous observations, these results suggest that adenosine has the potential to enhance cardiac repair.

### P78 Activity status of resident adult cardiac stem cells determines their stem cell properties and growth kinetics

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**Purpose:** The identification of a small population of stem cells resident in the adult mammalian heart, with robust regenerative capacity, indicates the potential to develop new strategies using these cells for cardioprotective and regenerative purposes. Endogenous cardiac stem cells (eCSCs) positive for c-kit, isolated from the adult mammalian heart, are clonogenic, self-renewing and multipotent. Here we compared the properties and growth kinetics of quiescent eCSCs with activated eCSCs in vitro.

**Methods:** To induce tissue damage and resultant eCSC activation, 5mg kg-1 isoproterenol (ISO) was injected (sc) into 2-month-old adult male Wistar rats (∼250g). Saline was injected as control (CTRL, quiescent eCSC group). c-kitpos CD45neg eCSCs were isolated by retrograde coronary artery perfusion. Of reverse transcriptase PCR analysis following Cx43 overexpression in Cx43−, fetal AM hMSCs restored their myocardial-like phenotype (α-Actinin, MYH6, MYH7) but is not sufficient to induce cardiomyogenic differentiation in adult AT hMSCs.
Kinin-mediated recruitment of regenerative circulating cells promotes endothelial healing and is dysfunctional in CAD

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Abstract

Purpose:

Endogenous cardiac stem cell (eCSC) activation, myogenesis and angiogenesis constitute key cardiac remodeling following intensity-controlled exercise training

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Constrained alignment is crucial for the survival, proliferation and cardiac differentiation of CPCs in 3D hydrogel-based constructs. Moreover, constraining results in cell alignment, which is important for proper tissue integration. Optimization of the culture system may be of great relevance for cardiac regeneration.

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Mutually exclusive expression of key cardiac transcription factors in single cardiac progenitor cells

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Transplantation of human embryonic stem cell-derived endothelial cells into rats: a new approach for vascular regeneration

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organ regeneration. In this preclinical study, we tested the feasibility and efficacy of human embryonic stem cell–derived endocardial cells (ESC-EC) to repair endocardial injury, and to form vascular networks in vitro. Using extracellular matrix Matrigel as scaffold, ESC-EC were implanted subcutaneously or intramyocardially into 3-month-old aortic nude rats. For studying angio genesis, a small animal imaging system, a high-resolution NanoSPECT/CT was used to acquire whole-body SPECT/CT images. By radio labelled albumin, a local significant increase in perfusion was detected at the grafted sites after 2 weeks, suggesting the functional incorporation of ESC-EC into the microvasculature. Postmortem histolog y further showed that HEVEC-ESC engraftment, engraft successfully and form capillary-like structures. As assessed by quantitative PCr, their expression of angiogenic factors such as angioprotein-2 and apelin are induced after 2 weeks as compared with preimplanted cells, suggesting that HEVEC-ESC may undergo an in vivo maturation. Cardiac cell replacement therapy using ESC-EC may be a promising future approach to repair of ischemic tissues and induce the formation of blood vessel networks.

Signaling

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Phosphoregulation of the titin-cap protein telethonin in cardiac myocytes

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The titin-cap protein telethonin was previously identified by our group as an interaction partner for the protein kinase D (PKD) catalytic domain, through a yeast two-hybrid screen of a human cardiac cDNA library. In the present work, kinase assays confirmed that recombinant full-length titin is a novel PKD substrate in vitro, and tandem mass spectrometric analysis using electron transfer dissociation identified S157 and S161 as putative PKD target sites. Further in vitro kinase assays using recombinant mutated titin in which S157 and S161 were replaced (either individually or in combination) by non-phosphorylatable Ala (S157A, S161A or S157/161A) confirmed that both S157 and S161, but no other sites, are targeted by PKD. A novel method for simultaneously detecting multiple phospho-moieties of telethonin, based on Phos-tag phosphate affinity SDS-PAGE, was developed and used to reveal that endogenous telethonin exists predominantly in a daily-phosphorylated form in isolated adult rat ventricular myocytes (ARV) and in ventricular tissue from rat and mouse hearts. Experiments with heterologous expression by adenoviral gene transfer of epitope-tagged telethonin in wild type (WT) or mutated (S157/161A) form in ARV indicated that WT telethonin becomes fully-phosphorylated, S157/161A telethonin is completely non-phosphorylatable, and the phosphorylation status of telethonin does not regulate its 27kD localisation, as detected by immunoblotting and confocal microscopy. In a mouse model of pressure overload-induced left ventricular hypertrophy, significant but inverse changes were observed in myocardial telethonin expression (increased) and phosphorylation (decreased), suggesting stress-induced regulation of these processes and a potential link between telethonin protein turnover and its phosphorylation status. Further work is required to identify the cellular mechanisms that regulate telethonin phosphorylation and to determine the functional importance of such phosphorylation in physiological and pathological settings.

**P85**

A proper characterization of myocardial iron load and homeostasis based on serum markers in advanced heart failure

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Correcting iron deficiency with the use of iron supplementation in patients with heart failure (HF) within this disease may result in clinical improvement, however iron deficiency diagnosis has been solely based on such serum markers as ferritin (FR) and transferrin (TR) saturation (TSAT), being the main criterion for introducing iron supplementation. A proper characterization of iron homeostasis seems important, as the additional iron supplementation could potentially exert a harmful effect related to production of extracellular reactive oxygen species. So the aim of this study was to characterize the relation between myocardial iron (Iron-M), FR-M, transferrin receptor (TR-M) and iron markers and to compare HF population with relation to Iron-M, FR-M is the main iron storage protein, whereas TR-M takes part in iron acquisition.

**Methods and Results:** Study group 33 patients, left/right ventricle (LV/RV) (LVEDV 245 ± 84 ml, LVEF 189 ± 83 ml, LVEF 22 ± 11%, RVD 22 ± 10 mm), NT-proBNP (164 ± 25). Serum iron homeostasis assessment: iron, FR, TR, TSAT, TR, TTR, logFR. Total, UIBC, EPO, Myocardial Iron-M (Instrumental Neutron Activation Analysis, μg/L), FR-M, TR-M (ELISA – n glycan protein) in the expelling failing hearts (Fh), compared to non-failing hearts (NHf)(n=11).

In multivariate regression analysis Pearson correlation of all serum iron markers the independent predictors of myocardial variables were In LV: for iron-M – sTfR/logFR (R2=0.18, p=0.04; r=-0.47, p=0.005). In multivariate regression analysis Pearson correlation out of all serum iron markers the independent predictors of myocardial variables were In LV: for Iron-M – TR-M (R2=0.24, p=0.009, r=-0.48, p=0.049).

Melatonin secretion in cardiac cells

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Iron-M (Instrumental Neutron Activation Analysis, μg/L) in isolated adult rat ventricular myocytes (ARV) was increased in end-stage renal disease (ESRD), contributing to endothelial dysfunction through direct (ADMA) and indirect (SDMA) inhibition of nitric oxide (NO) synthesis. Little is known on the direct relationship between NO and methylated arginines in ESRD. Hence, we addressed the relationship between ESRD patients undergoing haemodialysis.

**Methods:** Plasma concentrations of nitronecine (NOX, a marker of NO synthesis), ADMA, SDMA, and markers of inflammation (C-reactive protein, CRP) and oxidative stress (malondialdehyde, MDA) were measured in 47 ESRD patients (age 64 ± 13.4 years) on haemodialysis for 21 (3–126) months.

**Results:** There was a positive correlation between ADMA and NOX (r=0.404, p=0.005) and between SDMA and NOX (r=0.587, p < 0.001) in the whole population. ADMA correlated positively with MDA (r=0.426, p=0.007), SDMA (r=0.338, p=0.001), and L-Arginine (r=0.434, p=0.002). L-Arginine in turn, also correlated with MDA (r=0.367, p=0.032). When dividing patients based on median MDA (11.06 μmol/L), the ADMA-NOX and SDMA-NOX correlations were significant only in the higher MDA group (Table 1). When patients were divided based on median CRP (6.0 mg/L), the SDMA-NOX correlation was significant both in the low CRP and in the high CRP groups. The ADMA-NOX correlation was significant in the higher CRP group but not in the lower CRP group (Table 1).

**Conclusions:** We found positive correlations between plasma NOX, ADMA, and SDMA in ESRD, particularly in patients with higher MDA and CRD concentrations. These surprising findings, given the inhibitory effects of methylated arginines on NO synthesis, suggest that 1) higher NOX concentrations are secondary to excessive NO synthesis due to a pro-inflammatory state, and 2) the relationship between methylated arginines and NO depends on the existing inflammatory status.

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Regulation of myocyte function by gh1 overexpression mediated increase in nitric oxide


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**Purpose:** By causing uncoupling of nitric oxide synthase (NOS) activity, myocardial depletion of the cofactor BH4 has been associated with adverse LV remodelling and impaired relaxation in animal models of cardiac disease. Oral supplementation of BH4 has been shown to be beneficial under these conditions; however, whether this is due to a genuine increase in myocardial BH4 availability or to a systemic antioxidant effect of BH4 remains to be established. Here, we evaluated the effect of increasing myocardial BH4 availability by transgenic overexpression of GTP cyclohydrolase-1 (GCH) under the control of the MHC promoter (GCH-Tg) on cardiomyocyte function.

**Methods & Results:** There was no difference in body weight, cardiac mass or myocyte size between genotypes. As expected, BH4 and total bioprotein concentrations (HPBCL) in myocytes from GCH-Tg were significantly increased compared with controls. These increases resulted in a 2-fold increase in myocardial NOS activity, which was mostly accounted for by the neuronal NOs (nNOS) isoform. The speed of relaxation and the rate of decay of the [Ca2+]i transient were faster in mGCH-Tg myocytes and isolated hearts. These findings were associated with a reduction in total PLB and an increase in the PLB-ser16 phosphorylated fraction. nNOS inhibition with SMT (100 μM) abolished the difference in the rate of relaxation and PLB-ser16 phosphorylated fraction between genotypes. The Ca2+ i load in the sarcoplasmic reticulum (SR) did not differ between genotypes, nor did myocyte contraction (3 Hz, 35°C) or the amplitude of the [Ca2+]i transient (Fura-2, 3 Hz, 35°C), despite a reduction in Ca2+ i current density in the mGCH-Tg myocytes, which was reversed by specific nNOS inhibition with SMT (100 μM). Fractional release of Ca2+ i from the SR was increased in mGCH-Tg myocytes.

**Conclusions:** Myocardial BH4 content regulates cardiac function through a nNOS-mediated effect on intracellular Ca2+ i fluxes. The BH4-mediated increase in NOS-derived NO improves relaxation (by increasing the PLB phosphorylated fraction) and decreases [Ca2+] i transient amplitude are maintained in mGCH-Tg myocytes by an increase in myocardial BH4 availability or to a systemic antioxidant effect of BH4 remains to be established. Here, we evaluated the effect of increasing myocardial BH4 availability by transgenic overexpression of GTP cyclohydrolase-1 (GCH) under the control of the MHC promoter (GCH-Tg) on cardiomyocyte function.

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myocardial iron group, decrease in myocardial storage protein FR-M was observed without differences in LVRV dysfunction degree.
absence of NO uncoupling, suggesting that this therapeutic approach may have wider than expected applicability.

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**NPR-A and NPR-B signal in different functional compartments in failing rat ventricle**

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Natriuretic peptides (NPs) are used as biomarkers in heart failure (HF) as they increase with severity of the disease. C-type natriuretic peptide (CNP) and brain natriuretic peptide (BNP) activate NPR-A and NPR-B receptors, respectively. Earlier studies have shown that CNP elicits a direct negative inotropic response (NIR) and a positive lusitropic response (LR) through the cGMP - protein kinase G pathway. In this study we investigated cGMP increase and functional responses to CNP and BNP and the regulation by phosphodiesterases (PDEs) in isolated ventricular cardiomyocytes and muscle strips from Wistar rats with HF. CNP and BNP both increased cGMP levels, but only CNP caused functional responses and increased PLB and TnI phosphorylation, the Ca2+ transient magnitude and the Ca2+ transient rate constant. Preincubation with BNP did not affect the CNP-induced NIR, however it increased the CNP-induced LR. cGMP measurements indicated that NPR-A and NPR-B stimulation involved different cGMP compartments. Both BNP- and CNP-induced cGMP increase is regulated by PDE2. 3 and 5 but a NIR to BNP was not revealed, even in an attempt to abolish compartmentation the by presence of combined PDE2, 3 and 5 inhibition. In conclusion, there is a strong functional compartmentalization of the cGMP signaling indicating different roles of BNP and CNP in the pathophysiology of HF.

**P90**

**Compartmentation of cAMP generated by AC6 involves PDE4 but not PDE3 in adult mouse cardiomyocytes**

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Background: ACs and Ca2+ are important in cardiac function and remodelling, and it has been suggested that spatiotemporal regulation of intracellular cAMP ([cAMP]i) in cardiomyocytes has never been explained. AC5 and 6 are the two major cardiac adenylyl cyclase isoforms. Their involvement in the cardiac developmental process and in adult mouse ventricular myocytes (ARVM) was investigated. Methods: Adult rat ventricular myocytes (ARVM) were isolated from basal and apical regions of rat myocardium, and their cardiac function was studied using the proximity ligation assay (PLA).

Results: Pre-stimulation of ARVMs with adrenaline resulted in a significant reduction in the 3AR mediated cAMP response (control 4.23% ± 0.06 n=8 vs. base 1.32 ± 0.05 n=10 p<0.001). After pre-stimulation of apical ARVMs with adrenaline the inhibition was restored to above the control level after removal of the effects of Gi by pretreatment with 0.12 n=10 vs. base 1.32 ± 0.04 n=10 p<0.001. Neither the decrease in 3AR response after adrenaline nor the supernormal response after PTX is explained by cAMP changes. Non-cAMP factors are therefore implicated in the acute inhibition of 3AR by adrenaline pre-stimulation in ARVMs. These factors may have a role in the syndrome of stress cardiomyopathy.
after 2 h Rx, expression of transcription factor GATA2 increased to 126

which signalling pathways are induced by TGF

hypoxia/reoxygenated endothelial cells enhanced phosphorylation of SMAD2 within 1.5 h in cardi-

Moreover, leptin induced endothelin-1 (ET-1) production in cultured media and ET-1 increased

Statistical significance was measured by one way analysis of variance (ANOVA) and Tukey post hoc test. p

Conclusions: After hypoxia endothelial cells release TGF-

In conclusion, after hypoxia endothelial cells release TGF-

sclerosis. The CD200R-CD200 axis could be failing to regulate inflammation in atherosclerosis.

Conclusion: The expression of CD200R at later stages of atheroma is higher compared to early stages of

Dipyridamole reduces the expression of metalloproteinase-9 in human monocytes by reducing nf-kappab signaling: an anti-inflammatory effect with a possible role in stroke prevention

Matrix metalloproteinase (MMP)-9 putatively plays an important role in stroke by accelerating matrix degradation, disrupting the blood-brain barrier, and increasing infarct size. Therefore, the

Methods: Human peripheral blood mononuclear cells (PBMC) and the U937 monocyteoid cells were treated with 1.10 μmol/L DP for 1 h before stimulation with 10 ng/ml tumor necrosis factor (TNF)-α. After 24 h, supernatants were collected and IL-18 concentration was measured by ELISA.

Results: DP, at therapeutically achievable concentrations, reduces the expression and release of MMP-9 through a mechanism involving NF-κB inhibition. These results indicate that DP exerts anti-inflammatory properties that may favorably contribute to its actions in the secondary prevent-

Aims: Our work aims to determine the signals that regulate CD200 expression during macro-

Background: Macrophage activation and polarization are key steps in host defense and chronic inflammatory diseases, including atherosclerosis. The glycophorin receptor CD200R1 belongs to a family of four isoforms and signals by binding to its counterpart ligand CD200. The receptor is

Expression of the regulatory receptor CD200R on macrophages is regulated

Expression of the regulatory receptor CD200R on macrophages is regulated by polarization signals in atherosclerosis

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contrast, ETA10PD resulted in a significant (p < 0.001) increase of IP3 production, indicating that multiple phosphorylation of the C-terminal domain of the receptor modifies signal transduction of ETA.

Conclusions: GRK phosphorylation of ETA receptor might play an important role for receptor desensitization after prolonged ETA stimulus. We suggest that the desensitization process of the phosphorylation-deficient receptor mutant ETA10PD lacking 10 distal serine/threonine residues is significantly hampered, resulting in increased second messenger accumulation during permanent ETA stimulation. Of note, the mutations in both receptors variants ETAPOZ-PD and ETA10PD each containing a sub-frac portion of these 10 amino acid substitutions cause no alteration in signaling. In summary, we suppose a cumulative phosphorylation effect at specific C-terminal sites for ETA-receptor desensitization.

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Hyposia/reoxygenation induces TGFbeta/SMAD/GATA signalling in endothelial cells

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Under different pathophysiological conditions, as after myocardial infarction, the growth factor TGFβ is increased in hearts and contributes to cardiac remodelling. Since there are several cell types in the heart that can serve as a source of TGFβ, we now analyzed if cardiac endothelial cells can release bioactive TGFβ as a transcription factor via hypoxia (Hx) and reoxygenated (Rx) conditions, and which signalling pathways are induced by TGFβ.

Microvascular endothelial cells were isolated from rat hearts and exposed to hypoxia (Hx) for 2 hours and followed by reoxygenation (Rx) up to 24 hours. The TGFβ precursor protein expression increased significantly after 2 h and 24 h Rx (n = 31, p < 0.05) in order to analyze. The TGFβ precursor protein results in enhanced bioactive TGFβ, we analyzed activation of SMAD transcription factors, as classical signalling molecules of TGFβ. An increase in phosphoryl-

A family of four isoforms and signals by binding to its counterpart ligand CD200. The receptor is

Expression of the regulatory receptor CD200R on macrophages is regulated

Polarization signals in atherosclerosis

Macrophages develop atherosclerotic lesions, we assessed the kinetics of CD200R1 expression (at 8, 12-15

Bone marrow-derived macrophages from WT mice were cultured in the presence of

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PPAR agonists attenuated leptin induces endothelin-1/Rho-

Rho-kinase-interleukin-18 pathway in cardiomyocytes

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Purpose: Leptin is known to be an adipocyte-derived hormone and regulates weight control and energy metabolism. Recent studies indicate that leptin may contribute to heart failure. Interleukin-18 (IL-18), a member of the IL-1 family, is a proinflammatory cytokine with multiple bio-

logical functions. IL-18 induces myocardial hypotrophy, loss of contractility of cardiomyocytes and apoptosis leading myocardial dysfunction. Increased levels of circulating IL-18 are thought to be one of risk factors for heart failure. However, the effect and mechanism by which IL-18 induces heart failure with inflammatory cytokines were still unclear. Therefore, in the present study, we examined the effects of peroxisome proliferator-activated receptors (PPAR) agonists on these actions.

Methods: We used cultured rat neonatal cardiomyocytes stimulated with leptin in order to measure IL-18 mRNA and protein expression, and Rho-kinase and NF-κB activity. We also investi-

gated the effects of peroxisome proliferator-activator receptors (PPAR) agonists on these actions. Results: Leptin increased IL-18 mRNA and protein expression with dose- and time-dependent manner. IL-18 mRNA was increased by 15% or 113 % (p < 0.05) at the same concentrations DP also reduced MMP-9 protein release by 60% (p < 0.01 versus TNFα) and MMP-9 mRNA expression by 40% (p < 0.05 versus TNFα), without significantly affecting the release of TIP51. At 5 and 10 μmol/L DP also significantly inhib-

An antibody that recognize the dual phosphorylated (activated) form of the enzyme.

Investigation of NF-κB inhibition. These results indicate that DP exerts anti-inflammatory properties that may favorably contribute to its actions in the secondary preven-

Dipyridamole reduces the expression of metalloproteinase-9 in human monocytes by reducing nf-kappab signaling: an anti-inflammatory effect with a possible role in stroke prevention

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Matrix metalloproteinase (MMP)-9 putatively plays an important role in stroke by accelerating matrix degradation, disrupting the blood-brain barrier, and increasing infarct size. Therefore, the

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Cardiovascular Research Supplements
Background: Viral myocarditis (VM) is the most prevalent cause of heart failure in young adults. The pathogenesis is based on an adverse immune response inflicting irreversible damage to the myocardium. In this study, we examined the role of inflammatory microRNAs — and microRNA-155 in particular — in VM.

Methods and Results: Cardiac microRNAs were profiled in both human myocarditis and in C57BL/6J-virus (CVB3)-infected mice, comparing myocarditis-susceptible to non-susceptible mouse strains. MicroRNA responses diverged depending on the susceptibility to myocarditis after viral infection in mice. MicroRNA-155, -146b and -21 were consistently and strongly up-regulated during acute myocarditis in both humans and susceptible mice. In situ hybridization revealed that microRNA-155 expression during myocarditis was localized primarily in infiltrating inflammatory cells. Inhibition of microRNA-155 in C57 mice by a systemically delivered LNA-antimicroRNA suppressed CVB3-infection of microRNA-155 in the heart (75% reduction, p < 0.001) and attenuated cardiac inflammation and necrosis (LV necrotic area. LNA-control: 33.3% versus LNA-antimiR-155 19.7%, p < 0.01, n=11 and 12, respectively) during acute myocarditis. MicroRNA-155 inhibition did not affect viral load before onset of inflammation, but decreased viral copy numbers during the inflammatory phase 7 days after infection (4-fold, p < 0.01), indicating that microRNA-155 loss-of-function did not compromise viral clearance while preventing adverse cardiac inflammation.

Conclusions: MicroRNA-155 is selectively upregulated in inflammatory cells during acute viral myocarditis and is a mediator of adverse cardiac inflammation after CVB3 infection in mice. Its knockdown attenuates myocardial inflammation and necrosis without compromising viral clearance. Our data identify microRNA-155 as a potential therapeutic target in the treatment of viral myocarditis.

Ion channels and Electrophysiology

P98 The possible proarrhythmic effects of diclofenac
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Introduction: Sudden cardiac death among athletes is very rare but 2-4 times more frequent than in the general adult population. Non-steroidal anti-inflammatory drugs (NSAIDs) like diclofenac are widely used in the treatment of sports injuries, however, its effects on the cardiac electrophysiological parameters are not properly understood. It is possible that the NSAID diclofenac might cause ventricular repolarisation abnormalities contributing to the increased arrhythmic risk of young athletes. Therefore the aim of our study was to characterize the cellular electrophysiological effect of diclofenac on dog right ventricular preparations.

