Abstracts From the 13th Annual Meeting of the European Council for Cardiovascular Research (ECCR)

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ECCR Oral Presentations

01.03 Longitudinal study of resistance artery function during the development of diet-induced obesity
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This work aims to characterize changes in vascular reactivity of resistance vessels related to the development of diet-induced obesity (DIO). Four-week-old C57Bl/6J male mice were assigned either to a low-fat (LF: 10 kcal % fat) or to a high-fat diet (HF: 45 kcal % fat) during 8, 14 or 32 weeks. Reactivity of resistance vessels was characterized by pertussis toxin (200 ng/kg i.p.) and assessed by flow myograph

01.04 Skeletal myoblasts transfected with liposome carrying vascular endothelial growth factor-165 for treatment of hind limb ischemia
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The study aims to use cholesterol (Chol) + DOTAP liposome (CD liposome) based human vascular endothelial growth factor-165 (VEGF-GFP) gene transfer into skeletal (SkMs) for treatment of acute hind limb ischemia in a rabbit model. The feasibility and efficacy of CD liposome mediated gene transfer with rabbit SkMs were characterized using plasmid carrying enhanced green fluorescent protein (pEGFP) and assessed by flow cytometry. After transfection, SkMs were transfected with CD lipoplasms carrying plasmid-VEGF-GFP (CD-pVEGF-GFP) and transplanted into rabbit ischemic limbs. Animals were randomized to receive intramuscular injection of either Medium199 (M199; group-1), non-transfected SkM (group-2), CD-pVEGF-GFP transfected SkM (group-3). Flow cytometry revealed that up to 16% rabbit SkMs were successfully transfected with pEGFP on the optimized transfection condition, transfected rabbit SkMs expressed VEGF-GFP up to day-18 with peak at day-2. SkMs were observed in all cell-transplanted groups, as visualized with DAPI and BrdU. Angiographic blood vessel score revealed increased capillary density in group-3 (14.88±2.0; p<0.03) compared to LF animals. Moreover, a higher NO production was observed in animal group-3 (0.173±0.18 vs 0.04±0.03; p<0.01) compared to LF animals. We hypothesized that even a low dose of eplerenone 50 mg in this study.

03.02 Benefits in total mortality and cardiovascular events in The Hypertension In The Very Elderly Trial (HYVET) by major subgroups
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Introduction: The HYVET trial showed marked reductions in total mortality and cardiovascular (CV) events. We examined whether this benefit varied by sex, age, previous CV disease and initial systolic blood pressure (SBP). Methodology: HYVET was a randomised, double-blind, placebo-controlled trial recruiting patients aged 80 or more. Entry criteria included a SBP of 160–199 mmHg. Active treatment was based on indapamide (SR) 1.5mg. Results: The hazard ratios (HR) with 95% confidence intervals (CI) for total mortality and cardiovascular (CV) events were 0.69 (0.50–0.96), 0.65 (0.49–0.88), 0.63 (0.49–0.82) and 0.75 (0.51–1.2) for the subgroups below. The interaction terms between active treatment and the various subgroups were not significant for total mortality (0.30<p<0.1) or CV events (0.42<p<0.80). Conclusions: For both total mortality and CV events benefits were seen across sex, age, previous CV disease and initial SBP. This adds support for the use of eplerenone even at low dose is highly effective in reducing blood pressure in patients with resistant hypertension. Moreover, eplerenone lowered left ventricular mass independent from its BP lowering effects.

03.03 Subjects with premature cardiovascular disease have a diminished glycocalyx volume as compared to healthy controls
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Introduction: The inner surface of the vesselwall consists of a glycoprotein layer, which is called the glycocalyx. This surface protects the vesselwall from atherogenic stimuli and preserves endothelial function. Subjects with premature cardiovascular disease (CVD) possibly lack protection against atherogenic stimuli and are therefore more likely to develop atherosclerosis. Whether these subjects also have a diminished glycocalyx volume is still unknown. We therefore investigated glycocalyx volumes in subjects with premature CVD.

03.01 Effect of eplerenone (50mg) on left ventricular mass in resistant hypertension
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MR antagonists are effective in resistant hypertension and reduce left ventricular (LV) mass. We hypothesized that even a low dose of eplerenone (50mg) will be effective to lower blood pressure (BP) in resistant hypertension and will reduce left ventricular mass to an greater extent than BP lowering alone.

We performed a randomized, double-blinded, placebo-controlled, parallel group study in 50 non-diabetic patients with resistant hypertension. All patients at enrolment received ACE inhibitors or angiotensin receptor blockers and diuretics together with a third drug. We additionally treated for six months with eplerenone 50 mg or to reach BP control without the use of MR antagonists by optimizing antihypertensive treatment (placebo group). Primary endpoint of the study was reduction of LV mass as assessed by MRI. Comparisons of groups were done by t-Test. All values are given as M±SD. Eplerenone treatment reduced casual BP by 35±20/15±11 mmHg, in the placebo group BP was lowered by 30±18/13±7 mmHg (p=0.40 and 0.57, respectively)24-hour BP: 18±13/9±6 vs. 13±8±7±7; p=0.13 and p=0.33). Eplerenone treatment reduced LV mass by 12.1%, placebo treatment reduced LV mass by 1.8%. This difference was statistically significant (p=0.038). There were no safety issues (e.g. hyperkalemia) with the use of eplerenone 50 mg in this study.

We conclude that eplerenone even at low dose is highly effective in reducing blood pressure in patients with resistant hypertension. Moreover, eplerenone lowered left ventricular mass independent from its BP lowering effects.

Abstracts From the 13th Annual Meeting of the ECCR
Aortic stiffness is associated with silent cerebrovascular disease in hypertensive patients

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Aortic stiffness predicts an excess risk of stroke, supposedly via cerebral small-vessel disease. Therefore, we assessed whether aortic stiffness is related to white matter hyperintensities (WMHs), silent lacunar infarcts (LACs) and brain microbleeds (BMBs). In 167 hypertensive patients (85 males) without a history of cardio- or cerebrovascular disease, a mean age of 51.8 ± 13.1 years and untreated office blood pressure levels of 169.3 ± 25.1/104.2 ± 12 mmHg, we determined aortic pulse wave velocity (aPWV), office and ambulatory 24-hour pulse pressure (off-medication), as well as the volume of WMHs and the presence of LACs and BMBs using brain MRI. Linear and logistic regression analyses were performed to assess the relationships between the arterial stiffness measures and brain lesions. Aortic stiffness and pulse pressure were significantly related to each of the brain lesions in univariate analyses (p < 0.05). Multivariate analyses, adjusted for age, sex, brain volume, mean arterial pressure and heart rate, showed that a higher aPWV was significantly associated with a greater volume of WMHs (unstandardized regression coefficient, 0.04; 95% CI, 0.005–0.078; p = 0.05) and the presence of LACs (odds ratio per SD increase in aPWV, 1.78; 95% CI, 1.06–2.99; p = 0.05), but not with microbleeds (odds ratio, 1.13; 95% CI, 0.67–2.19). The models for pulse pressure failed to reach statistical significance in multivariable analyses. In conclusion, aortic stiffness is independently associated with cerebral small-vessel disease in hypertensive patients without a history of cardio- or cerebrovascular disease.

Aortic stiffness predicts an excess risk of stroke, supposedly via cerebral small-vessel disease in hypertensive patients without a history of cerebral small-vessel disease in hypertensive patients.
differences between the groups in blood pressure were observed. High salt intake during pregnancy accelerates maturation of glomeruli but reduces the final number of glomeruli. Low number of glomeruli is known to cause high blood pressure later in life.

### W3.03

**Superoxide formation in human intrarenal arteries is mainly due to NADPH oxidase activity and contributes to endothelium dependent vasodilation in distal artery segments**

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We investigated the contribution of the NADPH oxidase (NOX) activity to superoxide anion formation in human intrarenal arteries and the modulation of agonist-induced vasoconstriction as well as endothelium dependent vasodilation by superoxide anions. Interlobar, arcuate and interlobular artery segments were dissected from non-cancerous regions of kidneys obtained from thirteen patients who underwent nephrectomy because of a renal tumor. Using inhibitors of the major oxygen radical forming systems (xanthine oxidase, NO-synthase, mitochondrial respiratory chain) we found that in human intrarenal arteries 75% of the superoxide anion formation was NOX-dependent. NOX activity was two-fold higher in distal (interlobular) than in proximal (interlobar) artery segments. mRNA expression levels of the isozymes NOX2 and NOX4 and the small subunits p22phox and p47phox which similar in interlobar, arcuate and interlobular arteries. Phosphorylated (P) and endothelin-1 (ET-1) induced similar maximum tension in arcuate and interlobar arteries with a p22 of 6.25 ± 0.25 and 8.22 ± 0.07, respectively. PE and ET-1-induced vasoconstriction was not affected by the oxygen radical scavenger Tiron. When precontracted with 3 μmol/l PE, acetylcholine reduced vascular tone in both artery segments by 60 %, Tiron significantly blunted acetylcholine-induced vasodilation in both arcuate and interlobar arteries. We conclude that the major source of superoxide anion formation in human intrarenal arteries is NADPH oxidase activity. Superoxide formation contributes to endothelium-dependent vasodilation and vasodilation in distal intrarenal arteries of human kidneys.

### W3.04

**Angiostatin-(1–7) improves renal endothelial dysfunction by inhibiting nitric oxide bioavailability in apoE deficient mice**

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ApoE-deficient mice (−/−) are associated with endothelial dysfunction caused by a decreased NO bioavailability. Ang-(1–7), acting through the MAS receptor seems to have endothelial-dependent vasorelaxant properties. Thus, the aim of our study was to investigate whether impaired endothelial function improves under chronic treatment with Ang-(1–7) in apoE(−/−) mice. 6 week old ApoE(−/−) mice fed with a lipid rich Western diet were divided into 3 group and treated via osmotic minipump either with saline, Ang-(1–7) or Interlobar, arcuate and interlobar artery segments were dissected from non-cancerous regions of kidneys obtained from thirteen patients who underwent nephrectomy because of a renal tumor. Using inhibitors of the major oxygen radical forming systems (xanthine oxidase, NO-synthase, mitochondrial respiratory chain) we found that in human intrarenal arteries 75% of the superoxide anion formation was NOX-dependent. NOX activity was two-fold higher in distal (interlobular) than in proximal (interlobar) artery segments. mRNA expression levels of the isozymes NOX2 and NOX4 and the small subunits p22phox and p47phox which similar in interlobar, arcuate and interlobular arteries. Phosphorylated (P) and endothelin-1 (ET-1) induced similar maximum tension in arcuate and interlobar arteries with a p22 of 6.25 ± 0.25 and 8.22 ± 0.07, respectively. PE and ET-1-induced vasoconstriction was not affected by the oxygen radical scavenger Tiron. When precontracted with 3 μmol/l PE, acetylcholine reduced vascular tone in both artery segments by 60 %, Tiron significantly blunted acetylcholine-induced vasodilation in both arcuate and interlobar arteries. We conclude that the major source of superoxide anion formation in human intrarenal arteries is NADPH oxidase activity. Superoxide formation contributes to endothelium-dependent vasodilation and vasodilation in distal intrarenal arteries of human kidneys.

### W3.05

**Development of hypertension and vascular remodeling depend on the immunological background in mice**


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Hypertension is associated with inward remodeling of small arteries. We propose that vascular remodeling relates to inflammation of the vessel wall and depends on the immunological background. We determined cardiovascular parameters and the systemic and local inflammatory response in mice, which received Nω-nitro-L-arginine methyl ester (L-NAME) to induce hypertension. The wall-to-lumen ratio of small mesenteric arteries was the main outcome measure. Two strains of mice with a genetically different immune background were used: C57BL/6 mice, a T-helper 1 dominant phenotype (n=14); and BALB/c mice, a T-helper 2 dominant phenotype (n=13). After 4 weeks, mean arterial blood pressure was increased by only 11% in BALB/c (P<0.04) and by 53% in C57BL/6 (P=0.001). Arteries from C57BL/6 mice showed a 6.5-fold greater increase in the wall-to-lumen ratio with L-NAME treatment (from 0.14 ± 0.02 to 0.23 ± 0.03), than BALB/c mice (from 0.106 ± 0.004 to 0.110 ± 0.003; P<0.001). In both strains, a complex inflammatory response was found after 3 days of L-NAME treatment, which had returned to baseline values after 4 weeks. The inflammatory response was similar in the two strains, except for the leukocyte marker CD11b, which showed an increased expression in C57BL/6 only. Confocal microscopy confirmed the presence of CD11b+/C668 leukocytes in the vessel wall. These data show that vascular remodeling and hypertension are strain dependent. Mice with a T-helper 1 phenotype are highly susceptible to the development of vascular remodeling and hypertension. This effect is associated with the recruitment of CD11b+/C668 leukocytes in the vessel wall.

### W3.06

**Lack of circulating serotonin increases blood pressure in mice**

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Serotonin (5-HT) has generally been considered to be a vasoconstrictive and hypertensive factor. Cardiovascular hypersensitivity to 5-HT is a hallmark of hypertension, and plasma levels of free 5-HT are elevated in hypertension. The circulating levels of 5-HT are 90–95% reduced in mice deficient in tryptophan hydroxylase 1 (TPH1), since they are devoid of platelet serotonin. In this study, we investigated basic mean arterial pressure (MAP) and heart rate (HR) and the response to 5-HT, 5-HT receptor agonists and antagonists in TPH1−/− deficient mice on a C57BL/6 background. MAP in TPH−/− (n=21) mice was significantly (p<0.0005) higher under baseline conditions than in wild-type (n=18) mice (116.1±1.3 vs. 108.2±1.1 mmHg). One minute after the injection of the 5-HT receptor agonist, 8-OH-DPAT, MAP decreased in TPH1 knockout mice but not in C57BL6 mice (−10.3±1.4 vs. −2.2±1.5 mmHg). In the same time, HR fell more strongly in TPH−/− mice (−180.6±16.4 vs. −49.0±19.6 beats/min, p<0.002), which may explain the difference in blood pressure reduction. No significant differences in the response to the 5-HT4 receptor antagonist, WAY-100635, were observed. Alpha-methylserotonin, a nonselective 5-HT3 receptors agonist, induces a dose dependent decrease in MAP in all mice, but TPH−/− animals showed a more pronounced fall. On the other hand, only in C57BL6 mice, cyproheptadine, a 5-HT3 receptor antagonist, increased MAP.

Our data suggest that the unexpectedly elevated blood pressure in TPH1−/− deficient mice is caused by the chronic absence of the ligand for 5-HT, and especially for 5-HT1 receptors, which both show antihypertensive properties.
Novel and known Angiotensinogen promoter variants and tissue specific Angiotensinogen expression in human kidney and visceral adipose tissue

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Objective: Angiotensinogen (AGT), is involved in essential hypertension especially when obesity-related. Human AGT gene promoter polymorphism has been associated with altered AGT transcription in vitro and with essential hypertension. In this study we investigated the association among AGT promoter variants and AGT expression levels in human visceral adipose tissue (VAT) and kidney to verify whether AGT promoter variants are associated with different tissue-specific AGT expression in vivo.

Methods: Samples of tissue from 35 consecutive non-diabetic patients undergoing renal surgery. AGT gene expression was studied by RealTime Taq-Man assay and genomic sequence of the AGT gene promoter (from -306 to +36) were obtained for each patient to identify variants. Statistical models were constructed considering age, gender and BMI.

Results: Two novel SNPs (rs175GA and rs163GA) in strong linkage disequilibrium (LD = 0.99) were associated with lower AGT expression only in VAT (P = 0.033). Patients with the known -20C variant had 3-fold higher AGT expression only in kidney medulla (P = 0.038) when compared to -20A homozygotes. The other known SNPs (rs94271GA and rs175GA) were not associated with different levels of AGT expression.

Conclusions: Two novel AGT promoter variants in strong LD appear to down-regulate AGT expression in VAT. The proximity and linkage of -175A and -163A variants suggest that they might destabilize the binding of specific nuclear factors. On the contrary, the -20C variant is associated with higher AGT expression in kidney medulla. Our results support the hypothesis that AGT promoter variants affect transcriptional activity in a tissue-specific way in humans.

Hic-5 and Hsp27 regulate contractility in rat mesenteric small arteries

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Cytoskeleton remodelling is an important component of contraction and in smooth muscle p38MAPK and tyrosine kinases are implicated in actin polymerisation and contraction, through Hsp27 and the cytoskeletal protein paxillin respectively. We studied the roles of downstream targets of p38MAPK and tyrosine kinases in cytoskeletal reorganisation and contraction in rat mesenteric small arteries. We identified hydrogen peroxide-inducible clone-5 (Hic-5), a paxillin homologue, and showed that nonselective Nω-nitro-L-arginine induced its tyrosine phosphorylation in a Src dependent manner. Furthermore, NA induced an interaction of Hic-5 with proline rich tyrosine kinase (PYK2) but not Src or p125focal adhesion kinase. This interaction was Src dependent suggesting that Hic-5 was a substrate for PYK2 downstream from Src. In parallel, NA induced p38MAPK dependent Serine2 phosphorylation of Hsp27 causing its dissociation from actin filaments and p38MAPK dependent actin polymerisation. Additionally, NA induced an interaction between Hsp27 and Hic5 that required phosphorylation of both proteins and was within the same time frame as NA induced contractile response. Inhibition of either p38MAPK or Src blocked the NA induced interaction between Hsp27 and Hic5 and the contractile response. Combined inhibition of p38MAPK and Src had no greater effect on contraction than individual inhibition, suggesting that the two pathways act through a common mechanism. These data show that parallel activation of p38MAPK and tyrosine kinases by NA regulates actin cytoskeleton dynamics and contraction through interaction of the downstream effectors Hic-5 and Hsp27 in small arteries.