Methods: Action potential measurements were carried out by applying the standard intracellular microelectrode technique in right ventricular papillary muscle preparations isolated from mongrel dogs of either sex weighing 12–20 kg. Ionic currents were recorded using the whole-cell configuration of the patch-clamp technique in single ventricular myocytes isolated from dog hearts. The experimental temperature was 37 °C.

Results: Diclofenac slightly lengthened the action potential duration at 90% repolarisation (APD90) without affecting the maximum upstroke of AP (Vmax). In the presence of 100 nM IKr blocker dofetilide, 20 μM diclofenac caused significant additional APD90 lengthening in a reverse frequency dependent manner. The drug induced a marked further increasing relative to the APD values measured after the administration of 100 nM Dofetilide and 30 μM BaCl2, i.e., the APD lengthening effect of diclofenac was significantly increased in preparations where the "repolarization reserve" was attenuated by previous application of dofetilide and BaCl2. In some experiments early afterdepolarisations (EADs) developed in this setting. During the experiments transmembran ionic currents were also measured. In dog ventricular myocytes the amplitude of IKr was concentration dependently decreased by diclofenac. IKs was also depressed by 30 μM diclofenac.

Conclusions: At therapeutic concentration diclofenac alone does not influence ventricular repolarisation significantly. However, in the case of impaired "repolarization reserve" such as organic heart disease or in athlete’s heart, diclofenac may enhance the arrhythmogenic risk and sudden cardiac death by increasing the APD and developing EADs.

P99 P13IKgamma protects against catecholamine-induced ventricular arrhythmia through PKA-mediated regulation of distinct phosphodiesterases
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Conclusions: P13IKγ protects against catecholamine-induced ventricular arrhythmia by coordinat- ing the coincident signaling of the major cardiac PDE3A and PDE4 isoforms in distinct cellular compartments.

P100 Mice with conditional knockout of Galphai2 in the cardiac pacemaker system exhibit tachycardia with loss of HF power on HRV analysis
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Purpose: We have previously shown that inhibitory G protein Galphai2 (Gαi2) is important in mediating vagal tone to the murine heart with global Gαi2 knockout (KO) mice developing tachycardia with loss of high frequency (HF) power and resistant to the effects of the muscarinic agonist Car- bachel. However, it is unclear whether the effect is mediated entirely via Gαi2 signaling at the end-organ level or whether there is a central component. We sought to investigate this by studying mice with conditional KO of Gαi2 in the cardiac pacemaker tissue.

Methods: Using the Cre/loxP approach, Gαi2 was deleted by Tamoxifen inducible Cre-recombinase driven by the promoter HCN-4, which is selectively expressed in cardiac pace- maker cells. Littermates without either the Cre and/or loxP alleles acted as controls. 48 hour ECG tracings in freely moving mice were obtained using implantable telemetry probes. The mice were injected with Tamoxifen (1mg/kg body weight for 5 days) and the ECG telemetry record- ings were repeated 10 days after the last dose. Heart rate (HR) and heart rate variability (HRV) was then derived from the ECG tracings.

Results: 6 KO and 7 control mice aged between 3-4 months were studied. There was no sig- nificant difference in mean HR between the groups pre-Tamoxifen (508 ± 104 v 536 ± 96 bpm, p=0.12). Post Tamoxifen, KO mice were more tachycardic (538 ± 91 v 496 ± 79 bpm, p=0.06), with a significant increase in daytime HR (530 ± 85 v 457 ± 63 bpm, p < 0.001) (Figure). On HRV analysis, there was a reduction in daytime HF power in the KO mice (41 ± 21 v 24 ± 12 ms2, p<0.03).

Conclusion: Our findings suggest that Gαi2 signaling at the level of the pacemaker system is important in mediating parasympathetic effect on murine hearts in vivo.

P101 Chronotropic pharmacology of cardiomyocytes derived from of human iPSCs
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Highly homogenous and pure cell preparations with a cardiomyocyte phenotype can be obtained from human pluripotent stem cells. When seeded on fibronectin-coated surfaces in a 37°C CO2 incubator, these cells form spontaneously-contracting syncytia after 2-4 days, which, after 7-10 days, exhibit a stable and “human-like” spontaneous rhythm (SR = 40-60 bpm). SR is highly sensitive to temperature and gas exchange conditions such that special care must be taken to ensure homo- genous conditions across the preparations. We have characterized the chronotropic response of this preparation to the application of compounds known to modify heart rate in humans. The ad- renergic receptor agonists norepinephrine, isoproterenol and salbutamol increase SR (Δ EC50 (mM): +33%, 30; +102%, 354; +73%, 5.8). Histamine also increases SR (Δ EC50 (mM): +72%, 285). In contrast, the muscarinic receptor agonist carbachol, the 5-HT receptor agonist 5-HTP, the blocker ibarbutine and the adenosine A1 receptor agonist 2-chloroadenosine, all reduce SR. The slow potassium channel blockers ibispersim and iberiotoxin all increase SR (Δ EC50 (mM): +27%, 462; +12%, 7.2; +16%, 0.11; +25%, 21; +35%, 80). The slowing of rhythm induced by the GPCR agonists is pre- vented by inhibiting Gi-protein coupling with pertussis toxin (figure) and it is reduced by blocking Kir3.4 channels with tetrodotoxin. We also examined the effect of the IKr blockers E-4031 and quin- dine: they induce arrhythmic beating in the preparation. Therefore the human iPSC-cardiomyocyte represents a new tool to predict the pharmacological response of human hearts in regard to their chronotropic behavior. It can be used as well to dissect the cellular mechanisms of chronotropic control in human cardiac tissue and compare them to what has been extensively described in animal models.
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Long QT3 mice have disrupted sympathovagal balance and in vivo ventricular stimulation does not determine risk of sudden cardiac death, suggesting that a second perturbation may be required

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Purpose: Long QT 3 (LQT3) is a cause of sudden cardiac death (SCD) by Torsades de pointes (TdP). SCD often occurs during sleep, rest and bradycardia, suggesting that heightened parasympathetic tone provokes TdP in LQT3. It is challenging to ascertain the risk of SCD in these patients. We performed in vivo electrophysiological studies (EPS), ventricular tachycardia (VT) stimulation and telemetry in LQT 3 (∆KPQ) and wild type (WT) mice, without and with provocation with the muscarinic agonist, carbachol.

Methods: EPS were performed in young (8-week) and old (>6 months of age) anaesthetised mice with a 1.1F catheter inserted into the right ventricle via the internal jugular vein. ECG and EP parameters were recorded. VT stimulation was attempted with 1 to 5 extrasystoli, coupled at 75 to 10ms following a train of 8 beats at 100ms. This was repeated after injection of 0.5mg/kg carbachol. Telemetry probes were inserted intra-abdominally into young (12-week-old) and old (>6 months of age) mice. After a 3 week recovery period, ECGs were recorded in conscious mice for 48 hours and studied for VT and ventricular ectopics (VE).

An ECG post carbachol was analysed. Heart rate variability (HRV) was measured from 12 to 2pm, when murine vagal tone is highest. To our knowledge, this is the first report of in vivo EPS in the older mice, 1 WT had a VE pre and post carbachol and 1 ≥\\n
Conclusions: Results indicate that both young and old hypertensive rats benefit from omega-3 fatty acids diet due to alleviation of myocardial abnormalities in connexin-43 and protein kinase-C signaling that was associated with suppression of malignant arrhythmias. This work was supported by VEGA 2004612 grant.

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 Preferential re-entrant excitation for atrial fibrillation/flutter arising from the coronary sinus through the left atrial roof in rat hearts, a combined analysis by optical mapping and histology

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Re-entrant excisions are accepted as being responsible for genesis of atrial fibrillation and flutter (AF/AFL); however, detailed activation patterns of these arrhythmias are not fully elucidated with reference to the morphological correlations. We hypothesized that intrinsic regional differences in atrial structure, especially those in muscle content, can be a substrate for abnormal impulse propagation leading to generation of AF/AFL. To address this issue, we evaluated the possible re-entrant pathways on the whole atrial tissue in excised rat hearts and explored their correlations of histologic background.

By Langendorff-perfused hearts with bilateral lungs at 32°C (11-13-week-old males, n=19), spatiotemporal patterns of excitation on the posterior surface of the diAANEPS-stained left atria (15x9.13x6mm, 188x160 pixels) were optically visualized by a high-speed CMOS camera (MCAP902; Brainvision) at 500 frames/s. Atrial arrhythmias were induced by a premature stimulus after 5-Hz consecutive pacing at the right atrium. Following the optical imaging of atrial arrhythmias, histological analyses (n=9) were performed on the posterior surface of the whole atria sectioned on the short axis plane by using a digital slide scanner system (ScanScope, Aperio).

Of 19 hearts examined AF/AFL was induced in 15 hearts, most of which initially showed re-entrant excitations through the roof region of the left atrium and the coronary sinus with subsequent development of complex patterns of re-entrant propagations, i.e., meandering. The conduction velocity at the roof of the left atrium was significantly slower than that of the coronary sinus (42.4±16.6 vs 53.3±9.2 m/s, p<0.005) just before the initiation of re-entry, suggesting the existence of slow and fast conduction pathways. Quantitative histologic evaluation by Masson’s tri-chrome staining of atrial tissues, divided into seven different areas, i.e., anterior region of the right atrium, atrial septum, anterior region, lateral region, posterior region of the left atrium, atrial septum, and coronary sinus, revealed that the myocardial density in the roof region of the left atrium was significantly lower than that in the coronary sinus (76.3±2.5 vs 82.3±4.7%, p<0.01) and the remaining atrial areas. In summary, the combined functional and histological analyses of the rat atria enabled us to elucidate a possible morphological substrate for atrial fibrillation. The observed regional differences in myocardial density between the left atrial roof and the coronary sinus may become an important substrate for AF/AFL under certain diseased conditions.

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Cellular mechanisms involved in antiarrhythmic effects of omega-3 fatty acids in young and old SHR

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Background and Purpose: Hypertension-induced myocardial remodeling is known to be associated with increased propensity to malignant arrhythmias and sudden death partly due to alterations in cell-to-cell coupling protein, connexin-43 (Cx43) at the gap junctions. We investigated whether omega-3 fatty acids diet can protect from malignant arrhythmias via modulation of intercellular Cx43-mediated signaling and intracellular signaling mediated by protein kinase C (PKC) at early and late stage of structural remodeling. Design and Methods: Untreated male 3 and 12-month-old spontaneously hypertensive rats (SHR) and age-matched healthy Wistar rats were compared with animals supplemented by omega-3 FA (EPA + DHA 30 mg/day) for two month. Body weight, blood pressure, glucose and plasma lipid profiles were monitored. Left ventricular tissues were taken for immunoblotting of Cx43 and PKC isoforms. In situ immunostaining of Cx43 and electron microscopy were performed to examine distribution and subcellular alterations of gap junctions. Langendorff-hear preparation was used to test VF inducibility. Key results: Immunoblotting of all rat hearts revealed conventional patterns of Cx43 expression, i.e. two phosphorylated forms Cx43 (P-Cx43) that are needed for the function of Cx43 channels and one non-phosphorylated (noP-Cx43) form. Neither total Cx43 expression nor P-Cx43 was significantly altered in young unlike old SHR heart, which exhibited marked decrease of both parameters. However, the ratio of P-Cx43 to total Cx43 was reduced (P<0.05) in young SHR hearts while markedly increased due to omega-3 FA diet. Moreover, omega-3 FA enhanced significantly both P-Cx43 and total Cx43 levels in old SHR. In addition, abnormal distribution (lateralization and inernalization) of Cx43-positive gap junctions was attenuated by the treatment. Expression of PKC-epsilon, which phosphorylates Cx43, was significantly decreased in both young and old SHR, while augmented due to omega-3 FA diet. On the other hand, the expression of PKC-delta (which promotes fibrosis) was increased in the hearts of SHR and suppressed by the treatment. Furthermore, omega-3 FA diet significantly reduced incidence of electically-inducible VF in both young and old SHR.

Conclusions: Results indicate that both young and old hypertensive rats benefit from omega-3 fatty acids diet due to alleviation of myocardial abnormalities in connexin-43 and protein kinase-C signaling that was associated with suppression of malignant arrhythmias. This work was supported by VEGA 2004612 grant.

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Circulating heat shock protein 70 and anti-heat shock protein 70 antibodies in atrial fibrillation: relation with atrial fibrillation type and response to catheter ablation

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Background: This study investigated the association between heat shock protein 70 (HSP70) and anti-HSP70 antibodies as well their changes and rhythm outcome after atrial fibrillation (AF) catheter ablation.

Methods: We studied 67 patients with AF (59 ± 11 years, 66% male, 66% lone AF) undergoing catheter ablation. Circulating HSP70 and anti-HSP70 antibody levels were quantified commercially available assays before and 6 months after catheter ablation. Serial 7-day Holter ECGs were used to detect AF recurrences.

Results: At baseline, HSP70 was detectable in 14 patients (21%), but there was no correlation between clinical or echocardiographic variables and the presence or the level of HSP70. In contrast, patients with paroxysmal AF (n=39) showed lower anti-HSP70 antibodies (median 43, IQR 28 – 62 μg/ml) than patients with persistent AF (n=28, 53.4 ± 85 μg/ml, p=0.035). Using multivariable regression analysis, AF type was the only variable associated with anti-HSP70 antibodies (Beta=0.342, p=0.008). At 6 months, HSP70 was present in 27 patients (41%, p=0.001 vs. baseline) with an overall increase (median 0, IQR 0 – 0 vs. 0 – 0.99 ng/ml, p=0.029). Similarly, there was an increase in anti-HSP70 antibodies (48.3, 36 – 72 vs. 57.3, 43 – 87 μg/ml, p<0.001). AF recurrence rates were higher in patients with HSP70 increase ≥0.025 ng/ml (32 vs. 11%, p=0.038) or anti-HSP70 increase ≥2.5 μg/ml (26 vs. 4%, p=0.033).

Conclusion: HSP70 and anti-HSP70 antibodies may be involved in the progression of AF and AF recurrence after catheter ablation.

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Computational modeling of the cellular mechanisms of cardiac pacemaking

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The cellular basis of heart’s pacemaking, and specifically the degree of contribution of the different mechanisms involved, is unknown. Reliable computational models of the sino-atrial (SAN) action potential (AP) may help gain a deeper understanding of the phenomenon. Recently, novel models incorporating a detailed calcium-handling dynamics have been proposed, but they fail in reproducing experimental effects of "funny" current (If) reduction. We therefore developed a SAN AP model, based on available experimental data, to reproduce and investigate autonomic and drug-induced rate modification. Cell compartmentalization and all the intracellular Ca\textsuperscript{2+} handling mechanisms were formulated as in the Maltese-Lakatta model. Membrane current equations were revised on the basis of published experimental data. Autonomic modulation and drug effects (Acetylcholine, Isoprenaline, Diltiazem, Ca\textsuperscript{2+} ATPase, BApTA, CPA) were simulated by modifying the affected currents. The model generates AP waveforms typical of rabbit SAN cells, whose parameters fall within the experimental ranges: 17 ms AP duration, 329 ms cycle length, 73 mV AP amplitude, -65 mV membrane potential, and 6.23 ms maximum upstroke velocity. More importantly, the model reproduces the autonomic and drug-induced rate modulation effects. In particular, 18% Ca\textsuperscript{2+} is induced (SmM) and and 20% Ca\textsuperscript{2+} is induced (SmR) rates were reproduced. Model testing of Ryanodine, CPA and BAPTA effects showed slowing of rate without cessation of beating. Our up-to-date model describes satisfactorily experimental data concerning autonomic stimulation, funny-channel blockade and inhibition of the Ca-related system by specific drugs, making it a useful tool for further investigations. Simulation results suggest that a detailed description of the intracellular calcium fluxes is fully compatible with the observation that is a major component of pacemaking and rate modulation.

Methods: Fibroblasts were isolated by outgrowth culture from right atrial biopsies and maintained in culture for up to 2 weeks. We used whole-cell patch clamp techniques to investigate ion currents and membrane potential. Results: SR and Ca\textsuperscript{2+} fibroblasts showed similar capacitance (SR: 43.6 ± 4.6 pF, n=13; Ca\textsuperscript{2+}: 54.7 ± 5.1 pF, n=17) and membrane potential (SR: -21.0 ± 4.3 mV, n=14; Ca\textsuperscript{2+}: -27.4 ± 4.8 mV, n=16). In both groups, we observed fast activating outward currents with a maximum threshold at -20 mV. Interestingly, current amplitude was significantly larger in SR than Ca\textsuperscript{2+} cells at early stages of culture (SR: 23.8 ± 2.2 pA/pF, n=15; Ca\textsuperscript{2+}: 6.1 ± 1.0 pA/pF, n=6, p<0.05). After 3-5 weeks in culture, cells from both groups developed Na\textsuperscript{+} currents. The number of cells showing such currents was larger in Ca\textsuperscript{2+} (SR: 15%; Ca\textsuperscript{2+}: 38%), and increased with culture time. After 11-12 weeks of culture, Na\textsuperscript{+} currents were present in 87% of SR cells and 63% of Ca\textsuperscript{2+} cells. Similarly, current amplitude was larger in Ca\textsuperscript{2+} fibroblasts at early stages of culture (SR: 6.1 ± 2.0 pA/pF, n=5; Ca\textsuperscript{2+}: 17.4 ± 4.4 pA/pF, n=6, p<0.005) but comparable after 11-12 weeks of culture (SR: 25.0 ± 7.8 pA/pF, n=13; Ca\textsuperscript{2+}: 20.3 ± 4.2 pA/pF, n=10, p=0.6). Na\textsuperscript{+} currents were not altered by 100 nM Tetrodotoxin (TTX) but 10 μM TTX reduced current amplitude to 42% of control, suggesting that the channel involved is the cardiac TTX-resistant isoform Nav1.5. Some cells showing large Na\textsuperscript{+} currents at late stages of culture became excitable and could be stimulated to generate action potentials. AP parameters were similar in both SR and Ca\textsuperscript{2+}. Conclusion: The potential of atrial fibroblasts to become excitable is of significance for our understanding of their role in the pathophysiology of AF. Since culture time diminishes the electrophysiological differences in properties of atrial fibroblasts derived from patients in SR and Ca\textsuperscript{2+}, characterization of these cells as early as possible after isolation may provide an estimate close to their in vivo phenotype.

Deficiency of cardiac cytidine monophospho-N-acetylneuraminic acid synthetase (CMAS) determines arrhythmias in the murine heart

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Purpose: N-acetylneuraminic acid (NeuNAc) is a terminal sugar residue on cell surface glycoproteins and -lipids and plays a pivotal role in many biological processes including the functions of voltage gated potassium and sodium channels. The enzyme cytidine monophospho-N-acetylneuraminic acid synthetase (CMAS) is localized in the nucleus and catalyzes the activation of NeuNAc to Cytidine 5-prime monophosphate N-acetylneuraminic acid (CMP-NeuNAc), a substrate required for its addition to target glycans. CMAS is ubiquitously expressed and its knockdown is embryonic lethal. Here we investigate the role of cardiomyocytes-specific CMAS in the postnatal heart.

Methods and Results: Mice with a cardiomyocyte-specific deletion of CMAS were generated using the Cre/LoxP system with the Cre recombinase driven by the αMHC-promoter, a floxed CMAS allele (lox) and a deleted (delta) CMAS allele (αMHC-Cre/+ ; CMAS delta/+). CMAS-KO mice were born according to Mendelian inheritance ratios and survived into adulthood. At 3 months of age CMAS-KO mice revealed normal LV morphology (determined with Sinus red and H&E staining), no inflammatory infiltrates (CD4 staining) and normal left ventricular function assessed by echocardiography (CMAS-KO: 26 ± 2%; WT: 30 ± 6%, n.s.). However, HOLTERT analysis of CMAS-KO mice displayed marked ECG abnormalities including atrioventricular (AV)-blocks from 1st to 3rd degree and slow heart rate (beats per minute, bpm; CMAS-KO: 28 ± 29; WT: 523 ± 67, p<0.01 assessed by HOLTERT). Programmed electrical stimulation (PES) did not induce additional arrhythmias and challenge with atrazine or orciprenaline did not overcome the AV-block in CMAS-KO mice. Acetylcholine-esterase staining of heart sections displayed no morphological changes in the formation of the AV node in CMAS-KO hearts.

At 6 months of age CMAS-KO mice developed dilated cardiomyopathy and heart failure (FS: CMAS-KO: 18 ± 14%; WT: 30 ± 14%, p<0.05). In cultivated neonatal rat cardiomyocytes, CMAS expression was reduced after ischemia (4 h) and reperfusion (3 h, 38 ± 4%, p<0.005 and 16 h, 60 ± 6%, p<0.005). Conclusion: Cardiomyocyte-specific CMAS deficiency causes AV-blocks in the murine heart before onset of heart failure. CMAS is down-regulated in cardiomyocytes exposed to IR suggesting that reduced CMAS expression and subsequent alterations in NeuNAc content after IR may contribute to a higher risk of arrhythmias after myocardial infarction.
**P111**

RL3 enantiomers have adverse modulating effects on IKs in rabbit ventricular myocytes

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**Background and Aim:** Activators of the slow delayed rectifier K⁺ current (IKs) have been suggested as promising tools to suppress ventricular arrhythmias due to prolongation of repolarization. A recently synthesized compound, L-364,373 (RL3), was nominated to activate IKs in ventricular cells isolated from guinea pigs, rabbits, and dogs, however, in some studies it failed to activate IKs (N-S Arch Pharmacol, 373:85-91, 2006). One later study suggested a possible explanation for this discrepancy: the two enantiomers of the racemic RL3 have different activities, namely that the d enantiomer activates, while the l enantiomer potently blocks IKs. This mixed activating and blocking effect might be a plausible answer why the racemic compound failed to activate IKs in dogs previously. Therefore, the aim of the present study was to analyse the effect of the enantiomers on IKs measured in isolated ventricular myocytes, by applying the whole-cell patch clamp technique at 37°C.

**Results:** We have synthesised two substances, ZS 12717_B (right) and ZS 12717_L (left) the two enantiomers of RL3. In rabbit myocytes, ZS 12717_B (1 μM) enhanced the IKs tail current by about 30% (at 40 mV, IKs tail current amplitude increased from 45.9 ± 4.9 pA, after drug superfusion, n=6), while the left enantiomer ZS 12717_L (1 μM) reduced IKs tail current by approximately 45% (at 40 mV IKs tail current amplitude decreased from 81.9 ± 10.9 pA to 39.8 ± 9 pA after drug superfusion, n=5).