Vaspin - a new regulator of cardiac fibroblast proliferation

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The close relationship of epicardial fat to the heart and its secretory activity implicate a possible influence of epicardial fat on the heart. In addition, obesity is known to increase the risk of cardiac hypertrophy and heart failure. In the present study we investigated the expression pattern of the adipocytokine vaspin (visceral adipose tissue-derived serine-protease inhibitor) in epicardial fat during the development of obesity-mediated cardiac hypertrophy and the effect of vaspin on cardiac fibroblasts. In male C57Bl/6j mice no significant differences in cardiac hypertrophy were detected after 15 weeks of high fat diet (HFD) and in low fat diet (LFD) control mice. However, heart weight/lean bodyweight ratio

significantly increased from 6.42 ± 0.66/g to 6.88 ± 0.61/g between week 15 and 25 of HFD, (p < 0.05). In parallel, vaspin mRNA-expression in epicardial fat markedly increased 2.4-fold from 15 to 25 weeks HFD. To explore molecular interactions between vaspin and cardiac hypertrophy, HIC2 cardiomyoblasts were stimulated with vaspin and leucine uptake was analyzed. Vaspin did not regulate leucine uptake. Next we investigated murine primary cardiac fibroblasts proliferation by BrdU-assay which shows a potent induction by vaspin (1.8-fold, vaspin 1µg/ml, p < 0.05 vs. 0.5%FBS, and 2.0-fold 0.1µg/ml vaspin p < 0.005 vs. 0.5%FBS) compared to a 1.9-fold induction by angiotensin II (10µM). The present study indentifies a new adipocytokine, vaspin, which is upregulated in epicardial fat during the development of cardiac hypertrophy. Vaspin might be involved in the pathogenesis of cardiac hypertrophy by inducing cardiac fibroblast proliferation of murine cardiac fibroblasts in vitro.

The High Mobility Group A1 Protein - a new regulator of PPARgamma-dependent gene transcription in vascular smooth muscle cells

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The study aimed to identify new nuclear effectors for PPARgamma (peroxisome proliferator-activator receptor gamma)-dependent gene transcription in human aortic smooth muscle cells (HASMC) in order to develop new PPARgamma-ligands with improved clinical safety.

Using an Oligo GEArray® Human Nuclear Receptors and Coregulators Microarray, we identified the transcriptional regulator and chromatin modifying High Mobility Group (HMG) A1 protein expressed in unstimulated HASMC. PPARgamma-dependent gene regulation was studied by analysis of PMA-induced MMP-9 (matrix metalloproteinase 9) expression ± pioglitazone (pi 10µM), PMA (50ng/ml) stimulated MMP-9 mRNA expression by 46.3 ± 22.3-fold (p < 0.05 vs. vehicle) which was markedly blocked by pi (10µM) (14.7-fold 1.9-fold vs. PMA alone p < 0.05). Furthermore PMA-induced MMP-9 promoter activity by 45% in transactivation assays in HEK293 using a pRL3-MMP-9 construct.

To evaluate the role of HMG1A, gene-silencing experiments with siRNA for HMG1A were performed (91 % in HASMC and 80.2% in HEK293 reduction of HMG1A protein expression). HMG1A siRNA completely abolished PPARgamma-mediated MPP9-mRNA repression (control siRNA: po- mediated MPP9 regulation vs. PMA: -66.8% in HASMC and -59.3% in HEK293 p < 0.01; HMG1A siRNA: pi-mediated MPP9-regulation vs. PMA: +10.7 % in HASMC and + 14.7% in HEK293 vs. PMA p n.s.).

By using ChIP assay we could demonstrate that pi-induced PPARgamma activation leads to a potent recruitment of PPARgamma (3.0 fold vs. 1.15 fold PMA) and HMG1A complexes (1.24 fold vs. 0.0 fold PMA) to the MMP9 promoter in HASMC. In conclusion, HMG1A is required for PPARgamma-mediated repression of MMP-9 gene transcription. Ligand-induced HMG1A-PPARgamma interactions might be an important determinant for ligand-specific anti-atherosclerotic actions.

SHP2-dependent dephosphorylation of p190A Rho GAP induces RhoA activation by Angiotensin II in vascular smooth muscle cells

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Angiotensin II (Ang II) is a major regulator of blood pressure, that essentially acts through activation of Ang II type 1 receptor (AT1R) of vascular smooth muscle cells (VSMC). AT1R receptor activates numerous intracellular signaling pathways, including the small G protein RhoA that control several VSMC functions such as contraction, differentiation and proliferation. Nevertheless, the mechanisms leading to RhoA activation by AT1R are unknown. Here we assed the involvement of the p190A GTPase-activating-protein (GAP) in this process. Small interfering RNA(siRNA)-mediated p190A silencing in VSMC increased basal RhoA activity (9.3±3.0 vs. 4.3±2.9% PMA) which was significantly reduced by siRNA transfected cells (1.2±0.3% vs. 4.3±2.9% PMA p < 0.05). In parallel, vaspin mRNA-expression in epicardial fat markedly increased 2.4-fold from 15 to 25 weeks HFD. To explore molecular interactions between vaspin and cardiac hypertrophy, HIC2 cardiomyoblasts were stimulated with vaspin and leucine uptake was analyzed. Vaspin did not regulate leucine uptake. Next we investigated murine primary cardiac fibroblasts proliferation by BrdU-assay which shows a potent induction by vaspin (1.8-fold, vaspin 1µg/ml, p < 0.05 vs. 0.5%FBS, and 2.0-fold 0.1µg/ml vaspin p < 0.005 vs. 0.5%FBS) compared to a 1.9-fold induction by angiotensin II (10µM). The present study indentifies a new adipocytokine, vaspin, which is upregulated in epicardial fat during the development of cardiac hypertrophy. Vaspin might be involved in the pathogenesis of cardiac hypertrophy by inducing cardiac fibroblast proliferation of murine cardiac fibroblasts in vitro.
PA.01 Decreased microcirculation compromises diastolic heart function in acute myocardial infarction

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Objective: The aim of this study was to assess the relation between the coronary flow velocity reserve (CFVR) and the non-invasively estimated of left ventricular (LV) filling pressure in patients with a recent acute myocardial infarction (AMI). Materials and Methods: A median of 4 days (IQR: 2–7 days) after admission for AMI, echocardiograms were obtained in 102 consecutive patients. We excluded patients with prior myocardial infarction, significant valvular disease and a known stenosis of >70% in the left anterior descending coronary artery (LAD). LV filling pressure was estimated using the ratio of early transmural flow velocity (E) to early diastolic mitral annulus velocity (E/e'). Transthoracic echocardiographic Doppler recordings of coronary flow in the distal LAD were performed at rest and during Adenosine infusion (140 μg/kg/min). CFVR was calculated as the hyperemic-to-resting coronary diastolic peak velocity ratio. Results: In patients with CFVR below 2.0, an accepted limit for dysfunctional CFVR, E/e' was 11.9 and in patients with CFVR above 2.0 E/e' was 9.6 (P = 0.0073). In patients with E/e' below and above 12, CFVR was 1.65 and 2.00 respectively (P = 0.01). In multiple linear regression analysis, E/e' was independently associated with CFVR (P = 0.013) after adjustment for age, gender, type of infarction (STEMI vs. Non-STEMI), history of hypertension and diabetes. Conclusions: The study suggests the existence of an independent association between CFVR and LV filling pressure in patients with AMI. The pathophysiologic mechanisms and the clinical implications of this finding warrant further investigation.

PA.02 Is mean blood saturation (SmbO2) a useful marker of tissue oxygenation?
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Increasingly we are monitoring the distribution of oxygen through the microcirculation using optical techniques such as optical reflectance spectroscopy (ORS) and near infrared spectroscopy (NIRS). Mean blood saturation (SmbO2) and tissue oxygen index (T0i) derived by ORS and NIRS respectively, evoke a concept that we can measure oxygen delivery to the tissue. The aim of this study is to establish whether SmbO2 is an appropriate indicator of tissue oxygenation. Mean blood saturation (SmbO2) was measured at rest in the skin microcirculation of forearm or index finger in 30 healthy subjects (15 male, age 21–42 years). Fourier analysis was applied to the spontaneous fluctuations in SmbO2 measured by ORS as changes in concentration of oxyhaemoglobin [HbO2] and deoxyhaemoglobin [Hb]. Two distinctly different spontaneous falls in SmbO2 were observed and identified as Type I swings and Type II swings. Type I swings induced by fluctuations in arterial blood volume resulted from the effects of respiration, endothelial, sympathetic and myogenic activity. There was no apparent change in [Hb]. In contrast, Type II swings resulted from a fall in [HbO2] accompanied by a rise in [Hb] and were only induced by endothelial and sympathetic activity. Thus the same fall in SmbO2 can be induced by two distinct mechanisms. The Type I swing does not suggest an inadequacy in oxygen delivery whilst the Type II swing may indicate a change in oxygen delivery to tissue. Blood oxygen saturation cannot therefore be accepted as a definitive marker of tissue oxygenation.

PA.03 Upstream and downstream cardiovascular variables are “disconnected” in patients with severe sepsis
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The lethality of severe sepsis is not declining. This may be due to over-emphasis on normalising macrocirculatory parameters which does not ensure adequate microcirculatory function or effective tissue oxygenation. Aim: To examine the correlation between traditional macrovascular resuscitation targets (cardiac index, CI; Oxygen delivery (DO2), central venous pressure (CVP) and mean arterial pressure (MAP)) and downstream markers of microvascular or tissue function (lactate, arterial pH and urine output). Methods: Macro and microvascular data were concurrently collected from 22 intensive care patients with severe sepsis on admission, at 6 and 12 hours, using oesophageal Doppler cardiac output monitoring (Deltex), arterial and central venous sampling, and standard pressure monitors. Changes in macrovascular variables were correlated with changes in surrogate microvascular and tissue function markers using linear regression analyses. Results: (Table 1.) Changes in cardiac output, filling pressures or oxygen delivery values neither predicted nor correlated with changes in tissue perfusion in septic patients over 6 or 12 hours.

Conclusion: There is no significant relationship between changes in macrovascular variables and downstream markers of tissue wellbeing over the first 6 or 12 hours of intensive care admission in patients with severe sepsis.

TABLE 1. (6 hr results)

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<th>Downstream markers</th>
<th>Macrohemodynamic variables</th>
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<tr>
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<td>Mean arterial BP</td>
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<td>Lactate</td>
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<td>pH</td>
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<td>Urine output</td>
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PA.04 Fovea thickness and capillary pressure in non-diabetic individuals
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Diabetic retinopathy is the leading cause of blindness in Europe’s working age population. Assessment of macular oedema (thickness), an important sequelae of diabetic retinopathy, has recently been revolutionised by Optical Coherence Tomography (OCT) enabling the detection of subclinical thickness changes in the macula. This study aims to examine whether systemic or capillary blood pressures are associated with fovea (central region of the macular thickness in individuals without diabetes or overt cardiovascular disease. Fovea thickness was determined in the right eye of 28 subjects (age range 26–74y, 12males) by OCT. Finger nailfold capillaries were cannulated using a glass micropipette, and the pressure measured by a servo-nulling system. Mean (standard deviation) fovea thickness in the right eye was 211(20) μm. Geometric mean capillary pressure (CP) was 16.3 (confidence intervals: 14.9–17.9) mmHg, and mean (SD) systolic and diastolic blood pressure were 118(17) mmHg and 73(11) mmHg respectively. Systolic, diastolic and capillary pressure data were entered into a stepwise linear regression model. CP was associated with fovea thickness, with the data suggesting that a 1 mmHg increase in CP results in a 1.72 μm increase in fovea thickness in the right eye. Systemic blood pressure was not associated with fovea thickness. This study demonstrates that finger nailfold capillary pressure is independently associated with fovea thickness in individuals without diabetes or overt cardiovascular disease. Further research needs to explore whether lowering capillary pressure reduces fovea thickness and thus delay the progression of macular oedema.

PA.05 The effect of glibenclamide on acetylcholine and sodium nitroprusside induced vasodilatation in human cutaneous microcirculation
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Objective: KATP channels have an important regulatory role in resting vascular tone and during hypoxia. Their role in endothelium dependent and independent vasodilatation in human microcirculation is less known. Methods: We monitored the laser-Doppler (LD) response in 14 healthy male volunteers on the skin of the forearm. In the case of endothelium dependent (acetylcholine (ACh) induced) vasodilatation, saline solution (control) or solution of glibenclamide (KATP channel blocker) were randomly injected each into distinct forearm followed by the iontophoresis of ACh. We tested endothelium independent (sodium nitroprusside (SNP) induced) vasodilatation by random microinjection of glibenclamide or saline solution each into distinct forearm, followed by the iontophoresis of SNP. Results: In the case of ACh application, there was a significantly lower LD flux increase after the application of glibenclamide in comparison with saline solution (p < 0.05, paired t-test). SNP application caused a significantly lower LD flux rise after glibenclamide application in comparison with saline solution as well (p < 0.05, paired t-test). Conclusions: According to our results KATP channels play an important role in ACh and SNP induced vasodilatation in human skin microcirculation.

PA.06 Reproducibility of a system for the assessment of early cardiovascular risk markers
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Aim: In this work the reproducibility of a system for the automatic assessment of carotid intima-media thickness, diameter and distension from ultrasound image sequences, which was developed in our lab, is evaluated. Methods: Sequences of the right/left common carotid arteries of 10 healthy volunteers were acquired and analysed in two different sessions 7 days apart. In the first session, two observers (operator 1 and 2) were involved and both of them examined each vessel three times. After each measurement the probe was removed and repositioned. In the second session, only operator 1 repeated the analysis. Intima-media thickness (IMT), diastolic diameter (Dd) and distension (ΔDd) were evaluated on each image sequence. Variabilities were presented as the coefficients of variation. Results: The intraobserver inassraenality variation was 7%±6% for IMT,
2%±1% for D2, and 11%±7% for ΔD. The interobserver intrasession variability was 8%±5% for IMT, 3%±2% for D2, and 11%±10% for ΔD. The intraobserver intrasession variability was 6%±6% for IMT, 3%±2% for D2, and 11%±10% for ΔD. Conclusions: The reproducibility of the system is not influenced by the observers’ skill and it is comparable to that reported in literature for highly precise but more expensive and complex ultrasound RF based systems. Thus, the robustness of our device, its low-cost and flexibility (it can be used with any ultrasound equipment) make it suitable for large population studies.

Allopurinol improves endothelial dysfunction by ameliorating vascular oxidative stress in patients with chronic stable angina on optimum medical therapy

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Introduction: To study the effect of xanthine oxidase inhibition on vascular oxidative stress (OS) and endothelial function in patients with stable angina without heart failure and on optimum medical therapy. Methods and Results: Randomised, double blind, placebo controlled, cross over study in 80 subjects. Forearm venous occlusion plethysmography (FVOP), flow mediated dilatation (FMD) and pulse wave analysis were used to assess endothelial function. Intra arterial vitamin C and acetylcholine co-infusion was used to assess vascular OS. Allopurinol improved endothelial dependent vasodilatation significantly when compared to placebo (mean ± SEM: 93% ± 8% vs 115% ± 11%, p<0.006). FMD (mean ± SD: 41.1 ± 1.8% vs 54.1 ± 1.7%, p<0.001) and augmentation index (27.32 ± 4.98 % vs 24.69 ± 4.55%, p<0.001) improved significantly as well. Vascular OS seen during placebo with highly significant improvement in forearm blood flow (p<0.001) with vitamin C and acetylcholine infusion, was conspicuously absent during allopurinol treatment (p<0.4) indicating amelioration of vascular OS. Conclusion: Our study demonstrates that despite contemporary, evidence based treatment for stable angina, endothelial dysfunction and vascular OS remain still marked. The improvements seen with allopurinol raise the prospect that xanthine oxidase inhibition might reduce future atherothrombotic events in coronary artery disease over and above their current therapies.

Glimerular hemodynamics and arterial function in normal subjects

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Objective: To determine the relationship between arterial function (stiffness and wave reflection) and glomerular hemodynamics. Methods: In 49 healthy normotensive subjects, glomerular filtration rate (GFR) and effective renal plasma flow (ERPF) were measured by urinary isotopic clearances. Filtration fraction (FF) was computed as GFR/ERPF. Urinary albumin-creatinine ratio (UACr) was quantified by nephelometry. Arterial stiffness was estimated by carotid-femoral pulse wave velocity (PWV). Wave reflection was evaluated by carotid augmentation index (AIx), reflection magnitude (RM) and the round-trip travel time of the pressure wave (TR). Results: PWV and TR were not correlated with any renal hemodynamic parameter or UACr. AIx and RM were directly correlated with FF (r = 0.35, p = 0.026 and r = 0.37, p=0.020, respectively) and UACr (r = 0.43, p = 0.003 and r=0.53, p<0.001, respectively). When the population was divided into quartiles of RM, FF and UACr progressively increased from the lowest to the highest quartile and after adjustment for age, arterial pressure, but also gender for UACr (p linear trend = 0.007 for FF and <0.001 for UACr). Conclusion: It is suggested that in normal subjects, the amplitude of wave reflection but not arterial stiffness is associated with signs suggestive of increased glomerular pressure (FF and UACr), independently of systemic blood pressure.