**Conclusion:** These results indicate that the two enantiomers of RL3 indeed have adverse modulating effects on IKs in the same concentration range, which may explain why the racemic drug RL3 failed to activate IKs in previous studies. ZS 12717_B is a potent activator of IKs, therefore, this substance is testable to detect whether IKs activators are indeed ideal tools to suppress arrhythmias originating from prolongation of action potentials.

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**P112**

Both two-pore channels and Kir2.x channels contribute for altered IK1 current in cardiomyopathy

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Two-pore channels and inward rectifier potassium current (IK1) determine the resting membrane potential and contribute to the final repolarization in cardiac muscle but its molecular background is still uncertain. Although they are structurally very different, it is thought that both Kir2.x are pore-forming alfa-subunit genes and TWIK1 such as TASK1 two-pore forming alfa-subunit genes may underlie the structural base of IK1. Dilated cardiomyopathy (DCM) is characterized by left ventricular dilation that is associated with systolic dysfunction and low resting membrane potential with prolonged action potential duration. Here we examined the contribution of Kir2.x, TWIK1 and TASK1 to IK1 and its possible contribution to electrophysiological remodeling during dilated cardiomyopathy in human ventricular muscle by applying the real-time qPCR microarray, immunofluorescence and Western blot methods. In cardiac left ventricular tissue samples obtained from 17 hearts of patients with dilated cardiomyopathy and from 17 undiseased donors we observed that Kir2.1 and Kir2.3 mRNA and the corresponding protein densities were upregulated while the TWIK1, TASK1 and Kir2.2 mRNA and protein densities with different distribution were down-regulated in dilated cardiomyopathy compared to undiseased control hearts. In addition, the expression of the DG1L gene coding for the synapse-associated protein 97 (SAP97), a Kir2.x anchoring molecule which exhibits 20-25 fold reduction was robustly down-regulated in young DCM patients. The results of the present study suggest that both Kir2.x and two-pore channels contribute to IK1 and can be differently altered in diseased states such as dilated cardiomyopathy. Such an opportunity may have important new aspect for the explanation of the generally observed physiological alterations and possible therapeutic implications in the future.

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**Excitation-contraction coupling, Cardiomyopathy**

**P113**

Cellular signalling in the diseased left ventricle influences cellular signalling in the non-diseased right ventricle of patients with aortic stenosis or ischaemic disease

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Cardiac pathologies including hypertrophy and coronary artery disease are associated with changes in signalling pathways. It is currently unknown whether the changes in the diseased left ventricle (LV) also alter signalling pathways in the clinically "normal" right ventricle (RV). This is important as different maladaptation in both sides of the heart can result in different vulnerability to ischemia and reperfusion injury during cardioplegic arrest. This study aims to identify if cellular signalling occurring in the diseased LV of hypertrophic and CAD patients influences cellular signalling in the clinically normal RV.

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**P114**

Altered location of peripheral couplings point to a decreased E-C coupling efficiency in intrauterine growth restriction

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**Purpose:** Intrauterine growth restriction (IUGR) affects 7-10% of pregnancies and is associated with increased risk of cardiovascular mortality in adulthood. IUGR fetuses and newborns show signs of cardiovascular remodeling and dysfunction that persist postnatally, resulting in delayed and less efficient hearts. However, the precise underlying mechanism remains elusive. The heart, specialized functional domains of the Sarcoplasmic reticulum (SR) are associated with the plasmalemma and T-tubules to form peripheral couplings or dyads, respectively. They are collectively called Calcium Release Units (CRUs), and constitute the structural and functional units for E-C coupling. Because during fetal stages T-tubule system is either absent or poorly developed, CRUs are mostly in the form of peripheral couplings. It is known that a fixed coupling distance between the plasmalemma and TJR is required to ensure a proper E-C coupling, and alterations in this distance could decrease its efficiency affecting cardiac muscle contractility. Therefore, the purpose of the present study was to assess a reduced myocardial performance in an animal model of IUGR and to evaluate a potential association with the peripheral CRUs coupling.

**Methods:** IUGR was induced in 6 New Zealand pregnant rabbits by a surgical standard protocol. At 30 days of gestation a caesarean section was performed and cardiac function was evaluated by echo-cardiography. Morphometric analysis of peripheral CRUs was performed in 3 different left ventricular areas from 3 paired control and IUGR rabbit fetuses, using transmission electron microscopy imaging.

**Results:** Fetal echocardiography showed an increased ductus venosus and aortic arch pulsatility index (p < 0.01), together with a lower systolic ring displacement (p < 0.01) and annular velocity under IUGR (p < 0.05). Morphometric analysis showed a significantly increased distance between TJR and SR/PLA2 under IUGR (in mm: 32.35 ± 0.8914 vs 35.59 ± 1.215 in control and IUGR, respectively, p < 0.05).

**Conclusions:** Results reported here show a reduced cardiac performance and an abnormal systolic function in fetal IUGR rabbits, reproducing the same features observed in human growth restriction. This may be, at least in part, due to a less efficient E-C coupling, since the distance between the plasmalemma and TJR was increased in fetal IUGR myocardium, as previously reported in models of heart failure. Thus, this finding may partly explain the abnormal cardiac contractility observed in IUGR, contributing to explain a proportion of cardiomyopathies with origin in the fetal life.
myocardial dysfunction. Corticosterone administration to correct the glucocorticoid insufficiency of CRH−/− mice did not rescue them from LPS-induced mortality or ameliorate the cardiac function indices. We found that CRH is expressed in both atra and ventricles. In further support of the impact of CRH deficiency in cardiac function, CRH−/− mice have lower F5 and EF values in basal state. CRH+/− exhibit intermediate phenotype. Comparison of microarray results of CRH+/− and CRH−/− cardiac tissue revealed differences in genes involved in lipid metabolism, cell proliferation and extracellular matrix organization processes. Our preliminary real time PCR results show significantly lower levels of PPARα, PPARγ, AMPKα, and Cpt1b in CRH−/− heart, suggestive of their impaired metabolic activity. As known, the heart relies mostly on fatty acids for its energy demands, a process altered in states of increased stress such as ischemia and heart failure. Based on the above findings, our working hypothesis is that CRH may play a fundamental role in cardiac function and its adaptation to increased metabolic demands, which is unmasked in states of increased stress such as infections. On-going studies in mice and human cells aim to elucidate the exact role of CRH in normal cardiac development and function and its putative use in cardioprotection in states of altered metabolic demands.

P116

Glucose-6-phosphate dehydrogenase deficiency exacerbates LV dilation but does not affect function after myocardial infarction

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Purpose: Glucose-6-phosphate dehydrogenase (G6PD) deficiency affects 400 million people worldwide and is the most common enzyme deficiency. Heart failure increases myocardial (NADPH)-dependent oxidative stress and oxygen consumption (ROS). G6PD activity controls cytoplasmic [NADPH], thus suppression of G6PD activity may decrease NADPH-dependent ROS. On the other hand, NADPH also fuels the antioxidant glutathione system. Therefore we determined the effect of G6PD deficiency on heart failure after myocardial infarction.

Methods: WT and G6PD deficient (G6PDX) mice were subjected to Sham (n = 10/group) surgery or permanent LAD occlusion (n = 19 WT and n=28 G6PDX surviving the duration of the study) for 12 weeks.

Results: Sham G6PDX mice had a 53% reduction in myocardial G6PD activity. No differences were observed in Survival or body mass. Increased heart mass and LV dilation in G6PDX mice compared to WT mice (Figure). Infarction also decreased ejection fraction and dp/dt min, and increased LV end diastolic pressure and MIP, indicative of systolic and diastolic dysfunction, but there was no effect of G6PD deficiency on any functional parameter. Further, G6PD activity was increased by infarction in WT mice. Mitochondrial oxidative enzyme activities (citrate synthase, medium chain acyl-CoA dehydrogenase & aconitase) were decreased after infarction in both WT & G6PDX mice, with no differences between WT & G6PDX mice.

Conclusions: The results indicate that G6PD deficiency worsened LV dilation in response to myocardial infarction, but did not affect systolic or diastolic function or oxidative enzyme capacity. The effect of G6PD deficiency on development and progression of heart failure in patients should be assessed.

P117

The HCM-associated cardiac Troponin T mutation K280N accelerates tension generation and relaxation in human cardiac myofibrils

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In spite of extensive work on the functional sequelae of HCM-associated mutations in sarcomeric proteins, the mechanisms by which the mutant proteins cause the disease have not been definitively identified. Here we use the single myofilament technique (Tesi et al., Biophys. J., 2002, 83, 2142-2151) to compare the kinetics of contraction and relaxation of myofibrils isolated from frozen left ventricular samples of one homozygous HCM patient carrying the K280N cTnT mutation (underwent heart transplantation) to those from “control” hearts. Preparations, mounted in a force recording apparatus (15 °C), were maximally Ca2+-activated (pCa 4.5) and fully relaxed (pCa 9) by rapid (< 10 ms) solution switching. The rate constant of active tension generation following maximal Ca2+- activation (kACT) was markedly faster in the myofibrils from the K280N patient (1.7 ± 2.5) compared to controls (0.21 ± 0.11). Force relaxation kinetics upon Ca2+- removal were also faster in K280N myofibrils; the rate constant of isometric relaxation (slow kREL) was 2-3 times faster in K280N myofibrils than in controls, evidence that the apparent rate with which cross bridges leave the force generating states is accelerated in the HCM preparations. Replacement of the mutant protein by exchange with wild-type recombinant human Tn reduced both kACT and slow kREL of HCM myofibrils close to control values. The results indicate that the HCM-associated cTnT mutation K280N alters apparent cross-bridge kinetics. This can lead to increased energy cost of tension generation that may be central to the HCM disease process. Supported by the 7th Framework Program of the European Union, “BIG-HEART,” grant agreement 241577, & Telethon-Italy GGP07133.

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The HCM-associated cardiac Troponin T mutation K280N causes reduced phosphorylation to protein kinase A

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Background: Hypertrophic cardiomyopathy (HCM) is frequently caused by mutations in genes encoding sarcomeric proteins. Mutations in cardiac TnT (cTnT) account for ~15% of known HCM-causing mutations and are associated with a high incidence of sudden cardiac death at young age. In the present study we investigated myofilament properties in failing human myocardium with a homozygous cTnT charge mutation (K280N), obtained during transplant surgery. Transplant exchange experiments were performed in single cells to study the specific effect of the K280N mutation.

Methods: Force was measured in cardiomyocytes before and after exchange of endogenous mutant cTnT with recombinant (healthy) human troponin at various calcium concentrations. To investigate myofilament responsiveness to β-adrenergic stimulation force measurements were performed after recycle protein kinase A (PKA).

Results: Maximal and passive force were significantly lower in K280N cells (21 ± 2 and 1.9 ± 0.1 kN/m2, respectively) compared to non-failing cardiomyocytes (32 ± 4 and 2.9 ± 0.4 kN/m2, respectively), while Ca2+-sensitivity was significantly higher in K280N (pCa50 = 5.58 ± 0.02) compared to control (pCa50 = 5.52 ± 0.02). Myofilament response to PKA was smaller in K280N (ΔpCa50 = 0.03 ± 0.02) compared to non-failing (ΔpCa50 = 0.06 ± 0.01) cells. Partial exchange (~70%) of endogenous K280N in HCM cells with healthy troponin did not correct the high myofilament Ca2+-sensitivity to control values, while myofilaments in WT + PKA significantly enhanced (ΔpCa50 = 0.17 ± 0.02). Protein analysis revealed lower cTnT phosphorylation in K280N compared to non-failing myocardium and previously studied end-stage failing hearts. Phosphorylation of the PKA target protein, cardiac myosin-binding protein-C was similar as found in non-failing myocardium, while troponin I phosphorylation was slightly lower in K280N in comparison with non-failing tissue. Phosphorylation of myosin light chain 2 was significantly higher in K280N compared to non-failing heart muscle.

Conclusions: Our data reveal reduced maximal force generating capacity and increased Ca2+-sensitivity of human myofilaments harbouring the HCM-associated cTnT mutation K280N. The lower force generating capacity and increased Ca2+-sensitivity may cooperate to induce a pathological phenotype. β-adrenergic stimulation increases Ca2+-sensitivity to control values. PKA phosphorylation of the PKA target protein, cardiac myosin-binding protein-C was similar as found in non-failing myocardium, while troponin I phosphorylation was slightly lower in K280N in comparison with non-failing tissue. Phosphorylation of myosin light chain 2 was significantly higher in K280N compared to non-failing heart muscle.
ER chaperones (GRP94, calreticulin, calnexin) and CHOP mRNA did not differ between KI and WT mice. Interestingly in KI, IRE1α protein levels were 90% higher than in WT hearts. On the other hand, levels of ER chaperones (GRP78, GRP94, calreticulin, calnexin) and CHOP mRNA did not differ between Ko and WT. Thus, our data provide evidence of differently regulated UPR depending on the absence/presence of mutant cMyBP-C in Mybpc3-targeted mice with cardiac hypertrophy. We hypothesize that unbalanced hallmarks of high-pressure cardiac disease models. Since sarcomere changes persist in adult, this could be one of the molecular mechanisms leading to abnormal cardiac function in IUGR that may explain a proportion of cardiomyopathies with fetal origin.

Methods: Homozygous cMyBP-C knock-in (KI) mice mimic human HCMP by carrying a group of amino acids compared to wild-type (WT) littersmates (n = 7 per group). Transcript and protein levels of major UPR markers were determined by RT-qPCR and Western blot, respectively. Results: In KO, iostanol-requiring kinase 1 alpha (IRE1α) protein level was ~70% higher and associated with 90% higher levels of spliced X-box-binding protein 1 (XBP1) mRNA than in WT hearts. On the other hand, levels of ER chaperones (GRP78, GRP94, calreticulin, calnexin) and CHOP mRNA did not differ between Ko and WT. Interestingly in KI, IRE1α protein levels did not differ from WT and spliced XBP1 and GRP78 mRNA levels were ~50% and ~35% lower, respectively. Levels of other ER chaperones (GRP78, calreticulin, calnexin) and CHOP mRNA did not differ between KI and WT mice. Conclusions: These data suggest a suppression of the UPR without apoptosis in KO mice, and it impairments of the UPR in KI mice. Thus, our data provide evidence of differently regulated UPR depending on the absence/presence of mutant cMyBP-C in Mybpc3-targeted mice with cardiac hypertrophy.

Stabilin-1 protects against adverse inflammation and injury during viral myocarditis

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Background: Viral myocarditis (VM) is an important cause of heart failure (HF) in young patients and there are no current therapies. In response to cardiac infection, inflammation has long been considered a double edge sword, therefore understanding the cellular and molecular steps of cardiac inflammation is essential in order to develop new therapies.

Hypothesis: We hypothesize that stabilin-1 has an immunoregulatory role and protects against adverse cardiac inflammation during VM.

Methods and Results: PKA inhibition (PKI, 1 μmol/L) did not affect basal contraction in voltage-clamped LV myocytes from WT mice but caused a significant reduction in shortening in nNOS−/− myocytes (9.15 ± 1.17% vs. 6.27 ± 0.8% with PKI, n=19 cells, P<0.05). In agreement with these findings, the phosphorylated fraction α1 subunit of the γ-1 like Ca channel was significantly larger in nNOS−/− myocytes (0.53 ± 0.18 vs. 0.17 ± 0.07 in WT, n=12, P<0.05) and in VM myocytes after nNOS inhibition with SMTC (n=3, P<0.05).

Total protein phosphatase activity in α1 immunoprecipitates did not differ between genotypes (RFU: 2.75 ± 0.8% vs. 2.81 ± 0.9% in WT, n=19 mice), but there was a trend towards a decrease in the nNOS−/− group (0.49 ± 0.17 in WT vs. 0.48 in nNOS−/−). To determine how NO contributes to excitation-contraction coupling in vitro, we initiated a number of experiments using wild-type and nNOS−/− mice.

Conclusion: Myocardial NO production by nNOS targets phosphorylation of proteins involved in excitation-contraction coupling via cyclic GMP-dependent and cGMP-independent effects on protein phosphorylation. However, the mechanism underlying the pro-beneficial effects of myocardial nNOS−/− NO on isoprenaline and the sarcolemmal Ca2+ current (ICa) remains to be explored.

Methods: Intrauterine growth restriction (IUGR) is associated with cardiovascular remodelling and dysfunction persisting into adulthood, resulting in dilated and less efficient hearts. However, the underlying molecular mechanisms remain to be elucidated. Here we test the hypothesis that unbalanced calcium (Ca2+) homeostasis is a key contributor to cardiac fetal programming.

Methods: IUGR was induced in 6 pregnant New Zealand rabbits by a surgical standard protocol. Puppies were assigned to: i) fetal (30 days of gestation) or ii) young adult (70 days postnatally) groups. Fetal cardiac function was assessed by echocardiography. IUGR−gene expression profile was analyzed by a bioinformatic gene set analytic tool. Sarcommate quantitative morphometric changes were assessed by multiphoton microscopy based on the SHG signal, called second harmonic generation microscopy (SHG).

Results: Fetal echocardiography showed that ducus venous and aortic isthmus patility index were increased in IUGR rabbits, revealing a reduced cardiac performance. Additionally, both systolic and diastolic cardiac function was compromised by IUGR, illustrated by a lower systolic ring displacement and increased diastolic relaxation time. Gene set analysis suggested that the sarcomeric M-band (GO: 0013440) function was over-represented in IUGR hearts (p<0.001). Results provided by SHG showed that resting sarcomere length, defined by the distances between the two Z-discs, was shorter in IUGR fetuses (p<0.01). Distances between intrasarcromere A-bands were also shorter under IUGR (p<0.03). Additionally, this data suggests overlap with the shorter IUGR EF5 (p<0.05). We assessed the postnatal persistence of all sarcommative morphometric changes in adult myocardium (p<0.05). Conclusion: Results reported here suggest that IUGR induces permanent changes of cardiac sarcomeres in fetal life to cope with the adverse intrarenal environment. Importantly, these changes persist postnatally and might explain the stiffer and less contractile heart phenotype of IUGR, resembling hallmarks of high-pressure cardiac disease models. Since sarcomere changes persist in adulthood, this could be one of the molecular mechanisms leading to abnormal cardiac function in IUGR that may explain a proportion of cardiomyopathies with fetal origin.
P125

Translamininregulates affect viability and functional properties of cardiomyocytes
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Purpose: Senile systemic amyloidosis (SSA), is a sporadic disease whose main symptom is a severe cardiomyopathy associated with arrhythmia. SSA is characterized by the presence of extracellular amyloid fibrillar aggregates of transferritin (TTR), a plasma protein carrying the thyroid hormone and the retinol binding protein. The aggregates are deposited in several tissues and are responsible for tissue functional impairment. To date, liver and heart transplantation are the only medical treatments; so, a thorough investigation of the molecular basis functional and viability impairment induced by TTR aggregates is expected to identify new pharmacological targets and to develop novel therapeutic strategies.

Methods: Cardiomyocytes line (HL-1) and ventricular cardiac myocytes isolated from mouse heart were exposed to pre fibrillar and fibrillar aggregates of TTR. The TTR aggregates internalization into HL-1 is assessed by immunofluorescence detection whereas cell cytotoxicity is determined by JC-1 assay. Modifications of intracellular calcium levels were studied by fluorescence microscopy on HL-1 cells. The effect of TTR aggregates on action potential profile is determined by single cell patch-clamp technique on mouse ventricular cardiomyocytes.

Results: Only pre fibrillar aggregates were able to interact with cell membrane and were internalized. This resulted in a moderate impairment of cell viability at concentration of 10 μM in the same cells exposed to TTR pre fibrillar aggregates; the cysticolic content showed a progressive rise over time; it did not reach a steady state level and came back to its basal levels upon TTR removal from bath solution. By patch-clamp technique we investigated the effect of the enhanced intracellular calcium on the electrical properties of mouse ventricular myocytes. Action potential recordings were performed at increasing rate of stimulation (0.5, 1 and 2 Hz) before and after application of TTR. The results showed a progressive prolongation of the action potential that was associated with a marked increase of the duration of the plateau phase. These effects were seen at any frequency of stimulation.

Conclusions: TTR internalization is associated with rise of cytoplasmic calcium content and electrical abnormalities in exposed cells. These data demonstrate a proarrhythmic effect of TTR aggregates in cardiomyocytes, a possible cause of SSA cardiomyopathy.

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Chronic changes in cardiac load dynamically alter T-tubule morphology and local Ca
2+-induced Ca
2+ release in rat ventricular cardiomyocytes
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Purpose: Transverse (t) tubules enable tight coupling between L-type Ca
2+ channels and ryanodine receptors throughout the depth of cardiomyocytes, and are disrupted in several cardiac diseases. We have previously reported that mechanical unloading can influence t-tubule structure and Calcium-induced Calcium-release (CICR). In this study, we used graded mechanical unloading and mechanical unloading to comprehensively assess the effect of load on t-tube structure and CICR.

Methods: To test this hypothesis, rat hearts were unloaded for 4 or 8 weeks using heterotopic abdominal heart-lung transplantation, or overloaded for 6 or 10 weeks using thoracic aortic constriction. Left ventricular cardiomyocytes were isolated enzymatically and studied using confocal microscopy. Results: 10 weeks (TAC60) but not 6 weeks (TAC30) of chronic mechanical overload increased cell size (Control 45,348 ± 3091 m3, n=42; p < 0.01)x10^7, n=53 vs. UN 8 weeks 1.1 ± 0.3 x10^7, n=44 vs. OV 10 weeks 9.45 ± 1.6 x10^7, n=47; p < 0.01). T-tubule regularity (Control 1.69 ± 0.1x10^7, n=44 vs. OV 10 weeks 9.45 ± 1.6 x10^7, n=47; p < 0.01) of intact papillary muscle from ACTCE99K transgenic mice and NTG littermates and confirmed the energy depletion is associated with HCM.

Conclusions: Energy depletion between the link between glucose transport, its metabolism, and NOX2 activation under hyperglycemic conditions. Primary: cultured rat cardiomyocytes were incubated with high (HG, 21 mM) or low (LG, 6 mM) glucose concentration for 24 h. Cell mortality was evaluated using tetrazolium iodide. ROS production was measured using HepCFOFA, p47phox translocation was quantified by immunofluorescence and Rac1 activity by a pull down assay method. Protein co-localization was assessed by in situ Proximity Ligation Assay.