Evaluation of vascular parameters with a new real-time artery interfaces detection system

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Analyzing the artery mechanics is a crucial issue because of its close relationship with several cardiovascular risk factors, such as hypertension and diabetes. For this reason, an accurate temporal localization of the main vessel interfaces becomes a central task. The system which we developed is a stand-alone video processing system which automatically locate the position of the artery interfaces in real-time. Three clinical applications have been developed on the system and validated against gold-standard techniques: the flow-mediated dilation (FMD), the carotid intima-media thickness (CIMT) and the carotid arterial distension (CODIST).

The FMD method was tested on a total of 20 examinations. An expert analyzed twice the dataset, both manually and automatically. The regression analysis between automatic and manual %MD has slope 0.98 and intercept 0.01. The coefficient of variation of the automatic system is 3.0%. CIMT measurements were carried out on 150 ultrasound images. The comparison between the automatic and gold-standard measurements shows a bias of 0.001 mm with a SD of the differences of 0.035 mm. As regards the technical reproducibility, the intra-observer variability was 0.036% and the inter-observer variability was 0.52%. In 28 patients, we assessed carotid stiffness with our system and by means of applanation tonometry. The carotid-to-femoral PWV was significantly (p<0.0001) correlated with the parameter (r = 0.77) evaluated by our system.

In conclusion, the system we developed is a reliable and easy-to-use instrument that can help physicians in the evaluation of FMD, CIMT and CODIST.

Relationship between escalating atorvastatin dosage, flow mediated dilatation and biochemical markers of endothelial function in older men with vascular disease

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There is much evidence that the ‘pleiotropic’ effects of statins are of equal importance to their cholesterol-lowering effects and include up-regulation of endothelial nitric oxide synthase. The aims of this study are to determine if there is a dose-response relationship between ultrasonographic and biochemical markers of endothelial function and dosage of atorvastatin. A secondary aim was to identify if nitric oxide metabolites correlated with flow-dilatation (FMD).

Subjects (male, n=10) had a history of vascular disease and had elevated total cholesterol or low density lipoprotein at baseline. Brachial artery high frequency ultrasound was used to measure FMD following transient upper limb ischaemia. Data was collected at baseline and on treatment doses of 10, 20, 40 and 80mg of atorvastatin. FMD, lipids, urinary and serum nitric oxide metabolites were measured. High sensitivity CRP, microalbuminuria, adhesion molecules and selectins were also measured. Mean age was 72.5 ± 8.2 years.

There was a significant increase in FMD from the baseline statin naïve state to Atorvastatin 10mg once daily (1.1% to 4.6%, Z = -2.803, p<0.05). There was no further increase in FMD at higher doses. There was a significant increase in urinary nitric oxide metabolites measured at atorvastatin 80mg. There was no significant change in hsCRP, microalbuminuria, adhesion molecules or selectins.

This study identifies that low dose atorvastatin improves nitric oxide related FMD, a marker of endothelial function, while reducing cholesterol and LDL levels. The improvement in endothelial function was most marked at low dose statin therapy.

Escalating atorvastatin dosage does not augment flow mediated dilatation (FMD) in healthy subjects but does increase urinary nitric oxide metabolite production

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There is much evidence that the ‘pleiotropic’ effects of statins are of equal importance to their cholesterol-lowering effects. However there is conflicting evidence in the literature in relation to the augmentation of endothelial function in healthy adults with the use of various therapeutic agents, including statins. The aims of this study were to identify if changes in nitric oxide (NO) mediated flow dilatation and biochemical markers of endothelial function were seen at increasing doses of atorvastatin in previously healthy non-smoking adults.

10 male subjects were recruited. Brachial artery high frequency ultrasonography was used to measure flow mediated dilatation (FMD), following transient upper limb ischaemia. Data was collected at baseline and on treatment doses of 10, 20, 40 and 80mg of atorvastatin. FMD, lipids, urinary and serum nitric oxide metabolites were measured. High sensitivity CRP, microalbuminuria, and lipids were also measured. Mean age was 28 ± 5.4 years.

There was no significant alteration in FMD measurements as the dose of atorvastatin increased (FMD - Baseline 6.2%; Atorvastatin 80mg 7.1% - Z=-1.274, p=0.203). There was a small but significant increase measured in urinary nitric oxide metabolites at atorvastatin 80mg compared to baseline (60 to 72 micromol/mmol creatinine; Z=-2.803, p<0.005). There was no significant change in other markers of endothelial function.

This study suggests that FMD is not significantly augmented at increasing statin dosage in healthy young adults with normal baseline FMD, even in the presence of increased NO metabolism production.

Cardiovascular risk in young patients with rheumatoid arthritis: role of inflammation

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In order to evaluate the level of cardiovascular risk (CVR) and its relation to inflammatory activity in rheumatoid arthritis (RA) 71 young RA patients aged 18 to 44 years were
screened for CVR, according to European Society of Cardiology Guidelines, 2007. Results: 60.56% of patients were normotensive, 39.44% had mild/moderate arterial hypertension. Patients had no history of myocardial infarction, stroke or diabetess mellitus. 30.98% of RA patients had moderate and 69.02% – high RA activity (DSAS82), 26.76% RA patients had no risk factors (RF), 39.44% – 1 RF, 14.08% – 2 RF and 19.72% – 3 and more RF. Most frequent RF were: hypercholesterolemia, family history of CV disease, abdominal obesity, smokinghabit, hyperuricemia, 71.15% of patients had subclinical organ damage (OD): 25% - left-ventricular hypertrophy (LHV), 17.3% - increased intima-media thickness (IMT), 26.92% - combination of LHV and increased IMT, 7.69% - slight increase in plasma creatinine. Ankle-brachial index never was < 0.9. 61.29% of normotensive RA patients had subclinical OD and higher DSAS82 (P < 0.05) than normotensives with OD. 12.68% of patients had average CVR, 25.35% - low, 2.82% - moderate, 53.52% - high and 5.64% - very high CVR. It was found that age and DSAS28 were significantly associated with CVR in RA patients in a multiple regression model (adj. R2 = 0.53). Thus, RA patients have an unfavourable CV profile with high frequency of traditional RF and frequent subclinical OD. Inflammation evidently influences CVR level in RA. Therefore, control of inflammation is essential in RA patients in order to prevent cardiovascular disease onset and progression.

PA.13
Association between LDL subclasses and carotid intima-media thickness in coronary artery disease
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Background: Small LDL subclasses (sLDL) are known to play a causative role in atherosclerosis and its clinical manifestation—coronary artery disease (CAD). Previous studies show increased prevalence of sLDL in CAD patients with normal levels of traditional lipid risk factors. Carotid intima media thickness (IMT) is considered as a marker of atherosclerosis and in prediction of clinical coronary events. With increasing interest in the role of non-traditional lipid risk factors in CAD, we undertook this study to relate LDL subclass profile and carotid intima-media thickness in CAD patients. Methods: LDL subclasses were separated with 3–31% PAG Electrophoresis, and IMT was determined using high-resolution B-mode ultrason in 59 patients (age 40–69; 29 females and 30 males) with CAD, with normal levels of traditional lipid risk factors. Results: Mean value of left and right carotid artery measurement was selected as value for correlation with LDL subclass size in each patient. The mean LDL size was 24.97 ± 1.07 mm, and the mean IMT in all patients was 0.89 ± 0.13 mm (0.6–1.2 mm). LDL size was inversely correlated with IMT (r = −0.36; p < 0.01). Prevalence of subjects with increased IMT was higher among subjects with small LDL subclasses (d = 5.5 mm). Conclusion: LDL size showed a strong association with carotid IMT in CAD patients with normal levels of traditional lipid risk factors and may play an important role in assessment of coronary risk in addition to traditional coronary risk factors.

PB.01
Impact of gender and enhanced NO/cGMP signalling on aortic remodelling in experimental hypertensive nephropathy
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Cardiovascular complications represent the most important comorbidity in renal patients, with impaired NO production as key pathway. Estrogens seem to be cardioprotective in premenopause. This study investigates the effects of both gender and enhanced NO/cGMP signalling on aortic remodelling in a model of hypertensive nephropathy. Male (M) and female (F) Wistar rats were each assigned for 18 weeks into following groups: 1) subtotally nephrectomised (SNX); 2) SNX Bay 41–8543-treated; 3) SNX hydralazine-treated; 4) control. Prevalence of subjects with increased IMT was higher among subjects with small LDL subclasses (d = 5.5 mm). Conclusion: LDL size showed a strong association with carotid IMT in CAD patients with normal levels of traditional lipid risk factors and may play an important role in assessment of coronary risk in addition to traditional coronary risk factors.