Results: HG exposure activated Rac1. Rac1 induced p47phox translocation to the plasma membrane resulting in Rac1 activation, increased ROS production, insulin resistance and eventually cell death. Inhibition of the pentose pathway (PPP) by 6-aminonicotinamide (6AN) counteracted ROS production in response to HG but did not prevent Rac1 activation and p47phox translocation. Modulation of facilitated glucose uptake through GLUT4 by insulin (stimulation) or phloretin (inhibition) barely affected oxidative stress and toxicity induced by HG. Interestingly, non-metabolizable glucose analogues (i.e. 3-O-methyl-D-glucopyranoside and alpha methyl-D-glycero-pyranoside) reproducibly blocked ROS production under HG. Inhibition of sodium/glucose cotransporter SGLT1 by phlorizin counteracted HG-induced NOX2 activation and ROS production. p47phox and SGLT1 co-localised with Caveolin-3 (Cav3). Caveolae was required for glucotoxicity since caveolar disruption with methyl-beta-cyclodextrin completely blocked ROS production under HG. Phosphorylation of ERK proteins can stimulate Rac1. Under HG, ERK proteins were phosphorylated and also located closed to cav-3.

Conclusions: Increased glucose metabolism by itself does not trigger NOX2 activation, although PPP is necessary to provide NOX2 with NADPH and to produce ROS. The major finding of our study is that glucose transport through GLUT1 and metabolism are not required to induce glucotoxicity and NOX2 activation. NOX2 activation results from glucose transport through a sodium-glucose co-transporter, SGLT1 thus transducing a metabolic signal into intracellular ionic signal. SGLT1 activates ERK dependent signalling cascade located in the caveolar structure leading to NOX2 activation.

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Mechanical efficiency of hypertrophied rat papillary muscle related to phosphatidyglycerol/cardiolipin and cytosolic cytochrome c
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Hypertrophied hearts are inefficient, i.e. consume more oxygen per unit of myocardial work than normal. The inefficiency may increase myocardial oxygen demand several fold and can be a major contributor to the development of hypoxia in hypertrophied myocytes and reduced cardiac output. We hypothesize that the inefficiency is due to mitochondrial dysfunction. Papillary muscles were dissected from the right ventricle of control (n = 5) and monocrotaline-induced (60mg/kg sc) pulmonary hypertensive Wistar rats (n = 7). Mechanical efficiency was determined in 0.0388 g muscle from chamber work and oxygen uptake during 5 Hz sinusoidal pressure changes, peak-to-peak amplitude of 35 % of optimal length, as 37°C. The efficiency was 35 (SD 5 %) in control and 14 (SD 14 %) in hypophrophied preparations (P=0.006). Experiments using blebbistatin to inhibit cross bridge interaction demonstrated that the inefficiency is not due to sarcomere dysfunction. The right ventricular free wall was used to determine the phosphatidylglycerol/cardiolipin ratio using the HPLC mass spectroscopy and cytosolic cytochrome c by quantitative immunohistochemistry. Mechanical efficiency of papillary muscles correlated with phosphatidyglycerol/cardiolipin (r = 0.82, P < 0.01) and cytosolic cytochrome c concentration (r = 0.78, P=0.01). Cardiolipin is an essential component of the mitochondrial inner membrane - regulating its permeability, respiratory chain components and cytochrome c release - and is synthesized from phosphatidylylserine. Our results suggest that the mechanical inefficiency of hypertrophied myocardium is caused by mitochondrial dysfunction due to a disturbance of cardiolipin metabolism.
The effects of a non-obese high-fat diet on cardiac mitochondrial morphology and function

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High-fat diet can trigger cardiomyopathies that are associated with obesity-induced co-morbidities (e.g., diabetes, hypertension). High-fat diet can also directly trigger cardiac changes without obesity (e.g., altering cardiac metabolism) and has been shown to increase vulnerability to ischemia-reperfusion (IR) injury. The underlying mechanism for the latter could be due to changes in mitochondria. As it has been reported that larger, fused mitochondria are better able to survive IR. The aim of this work was to investigate the effects of high-fat diet, without obesity and associated co-morbidities, on cardiac mitochondrial morphology and function.

Male C57BL/6 mice were fed either a normal diet (13% kcal from fat) or a high-fat diet (45% kcal from fat) for 20 weeks. Isolated hearts and isolated mitochondria were used to study mitochondrial morphology and function, respectively. Hearts were fixed and sliced in longitudinal plane for electron microscopic imaging. Isolated mitochondria were supplied with either pyruvate and malate (P/M) or palmitoyl-coenzyme A (Pal-CoA) as substrates. Isolated mitochondria were assessed for oxygen consumption and H2O2 production. Data are presented as mean ± SEM and analysed using unpaired student’s t-test.

C57BL/6 mice fed a high-fat diet had elevated blood cholesterol and triglycerides but no evidence of hypertrophy or change in body weight and insulin sensitivity. Following high-fat feeding interfibrillar mitochondria were smaller, shorter and less dense (n = 4 hearts/group and ~1000 mitochondria/heart from >10 electron micrographs). The mitochondrial size decreased from 0.68 ± 0.06μm2 in the normal diet group to 0.47 ± 0.02μm2 in the high-fat group (P < 0.05). The amount of longer mitochondria (>1.2μm in length) in the high-fat group was lower compared to the normal diet (6.7 ± 0.1 vs. 11.8 ± 1.6% of total mitochondria, P > 0.05). The density of interfibrillar mitochondria was higher, 314 ± 0.8%, in the normal diet group compared to 26.6 ± 1.6% in the high-fat group (P < 0.05).

The oxygen consumption rates of isolated mitochondria were not different between normal diet and high-fat diet in state 3 and 3.5. H2O2 production in state 3 was not different when using P/M as a substrate. With Pal-Car H2O2 production in state 3.5 was higher in mitochondria isolated from hearts fed high-fat mice compared to normal diet. (46.0 ± 4 vs. 30.9 ± 0.9 pmol/min/mg protein, P > 0.05, n=3 isolations/group using 2 mice per isolation). This works shows changes in mitochondrial morphology and increased H2O2 production in high-fat diet hearts, which could contribute to the increased vulnerability during IR.

The myocardial postconditioning preserve the bioenergetics systems during ischemia-reperfusion

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Purpose: This study investigated the effect of myocardial ischemic postconditioning (PostC) and pharmacologic postconditioning by cyclosporine A on the changes in capacity of oxidative phosphorylation, and coupling between adenine nucleotide translocase (ANT) and mitochondrial creatine kinase (miCK).

Methods: Isolated perfused hearts from C57BL/6J mice were subjected to 40-min of ischemia followed by 20-min of reperfusion (Control group). Hearts were divided into 4 groups: 1) Control; 2) PostC (elicited by 5 cycles of 5 s ischemia–5 s reperfusion after 40 min ischemia); 3) cyclosporine A; and 4) sham-perfused heart (sparing the heart from ischemic–reperfusion injury). Parameters of mitochondrial function were assessed on saponin-skinned fibers from isolated hearts. The protocol of the respiration rate determination included a cytochrome c test to check the intactness of the outer mitochondrial membrane. The apparent affinity of the mitochondrial oxidative phosphorylation system for ADP (Km) in the presence and absence of creatine was also evaluated by determining the ANT-miCK functional coupling.

Results: Our results show that the capacity of oxidative phosphorylation was significantly reduced in control and cyclosporine A groups, and equivalent in PostC group in comparison to sham group. The cytochrome c test was negative in PostC and cyclosporine A groups, suggesting intact outer mitochondrial membrane; and positive in control group, suggesting some alteration of the outer mitochondrial membrane. In Sham and PostC groups, Km is high and addition of creatine significantly decreases Km, suggesting a high efficiency of the ANT-miCK functional coupling. Alterations in mitochondrial function in control and cyclosporine A groups were characterized by a significant decrease in Km and partial loss of the stimulatory effect of creatine.

Conclusions: PostC and cyclosporine A prevent the loss of integrity of the outer mitochondrial membrane after prolonged ischemia, moreover PostC contribute to the preservation of the ANT-miCK functional coupling.

Endothelial progenitor cells mobilization and increased levels in the injured myocardium after sevoflurane preconditioning

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Aim: Reactive oxygen species (ROS) are thought to be key players in ischemia-reperfusion (IR) injury. However, clinical trials using antioxidants to scavenge ROS failed to show a benefit. Thus, inhibition of ROS-production may be a superior therapeutic strategy. We recently identified NOX4 as a major source of ROS in ischemic stroke in mice. Therefore, we hypothesized that NOX4 may also play a detrimental role in IR of other organs such as heart and lung.

Methods: We performed ischemia-reperfusion (IR) of the heart in male and female NOX4 KO and matched WT mice by ligating the left descending coronary artery (LAD) for 45 min, followed by 24 h reperfusion and observation period. Basal and 24 h post-reperfusion ultrasound was performed. After 24 h, area at risk and infarct size (Evans’ Blue-TTC double-staining) and left ventricular haemodynamic function were determined.

Results: NOX4 knockout (KO) mice had fewer necrotic cells, smaller infarcts, and higher survival rates compared to wild-type (WT) mice. In the late phase of reperfusion, NOX4 KO mice had significantly increased post-reperfusion function compared to WT mice. In conclusion, NOX4 KO mice were protected against myocardial injury following IR, suggesting that NOX4 could be a potential therapeutic target for myocardial IR injury.

On the role of NOX4 in different forms of ischemia/reperfusion injury

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P115 The early markers of ischemic heart disease in cardiac remodeling in patients with metabolic syndrome and impaired glucose metabolism
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Material and methods: 345 patients were examined: 95 patients with arterial hypertension (AH) and dyslipidemia (DLP) without abdominal obesity (AO) – group 1 (control group), 50 women, 45 men; 49,85 ± 10,6 years. 103 patients with AH, DLP and AO (waist girth > 80 cm in men, > 94 cm in men) – group 2 (67 women, 66 men, 46,98 ± 10,97 years). 34 women with AH, DLP, AO and obesity (waist girth > 104 cm in women, > 113 cm in men) – group 3 with ischemic heart disease (IHD) (comparison group, 46 women, 69,42 ± 9,93 years). All patients went through transthoracic echocardiography with remodeling index calculation, lipid and glucose testing.

Results: groups 1 and 2 showed increase of linear dimensions of LV and myocardial mass (LVMPM: highly significant in group 1, 58,07 (56,24; 68,13) vs. 52,86 (52,01; 62,15) in group 2, p<0,001) in group 1 and 2, but no increase of LVSP (p>.05) and TAWP (p>.05). However, the role of CypD ablation in long-term cardioprotection after 72hrs reperfusion has not been examined. Whether lethal myocardial reperfusion injury progresses with increasing periods of reperfusion is controversial, and is also investigated here.

Methods: Using B6v8129 male mice, our first objective was to establish for the first time in our laboratory an in vivo recovery model of acute myocardial ischemia-reperfusion injury (IR) comprising 30min occlusion of the left anterior descending (LAD) artery followed by extended reperfusion for 2, 6, 24 and 72hrs. Infarct size was expressed as a % of the area-at-risk (AAR), Ischemic preconditioning (IPC, comprising one 5-min cycle of LAD occlusion and reflow prior to IR) was used as a positive control. Male mice deficient in CypD (CypD+/-) and wild-type littermates (CypD+/?+ ?) were subjected to IR.

Results: There was no increase in AAR size as the reperfusion time was prolonged over the 72hr period (38.4 ± 3.4% in rats, 37.5 ± 1.5% at 24hrs, 31.4 ± 3.3% at 72hrs; 30.7 ± 2.8% at 72hrs P>0.05, N=8/group). As expected, IPC significantly reduced AAR size after 72hrs reperfusion (30.2 ± 3.7% in control versus 16.2 ± 2.7% with IPCP 0.05% N=8/group). Male mice deficient in CypD sustained smaller AAR size (35.4 ± 4.3% in male CypD+/- + IPC versus 22.6 ± 2.4% in male CypD+/? P>0.05 N=8/group).

Conclusions: Myocardial infarct size did not enlarge with increasing duration of reperfusion suggesting that in the murine model lethal myocardial reperfusion injury does not progress over time. Genetic ablation of CypD confers long-term protection against IR in both male and female mice.

P138 S-nitrosoylation-mediated inhibition of protein tyrosine phosphatases attenuates ischemia-induced cardiac injury
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Ischemia-induced injury contributes to the progression of coronary artery disease. To date it is not known what governs ischemia-mediated perturbation of signaling in heart, or what strategy might be used to counteract such perturbation thus protecting heart against ischemic insults. It has been proposed that dysfunctions of protein kinases or phosphatases might lead to injury in heart suffering from ischemia. However, it remains elusive how ischemia-induced perturbations of intrinsic phosphorylation signaling contribute to cardiac damage. In the present study, using surgical ligation of the left anterior descending coronary artery (LAD) as a model, we have shown for the first time that the levels of phosphotyrosine (pTyr) signaling were decreased significantly in the left ventricular tissue of mouse heart, concomitant with a burst of creatine phosphokinase (CPK) and lactate dehydrogenase (LDH) released to the circulation. To gain insights into this disease process at the molecular level, we examined signaling events in rat myocardial H9c2 cells. Interestingly, H9c2 cells underwent a drastic decrease of pTyr signaling in response to hypoxia. Such results recapitulated the ischemia-induced perturbed phosphorylation state of eNOS1. Taken together, our data suggest a protective role of NO under hypoxic condition. Employing a pan-PTP inhibitor phenyl vinyl sulfone in H9c2 cells, we showed a critical role of PTP activation via S-nitrosylation on the active-site Cys residue leading to a significant increase of their activity.

Conclusion: Our data confirm that NO is a key signal in ischemia-reperfusion injury. It has been proposed that NO can decrease cardiac function, have proarrhythmic effect and increased cardiac mortality. We found that 1 mg/kg/day farnesol pretreatment significantly decreased infarct size (22.3 ± 3.9% vs. 40.0 ± 4.1%, p<0.05). However, 0.2, 5 and 50 mg/kg/day farnesol remained ineffective (37.7 ± 3.3% in group 1, 31.9 ± 3.1%; 43.3 ± 5.1% vs. 40.0 ± 4.1%, respectively). The effective dose of farnesol significantly increased cardiac N-acetyl-seryl-tyrosyl-L-cysteine level (68.6 ± 11.1 vs. 56.0 ± 9.0 ng/mg tissue, p<0.05), but did not influence cardiac N-acetyl-seryl-L-cysteine level (21.4 ± 15 vs. 30.7 ± 5.9 ng/mg tissue). One mg/kg/day farnesol significantly increased cardiac cholesterol level (0.73 ± 0.02 vs. 0.56 ± 0.04 mg/g tissue p<0.05), and caused a non-significant increase in cardiac cTnT and cTnI levels (94.7 ± 6.9 vs. 68.9 ± 15.4; 44.6 ± 6.5 mg/g tissue, 0.56 ± 0.52 mg/g tissue, respectively). Cardiac 3-nitrotyrosine level was modified significantly only by the highest dose 50 mg/kg/day farnesol (1.16 ± 0.18 vs. 2.44 ± 0.47 mg/g protein, p<0.05).

Conclusion: Farnesol dose-dependently decreased infarct size and 1 mg/kg/day farnesol was the most effective dose. Our data suggest that farnesol significantly decreases infarct size and modulates cardiac oxidative/nitrosative stress and seems to be independent of the antioxidant effect of farnesol.
Methods: Sixteen male Wistar rats were used. In each rat, either rosiglitazone (1mg/kg) or normal saline solution was administered intravenously. Then, the left anterior descending coronary artery was ligated for 30 min and released to permit reperfusion for 120 min. Cardiac function before ischemia and during I/R was determined using the pressure-volume recording system. The level of phosphorylated connexin 43 at serine368 residues, Bax and Bcl-2, and the infarct size were also determined.

Results: Rosiglitazone increased both the arrhythmia score and VF incidence in I/R rats (Figure), without preventing cardiac dysfunction. The phosphorylated connexin 43 level in rosiglitazone-treated hearts was markedly decreased in both ischemic and non-ischemic myocardium, compared to the vehicle-treated rats (Figure). The infarct size was also markedly decreased in rosiglitazone-treated rats. However, the Bax/Bcl-2 ratio was not different between the two groups.

Conclusions: Rosiglitazone facilitated the occurrence of VF during I/R by decreasing the phosphorylation of connexin 43 in the heart. This proarrhythmic effect of rosiglitazone could be responsible for increased mortality reported previously.

P142
Activations of Oxytocin receptors protects the myocardium via recruitment of PI3K-AKT intracellular signaling pathway during reperfusion
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Recent studies have emphasized an important role for Oxytocin in protecting against myocardial ischemia-reperfusion injury. In this study, we assessed whether phosphatidylinositol-3-kisane (PI3K-AKT) pathway was involved in Oxytocin mediated cardioprotection. Isolated perfused rat hearts were subjected to 35minutes of ischaemia and 120minutes of reperfusion. Hearts underwent triphenyl-tetrazolium staining for infarct size assessment and calculated as a percent of area at risk (MI/AR, %). Oxytocin (1nM, 10nM, 100nM) was preincubated for 3 minutes before reperfusion started. Oxytocin (10nM, 100nM) treatment reduced MI significantly compared with controls (22.7 ± 1.6% (1nM), 10.2 ± 7.1% (10nM), 37.3 ± 1.5% (100nM)). p = 0.001, control (p > 0.05). The protective effect of Oxytocin (1nM) was abrogated by administration of the PI3K inhibitor Wortmannin (100nM) (49.2 ± 3.1%, p < 0.0001), Wortmannin (100nM) (59.1 ± 4.0%, p < 0.0001). Western blot analysis further demonstrated that Oxytocin receptor activation during reperfusion induced a significant increase in p-AKT(ser473) compared to non-treated control hearts. Oxytocin dependent phosphorylation of Akt was abrogated by Wortmannin. This is the first study to show that Oxytocin receptor activation can protect the ischemic-reperfused myocardium via recruitment of the PI3K-AKT cell Survival pathway.

P143
Role of NO in apelin-induced protection against myocardial ischemia and reperfusion injury
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Background: Cardioprotective activity of the adipocytokine apelin is attributed to upregulation of endothelial nitric oxide synthase (NOS). This study was designed to examine effects of a synthetic 12 C-terminal residue of apelin (A-12) and NG-nitro-L-arginine methyl ester (L-NNAME), a non-selective NOS inhibitor, in ex vivo and in vivo models of ischemia/reperfusion injury.

Methods: Isolated working rat hearts were subjected to 35-min global ischemia followed by 30-min reperfusion. 140 µM A-12 and 100 µM L-NNAME were administered separately or in combination before global ischaemia. Metabolic state of reperfused hearts and lactate dehydrogenase (LDH) leakage in perfusate were assessed by enzymatic methods. Anaesthetized open-chest rats were subjected to 40-min regional ischemia and 60-min coronary reperfusion. L-NAME (37µmol/kg 10 min prior to reperfusion) and A-12 (0.35 µmol/kg at the onset of reperfusion) were injected intravenously; control rats received saline. Myocardial injury was evaluated by MB-creatinine kinase (MB-CrK) and LDH activities in plasma. Infarct size was determined by the Evans Blue/2,3,5-triphyl tetrazolium chloride staining method.

Results: Prescission infusion of A-12 increased recovery of cardiac function during reperfusion compared with control and resulted in enhanced restoration of myocardial ATP, adenosine nucleotide pool, phosphocreatine and reduction of myocardial lactate and lactate/pyruvate ratio. Cardioprotection of A-12 and L-NAME aggravated recovery of coronary flow and cardiac function compared with these indices after A-12 treatment. Cardiac dysfunction was associated with increase in LDH release in myocardial effluent, reduction of glucose oxidation and abolition of augmented restoration of high energy phosphates. A-12 administration, or freeze for Western blot analysis. Apelin (1nM, 10nM, 100nM) was administered throughout reperfusion in the presence and absence of the Oxytocin receptor antagonist of LST752 (10µM), PI3K inhibitor Wortmannin (100nM). Data was analyzed using one way ANOVA followed by Tukey’s test (n = 4–6). Oxytocin (1nM, 10nM, 100nM) when administered during reperfusion significantly reduced infarct size when compared to control (22.7 ± 1.6% (1nM), 10.2 ± 7.1% (10nM), 37.3 ± 1.5% (100nM)). p = 0.001, control (p > 0.05).

Conclusions: The results demonstrate the principal role of NO as a mediator of overall cardiac protection afforded by apelin.

P144
The role of prolyl-hydroxylation inhibition in rat aorta during ischemia/reperfusion
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Purpose: Although vascular grafts are frequently applied in the cardiac surgery, the storage protocols need further improvement against ischemia/reperfusion (IR) injury. Endothelial dysfunction may result in postoperative graft failure and promote late graft vasculopathy. Low oxygen tension elicits a variety of complex cellular responses by altering the activity of many signaling pathways, such as the oxygen dependent prolyl-hydroxylase-domain containing enzymes (PHD). Reduction of PHD activity during reperfusion leads to stabilization and accumulation of hypoxia inducible factor 1-a, a transcription factor which regulates the expression of target genes in response to hypoxia. We investigated the effects of PHD-inhibitor dimethylafoxylamine (DMOG) on the
vasomotor responses of isolated rat aorta and aortic vascular smooth muscle cells (VSMC) in a model of physiological thrombosis in vivo. The aim of our study was to address these questions.

Methods: Methods: Isovolumetric pressure-volume loops were performed in Sprague-Dawley rats (120-150 g) using a pressure transducer and a Statham pressure transducer. The heart rate was monitored using an electrocardiogram (ECG) and the left ventricular pressure (LVP) was recorded using a pressure transducer. NO production was assessed by measuring the formation of the NO-dependent metabolite, nitrite, in rat aorta segments using the Griess reaction.

Results: Results: NO production in the NO-sensitive isolated aortic segments was significantly increased in the diabetic rats compared to the control rats (P<0.05 for both). The increase in NO production was associated with a decrease in blood pressure (P<0.05 for both). NO production was also increased in the diabetic rats compared to the control rats (P<0.05 for both). The increase in NO production was associated with a decrease in blood pressure (P<0.05 for both).

Conclusion: Conclusion: Our results suggest that the increased NO production in the diabetic rats is associated with a decrease in blood pressure. This may provide a potential mechanism for the higher NO production in the diabetic rats and may contribute to the maintenance of arterial function in diabetes.
Purpose: The aim was to investigate the mechanisms behind the vasodilation seen in response to acute aldosterone in porcine coronary arteries. We hypothesized that hypoxia results in KC+ channel opening in porcine large coronary arteries thereby leading to vasorelaxation.

Experimental Approach: Porcine left anterior descending coronary artery segments without endothelium were mounted in myographs for isometric tension recording. Functional studies examining the influence of KC+ channels were performed and the presence of KC+ channels was examined by PCR and immunodetection. 

Key Results: In prostaglandin F2α (PGF2α)-contracted arteries relaxations induced by gradually reducing oxygen from 95% to 1% were associated with lowering of smooth muscle calcium a blocker of large-conductance calcium-activated K+ channels, by 4-aminopyridine, a blocker of voltage-dependent KC+ channels, by glibenclamide, a blocker of ATP-sensitive KC+ channels, but the largest effect was seen by XE991 and linopirdine, blockers of the Kv7.1-7.5 channels, while no effect was seen by chromanol 293B a blocker of Kv7.1, Kv7.4, Kv7.5 and the large-conductance calcium-activated BKCa channels were expressed in porcine coronary arteries.

Conclusion: Our findings suggest that hypoxia induces KC+ channel opening in isolated porcine coronary arteries. The effect is mainly mediated through Kv7 channels, which for the first time has been identified in porcine coronary arteries.

P150 Hyperpolarizable phenotype of the arterial wall of spontaneously hypertensive rats: involvement of vascular smooth muscle cells

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The anticoagulant role of the vascular wall suggests that there are connections between coagulation and the phenotype of vascular smooth muscle cells (VSMCs). Our objective was to determine whether hypoxia confers a hyperpolarizable state. We used the model of spontaneously hypertensive rats (SHR) compared with Wistar rats. The reactivity of the coagulation system was studied in vitro by monitoring the kinetics of thrombin generation (Thrombography) in two experimental conditions: (i) the plasma from SHR versus Wistar rats and (ii) on the surface of cultured VSMCs from aortas of rats in the presence of platelet-poor plasma of Wistar rats.

At baseline, plasma from SHR has a rate of thrombin generation significantly lower (p < 0.05) than the control. The addition of aortic rings of SHR to a pool of plasma from Wistar rats results in a significant increase in thrombin generation compared to the addition of rings of Wistar rats (699 ± 23 vs. 637 ± 8 mU/min, p < 0.05) to the same pool control. At the surface of cultured VSMC from SHR, thrombin generation was significantly higher than at the surface of VSMCs from Wistar rats (635 ± 12 vs. 568 ± 3.4 mU/min, p < 0.05). These results show that the wall of the SHR is more thrombotic than the vessel wall of Wistar rats. This phenotype is partly due to the ability of VSMCs to serve as a cellular support for the generation of thrombin. These results, obtained in vitro without hemodynamic stresses suggest a specific structural effect of the arterial wall producing an increased stiffness.

P151 Different potassium channels are involved in relaxation of arterial graft induced by nicorandil

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The drug nicorandil is a vasodilator approved for treatment of anogia. In addition to its well-known effect on the opening of ATP-sensitive KC+ (KATP) channels, nicorandil-induced vasorelaxation also affects the opening of KC+, activated KCa (KCa) channels.

Purpose: The aim of this study was to investigate the effects of nicorandil on KC+ channel opener, on the isolated human internal mammary artery (HIMA) and to define the contribution of different KC+ channel subtypes in nicorandil action on this blood vessel.

Methods: The HIMA segments were collected from male patients suffering from coronary artery disease who were undergoing coronary artery bypass surgery and studied in organ bath. HIMA rings were pre-contracted with phenylephrine (10 μM). Endothelium was removed mechanically.

Results: Our results show that nicorandil (0.001 μM – 300 μM) induced a concentration-dependent relaxation of HIMA rings pre-contracted by phenylephrine. Glibenclamide (10 μM), a selective blocker of ATP-sensitive KC+ channels as well as iberiotoxin (100 μM), a most selective blocker of large-conductance KCa (BKCa) channels, partly antagonized relaxation of HIMA induced by nicorandil. In contrast, a non-selective blocker of voltage-gated KC+ (Kv) channels, 4-aminopyridine (4 μM, 0.5 mM), as well as margetuxin (10 μM), a potent inhibitor of KV1.3 channels, did not abolish the nicorandil-induced relaxation of HIMA rings.

Conclusions: Our results showed that nicorandil-induced strong endothelium-independent relaxation of HIMA. It seems that KATP and BKCa channels located in the smooth muscle of HIMA mediated relaxation induced by nicorandil.

P152 Gal-3 is a potent mediator of aldosterone effects in vascular remodeling

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Background: Aldosterone (AlD) is involved in extracellular matrix (ECM) remodeling and inflammation, but its mechanisms remain unknown. Galectin-3 (Gal-3), a β-galactoside-binding lectin, plays an important role in inflammation and HF. We have investigated whether Gal-3 mediates AlD-induced ECM remodeling in vascular smooth muscle cells (VSMCs) in vitro and in vivo.

Methods: In vitro, primary cultured VSMCs were stimulated with Aldo (10-8M) for 24h, with or without mineralocorticoid receptor (MR) antagonists (eplerenone, RU28318) and Gal-3 inhibitors (modified citostine, N-acetylcysteamine, lactose). Gal-1 was over-expressed (transfection) and knocked-down (siRNA). In vivo, Wistar rats were treated with Aldo (1mg/kg/day) + sal or Aldo + sal + spironolactone (200mg/kg/day) for 3 weeks. Gal-3 expression, ECM production (collagen type I, III, fibronectin and elastin deposition and degradation) were evaluated by RT-PCR, Western blot, zymography and immunohistochemistry in VSMCs and aorta.

Results: Gal-3 was spontaneously expressed in cultured VSMCs. Its over-expression enhanced collagen type I production. Aldo up-regulated Gal-3 levels in a dose- and time-dependent manner via the mineralocorticoid receptor. Gal-3 chemical inhibitors blocked Aldo-induced ECM protein production. In addition, Gal-3 silencing abolished Aldo-induced collagen type I synthesis. In Aldo-salt hypertensive rats, aortic Gal-3 expression, ECM proteins and MMP activities were enhanced. Spironolactone treatment reversed all the above effects. Aortic Gal-3 expression was positively correlated with vascular collagen type I, elastin, MMP-2 and MMP-13 activities.

Conclusions: Aldo up-regulates Gal-3 expression via its mineralocorticoid receptor in VSMCs in vitro and in vivo. Gal-3 over-expression induces collagen type I synthesis. Moreover, Gal-3 is required for the fibrinotic response to Aldo. Our data suggest a key role for Gal-3 in Aldo-induced vascular collagen accumulation.
via immunoblotting, quantitative-PCR, functional assays and gene expression measurements in the Tampere Vascular Study cohort (n = 72).

**Results:** AtSMC exhibited protein signatures of increased oxidative stress and mitochondrial damage or dysfunction when compared to AoSMC, e.g. increased oxidized peroxiredoxin-4 (PRDX4, p = 0.019) and decreased mitochondrial proteins ATP Synthase subunit-beta (ATP5B, p = 0.0009) and Aldehyde dehydrogenase 2 (ALDH2, p = 0.011). Differences in the gene expression of the genes encoding for these proteins were also confirmed in the Tampere Vascular Study (PRDX4, p = 0.0001; ATP5B, p = 0.013; ALDH2, p = 0.0001). Accordingly, a decrease in mitochondrial mass was observed in AtSMC vs. AoSMC determined by citrate synthase assay (p = 0.0001). Surprisingly, a comparison between AtSMC isolated from patients with or without recent acute cerebrovascular symptoms revealed further differences, including an increase in Annexin 1 in the asymptomatic group (p = 0.030). This difference was also confirmed in the Tampere vascular Study (p < 0.0001). Inhibition of Annexin 1 function was associated with increased cytokine production both at baseline (p < 0.01), and after stimulation with the proinflammatory cytokine TNF-α.

**Conclusions:** Our study shows that atheroma-derived SMC display signatures of increased oxidative stress and mitochondrial damage/dysfunction. Moreover, we found an increased expression of the anti-inflammatory protein Annexin 1 in SMC in asymptomatic plaques, that may represent an additional mechanism through which SMC promote plaque stability through the modulation of inflammation.

**P155**

**Targeting myocardial edema: The depletion of extracellular RNA reduces vascular permeability and myocardial infarction size**

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**Purpose:** Angiotensin-converting enzyme 2 (ACE2) is the main enzyme responsible for the degradation of Ang II and generation of Ang-(1-7), peptides which play critical roles in the regulation of blood pressure (BP) and endothelial function. The aim of the present study was to evaluate the effects of chronic Ang-(1-7) treatment and AT1 receptor blocking by losartan on BP in ACE2-deficient (ACE2−/−) mice and to assess the endothelial function and the vascular redox balance in these animals.

**Methods and Results:** The study was conducted in 20-week-old ACE2−/− male mice on C57Bl/6 background. Telemetric blood pressure measurements confirmed an increase in mean arterial pressure (MAP) in these mice (ACE2−/−: 112.5 ± 3.3 vs. C57Bl/6: 106 ± 2.3 mmHg, p < 0.01). Chronic Ang-(1-7) infusion led to a 2 mmHg decrease in BP in both ACE2−/− and C57Bl/6 animals, whereas the BP lowering effect of losartan was more pronounced in ACE2−/− than in control animals (MAP post-losartan: ACE2−/−: 101.8 ± 1.9 vs. C57Bl/6: 1017 ± 2.6). Endothelial function was evaluated by measuring changes in MAP in response to bolus intra-aortic acetylcholine (ACH) and sodium nitroprusside (SNP) administration. The endothelium-dependent vascular reactivity was impaired in ACE2−/− mice compared to C57Bl/6 animals (Fig. 1, p < 0.001). ACE2−/− mice presented a lowered plasma and urine nitrite concentration, and reduced aorta NO levels. Lipid peroxidation was significantly increased and superoxide dismutase activity decreased was decreased in the aorta homogenate of ACE2−/− mice in comparison to controls, indicating impaired antioxidant capacity in these animals.

**Conclusion:** These data demonstrate that oxidative stress and NO imbalance, induced by increased Ang II levels may cause elevated blood pressure and endothelial dysfunction in ACE2−/− mice.

**Fig.1 Endothelial function in ACE2−/− mice**

**P157**

**Nitroto-redox imbalance contribute to vascular dysfunction and elevated blood pressure in ACE2-deficient mice**

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**Fig.1 Endothelial function in ACE2−/− mice**

**P158**

**EDN1 Lys198Aan is associated with microvascular angina or cardiac syndrome X in women**

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**Relevance:** In women with a diagnosis of ischemic heart disease (IHD) besides atherosclerosis of coronary artery uncharged coronary vessels are revealed by angiography. The one reason of smooth coronary artery is microvascular angina or “cardiac syndrome X”. Leading role in the development of this disease belongs to endothelial dysfunction, which may be because of genetic polymorphism of the endothelin-1 and NO synthase.

**Purpose:** To study the distribution of allele and genotype frequencies of polymorphisms Lys198Aan gene endothelin-1 and 4u/b gene eCHNOS in women of young and middle age with IHD compared with women of similar age without clinical manifestations of coronary artery disease and family history of cardiovascular disease.

**Materials and Methods:** 222 women were included. 115 women had coronary atherosclerosis (stenosis of coronary artery more than 70%) (average age 52.7 ± 0.5 years), 36 women had diagnosis “cardiac syndrome X” (mean age 53.6 ± 1.0 years) and 71 women were without clinical evidence of coronary heart disease (average age 51.3 ± 1.0 years). Detection of polymorphisms Lys198Aan EDN1 and 4u/b gene eCHNOS was conducted by PCR followed by restriction analysis.

**Cardiovascular Research Supplements**
Inhibitors of known mechanisms of action were used: NIsolated middle cerebral arteries (MCA) and basilar arteries (BA) of rats were studied in a

Methods:

Purpose: In most, if not all peripheral arteries increases in intraluminal flow elicit dilations. Much less is known regarding the nature of flow-induced responses in cerebral vessels. Previous studies reported both dilations and constrictions to flow in vessels isolated from different regions of the brain. We hypothesized the nature of the responses depend on the origin of cerebral vessels.

Methods: Isolated middle cerebral arteries (MCA) and basilar arteries (BA) of rats were studied in a pressure flow chamber. Changes of inner diameter to stepwise increases in intraluminal flow (at a constant intraluminal pressure of 80 mmHg) were measured by a laser Doppler velocimeter. Intraluminal flow was established by increasing the pressure difference between the vessels (SP = from 0 to 60 mmHg). Inhibitors of known mechanisms of action were used: Nω-nitro-L-arginine methyl ester (LNAME) and indomethacin (INDO) to inhibit the synthesis of nitric oxide and prostaglandins, respectively. At the end of experiments the passive diameters (PD) of vessels (in Ca²⁺ free solution) were measured.

Results: In the presence of 80 mmHg intraluminal pressure the following data were found: MCA and BA were (181 ± 6 µm and 338 ± 9 µm, which were ~58% and ~78% of PD, respectively. Increases in flow elicited significant constrictions in MCA (from 61 ± 2 to 50 ± 1.3% of PD, p < 0.05) and dilations in BA (from 281 ± 36 to 371 ± 21% of PD, p < 0.05). L-NAME, which reduced the dilation to acetylcholine, did not affect flow-induced constrictions of MCA and dilatation of BA. Indomethacin inhibited the constriction of MCA, but did not affect the dilation of BA to increases in flow, confirming the findings of previous studies.

Conclusions: We propose that the nature and the mediation of responses of cerebral arteries to increases in flow are depending on the location of vessels in the brain. Furthermore, the opposite response to the same stimuli may be explained by different reactivity of different brain regions.


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P162

Aquaporin-1 induces actin-remodeling via activation of beta-catenin in human induced pluripotent stem cells following exposure to glucose-induced hyperosmolarity

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Background and Objectives: Diabetic hyperglycemia increases plasma osmolarity, leading to activating cellular processes. Aquaporin-1 (AQP1) is induced by hyperosmolarity and plays a role in the vascular permeability. Induced pluripotent stem cells (iPSCs) offer a disease cellular model for the interplay of risk factors in the vascular complications of diabetes. We tested the hypothesis that glucose induced hyperosmolarity promotes angiogenesis in human iPSCs through activation of AQP1 and a downstream osmooxic pathway, thus orchestrating cell sprouting and migration.

Methods and Results: Human iPSCs were generated from skin fibroblasts by lentiviral transduction of 3 weeks compact refractive embryonic stem cell-like colonies emerged. All the iPSC colonies expressed OCT4, Nanog, c-Myc and SOX2, characteristic of self-renewal differentiation. After reprogramming, iPSCs were transfected with sRNA-AQPI or scrambled controls, and exposed to 5.5 mmol/L glucose (normoglycemia), high glucose (HG) at 12.5, 25 and 45 mmol/L, or with high mannitol (HM) at 12.5, 25 and 45 mmol/L for 24-72 hours. Exposure to either HM or HG increased expression of AQPI and toxicity enhancing binding protein (TonEBP). In iPSCs, AQPI could be co-immunoprecipitated with β-catenin. HG and HM strongly induced the expression of β-catenin (n = 3, p < 0.01 vs normal glucose by ANOVA). Under these conditions, proteins co-immunoprecipitated with anti-AQPI and β-catenin showed increased ratio of F-actin vs G-actin (n = 3, p = 0.01 vs normal glucose by ANOVA). iPSCs formed bundles in methacrylcellulose matrix and tubing networks in matrigel, especially when they were exposed to HG and HM (HG 2.8 ± 0.2 fold; HM 3.3 ± 0.5 fold, n=4, p < 0.01 by ANOVA). SirNA to AQPI or to TonEBP all reverted the inducing effects of HG and HM on β-catenin expression, actin polymerization and angiogenic activities.

Conclusions: The hyperosmolarity serves as a biophysical factor that promotes angiogenesis in human iPSCs. This effect may occur through an AQPI-associated cytoskeleton remodeling. Targeting the osmooxic pathway offers a novel strategy to reduce vascular complications of diabetes.

P163

Angiotensin-2: mediator of septic hypercorticosteronism

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Background: Angiotensin plays a central role in the quiescence of the endothelium. Pathalogical stimuli, such as inflammation, lead to a destabilization of the endothelial cell layer via Angiotensin-2 and its receptor Tie-2. In clinical studies, elevated Angiotensin-2 levels in sera of patients suffering severe sepsis have been described (Kumpers 2008). Recently, it has been demonstrated, that treatment with Ang-1 adenovirus in septic mice resulted in improved cardiac function as well as enhanced survival (Wirtzbandzik 2005). Here, we assessed the protective effect of Ang-2 anti-bodies (Ang-2ab) in a murine model of lipopolysacharide (LPS)-induced sepsis.

Methods: To induce Sepsis, LPS [20ng/kg] was injected intraperitoneally into C7SL/SLE mice. 24 hours before sepsis induction, groups were pre-treated with Age2-ab or an unspecific antibody (control-ab) as a control. After sepsis induction a sepsis-severity-score was assessed after 6 and 12 hours, followed by invasive and non-invasive hemodynamic measurements or observation of further survival. The sepsis-severity-score includes five different parameters (behaviour, pain, ascites, dispnea, weight loss), according to the severity points between 0 and 20 are given. Blood samples and organs were harvested for histological and molecular analysis.

Results and Conclusion: Whereas in control-ab treated mice, hemodynamic function was severely depressed 12 hours after LPS injection, as seen in decreased left ventricular developed blood pressure (74 ± 9 mmHg) and severely reduced systemic blood pressure (45 ± 3 mmHg), in
Ang2-ab treated mice left ventricular developed blood pressure fell only to 92 ± 6 mmHg. Similarly, the left ventricular systolic blood pressure was less pronounced (77 ± 10 mmHg). This resistance to LPS-induced hemodynamic changes was reflected by reduced sepsis-severity score results after 6 (5 ± 1 points vs. ± 1 points) and 12 hours (10 ± 1 points vs. ± 1 points) and at least by trend improved survival rate of the Ang2-ab treated mice. Histological analysis revealed that the drop in perfusates in septic mice is abolished by Ang2-ab treatment. These findings highlight the pivotal role of Ang2 in the onset and progression of sepsis and identify Ang2 as a potential target for treatment of Sepsis.

P164 Adenosine up-regulates thrombospondin-1 production by macrophages
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Background: Increase of blood capillary density at the interface between normal and ischemic tissue after acute myocardial infarction reduces infarct size and improves cardiac function. Cardiac injury triggers the production of the matricellular component thrombospondin-1 (TSP-1) and adenosine. Adenosine is thought to be involved in cardiac repair and is known to stimulate angiogenesis. The role of TSP-1 in angiogenesis is less clear, since both anti- and pro-angiogenic activities have been reported. We hypothesized that adenosine controls angiogenesis through modulation of TSP-1 production.

Methods: Primary human macrophages were obtained by differentiation of peripheral blood monocytes from healthy volunteers, and were treated with adenosine (0.1-50 μM/L) under ischemic conditions (hypoxia and starvation) or stimulation by cytokines (IL-1β and TNF-α). A rat aortic ring assay was implemented to evaluate angiogenesis.

Results: Adenosine dose-dependently increased the production of TSP-1 by macrophages, reaching a 4-fold increase at 10 μM (n = 11, P < 0.001). A 13-fold induction of TSP-1 mRNA expression was measured. These effects were observed both under basal conditions, during ischemia, and after stimulation with cytokines. Use of agonists and antagonist of adenosine receptors, coupled to RNA interference experiments suggested that the A2A and A2B receptors mediate the effect of adenosine on TSP-1. This effect was reproduced by cholera toxin (Gs protein activator) and forskolin (adenylate cyclase activator), and blocked by the PKA inhibitor H89. Low doses of purified TSP-1 (1-50 μg/mL) increased microvascular outgrowth from rat aortic rings, whereas high doses (>500 μg/mL) decreased this outgrowth. Conditioned medium from adenosine-treated macrophages (containing approximately 0.2 μg/mL of TSP-1) enhanced microvascular outgrowth. Addition of anti-TSP-1 antibodies to conditioned medium blocked angiogenesis in this model.

Conclusions: We show for the first time that adenosine up-regulates TSP-1 production by macrophages. This effect involves the A2-type adenosine receptors and is mediated through the cAMP/PKA pathway.

P165 MicroRNA-15a and mir-16 regulate sdf-1 migration of endothelial progenitor cells in diabetic patients with limb ischemia and healthy controls
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Purpose: Circulating endothelial progenitor cells (EPCs) are reduced and functionally impaired in diabetic patients, possibly contributing to the number and severity of ischemic events observed in this population. The molecular mechanisms underlying diabetes-associated EPC impairment are incompletely understood. microRNAs (miRs) post-transcriptionally inhibit the expression of target genes. We aim to obtain mechanistic insights of diabetic EPC impairment and to provide the first characterization of the EPC-associated miRNAs with special respect to those potentially involved in the control of angiogenesis. Here we report results on miR-15a-16.

Methods: EPCs were prepared from the peripheral blood of type-2 diabetic patients undergoing revascularization for limb ischemia (Li) and from healthy individuals. The migratory activity of EPCs was measured in a transwell migration assay using SDF-1 (100ng/ml) as a chemo-attractant. A miR screening was performed (TaqMan PCR) in diabetic Li EPCs (n = 55) (controls healthy donors n=17) to select some differentially expressed miRs to be further analyzed. miR expression in EPCs was regulated using pre-miR, anti-miR and scramble control (all from Ambion).

Results: As expected, EPC migration (modified Boyden chamber) to SDF was reduced by disease (migration index — ratio of SDF-1 to BSA migrated EPCs : 1.08 ± 0.02 vs. 1.87 ± 0.09 for healthy EPCs, P < 0.01, n=6 patients and n=7 controls). The miR-15a-16 expression (relative to rmiR6) was increased in “diseased” EPCs in comparison to controls (miR-15a: 5.19 ± 0.85 vs. 1.4 ± 0.15, p < 0.05; miR-16: 4.91 ± 0.91 vs. 1.13 ± 0.13, p < 0.05). To understand the effect of miR-15a-16 up-regulation on EPC capacities, we transfected pre-miR-15a and pre-miR-16 in healthy EPCs. miR-15a-16 overexpression inhibited EPC migration to SDF-1 (migration index: 1.24 ± 0.04 in control vs. 0.85 ± 0.15 in miR-16 transfected EPCs; p < 0.05). Next, to understand if increased endogenous miR-15a-16 contributes to impaired EPC migration observed in patients, we transfected “diseased” EPCs with anti-miR-15a and anti-miR-16. This corrected the migratory capacity of patient-derived EPCs (migration index: 1.51 ± 0.14 vs. 1.1 ± 0.09 in scramble, p < 0.05 and PNS vs. healthy EPCs group scramble). Bioinformatic analyses predict BCL2, VEGFA, and AKT3 to be target genes of both miRNA-15a and mir-16, which additionally suggest anti-angiogenic and pro-apoptotic roles of these miRs.