PB.02
Pressure-induced remodelling in resistance sized arteries cultured for three days in a culture myograph system
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Objectives: Culture myography is an in-vitro ex-vivo technique that allows to study the effects of different stimuli on isolated resistance arteries. It is well known that sustained hypertension determines small arteries remodelling; to date, different roles of wall stress and shear stress in this process are not completely elucidated. Aim of this study was to assess whether different culture pressures can cause different degrees of remodelling in a 3-day-experiment. Methods: Eighteen rats, 6 Spontaneously Hypertensive Rats (SHR) and 12 Wistar-Kyoto rats (WKY), 12-weeks-old were used for the present study. First order mesenteric arteries were mounted in a culture myography system. Vessels were incubated at different pressures (SHR 60 mmHg, WKY 60 mmHg and WKY 35 mmHg) for 3 days. Every day pressure-diameter (P/D) curves (10–140 mmHg) were recorded in the absence of smooth muscle tone. Vessel viability was assessed by norepinephrine-induced constriction. Results: SHR did not show any remodelling during the three days of culture as well as WKY kept at 35 mmHg. WKY cultured at 60 mmHg showed a significant remodelling (p < 0.05) from day 0 and day 3 as compared to WKY left at 35 mmHg. W/L ratio significantly increased in WKY cultured at 60 mmHg from day 0 to day 3 (day 0: 0.98 ± 0.007; day 3: 0.93 ± 0.006; p < 0.05), but not in WKY cultured at 35 mmHg and SHR. Conclusion: Increase in wall stress per se induces small resistance arteries remodelling, at least in WKY kept at 60 mmHg, possibly due to sustained increase in myogenic tone.

PB.03
Cure culture myograph is not a good model to study Angiotensin II-induced remodelling in resistance arteries of Spontaneously Hypertensive Rats
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Objectives: Culture myography is an in vitro ex vivo technique that allows the study of the effects of prolonged administration of different molecules on small resistance arteries remodelling. In vivo chronic angiotensin II (AT-II) infusion is a well known model of experimental hypertension in rodents. Aim of this study was to assess whether a 3 days AT-II administration in an organ culture model is able to induce remodelling of mesenteric resistance arteries of Spontaneously Hypertensive Rats (SHR). Methods: Twelve SHR, 12 week old were included in the present study. First order mesenteric arteries were isolated and mounted in a culture myography system. Vessels were incubated for 3 days in the presence or absence of AT-II (1 μM) at a pressure of 60 mmHg. Every day pressure-diameter (P/D) curves (10–140 mmHg) were recorded in the absence of smooth muscle tone. Vessel viability was assessed by norepinephrine-induced constriction on day 3. Results: Exposure to AT-II failed to induce any statistically significant change in P/D curves, in M/L ratio (Cnt: 0.08768 ± 0.00230; Ang: 0.08799 ± 0.00763; p < NS) and in stress/drain curves. Conclusion: Culture myograph does not seem to be a good model to study AT-II-induced remodelling, at least in SHR after development of hypertension. Further studies are needed in order to clarify whether these results are related to limitation of the technique (short duration of culture) or to pre-existing renin-angiotensin-aldosterone system activation in SHR.

PB.04
Role of angiotensin II in the remodelling induced by chronic changes in blood flow in rat mesenteric resistance arteries in vivo
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Flow (shear stress)-induced remodelling of resistance arteries occurs in physiological (growth, pregnancy, exercise) and pathological processes such as ischemic diseases, atherosclerosis or hypertension. Angiotensin II has trophic properties and flow activates its production. We investigated the effect of angiotensin I converting enzyme inhibition (perindopril) and angiotensin II type 1 receptor blockade (candesartan) on outward hypertrophic remodelling induced by high blood flow in mesenteric resistance arteries. Arteries were ligated in vivo in order to generate high (HF) flow arteries and analyzed after 1 week. HF arteries diameter increased in association with medial hypertrophy, eNOS overexpression, hypercontractility to angiotensin II and superoxide overproduction. ERK1/2 phosphorylation increased in HF arteries. Perindopril and candesartan, not hydralazine, prevented hypertension in HF arteries without affecting diameter enlargement, reduced hypercontractility and improved endothelium-dependent dilatation. Super oxide scavenging with tempol prevented both hypertrophy and diameter enlargement due to high flow. Hypercontractility to angiotensin II and ERK1/2 activation were prevented by perindopril and candesartan. ERK1/2 inhibition in vivo (01261) prevented HF remodelling.
PB.05
Severe defect in structural and functional adaptation to chronic blood flow changes in vivo in type 2 diabetic rats resistance arteries
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Endothelial dysfunction in resistance arteries leads to end organ damages in type 2 diabetes. In healthy subjects, increasing blood flow with exercise or vasodilator treatments enhances shear stress leading to a rise in arterial diameter and endothelium-dependent dilation. Nevertheless, in diabetes, impaired sensitivity to shear stress and oxidative stress might affect remodeling. Thus, we investigated flow-induced remodeling in Zucker diabetic fatty (ZDF) and lean (LZ) rats. Mesenteric arteries, alternatively ligated in vivo, were submitted to high (HF) or normal flow (NF) for 21 days and isolated for structural and functional analysis in vitro.

By opposition with LZ rats, diameter and endothelium (flow- and acetylcholine)-dependent dilation decreased in HF arteries from ZDF rats. The chronic increase in flow induced a rise in eNOS and NADPH-oxidase subunits (p91 an eN7) expression as well as a rise in superoxide production in LZ and ZDF rats. Acetylcholine-induced dilation in NF and HF arteries from ZDF rats was improved by an acute antioxidant (tempol). In ZDF rats chronically treated with tempol HF arteries diameter increased and endothelium-dependent dilation was restored to a level similar to that observed in LZ rats HF arteries. Thus, in diabetic rats increasing flow chronically failed to induce the expected remodeling and to improve endothelium-dependent dilation due to ROS overproduction. Consequently, increasing flow in diabetes is beneficial if associated with an antioxidant treatment.

PB.06
Blood pressure-independent effects of salt on vascular structure – fetal programming and adult remodeling
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High salt intake leads to hypertension and adverse cardiovascular outcomes, but some forms of salt-mediated target organ damage are blood pressure-independent. High salt intake during pregnancy may influence blood pressure in the offspring. We investigated whether high salt intake in pregnant rats would alter vascular morphology in the offspring. Sprague-Dawley rats were fed low (1.0%, LS), medium (3.2%, MS), or high (8.0%, HS) salt diet during pregnancy and weaning. The offspring were weaned at 4 weeks of age and maintained on the same diet or changed to low or high salt respectively. Systolic blood pressure was measured by telemetry. Vascular geometry was assessed at 7 and 12 weeks postnatal. No differences in blood pressure were observed between the offspring groups. There was no difference in vascular geometry at 7 weeks postnatal. At 12 weeks, however, wall thickness of central arteries (aorta, carotid) was significantly greater in HS mothers irrespective of the diet post-weaning (HS, LS), the same was true for muscular arteries in the systemic (mesenteric) and pulmonary circulation. Serum ADMA was significantly higher in offspring of HS mothers irrespective of the diet post-weaning (HS, LS), and in offspring of LS mothers switched to HS compared with offspring of MS and LS mothers maintained on LS post-weaning. High salt intake in pregnant rats has long-lasting effects on modeling of central and muscular arteries independent of postnatal salt intake and blood pressure.

PB.07
Regulation and actions of Cardiotrophin-1 in cultured rat vascular smooth muscle cells
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Cardiotrophin-1 (CT-1) is a cytokine belonging to the interleukin-6 family that exhibits trophic and survival properties in a number of cell types. CT-1 protein expression has been identified within the media of athereosclerotic arteries, but its role in the vessel is still unknown. This study aims to characterize the actions and regulation of CT-1 in vascular smooth muscle cells (VSMC). Rat aorta VSMC were stimulated with vehicle or CT-1 (10-11-10-9M) for up to 48 hours, without and with antibodies against CT-1 receptors. In addition, the effects of aldosterone (10-10-10-5M) and angiotensin II (10-10-10-5M) on CT-1 expression were also evaluated. Cell proliferation was assessed by MTT assay. The expression of CT-1, collagen type I and fibronectin was quantified by Western blot. Matrix metalloproteinases (MMPs) activities were assessed by gelatin and casein zymographies. A 48-hour treatment with CT-1 induced VSMC proliferation in a dose-dependent manner (p<0.01) and inhibited 1,24-hour incubation with CT-1 led to an increased expression of collagen type I (p<0.01) and fibronectin (p<0.05), with a parallel and dose-dependent increase in active MMP-2 (p<0.01), MMP-3 (p<0.05) and MMP-9 (p<0.01), all of these effects being reversed in the presence of antibodies against CT-1 receptors. Whereas VSMC spontane-ously expressed CT-1, both aldosterone and angiotensin II enhanced (p<0.01) CT-1 expression in a dose- and time-dependent manner. CT-1 induces proliferation and a secretory phenotype in VSMC. Uprogation of CT-1 expression by angiotensin II and aldosterone in VSMC suggests a mediator role for this cytokine in alterations of these cells caused by the RAAS in vascular diseases.