Conclusions: miR-15a-16 are involved in SDF-1 migratory impairment of EPCs from diabetic patients with Li.

P166 Tissue factor induces endothelial neovessel formation through Akt signaling
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P167 Dynamic course of serum-induced endothelial cell apoptosis in ST-segment elevation myocardial infarction patients treated with primary angioplasty: implications for a deregulation of immune response
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Purpose: Acute loss of endothelial cells may play a role in the ischemia-reperfusion injury process. The aim of this study was to evaluate the pro-apoptotic effect of blood serum on endothelial cells in reperfused ST-segment elevation myocardial infarction (STEMI) patients.

Methods: Human umbilical vein endothelial cells (HUVEC) were incubated with serum of 20 patients with a first STEMI treated with primary angioplasty drawn before reperfusion and 24h, 96h and 3 days afterwards. Apoptosis, necrosis and viability percentages were evaluated by flow cytometry. Cytokine levels and lymphocyte subtypes were evaluated by multiplexed immunoassay and flow cytometry respectively. Values were compared with serum of 12 age- and sex-matched control subjects with normal coronary arteries.

Results: In comparison with controls, serum of STEMI patients induced a loss of HUVEC viability mainly due to apoptosis but not necrosis. In patients, the pro-apoptotic effect of serum was maximum at 96h post-reperfusion (Figure). A pro-inflammatory response paralleled the pro-apoptotic effect of serum. In comparison with controls, at 96h anti-inflammatory cytokines IL-4 and IL-10 did not vary but pro-inflammatory cytokines IL-6 (p < 0.001) and IL-1β (p < 0.001) and pro-apoptotic cytokines TNF-α (p < 0.001) and TGF-β (p < 0.05) increased. Similarly a pro-inflammatory response in adaptive immune cells occurred: CD4+ cells count and Th17/Tc2 ratio increased but FOXP3+ T regulatory cells count diminished (p < 0.05 in all cases).

Conclusion: Serum of STEMI patients induces apoptosis on endothelial cells. This effect progressively increases in the days following reperfusion and it is accompanied by an acute pro-inflammatory deregulation of the adaptive immune system.

Lipids, Atherosclerosis

P168 Metabolic hormone levels in patients undergoing on pump coronary artery bypass grafting
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Prone to rupture atherosclerotic plaques (AT) show significant angiogenesis. The mechanism of neovessel formation in the growing AT is still unknown. We have previously shown that tissue factor (TF) and its signaling mediators have a significant role in mature neovessel formation.

Objective: To define the signaling pathways involved in TF-induced angiogenesis.

Methods and Results: We analyzed the Akt, a downstream effector of the TF-induced signaling via activation of PI3K. The following results were obtained: a) TF-RNA inhibition of TF in the human endothelial cells (HMEC-1) resulted in a highly significant inhibition of endothelial-tube-like formation (Etub) with stable phenotype in 3D cultures (2500 ± 25 μm random-stRNA versus 260 ± 45 μm TF-stRNA). The inhibition in Etub was associated with a down-regulation of Akt expression (2x) and with increased Raf-phosphorylation at Ser259. b) Overexpression of TF resulted in an increase in Etub (1320 ± 15 μm versus 2500 ± 25 μm) and in the up-regulation of Akt protein; c) Immunosuppression of Akt revealed that TF is directly associated with Akt; d) The effect of silencing TF was only reversed by PAR2 agonist (H-SK42) to a 65% of control Etub (1630 ± 25 μm versus 260 ± 45 μm); e) Enforced expression of Akt by pcDNA3-Myr-HA-Akt1 plasmid in TF-silenced endothelial cells rescued Etub formation (2250 ± 45 μm versus 260 ± 45 μm) and induced Ets-1 phosphorylation.

Conclusion: TF and Akt form a complex that regulates Etub formation signaling through Raf/Erk/ Ets-1. The triggering effect of TF on the formation of endothelial-tube-like formation may have a significant impact not only in atherosclerotic plaque neovessel formation but in cancer metastasis and other processes curing with neovascularization.
Purpose: To explore the response pattern of plasma adiponectin and ghrelin levels to coronary artery bypass graft (CABG) surgery in patients receiving cardiac pulmonary bypass (CPB) with glucose-insulin-potassium (GIK) infusion.

Methods: 16 consecutive patients (age 63 ± 10 years, male: 16) with obstructive coronary artery disease (CAD) who underwent elective CABG surgery with CPB and intraoperative GIK infusion were enrolled. Blood samples were taken before, during and after surgery. Intraoperative samples were withdrawn simultaneously for peripheral and sinus (SC) cardiac plasma. Adiponectin concentrations were measured by ELISA, those of ghrelin by RIA kits.

Results: The baseline values of insulin and leptin were higher (p < 0.01), those of resistin and ghrelin were lower (p < 0.01) in patients undergoing CABG with CPB than the respective values of controls. In response to surgical intervention, there was an early transient fall in plasma levels of leptin (p < 0.06), adiponectin (p < 0.001) and resistin (p < 0.002) followed by an increase to approach their initial values. Plasma ghrelin also increased (p = 0.045), this increase, however, was confined to the period of GIK supported CPB. Moreover, it was demonstrated that plasma lep tin, adiponectin and ghrelin levels did not change in general versus after those from patients without surgery. By contrast, plasma insulin (p < 0.003) and resistin (p < 0.009) was significantly higher in the periphery than in SC.

Conclusions: Adipose tissue-derived factors may mediate the metabolic and vascular effects of insulin in patients with CABG surgery. Epicardial adipose tissue is unlikely to have major contribution to the development of CAD as adiponectin are not elevated in SC.

P149
High dose statines reduce carotid artery intima-media thickness (IMT) and decrease the risk for general cardiovascular disease
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Purpose: To use High dose Statins in non symptomatic patients with increased Carotid Intima-Media Thickness (IMT) to reduce the incidence of Cardiac Arrhythmias and of fatal or non-fatal Coronary Artery Disease. The patients were divided in two groups. Group 69 patient was treated with 80 mg Simvastatin per day and second group (101patient) with 20mg simvastatin per day. ACS and Aspirin 100mg were prescribed to both groups.

Methods: We investigated 170 patients who had increased IMT 1.1-1.4mm, had low-density lipoprotein (LDL) cholesterol levels of 100 to 190 mg per deciliter (2.6 to 4.9 mmol per liter), and had not known Cardiac Arrhythmias and CAD. The patients were divided in two groups. One group (69 patient) was treated with 80 mg Simvastatin per day and second group (101patient) with 20mg simvastatin per day. ACS and Aspirin 100mg were prescribed to both groups.

Results: The mean LDL cholesterol level during the trial was 73 mg per deciliter (1.9 mmol per liter) among patients receiving simvastatin 80mg per day and 117 mg per deciliter (2.9 mmol per liter) among patients receiving 20mg simvastatin per day. IMT in patient receiving 80mg simvastatin pretreatment was diminished 0.189, whereas in patients receiving 20mg simvastatin pretreatment it increased after 1.9 years. During a median follow-up of 1.9 year, 15 patients (21.7%) receiving simvastatin 80mg per day and 30 patients (29.75%) receiving 20mg had a fatal or nonfatal CAD and atrial and ventricular arrhythmias.

The group received simvastatin 80mg had 3 atrial fibrilacion,1 ventricular arrhythmia,7 NONSTEMI and 4 STEMI, whereas the group, received 20mg simvastatin had 8 atrial fibrilacion and other atrial arrhythmias,3 ventricular arrhythmia,12 NONSTEMI and 7 STEMl.

4 (5.76%) patients from 80mg simvastatin group and 9 (8.9%) from 20mg simvastatin group died from cardiac and cardio-cerebral event.

The five-year absolute reduction in the risk of major cardiovascular events was 3.5 percent.

The overall mortality rate was low in patient received high dose simvastatin.

IMT also that is one of them marker of cardiovascular disease, did not changed or improved. Elevated liver enzyme values were more common in patients taking simvastatin in high dose.

Conclusions: In patients with increased Carotid IMT and increased risk for cardiovascular disease, high dose statines reduce carotid artery intima-media thickness (IMT) to reduce the incidence of Cardiac Arrhythmias and of fatal or non-fatal Coronary Artery Disease.

P171
P. gingivalis-induced aggregation and ros production in whole blood is dependent on ghrelin
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A large body of data accumulated over the past several years suggests that the periodontal pathogen Porphyromonas gingivalis is associated with cardiovascular disease. Circulating bacteria may contribute to cardiovascular disease (CAD) by promoting CD11b/CD18-mediated interactions between neutrophils and platelets, causing reactive oxygen species (ROS) production and aggregation. We have previously demonstrated that P. gingivalis induces aggregation and ROS production in whole blood, and that the anti-inflammatory mediator lipoxin A4 (LXA4) inhibits these responses by modulating platelet-neutrophil aggregation through a direct regulation of the bacterially induced surface expression of CD11b/CD18 on neutrophils, likely by inhibiting Rac2 and Cdc42 signaling pathways. Furthermore, P. gingivalis, unlike other periodontopathic bacteria, has been shown to trigger platelet aggregation, mainly through the interaction between bacterial lipoproteins and platelet activating receptors (PARs) on the platelet. Since platelet aggregation triggers thromboembolic events, this is an important reperfusion feature of the bacterial infection. The goal of the aim of this study was to investigate the effect of ghrelin on P. gingivalis-induced cell activation in whole blood. Platelet/leukocyte aggregation and ROS production was examined by lumizegregometry. This study shows that leupetin, a protease inhibitor of ghrelin, inhibits P. gingivalis-induced aggregation and ROS production in whole blood. Supersaturants of bacteria suspensions induced no ROS-production, but an aggregatory response that was also inhibited by leupetin. In conclusion, P. gingivalis-induced aggregation and ROS production in whole blood is mainly dependent on ghrelin. However, since bacterial supersaturants (containing soluble ghrelin) stimulate only aggregation, this suggests that a ghrelin PAR-mediated mechanism in combination with phagocytosis of whole bacteria is a prerequisite for inducing a respiratory burst and an inflammatory response. These findings may contribute to new strategies in the prevention and treatment of periodontitis-induced inflammatory disorders, such as atherosclerosis.

HPSA1A phenotypes and risk of cardiovascular disease
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Purpose: HPSA1A is a serum and intracellular heat shock protein with antiapoptotic / antithrombotic possible properties. Possible polymorphisms of the regulatory region of HPSA1A could affect HPSA1A protein synthesis, determining diminished, normal or increased HPSA1A-producing phenotypes. The hypo-producing phenotype would entail a greater risk of developing atherosclerotic disease. The present study was made to identify SNPs in the HPSA1A regulatory region and evaluate whether any of them could affect HPSA1A synthesis in a randomly selected population which was later stratified into different groups according to the degree of vascular risk.

Methods: Serum and intragranulocytic HPSA1A was quantified by ELISA and direct Sanger sequenc ing performed in all subjects. An analysis was made of the association of two SNP (db ns1008438 -110AC and db ns104318 +190GC) with circulating and intragranulocytic HPSA1A and the risk of atherosclerosis. Data were analyzed using a nonparametric Mann-Whitney rank sum test or a Kruskal-Wallis test. A Chi-square test or Fisher’s exact test was used to assess the goodness-of-fit between the observed allele frequencies and the expected counterparts by Hardy-Weinberg equilibrium, and to evaluate differences in allele distributions between groups.

Results: The study population consisted of 452 randomly selected subjects, 234 females (49.5 ± 6.9 years), and 218 males (48.6 ± 7.7 years). They were stratified into three groups according to Task Force Chart Criteria: no vascular risk or risk < 5%, n=239; moderate vascular risk (10-20%) without clinical atherosclerosis, n=116; and overt atherosclerosis n=52. The greatest percentage of intragranulocytic HPSA1A hypo-producers corresponded to the subjects with the CC genotypes of -110AC and +190GC SNPs. The assignment to a given vascular risk group revealed differences in the percentages of hyper-, normal- or hypo-producing individuals. The CC genotype entails a risk of being a low intracytoplasmic HPSA1A producer with respect to normal production of 1.673 (95% CI 1.191-2.351, p=0.004) versus the AC genotype, and of 1.491 (95% CI 1.034-1.947, p=0.033) versus the AA genotype.

Conclusions: Based on our results, -110A/C and +190GC homozygous carrier state entails a risk of presenting modest vascular risk or declined atherosclerosis — probably as a result of diminished intracellular HPSA1A synthesis and a consequent partial loss of its antiinflammatory and antithrombotic properties. In coincidence with other authors, this leads us to postulate the -110 A and + 190 G alleles as possible genetic markers of less severe clinical phenotypes.

P173
Interleukin-33 induces urokinase-type plasminogen activator and plasminogen activator inhibitor type-1 in human endothelial cells in vitro
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Interleukin-33 (IL-33) is a member of the IL-1 family of cytokines and is known to be involved in several inflammatory processes by inducing the release of pro-inflammatory and anti-inflammatory cytokines. However, the mechanisms by which IL-33 induces the release of pro-inflammatory cytokines remain unknown. In this study, we investigated the effect of IL-33 on the release of plasminogen activator (PA) and plasminogen activator inhibitor (PAI) type-1 in human endothelial cells (HMEC-1) in vitro. HMEC-1 cells were treated with IL-33 and the release of PA and PAI-1 was measured using enzyme-linked immunosorbent assay (ELISA). Our results showed that IL-33 induced the release of PA and PAI-1 in a dose-dependent manner. These results suggest that IL-33 may play a role in the regulation of endothelial cell function and contribute to the pathogenesis of inflammatory diseases.
Background: Urokinase-type plasminogen activator (u-PA) and its inhibitor, PA inhibitor type-1 (PAI-1), regulating extracellular proteolysis are involved among other pathophysiological events in vascular remodeling and in plaque angiogenesis and stability in atherosclerosis. IL-33 is a novel member of the IL-1 cytokine family and is a ligand of the ST2 receptor. IL-33 has recently been implicated in the pathogenesis of atherosclerosis. It was shown to induce vascular permeability and angiogenesis and the production of inflammatory cytokines in endothelial cells. Here we aimed to study a possible regulation of u-PA and PAI-1 by IL-33 in human endothelial cells (EC).

Methods: Human umbilical vein EC (HUVEC) and human coronary arterial EC (HCAEC) were treated with IL-33 alone or together with soluble ST2 fusion-protein (sST2Fc) or together with simvastatin. Specific mRNA levels for u-PA and PAI-1 were determined by RT-PCR and u-PA and PAI-1 antigen levels and activities were measured by specific ELISA.

Results: u-PA mRNA was up-regulated up to 5-fold in HUVEC and up to 2.4-fold in HCAEC, when these cells were treated with 100 ng/ml IL-33 for 9h whereas PAI-1 mRNA increased up to 2.5-fold and up to 2.4-fold, respectively. IL-1 receptor antagonist had no effect on IL-33-induced increase in u-PA and PAI-1, which suggested that these effects are IL-1-independent. PA-PA antigen increased up to 30-fold and PAI-1 antigen increased up to 2.4-fold after 48h of incubation with 100 ng/ml IL-33 in HUVEC. In HCAEC u-PA increased up to 10-fold after 4h and PAI-1 increased up to 3.5 fold after 24h of incubation with 100 ng/ml IL-33. PAI-1 activity increased up to 4.5-fold after 4h of incubation with 100 ng/ml IL-33 in HUVEC and up to 5-fold after 6h of incubation in HCAEC. The increase in u-PA and PAI-1 antigen was concentration-dependent when the cells were incubated with IL-33 concentrations ranging from 1 to 100 ng/ml. sST2Fc abrogated the IL-33-induced increase in u-PA and PAI-1 antigen suggesting that these effects of IL-33 are ST2 receptor-mediated. Simvastatin at concentrations ranging from 0.5-2.5 µM also abrogated IL-33-induced increase of u-PA and PAI-1 antigen, thus providing further evidence that statins have effects beyond reduction of cholesterol.

Conclusion: Via induction of u-PA and PAI-1 antigen was concentration-dependent when the cells were incubated with IL-33 ranging from 1 to 100 ng/ml. sST2Fc abrogated the IL-33-induced increase in u-PA and PAI-1 antigen suggesting that these effects of IL-33 are ST2 receptor-mediated. Simvastatin at concentrations ranging from 0.5-2.5 µM also abrogated IL-33-induced increase of u-PA and PAI-1 antigen, thus providing further evidence that statins have effects beyond reduction of cholesterol.

P176
Relationship between natural killer cells and platelet reactivity in patients with acute coronary syndrome
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Purpose: Preliminary studies show that patients with Coronary Artery Disease (CAD) present reduced NK cell compartment and low cytotoxic NK cell functions may be affected by inflammatory processes with consequent impairment of immunological response leading to progression of CAD. High levels of inflammation associated with enhanced platelet aggregation are typical of acute phases of coronary disease. Thus, we analyzed the relationship between thrombogenicity and NK cell distribution in patients with non-ST-elevation acute coronary syndrome (NSTEMACS).
Methods: Peripheral blood samples were taken from 50 statin-naive patients with NSTEMACS and analyzed for the distribution of NK cells by Flow Cytometry (using anti-CD3, -CD8, -CD16 + 56, -CD57 monclonal antibodies) and for platelet reactivity by the VerifyNow P2Y12 assay. Platelet reactivity was assessed as residual ADP-induced aggregation (PRU) after P2Y12 receptor blockade and as thrombin-induced aggregation (Base PRU) which provides an estimation of total platelet reactivity.
Results: Overall, there were a significant positive correlation between PRU and Base PRU values (r = 0.38, p<0.02) and inverse correlations between the absolute number of NK cells (CD3+CD16 + 56 + CD8+CD57 + ) and PRU (p<0.05) and Base PRU (p<0.01). We divided the patient population into two groups: Low Reactivity (Base PRU < 305) and High Reactivity (Base PRU ≥ 305). NK cell frequencies were significantly lower in the High Reactivity than the Low Reactivity group (Figure1).
Conclusions: This study shows that in patients with NSTEMACS there is a link between platelet reactivity and NK cell distribution. As PRU values correlate with clinical outcome, our results may suggest a possible link between NK cell frequencies and prognosis in patients with ACS.

NK cells by platelet reactivity in ACS

P177
Apopotosis pathways are deregulated in CD4 + CD28null T cells from patients with acute coronary syndrome
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Purpose: T lymphocytes, the main effectors of adaptive immunity, have key roles in the development and progression of atherosclerosis. One subset of T lymphocytes that is altered in patients with acute coronary syndrome (ACS) is the CD4 + CD28null T cells. These cells expand significantly in ACS patients compared to stable angina and healthy controls and have been suggested to mediate plaque instability and recurrence of acute coronary events. CD4 + CD28null T cells characteristically do not express the CD28 receptor which has pivotal roles for the activation and survival of T lymphocytes. Apoptosis is a homeostatic process that regulates the size of the T cell compartment. Very little is known about the mechanisms that lead to the accumulation of CD4 + CD28null T cells in ACS. Our aim was to characterise apoptosis pathways in CD4 + CD28null T cells in ACS patients to identify alterations that could explain the persistence of this cell subset.
Methods: We first identified the expression of death receptors (Fas) and ligands (FasL), as well as the levels of anti-apoptotic (Bcl-2, Bcl-xl, survivin) and pro-apoptotic (Bax, Bim) proteins in CD4 + CD28null T cells in patients with ACS using flow cytometry. In addition, we have quantified apoptosis sensitivity of in vitro activated CD4 + CD28null T cells using Annexin V and 7-AAD staining and detection of activated caspase-3.
Results: We found that CD4 + CD28null T cells express significantly lower levels of the death receptor Fas compared to conventional CD4 + CD28+ T cells. Furthermore, the pro-apoptotic protein Bim was significantly decreased in CD4 + CD28null T cells compared to their CD28+ counterparts. Interestingly, CD4 + CD28null T cells failed to upregulate Bim following activation, in stark contrast to CD4 + CD28+ T cells. No differences were found in the levels of anti-apoptotic proteins between the two CD4 + T cell subsets.

Conclusions: The increased expression of death receptors in these cells may result in their prolonged survival, leading to their accumulation in the ACS patients. Further studies are needed to investigate the relationship between apoptosis pathways and the persistence of CD4 + CD28null T cells in ACS patients.
Conclusion: We identified defects in the death receptor Fas and the pro-apoptotic protein Bid in CD4+ CD28null T cells from ACS patients which suggest that these cells are resistant to apoptosis. These findings could open the way for novel therapies aimed at targeted induction of apoptosis in CD4+ CD28null T cells to stabilise atherosclerotic lesions.

P178 Cardiac expression and activation of NF-κB in association with enhanced spleen inflammation in mice with obesity and diabetes

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Inflammation serves as a risk factor for atherosclerosis. In diabetes, abnormal production of inflammatory factors contributes to cardiovascular dysfunction. The nuclear factor NF-κB plays an important role in inflammatory signal transduction.

Objective: To test the association of spleen inflammatory responses and activation of CD1d-restricted NKT cells, with expression and activation of NF-κB in the hearts of obese hyperinsulinaemic diabetic mice.

Db/db and C57Bl/6 mice of both genders, in comparison with their respective background wild-type C57Bl/6 and BAlb/c were examined. The spleen tissues of db/db mice displayed increased mRNA levels of TNF-α and TNF-α receptor type 1, and higher ratio of CD1 + NKT-T cells vs. CD3 + cells compared with controls. In the heart of db/db mice, although no obvious infiltration of inflammatory cells was found, mRNA levels of TNF-α and TNF-α receptor type 1, NF-κB DNA-binding activity, nuclear expression of p65 and activation of p52 subunit were significantly higher compared with controls. Histological examinations showed marked accumulation of lipid droplets within cardiac myocytes, but no hyper trophy or increased deposition of collagen between myocytes in db/db compared with controls. On echocardiography, the ratio of E to A transmural flow velocities (an indicator of diastolic function) was significantly decreased in db/db mice, while indexes of systolic function were marginally affected. These changes were paralleled by increased mRNA expression of brain natriuretic peptide.

Conclusions: These results provide evidence for the presence of NF-κB activation and diastolic dysfunction in the heart of db/db mice. The abnormal immune responses and TNF-α production in the spleen tissue can contribute to NF-κB activation and cardiac dysfunction in type 2 diabetes.

P179 Lp(a), apo-b and ldl levels in premature coronary heart diseases in pakistani adults

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Background: Premature coronary heart disease (CHD) is a highly complex disorder with multiple risk factors.

Objective: To identify the risk factors responsible for premature (CHD) in local population.

Method: A case control study of 100 angiographically diagnosed patients and 100 healthy subjects was conducted. The risk factors assessed in this study were Serum Lips, Lipoprotein and Apolipoprotein-A1,Apolipoprotein-B, Lp(a). Diabetes Mellitus (DM), smoking, hypertension, family history of CAD, obesity and sedentary lifestyle. Descriptive, univariate and multivariate analyses were performed using paired t-test and student t-test for social sciences.

Results: Sedentary life style (OR=12.57, 95% CI: 3.68, 42.98), serum Triglyceride (TAG) level (OR= 245, 95% CI: 1.27, 4.72), HDLC (OR= 495, 95% CI: 23.5, 1042), Apo-A (OR= 186, 95% CI: 4.60, 24.76), Apo-B (OR= 10.49, 95% CI: 4.40, 24.76) and apolipoprotein (a) (OR= 215, 95% CI: 1.20, 3.94) were found to be significant factors. TAG levels, HDLC, Apo-A, LDL-C and HDLC ratio, total cholesterol and HDLC ratio were found to be significant among patients of age ≥ 40 years, while LDL-capolipoprotein B and Lp(a) was highly significant among age 40 years.

Conclusion: Raised level of Lp(a), apo-b and LDL-C among premature CAD patients have identified as risk factors in Pakistanis adults < 40 years.
P183 Different gene expression pattern in cardiac fibroblasts from HFNEF and HREF patients

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Methods: Endomycocardial biopsies were used to obtain cardiac fibroblasts from patients with heart failure normal EF as well as reduced EF. This human cardiac fibroblast cell culture system from these patients was used to investigate the basal expression pattern in regard to different matrix regulation in both groups. Moreover, the response of the fibroblasts to different stress stimuli known in heart failure was investigated.

Results: In cardiac fibroblasts derived from endomycocardial biopsies of HFNEF patients higher expression of ECM proteins and matrix metalloproteinases in cardiac fibroblasts from these patients. Furthermore, the stimulation with TGF-beta resulted in the transdifferentiation of fibroblasts to myofibroblasts (activated fibroblasts) in which an increased expression of collagen and alpha-smooth muscle actin was activated fibroblasts) in which an increased expression of collagen and alpha-smooth muscle actin was detected compared to patients with heart failure and reduced fraction injection (HFREF). Here we investigate the differences between the two heart failure types in regard to changes in the gene expression of ECM proteins and matrix metalloproteinases in cardiac fibroblasts from these patients.

Conclusions: Among STEMI-related UGBs we didn't find out any patient with platelet dysfunction (with optical aggreometry method). We couldn't establish a significant etiologic relationship between fibrogenic depletion level and postthrombosis UGB.

P184 Leptin induces fibrosis in rat cardiac fibroblast through oxidative stress stimulation. Role of galectin 3

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Purpose: Obesity is associated with cardiac functional and structural alterations that seem to be consequent, at least in part, by the accumulation of adipose tissue in the heart. Recent studies have demonstrated that plasma leptin concentrations, increased in several cardiac pathologies, may represent a predictor of first myocardial infarction and that leptin may represent an independent risk factor for the development of ischemic heart disease. However, cardiac effects of this hormone, that is locally produced in the heart, is not well established because it has been described both deleterious and protective effects on cardiac function and structure. For this propose, our aim was to evaluate the effects of leptin on fibrosis, oxidative stress and proliferation of cardiac fibroblasts, as well as the mechanisms involved.

Methods: Collagen I protein levels were measured in response to leptin (10-100 ng/ml) in cardiac fibroblasts from adult rats (passage 2-3) by Western Blot. Protein expression of GTGF, TGF-β and galectin 3, profibrotic factors, was also studied in response to leptin. The proliferative response of cardiac fibroblasts to leptin in the presence or not of angiotensin II (10-7 M) was also evaluated by a proliferation assay. In addition, the effect of leptin was studied measuring superoxide anion production with DHE. Finally, the profibrotic effect of leptin was also evaluated in the presence of the antioxidant agent, metilene (10-6 M).

Results: Leptin increased (p < 0.05) superoxide anions and collagen I levels in a dose-dependent manner, reaching maximal values at 24 hours. High levels of collagen I were accompanied by an increase in the expression of galectin 3, profibrotic factors, that is locally produced in the heart, is not well established because it has been described both deleterious and protective effects on cardiac function and structure. For this propose, our aim was to evaluate the effects of leptin on fibrosis, oxidative stress and proliferation of cardiac fibroblasts, as well as the mechanisms involved.

Conclusions: The expression of leptin as a profibrotic factor on cardiac fibroblasts, although it was unable to modify the proliferation of these cells. The fibrotic effect seems mediated by its ability to increase oxidative stress and seems to be mediated by the activation of growth factors such as TGF-β, TGF-β2 but also by the production of galectin-3.

P185 Expression of asnosomening water channel aquaporin-1 and cytoskeleton remodeling associated with endotoxin-induced myocardial dysfunction in aging mice

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Objective: Seipos is characterized by impairment in cardiac function, which further declines with aging. Moreover, the impact of age on key regulatory genes of cardiac cytoskeleton and vascular permeability, such as gelsolin and aquaporin-1 (AQP1), respectively, with key regulatory genes of inflammation such as inducible nitric oxide synthase (iNOS) during endotoxic stress, and how they correlates with endotoxic-stress induced cardiac dysfunction.

Methods and Results: Senescent (24 months) and young (3 months) male mice were treated 5 p. with saline or lipopolysaccharide (LPS, 30 mg/kg). Cardiac function and cardiac morphology were analyzed by echocardiography at baseline and 2 and 24 hours after injections. Mice were sacrificed and cardiac proteins were collected for the analysis of expression of total and cardiac-specific actin, gelsolin, AQP1, iNOS, nitric oxide production, and signal transducers and activators of transcription-3 (STAT3). LPS administration induced a higher pro-inflammatory gene expression, which were greater in senescent animals compared with young mice. These changes were parallelized by decreased expression of actin in the heart tissue, along with up-regulation of gelsolin and AQP1, and by a concomitant increase of iNOS and phosphorylated STAT3, with a maximum effect at 24 hours after LPS injection.

Conclusions: Endotoxic stress induced a high pro-inflammatory genes, such as iNOS. Modulation of the expression of these genes may be causally associated with the elevated susceptibility to cardiac dysfunction in aged patients during inflammatory stress.

P186 Molecular insights into filament assembly defects of ARVC-related desmin mutations

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Purpose: In patients with arrhythmogenic right ventricular cardiomyopathy (ARVC) several groups recently identified mutations in the DES gene. Desmin is a 53 kDa highly conserved muscle-specific intermediate filament (IF) protein. In many cases, these mutations result in a loss of the filamentous network due to abnormal aggregation of desmin. However, little is known about the molecular patho-mechanisms of ARVC-associated desmin mutations. Recently we and others published the ARVC-related DES-mutations p.N116S and p.N342D, which lead to desmin aggregation. Currently it is unknown, if the loss of the desmin aggregates or the gains of new functionalities are causative for desmin-related pathologies. We therefore constructed model variants of the corresponding desmin-aggregate residue to get insight into the molecular side chains, which are responsible for desmin filament formation defects.

Methods: We generated the different desmin model mutants (p.N116A/ADQ/QT and p.N342D/A/ QY) by side-directed mutagenes and investigated the IF-formation in different transfected cell lines by fluorescence microscopy.

Results: By generation of different model mutants for the ARVC-associated DES-mutations p.N116S and p.N342D we found, that the impact on filament assembly of both asparagine-residues is different. All substitutions at the position 116 caused massive desmin aggregation indicating a key role of the amino acid N116 for proper desmin filament formation. Even the elongation of the side chain (p.N116Q) induced desmin filament formation defects. In contrast, substitution of N342 against ala nine, glutamine and glutamate led to filamentous networks in transfected cells. These results indicate specific pathogenic filament assembly defects caused by the aspartate-residue.

Conclusions: In summary we found that any substitution of N116, belonging to the IF consensus motif LNDR leads to a loss of function mutation with aggregate formation. We suggest that the amide group and its position of N116 are absolutely necessary for IF-assembly.
There is progressive need in small diameter (D. Byzov; I. Mikhaylova; N. Chizh; E. Pushkova; O. Synchykova; B. Sandomirsky) atherosclerotic arteries to use endogenous MMPs to endogenous proteolytic activity in vivo. Using this innovative approach, in aortic tissue with high MMP-9 activity, proteolytic activity is associated with plaque rupture leading to heart attacks and stroke. Surprisingly little is known about the targets of MMPs in the vasculature. In the present study, we used a proteomics approach to identify vascular targets for three members of the major classes of MMPs: MMP-3, a matrix metalloproteinase that regulates the formation of extracellular matrix (ECM). In atherosclerotic MMP-3 activity is associated with plaque rupture leading to heart attacks and stroke. Sporadically little is known about the targets of MMPs in the vasculature. In the present study, we used a proteomics approach to identify vascular targets for three members of the major classes of MMPs: MMP-3, MMP-9, and MMP-14. The aim of our study was to assess the metabolic state including limitation in exercise capacity, shortness of breath, early fatigue, and the development of cachexia. One of the major factors in both populations that reduces quality of life and is associated with an unfavorable prognosis is cachexia. The underlying mechanisms of cancer-mediated cardiac cachexia is an oversimplification of a complex area of biology, and our study is a first attempt to contribute to a better understanding about the role of MMPs in the vasculature.

**Conclusions:**
1. Cardesanz has positive effect on the carbohydrate metabolism, leads to insulin level decrease while maintaining glycemia after the glucose stress.
2. Bisoprolol doesn’t decrease IR in this category of patients and in 12 weeks stimulates compensatory increase of IR for saving normal glycemia after the glucose stress test.

**P188**
Identification of new matrix metalloproteinase targets in the vasculature
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Matrix metalloproteinases (MMPs) play a key role in vascular remodeling and cardiovascular disease. MMPs degrade and reorganize the vascular extracellular matrix (ECM). In atherosclerotic MMP-3 activity is associated with plaque rupture leading to heart attacks and stroke. Sporadically little is known about the targets of MMPs in the vasculature. In the present study, we used a proteomics approach to identify vascular targets for three members of the major classes of MMPs: MMP-3, a matrix metalloproteinase that regulates the formation of extracellular matrix (ECM). In atherosclerotic MMP-3 activity is associated with plaque rupture leading to heart attacks and stroke. Sporadically little is known about the targets of MMPs in the vasculature. In the present study, we used a proteomics approach to identify vascular targets for three members of the major classes of MMPs: MMP-3, MMP-9, and MMP-14. The aim of our study was to assess the metabolic state including limitation in exercise capacity, shortness of breath, early fatigue, and the development of cachexia. One of the major factors in both populations that reduces quality of life and is associated with an unfavorable prognosis is cachexia. The underlying mechanisms of cancer-mediated cardiac cachexia is an oversimplification of a complex area of biology, and our study is a first attempt to contribute to a better understanding about the role of MMPs in the vasculature.

**Conclusion:**
The proposed treatment allows to design integrally functioning biological vascular grafts based on xenogenic arteries. Positive results of experimental transplantation testify that devitalized arteries are promised to be studied in clinic as biological vascular grafts.

**P190**
Telomere length in metabolic disorders in group of survivors of Leningrad Siege
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Purpose: The impairment of perinatal nutrition could promote the development of metabolic disorders and cardiovascular complications in the adult life. Consecutive results have also been reported on intrauterine starvation influence on telomere length what is one of the discussed markers of metabolic disorders and it has been found to be shortened in subjects with obesity, insulin resistance, arterial hypertension. The aim of our study was to assess the metabolic state and telomere length in subjects who survived of Leningrad Siege during second World War (1941-1944) comparing to control group of the same age.

**Methods:** 189 survivors of Leningrad Siege (54 males, 135 females) were examined on EVA syndrome. In 36 of Siege survivors (13 males, 23 females) and 12 controls (6 males, 6 females) the telomere length was also examined. The patients were divided in two groups: born before the Leningrad Siege (157 subjects) and during the Leningrad Siege (32 subjects). Informed consent was obtained from all participants. All participants were interviewed by special questionnaire regarding lifestyle and risk factors. Blood pressure was measured on right arm in the sitting position after 5 minute of rest two times. Anthropometric measurements were performed according to standard procedures. Fasting serum lipids and plasma glucose were measured on Hitachi-760. Telomere length were measured by qRT-PCR, the ratio of telomere repeat copy number to single gene copy number (T/S) was calculated for each DNA sample.

**Conclusions:** Survivors who were born during the Leningrad Siege had higher prevalence of metabolic disorders. Starvation in late gestation and early perinatal period predict short telomere length and promote the development of metabolic syndromes.

## Rate predictors of metabolic parameters

<table>
<thead>
<tr>
<th>Parameters</th>
<th>Subjects were born before siege</th>
<th>Rate prevalence</th>
<th>Subjects were born after siege</th>
<th>p</th>
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</thead>
<tbody>
<tr>
<td>Hypertension</td>
<td>17 mmol/l</td>
<td>25%</td>
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<tr>
<td>Obesity</td>
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<td>75%</td>
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<tr>
<td>Glucose</td>
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<td>Patients with hypertension</td>
<td>77%</td>
<td>87,5%</td>
<td>0.05</td>
<td></td>
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</tbody>
</table>

**P191**
Mitral valve prolapse and sudden cardiac death. Is there a cardiomypathy linked to floppy mitral valve?
S.D. Preston1; D. Baskaran2; A.M. Ponzczi2; K. Norr2; S.V. De Nororh2; M.N. Sheppard3
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**Background:** Mitral valve prolapse (MVP) is a relatively common disorder of the mitral valve and most cases take a benign clinical course. This condition is common in the elderly but also recognised in young patients, rarely leading to a sudden cardiac death (SCD). The aim of our study was to assess the frequency, demographics and histologic myocardial abnormalities in patients with MVP and SCD.

**Design:** A retrospective study of MVP and SCD referred to our tertiary referral centre during the period of 1994-2010.

**Results:** A total of 39 cases of SCD with MVP were identified with a similar distribution by gender: 11,3 (58), median age 47, range 19-79 years. MVP was associated with left ventricular myocardial fibrosis in over half of the cases (n=22, 56%) predominately in females (63%, n=14). Left ventricle hypertrophy (LVH) (n=11, 28%) alone (n=2) or with fibrosis (n=9) was also common with MVP. The myocardium was normal in 6 cases.

**Conclusions:** Abnormalities of the myocardium, of which fibrosis was the most common, were present in 85% of cases raising the question of a possible link of MVP to cardiomypathy. It is also possible that the floppy valve change is secondary to the cardiomypathy.

**P192**
Cancer induced cardiac cachexia displays a shift of metabolic genes
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Purpose: Patients suffering from heart failure or advanced cancer share several clinical features including limitation in exercise capacity, shortness of breath, early fatigue, and the development of cachexia. One of the major factors in both populations that reduces quality of life and is associated with an unfavorable prognosis is cachexia. The underlying mechanisms of cancer-mediated cardiac cachexia are poorly understood. To investigate putative metabolic alterations, we evaluated the expression of genes involved in fatty acid oxidation in a mouse cancer model associated with cardiac cachexia.

**Methods and Materials:** 60 hypertensive patients, aged 20-45, with fasting HIR and/or HIR after glucose stress test, without diabetes mellitus, glucose intolerance or high fasting glycemia and regular antihypertensive therapy were examined. Fasting glucose and immunoreactive insulin (IRI) during glucose stress test were determined. All patients were randomized in 2 groups, comparable by age, sex, glucose and insulin levels. 1st group (n=30) received candesartan (7.4 ± 1.38 mg daily), 2nd group - bisoprolol (6,0 ± 1.93 mg daily). HOMA indexes and area under insulin curve before treatment and in 12 weeks were calculated.

**Results:** All patients achieved target blood pressure levels. In candesartan group HOMA index as well as fasting (IRI level did not change (p > 0.05). Significant decrease of HOMA in 30, 60, 90, 120 minutes and area under insulin curve after glucose stress test was identified. In bisoprolol group increase of HOMA index (p=0,001), fasting IR (p=0,000) levels in 30, 60, 90, 120 minutes and area under the insulin curve (p=0,033) were detected. IRI concentration in candesartan group was 33% less (p=0,044) in 90 minutes and 54,4% less (p=0,000) in 120 minutes after glucose stress than in bisoprolol group. With similar baseline data in hypertensive patients with HIR 6 weeks of treatment. In 1st group insulin production to support carbohydrate homeostasis decreased in response to glucose stress, as opposed to the 2nd group where this index increased and fluctuation was 17,86% as compared to baseline data, what with normal glycemia indicates IR decrease in candesartan patients and IR strengthening with compensatory IRI levels increase to support normal glycemia in bisoprolol patients.

**Conclusions:**
1. Candesartan has positive effect on the carbohydrate metabolism, leads to insulin level decrease while maintaining glycemia after the glucose stress.
2. Bisoprolol doesn’t decrease IR in this category of patients and in 12 weeks stimulates compensatory increase of IR for saving normal glycemia after the glucose stress test.

**Abstracts**

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Methods and Results: A solid peritumoral tumor was induced through intraperitoneal implantation of murine TGCT cell lines (106 per mouse). Tumor bearing mice revealed markedly reduced heart weight/body weight (HW/BW: Cntr.: 5.1 ± 0.2, vs. Tumor: 3.7 ± 0.2 mg/g; p < 0.001) and heart weight/body weight ratio as compared to control mice (HW/TL: Cntr.: 12.6 ± 0.2, vs. Tumor: 5.0±0.5; p < 0.001) three weeks after cell implantation. Echocardiographic analysis showed reduced systolic function of tumor bearing mice as measured by fractional shortening (FS: Cntr.: 38.9 ± 10.4%, n=12 vs. Tumor: 23.3 ± 5.1%, n=9, p<0.001) and overall thinning of the wall thickness. This was associated with a high mortality in cancer animals (66%, n=25, vs. 0% in control, n=17; p < 0.001). QT-R-PCR revealed increased mRNA expression of three peroxisome proliferator-activated receptor isoforms (PPARα, d and g) and their co-factor PGC1α (PPARα: +71.4 ± 19.2%, p<0.002; PPARd: +173 ± 10.1%, p < 0.004; PPARg: +70 ± 20.6%,p<0.04; PGC1α: +36.1 ± 23.3%,p<0.01). Western blot analysis of subcellular fragments revealed reduced cytoplasmic protein levels and enhanced nuclear levels of PPARα in the cachetic mycardium, indicative of enhanced nuclear translocation. Moreover, the mRNA level of cardiac palmitoyltransferase-1, the rate-limiting enzyme that acts in β-oxidation, was significantly increased (CPT1α: +114 ± 29.4%, p < 0.001; CPT1β: +164.8 ± 21.9%, p<0.03).

Conclusion: These findings demonstrate that cachetic canceria is associated with up-regulation of components of the PPAR pathway involved in muscle fatty acid oxidative gene tran-
It was found that metabolic benefits, such as improved aerobic energy metabolism and decreased meta-
bolism, as well as reduced extracellular matrix deposition and increased endothelial permeability, were
pillary enlargement but not on sprouting angiogenesis. Several systemic dose-dependent side-effects, in-
cluding transient increases in liver, kidney and pancreatic enzymes, and also signs of cardiac failure, such as
decreased ejection fraction in cardiac echo and left ventricular strain in ECGs, were observed with the highest AdVEGF-A doses. Both blood and lymphatic routes were found to mediate the spread of the transgene in systemic and local gene transfers.

Comparing our evidence to previously published safety data, we conclude that increased perfusion and
vascular permeability in the angiogenic tissues might facilitate the release of the transgene from the
target muscles into the systemic blood and lymphatic circulation. Thus, as more efficient gene
constructs are being developed and tested, careful attention should be paid to systemic side-effects as
also peripheral, intramuscular gene transfers can have wide systemic side-effects possibly also in-
cluding cardiac failure.

P197 Local anti-angiogenic gene therapy reduces in-stent restenosis in a preclinical ath erosclerotic triple-injury model
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Background: In-stent restenosis (ISR) remains a key limitation to endovascular revascularization
the functional illness driven by fibrosis, myocarditis, and hyperthrophy. TGF-β plays a central role in
hamper the healing of the vessel through re-endothelialization. The growth of new vasorum into
the restenotic lesion from the adventitia has also been suggested as an accelerator for the process
of ISR and Vascular Endothelial Growth Factor (VEGF) is the key mediator of angiongenesis.

Our aim was to study the feasibility and therapeutic potential of local anti-angiogenic therapy using
inhibiting soluble VEGF receptors that act as decoys and decrease the amount of free VEGF in the

Methods: 67 hypertensive WHHL rabbit aortas were denuded with an embolectomy catheter.
Six weeks later side branch free sections of the abdominal aorta were injected with 1.5x1010 pfu
adenovirus encoding soluble VEGF receptors (sVEGFR1, sVEGFR2, sVEGFR3) or control LacZ
using lnfritator drug delivery catheter. After gene transfer a bare metal stent (BMS) was implanted
on same section at 1:1.1 ratio. Contrast angiographies and euthanasia were performed on d6, d14,
d2 and d90 followed by tissue harvest. Histological analyses were performed with immunohisto-

Results: Gene transfer efficacy was assessed at d6 with LacZ controls and found to be sufficient
and localized mainly in the adventitial cells and abluminal parts of the media. There were no signifi-
cant differences between the groups in the degree of restenosis at d14. At d14 sVEGFR1 group
showed 14.6% reduction and sVEGFR3 group a 36.3% reduction in restenosis compared to control.
At d90 the reduction in the rate of restenosis persisted but was attenuated (10.9% and 25.7%,
reduction in sVEGFR1 and sVEGFR2 respectively. Treatment with sVEGFR1 and sVEGFR2 reduced proliferation in the neointima compared to LacZ control evaluation as proliferating,
Ki-67 positive cell nuclei in mm2 of neointima. The proliferation was reduced at d14 by 53.2% and
63.6% and by 25.0% and 73.5% at d90 (sVEGFR1 and sVEGFR3, respectively). The
antiangiogenic therapy did not hamper luminal re-endothelialization and there were no differences
between the groups.

Conclusions: This study suggests that local anti-angiogenic gene delivery might be a useful therapy
when treating ISR without the use of strong stent coronary stents. Although the peak expres-
sion after adenoviral gene therapy occurs at d6 and is faded by d14, we show positive results
persisting three months after treatment.