PB.08
Nitric oxide activates the Vav3-Rac1 pathway via RhoA phosphorylation to promote aortic smooth muscle cell migration
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Nitric oxide (NO) is well known for its ability to induce angiogenesis and arteriogenesis. Although numerous studies described its effects on endothelial cells, very little is known about its role on vascular smooth muscle cells (VSMC) migration. We previously shown that in VSMC, RhoA is phosphorylated on Ser188 by N0-stimulated cGMP-dependent kinase (PKG). This Ser188 phosphorylation of RhoA leads to inhibition of RhoA-Rho kinase pathway. To assess whether wild-type Rhoa (WT), constitutively active (Q63L), phosphomimetic (S188A), double-mutants Q63L+S188A and Q63L-188E could regulate VSMC migration, we used a scratch-wound repair assay in VSMC monolayers. Expression of phosphoresistant mutants reduced the wound-closure, while, in contrast, phosphomimetic mutants greatly accelerated it. The N0 donor sodium nitroprusside (100 μM) accelerated the wound-closure in VSMC expressing the WT Rhoa but not in VSMC expressing the phosphoresistant mutant S188A. 8pCPT-cGMP induces Rac1 localization at the plasma membrane and phosphorylation of the Rac1 effector PKC in VSMC expressing the WT Rhoa but not in cells expressing the S188A. Expression of Q63L-S188E is sufficient to induce Rac1 activation, and using silencing RNA we shown that the Rho exchange factor Vav3 is necessary for this activation. 8pCPT-cGMP- or Q63L-S188E-induced wound healing is inhibited by a dominant negative Rac1 mutant or by Vav3 siRNA. Our work demonstrates that the effect of N0 on arteriogenesis, may result not only on its effect on the endothelium but also, through RhoA phosphorylation and activation of the Vav3-Rac1 pathway, to a positive effect on VSMC migration.

PB.09
The ouabain-sensitive isofrm of Na+/Ca2+-pump regulates vascular gap junctions via interaction with the Na+/Ca2+ -exchanger in membrane microdomain
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Ouabain, an inhibitor of the Na+-pump, has been shown to inhibit intercellular communication. We have recently shown that gap junctions between vascular smooth muscle cells (SMCs) are regulated through an interaction between a ouabain-sensitive isofrm of the Na+-pump and the Na+/Ca2+ -exchanger leading to increases in [Ca2+]i in discrete areas near the plasma membrane. This suggests close association of these transport proteins in microdomains. Using PCR and co-immunoprecipitation we aimed to test this hypothesis in SMCs from mesenteric small arteries and in A7r5 cell line. Intercellular electrical coupling was evaluated in functional studies. SMCs were electrically uncoupled when the ouabain-sensitive Na+-pump was inhibited by 10 μM ouabain. Inhibition of the Na+-Ca2+ -exchanger with 1 μM SEAD400 also uncoupled the SMCs. Depletion of [Na+]i and clamping [Ca2+]i, at low levels prevented the uncoupling. Ten μM ouabain evoked spatially restricted [Ca2+]transients along the cell periphery but not in the center of the cell. mRNA for all three isoforms of the Na+-pump α subunit were found in SMCs but only ouabain-sensitive α2 subunit was specifically co-immunoprecipitated with the Na+/Ca2+ - exchanger-1 and connexin-43. The α2 Na+/Ca2+ -pump subunit was not associated with these proteins but co-immunoprecipitated with caveolin-1. Based on these experiments we suggest that α2 Na+/Ca2+ -pump subunit is involved in regulation of the intercellular communication via interaction with the Na+/Ca2+ -exchanger-1 leading to local [Ca2+]transients near the membrane which block the closely associated connexin-43 containing gap junctions.
Vascular smooth muscle cells are potential players in thrombin generation and inhibition

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We investigated whether vascular smooth muscle cells (SMCs) are implicated not only in the formation but also in the inhibition of thrombin by the activated protein C (APC) system. Rat cultured SMCs between passages 2 and 18 or platelet microvesicles (PMV) were incubated with recalculated human platelet-free citrated plasma and recombinant human tissue factor at 0.25 pm. Thrombin formation and its deactivation by APC were assessed by the endogenous thrombin potential without APC (ETP0) and APC concentration reducing ETPs, by 50% (IC50-APC) using thrombography. Procoagulant phospholipids on SMCs and PMV were quantified by phospholipid-related procoagulant activity (phospholipidase A2 equivalents). Both thrombin generation and inhibition were supported by SMCs. Mean ETP0 were 671±92 nM.min for passages 2–14 and 185±40 nM.min for passages 15–18. Similarly, means amount of procoagulant phospholipids brought by SMCs were 251±48 nM phospholipase A2 equivalents for passages 2–14 and 601±55 nM for passages 15–18, suggesting a role of cell differentiation. No significant correlation was observed between this amount and OERs. Similar OERs were observed with SMCs and PMVs indicating that the IC50-APC values were higher with SMCs. In conclusion, SMCs provide a membrane binding site on which all of the plasma-derived procoagulant and anticoagulant complexes can be trapped. However, inhibition of thrombin by APC was less efficient on SMCs than on PMVs. Thus, SMCs may act as additional contributors to thrombin formation and represent potential targets for new antithrombotic developments.

Cyclical mechanical stretch-induced contractile differentiation is coupled with an increased expression of integrin β1, β3 and αv in vascular smooth muscle cells

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We have recently shown that α1 integrin plays a major role in hypertrophic response to angiotensin II in smooth muscle cells (SMCs) and in mechanical properties of the vascular wall (Louis H et al, Am J Physiol Heart Circ Physiol. 2007). In this study, the objective was to investigate the expression of various integrins during SMC differentiation in response to cyclical mechanical stretch. Rat SMCs were plated on silicone elastomer–bottomed culture plates precoated with collagen type I (Flexcell), and subjected to cyclic stretch with a Cyclic Stress Unit (FX4000). We investigated whether vascular smooth muscle cells (SMCs) are implicated not only in the formation but also in the inhibition of thrombin by the activated protein C (APC) system. Rat cultured SMCs between passages 2 and 18 or platelet microvesicles (PMV) were incubated with recalculated human platelet-free citrated plasma and recombinant human tissue factor at 0.25 pm. Thrombin formation and its deactivation by APC were assessed by the endogenous thrombin potential without APC (ETP0) and APC concentration reducing ETPs, by 50% (IC50-APC) using thrombography. Procoagulant phospholipids on SMCs and PMV were quantified by phospholipid-related procoagulant activity (phospholipidase A2 equivalents). Both thrombin generation and inhibition were supported by SMCs. Mean ETP0 were 671±92 nM.min for passages 2–14 and 185±40 nM.min for passages 15–18. Similarly, means amount of procoagulant phospholipids brought by SMCs were 251±48 nM phospholipase A2 equivalents for passages 2–14 and 601±55 nM for passages 15–18, suggesting a role of cell differentiation. No significant correlation was observed between this amount and OERs. Similar OERs were observed with SMCs and PMVs indicating that the IC50-APC values were higher with SMCs. In conclusion, SMCs provide a membrane binding site on which all of the plasma-derived procoagulant and anticoagulant complexes can be trapped. However, inhibition of thrombin by APC was less efficient on SMCs than on PMVs. Thus, SMCs may act as additional contributors to thrombin formation and represent potential targets for new antithrombotic developments.

Telmisartan prevents cytokine-induced release of MMP-9 in the vascular smooth muscle cells

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We have recently shown that the AT1 receptor blockade with Telmisartan protects aneurysm formation in rats by preventing proteolytic processes in aorta. Increased activity of matrix metalloproteinases (MMPs) in the aortic wall, leading to degradation of extracellular matrix components, plays a crucial role in aneurysm formation. The present study was to analyze the effect of high glucose and IL-1β stimulation in the regulation of MMP-9. Furthermore, the increased release of MMP-9 by aortic SMCs could be prevented by the AT1 receptor antagonist Telmisartan, thus, contributing to vascular protection.

In vitro and in vivo induction of adhesion molecules and leukocyte recruitment: interaction between high glucose and inflammation

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Vascular diseases are the main cause of mortality in diabetic patients. Diabetes is considered as a chronic inflammatory disease characterized by increased levels of circulating pro-inflammatory cytokines, including interleukin-1β (IL-1β). Leukocyte recruitment requires the expression of vascular and intercellular cell adhesion molecule-1 (VCAM-1 and ICAM-1), being an early step on the onset of atherosclerosis. The aim of this study was to analyze the effect of high glucose and IL-1β, either alone or in combination, on adhesion molecules induction and leukocyte recruitment in endothelial cells. Cultured
human umbilical vein endothelial cells (HUVEC) were treated with 5.5 mM (basal) or 22 mM D-glucose (HG), or with or without IL-1β (5 ng/ml) for 18 h. IL-1β increased basal VCAM-1 and ICAM-1 expression. HG alone did not modify basal VCAM-1 or ICAM-1 expression; however co-incubation of IL-1β and HG increased both molecules levels induced by IL-1β. These results were confirmed by in vitro leucocyte adhesion to HUVEC under flow conditions, in vivo leucocyte trafficking was analyzed by intravital microscopy in Sprague-Dawley rat after IL-1β (200 ng/kg) injection, alone or with D-glucose (40 mg/kg), IL-1β increased leucocyte trafficking mesenteric venules. While glucose injection had a limited effect on leucocyte trafficking, co-administration with IL-1β resulted in a higher increase of leucocyte-endothelium interactions compared to IL-1β alone. These results suggest that HG poorly modifies leucocyte recruitment by itself, but exacerbates the effects of IL-1β, providing us new strategies to treat vascular complications in diabetes.