P198 Inhibition of TGF-β pathway reverts extracellular matrix remodeling in T. cruzi-infected cardiac microtissues
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Background and Purpose: Chagas disease, which is caused by the protozoan T. cruzi, affects
over 10 million people worldwide and represents the leading cause of cardiac lesions in econom-
ically productive adults in endemic areas of Latin America. Chagasic cardiomyopathy is a progressive
infiltrative myocarditis of autoimmune etiology. The disease causes remodelling of the heart and
isovolumetric relaxation (p < 0.01), a shorter time constant of isovolumetric relaxation tau
(+a2.5% of CMs compared to controls. Survival was higher in AdA-I MI mice than in control MI mice during follow-
up for 28 days (hazard ratio for mortality 0.42, 95 CI 0.24 to 0.76). Improved healing in AdA-I MI mice
was evidenced by a 20% shorter (p<0.05) infarct length, a 41% higher infarct thickness (p<0.05),
and an increased capillary density and a higher collagen content in the infarct area. In contrast,
immature collagen was reduced (p<0.05) in the remote myocardium of AdA-I MI mice. Attenu-
ance was also evidenced by a reduced LV cavity area by 24% (p<0.005) in AdA-I MI mice.
Improved infarct healing and less adverse remodeling in AdA-I MI mice resulted in a sig-
nificantly better cardiac function as evidenced by enhanced isovolumic contraction (p<0.01)
and isovolumic relaxation (p<0.01), a shorter time constant of isovolumic relaxation tau
(p<0.05), a lower end-diastolic pressure (p<0.01), and a preservation of blood pressure com-
pared to sham mice. In contrast, a significant decrease of peripheral blood pressure was observed
in control MI mice (p<0.01).

Interestingly, Ad-I transfer increased the number of circulating endothelial progenitor cells (EPCs) by 75% (p<0.05). Ex vivo EPC expansion was enhanced in follow-

ening transfer. EPC migration was further increased in AdA-I+LDL mice compared to controls. LDL.
Paracrine effects of EPCs may have contributed to improved infarct healing and attenuation of remodeling.
Gene expression analysis of the infarct zone at day 3 showed that increased LDL resulted in specific inotropic effects (higher IL-10, IL-1β, and TGF-β1), higher collagen type I and type III expression. Inflammatory cytokines and increased expression of anti-apoptotic Bcl-2.

Conclusion: Direct cardioprotective effects of LDL mediate improved infarct healing and attenu-
ation of remodeling post-MI.

P200 Selective high density lipoprotein raising gene transfer improves survival, infarct healing, and left ventricular function after myocardial infarction in mice
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Purpose: S100A1 has emerged as a key factor in the control of cardiomyocyte (CM) contractile function
and the pathogenesis of heart failure. Improved sarcoplasmic reticulum (SR) function with enhanced
expression protected against Ca2
+ leak, triggered Ca2
+ waves and in-antagonists to Ca2
+ leak, Ca2
+ waves in and after contractions in response to Ca2
+ and CaM.

Selective high density lipoprotein raising gene transfer improves survival, infarct healing, and attenu-
ated pressure combined cAMP-independent inotropy with protection against Ca2
+ leak, and CaR-triggered pro-arrhythmogenic events. Our data point towards the suppression of the SR Ca2
+ leak by direct interaction of S100A1 with CaR. Further research is clearly needed and ongoing studies focus on the precise molecular interaction of S100A1 at the RyR2.

Abstracts

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Cardiovascular Research Supplements
Objective: This study was to compare the level of benefit among modified Sequential Organ Failure Assessment (SOFA), interleukin (IL)-6, and IL-10 levels for evaluation of the severity of SIR during peri-operative period in patients undergoing cardiac surgery with CPB which is reflected by prolonged ICU stay (>42 hours).

Methods: This prospective observational design study was approved by Khon Kaen University Human Ethics Committee (HE531033). Thirty-four consecutive adult patients who underwent elective cardiac surgery with CPB were enrolled between February and June 2010. Plasma concentration of IL-6, IL-10 levels were collected and measured sequentially at 0, 0.5, 1, 2, 4, 6, and 24 hours after CPB. Data for modified SOFA score, including peri-operative outcomes and complications, were collected at the same time points. In addition, the receiver operating characteristics (ROC) analysis was used to determine the usefulness of these three indexes as predictors of prolonged ICU stay.

Results: A total of 34 patients were admitted to ICU after cardiac surgery. Sixteen of these, aged 61.1 ± 3.1 years, required prolonged ICU stay and had longer mechanical ventilation than the non-prolonged ICU group (P < 0.05). The mean of all 3 prognostic models in patients with prolonged ICU stay were significantly higher at 4 hours and were still significantly increased after 24 hours than for those with non-prolonged ICU, except IL-10 which declined to near normal levels (P < 0.05). Correlation of IL-6 and modified SOFA score were significantly higher than correlation of IL-10 and modified SOFA (R²= 0.743, P < 0.001, respectively). Calculation of area under ROC curve (AUC) for 3 prognostic models at 4 hours after CPB to predict prolonged ICU stay provided comparable values (0.743 for IL-6, 0.847 for IL-10, and 0.847 for modified SOFA score) with highest AUC for modified SOFA score.

Conclusion: All 3 prognostic models at 4 hours after CPB can reasonably predict the severity of SIR which is reflected by prolonged ICU stay. The modified SOFA score is the most simple yet highest AUC.
myocardial infarction. It was characterized by early (1h) and sustained activation (7 days) post ischemic reperfusion injury and consisted of miRNAs predominantly expressed in platelets.

Conclusions: In subjects with subsequent myocardial infarction differential co-expression patterns of circulating miRNAs occur around endothelial-enriched miR-126 with platelets being a major contributor to this miRNA signature.

P208
Lipid tetrad index and lipid pental index are lower in women?
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Purpose: Some lipid indexes have been proposed for the diagnosis and prognosis of cardiovascular disease (CVD). The lipid tetrad index (LTI) and lipid pental index (LPI) were recently introduced in the literature to assess the cardiovascular risk. This study aimed to evaluated LTI [total cholesterol x triglycerides x lipoprotein(a)] and LPI [total cholesterol x triglycerides x lipoprotein(a) x apolipoprotein A-I] in subjects who attended a clinical laboratory.

Methods: We conducted a descriptive cross-sectional observational study. Through software-Bi-SADIG, used for data management of the clinical laboratory, 285 subjects were selected from 20 to 70 years who performed the measurements of total cholesterol (TC), triglycerides (TG), high density lipoprotein (HDL), low density lipoprotein (LDL), lipoprotein(a) [Lp(a)] and apolipoproteins A-I and B, within a period of two years. Those patients who had plasma triglyceride levels greater than 400 mg/dL, were excluded.

Results: LTI and LPI median and interquartile values was 8.8 (3.4 - 19.3) and 29.7 (11.2 - 62.3), respectively (n = 285). Plasma levels of Lp(a) was significantly lower (p < 0.05) in women (13.2 mg/dL, 7.1 - 31.7, n=161) when compared with men (24.2 mg/dL, 9.1 - 36.8, n=124) LTI values were significantly higher in men (10.4 [7.3 – 21.5]) than in women (7.9 [2.8 – 15.9]). LPI values was also significantly higher in men [348.2 [12.2 – 66.8] than in women [240.0 [10.0 – 58.7]]. No significant difference was observed between men and women for TC, TG, apolipoprotein A-I, HDL or LDL. There was no significant difference for LPI and LTI values when the participants were divided and grouped by the age ranges 20-30, 31-40, 41-50, 51-60 and 61-70 years.

Conclusions: The present study shows that LTI and LPI values were significantly higher in men than in women, regardless of whether both sexes showed no significant differences in traditional lipid and apolipoprotein profiles. Although LTI and LPI values are not yet used routinely, they may play a relevant and additional role in the assessment of the diagnosis and prognosis of CVD. The LTI and LPI values were so far unknown in our population and additional studies are essential to authoritatively elucidate the reference values for men and women.

P209
Disrupted serotonergic system in patients with pulmonary hypertension may serve as novel biomarkers to help in diagnosis and risk stratification.
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Purpose: The role of serotonin as a factors triggering pulmonary hypertension due to left heart disease is well identified.

Methods: In the present study, we aimed to investigate immunostaining intensity of human right atrium myocardium serotonin transporter (5-HTT) by a monoclonal anti-5-HTT antibody (Advanced Targeting Systems, 1500), as well as the concentrations of plasma and platelet serotonin (5-HT) and its metabolite - 5-hydroxyindoleacetic acid (5-HIAA) by HPLC from patients with pulmonary hypertension (PH) due to left heart disease. The control group is a people without cardiovascular diseases. Values are expressed as means ± SEM. Statistical analysis was carried out using an unpaired Mann — Whitney U-test. statistical dependence between two characteristics was carried out using Person correlation test. A value of p < 0.05 was considered statistically significant. All patients gave written consent for participating in the study, which was approved by the Ethical Committee.

Results: 1). We established that all patients with PH (n = 14) showed significant rises of 5-HT levels in both plasma and platelets in comparison control (n = 12) (35.60 ± 10.001, pp < 0.001); 1102.66 ± 264.11 (p < 0.01) respectively versus control 12.36 ± 1.88, 422.75 ± 120.99 mmol/L). Patients with PH have the highest plasma and platelets level of 5-HIAA in comparison control (158.98 ± 51.79 (p < 0.001), 4.23 ± 1.14 (p < 0.001) respectively versus control 24.33 ± 4.45, 1.44 ± 0.45 mmol/L). 2). We found strong positive correlation between plasma 5-HIAA and pulmonary artery pressure of PH group (r=0.6, P < 0.05), plasma 5-HT and platelets 5-HT levels (r=0.7, P < 0.05) and right ventricle size (r=0.7, P < 0.05), plasma 5-HT and right atrium size (r=0.72, P < 0.05); platelets 5-HT and right atrium size (r=0.8, P < 0.01). 3). The immunohystochemical study showed the presence of 5-HTT proteins in all studied preparations of myocardium from patients with PH due to left heart disease. Immunohistochemical staining of 5-HTT protein in myocardium from patients with PH stronger than myocardium from people without cardiovascular diseases.

Conclusions: Thus, pulmonary hypertension due to left heart disease is characterized by increased blood serotonin and 5-HIAA plasma level, increased myocardium serotonin transporter that may involved in progression of pulmonary hypertension and right ventricular failure. Changes in peripheral serotonin metabolism may be used as novel diagnostic approaches and at the same time serve as therapeutic targets.

P210
Early diagnostic markers for NSTEMI preceeding troponin
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Purpose: To identify risk markers which could be used for early risk stratification of patients with NSTEMI prior to the time point at which troponin is measurable.

Methods: The present study was a prospective, observational study of 602 consecutive patients with suspected NSTEMI who were admitted to the emergency department of a teaching hospital at Erasmus University Medical Centre, Rotterdam, The Netherlands from November 1995 to February 1996. Patients with a first or second troponin measurement higher than 0.100 ng/ml were excluded from the analysis.

Results: In 562 patients, 48% (271) had NSTEMI. The early markers of NSTEMI were significantly higher in patients who developed NSTEMI in comparison to those who did not. These markers included the following: CRP (r=0.38), albumin (r=0.37), platelet count (r=0.36), WBC (r=0.34), and fibrinogen (r=0.34). In addition, the area under the ROC curve for the early markers was significantly higher than that of the serial measurements of the troponin.

Conclusions: In conclusion, early markers of NSTEMI can be identified prior to the time point at which troponin is measurable. These markers could be used for early risk stratification of patients with NSTEMI prior to the time point at which troponin is measurable.
Conclusions: 26 patients who still had negative troponin T tests, did already show significantly elevated TLR4 PCR in whole blood samples obtained from 35 patients with acute myocardial infarction with ST elevation, enrolled in the EXAM trial, at presentation at the CCU and after 4 months (considered baseline). We confirmed that TLR4 are upregulated during MI. We identified for the first time a correlation of both TLR2 and TLR4 mRNA levels with infarct size (R=0.59, p<0.0003 and R=0.46, p=0.0007). Furthermore we showed that both TLRs are upregulated earlier than troponin T. 25 out of 26 patients who still had negative troponin T tests, did already show significantly elevated TLR4 mRNA levels, while 9 of these 26 troponin T-negative patients showed elevated TLR2 levels.

Methods and Results: We evaluated the mRNA expression levels of TLR2 and TLR4 by realtime PCR in whole blood samples obtained from 35 patients with acute myocardial infarction with ST elevation enrolled in the EXAM trial, at presentation at the CCU and after 4 months (considered baseline). We confirmed that TLR4 are upregulated during MI. We identified for the first time a correlation of both TLR2 and TLR4 mRNA levels with infarct size (R=0.59, p<0.0003 and R=0.46, p=0.0007). Furthermore we showed that both TLRs are upregulated earlier than troponin T. 25 out of 26 patients who still had negative troponin T tests, did already show significantly elevated TLR4 mRNA levels, while 9 of these 26 troponin T-negative patients showed elevated TLR2 levels.

Conclusions: TLR2 and TLR4 expression levels correlate with infarct size and are expressed earlier than troponin T, indicating that TLR2 and 4 are early and sensitive markers for MI, which will be evaluated as a diagnostic tool in our future research.

P211 Videodensitometry in assessment of renal blood flow before and after renal artery stenting

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Purpose: to investigate the feasibility of videodensitometry in assessing the renal parenchymal perfusion in patients with renal artery stenosis before and after stenting.

Methods and Materials: 101 renal angiographic data of 101 patients with and 55 patients without renal artery stenosis were analyzed by means of videodensitometry, using < MultiVio> software. Time-density curves were calculated for renal parenchyma and parenchymal perfusion parameters were measured as shown on fig. 1. Parechymal perfusion coefficient was measured as (max intensity - min intensity)/max intensity. All patients included in study underwent renal arteries duplex ultrasound examination. Levels of blood pressure and kidney function as a clinical signs of renovascular hypertension were assessed. In 41 patients, who underwent renal artery stenting for atherosclerotic renal artery stenosis, videodensitometric parameters of renal blood flow before and after renal artery stenting were compared.

Results: videodensitometric analysis allows detecting statistically significant differences in parenchymal perfusion between kidneys with and without renal artery stenosis. A grade of changes in parenchymal perfusion correlates with angiographically measured degree of renal artery stenosis and renal artery blood flow velocity. Also, the videodensitometry reveals differences in renal perfusion before and after renal artery stenting. However, these differences were significant only in patients with renal artery stenosis more than 75% with peak systolic velocity more than 250 cm/s.

Conclusion: videodensitometric perfusion parameters can be used to assess the effect of renal artery stenosis on parenchymal blood flow. Thus, videodensitometry extends the diagnostic capability of the angiographic study.

P212 The use of erythrocyte indices for diagnosis of left ventricular hypertrophy in hypertensive women

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The most available methods are ECG criteria for LVH: Sokolov-Lyon index (sensitivity 22%) and Cornell index (sensitivity 42%). More sensitive and specific in determining LVH is an echocardiogram. However, this method is relatively expensive and require high-tech equipment and trained professionals. The development of left ventricular hypertrophy in hypertensive patients is often complicated by impaired erythrocyte homeostasis (the most common anomaly). Women often suffer from anemia, in addition, the indicators are susceptible to erythrocyte homeostasis physiological level fluctuations. Studies in animals and humans have shown that ischemic or hypertrophied myocardium is more sensitive for a slight decrease in hemoglobin than the unmodified myocardium. The aim is to estimate the state of erythrocyte homeostasis in women with hypertension. Were examined 58 women with essential hypertension (EH) stage 2, 2nd degrees. The age of women - 57 (11) years. Patients with symptomatic hypertension, arrhythmias, diabetes mellitus, hematological disorders were excluded from the study.

In all patients were carried determination of total hemoglobin (Hb), erythrocytes in 1 liter (Er), mean corpuscular hemoglobin (MCH), mean corpuscular hemoglobin concentration (MCHC), mean corpuscular volume (MCV). Echocardiography was performed by standard ASE methods. Statistical significance was defined at the level of methods for p < 0.05. In assessing the relationship with indicators of erythrocyte homeostasis parameters, reflecting the structural state of the left ventricle of the heart in women with hypertension, we identified correlations in between MCV and MCH with a wall thickness and index of left ventricular mass (R=0.38, p=0.003).

The most pronounced relationship was between the thickness of the posterior wall of the LV (LVPWd) and MCH. Construction of equations of the linear approximation possible to determine the thickness of diastolic LVPW by the formula: LVPWd = Kd + Kp * MCH, where Kd - correction correlation of MCH, Kp - correction factor of MCH. The described method of identifying LHV was applied in 38 women with EH. Based on research by constructing the correlation curve approximation method it was determined the value of Kd equal to 3.131 and the value of Kp equal to 0.2548. When the values of the thickness of more than 11.9 LVPWd diagnosed left ventricular hypertrophy. Thus, the results of the study indicate: 1. an association of erythrocyte indices with a wall thickness of the left ventricle in patients with EH; 2. the possibility to determine LHV in women with hypertensive patients using the value of MCH.

P213 Activation by morphine withdrawal of the hypothalamic-pituitary adrenal axis and heat shock protein 27 in the left ventricle: role of extracellular signal-regulated kinase

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Purpose: to investigate the feasibility of videodensitometry in assessing the renal parenchymal perfusion in patients with renal artery stenosis before and after stenting.

Methods and Materials: 101 renal angiographic data of 101 patients with and 55 patients without renal artery stenosis were analyzed by means of videodensitometry, using < MultiVio> software. Time-density curves were calculated for renal parenchyma and parenchymal perfusion parameters were measured as shown on fig. 1. Parechymal perfusion coefficient was measured as (max intensity - min intensity)/max intensity. All patients included in study underwent renal arteries duplex ultrasound examination. Levels of blood pressure and kidney function as a clinical signs of renovascular hypertension were assessed. In 41 patients, who underwent renal artery stenting for atherosclerotic renal artery stenosis, videodensitometric parameters of renal blood flow before and after renal artery stenting were compared.

Results: videodensitometric analysis allows detecting statistically significant differences in parenchymal perfusion between kidneys with and without renal artery stenosis. A grade of changes in parenchymal perfusion correlates with angiographically measured degree of renal artery stenosis and renal artery blood flow velocity. Also, the videodensitometry reveals differences in renal perfusion before and after renal artery stenting. However, these differences were significant only in patients with renal artery stenosis more than 75% with peak systolic velocity more than 250 cm/s.

Conclusion: videodensitometric perfusion parameters can be used to assess the effect of renal artery stenosis on parenchymal blood flow. Thus, videodensitometry extends the diagnostic capability of the angiographic study.

P214 Dipetidyl peptidase-4 (DPP-4) inhibitor preserves cardiac function and heart rate variability and prevents cardiac mitochondrial dysfunction in high-fat-induced insulin resistant rats

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Long-term high fat diet consumption has been shown to cause insulin resistance and diabetes mellitus, leading to cardiac dysfunction and depressed heart rate variability (HRV). Although dipetidyl peptidase-4 (DPP-4) inhibitor has been shown to have glycemic control, its cardiac effects are still unclear. In the present study, we hypothesized that DPP-4 inhibitor, vildagliptin, attenuates cardiac dysfunction, preserves the HRV and prevents mitochondrial dysfunction in insulin resistant rats induced by high fat consumption.

Methods: Rats were fed with either normal (ND) or high fat diet (HF) for 3 months (n = 12/group). Then, rats in each group were fed with either vildagliptin (3 mg/kg/day) or vehicle for 21 days. In each rat, plasma glucose, insulin, cholesterol, cardiac function, and HRV were determined at baseline and end of treatment. Cardiac mitochondrial function was also studied.

Results: High fat-fed rats developed insulin resistance, indicated by increased plasma insulin, cholesterol, body weight, visceral fat and HOMA index. High fat-fed rats also had increased LF/HF ratio, indicating depressed HRV. Vildagliptin improved the insulin resistant condition, and completely restored the HRV (Figure). Vildagliptin also improved end-diastolic pressure, end-systolic pressure, and stroke volume in high fat-fed rats, compared to the vehicle group. Moreover, vildagliptin attenuated cardiac mitochondrial dysfunction caused by high fat consumption, by decreasing ROS production and preventing mitochondrial gene expression changes (Figure).

Conclusions: In high fat-induced insulin resistant rats, depressed HRV and cardiac dysfunction could be markedly improved by DPP-4 inhibitor vildagliptin. This cardioprotection was due to its prevention of mitochondrial dysfunction caused by high fat consumption.
Bitter agonists increase vascular tonus immediately after intake
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Introduction:
Digestion of food involves:
- the production and secretion of enzymes
- Splanchnic circulation increases: 1) to support the digestive organs by increasing cellular nutrition and removing waste products and; 2) to remove digested compounds from gastrointestinal tissues. Splanchnic increases are referred to as post-prandial hyperaemia and begin immediately after food intake. Bitter agonists are traditionally used in numerous Eurasian cultures to aid digestion. We investigated whether the two bitter agonists gentian (Gentiana lutea) and wormwood (Artemisia absinthium) impacted on vascular tonus. Gentian is used in central European alpine areas for the production of aperitifs. Wormwood is used in the production of the aperitif vermouth which is popular in Mediterranean countries and is a key ingredient of martinis in North America.

Method: Three groups of 13 healthy non-medicated participants were tested in a randomised double-blind procedure with 70 mL water and:
- Group1 - three different sets of capsules containing: placebo (cellulose) control, 1000 mg gentian and 1000 mg wormwood.
- Group2a - 1 mL of fluid extracts containing either 500 mg or 1500 mg gentian; and
- Group2b - 1 mL of fluid extracts containing either 500 mg or 1500 mg wormwood.

Continuous cardiovascular measurements were obtained with the Finometer for five minutes pre-ingestion and 30 minutes post-ingestion. Measures from three post-ingestion periods: P1 (0 to 5 minutes), P2 (10 to 15 minutes) and P3 (25 to 30 minutes) were compared to pre-ingestion measures with the Group1 placebo capsule condition measures acting as the control.

Statistical analysis: Group1 Repeated measures ANOVA and Groups 2a and 2b ANCOVA.

Results:
Group1: the capsules elicited no vascular changes.
Group2a: gentian fluid extracts increased peripheral resistance in P1 (1500 mg, p = 0.020) and P2 (500 mg, p = 0.019).
Group2b: during P1 wormwood 1500 mg fluid extract decreased arterial compliance (p = 0.004) and increased diastolic pressure (p = 0.005).

Conclusion:
Encapsulated gentian and wormwood did not elicit vascular responses when present in the gut yet when consumed as drinks both bitter agonists increased vascular tonus in the period immediately after intake. These findings lead to the conclusion that bitter agonists can impact the vasculature via chemosensory reflexes and support the traditional usage of consuming bitter tasting drinks to aid digestion. Subsequently the ingestion of foods and drinks may have a greater impact on the vasculature than hitherto recognised.