PC.03

Telmisartan attenuates outward aortic remodeling associated with diet-induced obesity

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We investigated the impact of the AT1 receptor antagonist Telmisartan on vascular outward remodeling in a diet-induced obesity-model in C57Black6J mice. Mice were fed with a high-fat diet (HFD, 25% kcal from fat) for 14 weeks. One group received additional treatment with telmisartan (3mg/kg/d) for the last 4 weeks of diet. Animals with a low-fat diet (LFD, 10% kcal from fat) served as controls (n=10 in each group). Aortic diameter was measured by ultrasound biomicroscopy. Aortic tissues were studied using morphometric histology, immunohistochemistry, quantitative real-time RT-PCR and immuno-blotting. In the HFD group, the aortas were dilated compared to controls (1:1.2: 0.04mm vs. 0.9±0.03mm, p<0.001). MMP2 mRNA expression was strongly up-regulated in the media (4-fold, p<0.05). MMP9 was increased in media and adventitia (-4- and 10-fold, respectively, p<0.05). TNF-α was strongly up-regulated in the adventitia of the fat-diet group as compared to LFD-treated animals (10-fold, p<0.05). In addition, TNF-α was co-localised with adipocytes in the adventitia, suggesting that mainly these cells contribute to inflammatory processes during vascular remodelling. Telmisartan abolished aortic dilation (0.88±0.04mm vs. 1.1±0.04mm, p<0.001), prevented cystic media degeneration, preserved elastic lamina and significantly decreased elastin fragmentation (p<0.05). Furthermore, HFD-induced changes concerning increased expression of MMP2, MMP9 and TNF-α were completely abolished by Telmisartan-treatment. Taken together, HFD induced significant aortic dilation, possibly triggered by upregulation of inflammatory pathways, which was abolished by AT1 receptor blockade.

PC.04

High prevalence of prolonged QTc interval in Type 2 Diabetes in the South Asian population in UK: The UK Asian Diabetic Study

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Objective: Corrected QT (QTc) prolongation is a promising predictive value of cardiovascular mortality in Type1 diabetic. As part of the UKADS Prospective Study, we have assessed the prevalence of prolonged QTc in South Asian people cohort with type2 diabetes in a local population attending diabetic services at a teaching hospital setting.

Research Design and Methods: Of the 393 type 2 diabetic South Asian subjects, a total of 345 patients were included in the study. As a baseline, demographic data, HbA1c, duration of diabetes, blood pressure and ECG were done. All the anterior chest leads of the ECG were independently assessed by five different clinicians. QTc intervals were obtained and analysed using SPSS.

Results: Cumulative prevalence of prolonged QTc in the South Asian cohort was 35.07%, which is significantly higher than Caucasians. Interestingly there was significant female predilection (women vs. men: 42.44 vs. 27.74 %, P< 0.004).

Conclusions: In type 2 diabetic subjects from the UKADs cohort, there is strong correlation between female sex and QTc interval. There was also direct correlation between duration of diabetes and hypertension. However, there is no demonstrable correlation between QTc and age.

PC.05

In vitro hypercoagulability in an early phase of the metabolic syndrome in Zucker rats

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We investigated whether an increased reactivity of the clotting system was correlated with changes arterial stiffness in 22-week-old male Zucker rats used as a model for the metabolic syndrome. Obese rats (fa/fa, n=25) were compared with age-matched lean controls (FA/FA, n=24). Aortic stiffness was assessed by the carotid-femoral pulse wave velocity (PWV). Carotid distensibility and the elastic modulus were measured by an echotracking system. Thrombin formation and decay were assessed using plasma recalculated in the presence of a low concentration of tissue factor. Systolic blood pressure (tail-cuff) of conscious fa/fa rats was slightly but significantly elevated compared to FA/FA (165 ± 5 vs. 158 ± 2 mmHg). Cholesterolemia and glycerina were increased in fa/fa rats. Carotid arterial diameter and arterial thickness were not significantly different between both groups. Elastic content in the aorta was significantly reduced in fa/fa but this was not associated with changes in distensibility, elastic modulus and PWV. In contrast the total thrombin activity was significantly different between fa/fa and FA/FA rats (420 ± 29 versus 252 ± 43 mm.min). This occurred independently of platelet activation, was associated with an increase in fibrinogen (4.9 ± 0.2 versus 3.2 ± 0.3 g/l) but was not paralleled by a modification of traditional plasma markers of thrombin generation in vivo. In conclusion, at this early phase of the metabolic syndrome, thrombography demonstrates an increase in the thrombin-forming capacity that appears independent of vascular functional changes. These data suggest a causing role of thrombin generation in delayed cell-wall modifications and thrombosis in the metabolic syndrome.

PC.06

Obesity increases the effect of sodium intake on left ventricular mass in hypertensive patients

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Background: Dietary sodium intake is involved in the pathogenesis of left ventricular hypertrophy (LVH), also independently of blood pressure. Nonetheless, it has not been investigated yet whether obesity can influence the relationship between salt intake and left ventricular mass (LVM). Obesity may influence LVM through inappropriately “normal” aldosterone levels. Methods: 150 overweight-obese essential hypertensives (<65-years-old, glomerular filtration rate >55 ml/min) were studied. Anthropometric and echo-cardiographic parameters, plasma aldosterone, 24 urinary sodium (NaU) and aldosterone were collected. Patients were classified as overweight (OW), grade 1 obesity (OB), and grade 2-3 (OB+). LVM was indexed by body surface area or height2/3, and LHU was defined by 3 different criteria. NaU was considered as continuous or categorical [cut-off: median value 114 mEq/24h] variable. Logistic and linear regression analysis were used to estimate the effect of 24h NaU on LVM, adjusting for covariates and separately for BMI classes.

Results: With each LVM definition, 24h NaU was a significant and independent factor of LVH (OR 1.80, r=0.16 in OW, r=0.21 in OB, r=0.29 in OB+). Plasma and urinary aldosterone levels were not reduced in the patients with higher NaU.

Conclusions: Dietary sodium intake appears a significant and independent factor of LVH. The effect on LVM is increased by adiposity, most likely because plasma and urinary aldosterone levels were not adequately reduced in OW/OB patients with higher NaU.

PC.07

Diabetes-accelerated elastocalcinosis is dependent on advanced glycation endproducts accumulation

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Duration and severity of diabetes is associated with advanced-glycation endproducts (AGEs) accumulation and an acceleration of elastocalcinosis. We propose that the acceleration of elastocalcinosis is related to AGEs deposition and AGEs receptor (RAGE) activation. Male Wistar rats received a high fat diet during 2 months and then a low dose injection of streptozotocin (30mg/kg i.p.) to induce diabetes (D). Elastocalcinosis was induced by warfarin and vitamin K (WK). We started the WK treatment 28 days after the injection of streptozotocin for 3 or 7 weeks. Pyridoxamine or ALT711 were administrated before the streptozotocin or 3 weeks after the beginning of WK treatment, respectively. Ex vivo, femoral arteries from normal Wistar or streptozotocin-induced diabetic rats were incubated in normal media or a calcifying media (CM), N-methylpyridinium, an agonist of RAGE, was added to normal media and CM. Inhibitors of several signaling pathways of RAGE were added. In this animal model, femoral arteries presented simplified calcification and AGEs deposition on collagen. These two parameters were significantly reduced by pyridoxamine and with ALT711. Ex vivo, calcification was significantly enhanced by N-methylpyridinium only in arteries of diabetic rats. Only inhibitors of ERK1/2, Jak2 and Ras were able to limit the augmentation of elastocalcinosis. Diabetes-accelerated elastocalcinosis was prevented with pyridoxamine and limited by ALT 711. Moreover, the stimulation of RAGE enhanced calcification ex vivo. It suggests that, in diabetes, elastocalcinosis is linked to AGEs formation and to its interaction with RAGE.
PC.08

Endothelial and microcirculatory dysfunction occur in impaired glucose tolerance
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Impaired flow mediated dilation (FMD) reflects endothelial dysfunction and has been shown to occur in conditions associated with cardiovascular disease including type 2 diabetes. Shear stress is the stimulus for the endothelial-dependent mechanism which elicits FMD. It is reliant on the forearm microcirculation which may exhibit structural and functional abnormalities prior to development of vascular disease. We investigate the relationship between diastolic shear stress (DSS), FMD and forearm microcirculatory haemodynamics in impaired glucose tolerance (IGT).

Method: Brachial artery FMD was performed on 40 IGT patients and 24 controls using real time ultrasound analysis software (WinFMD) with a baseline ultrasound of the brachial artery obtained immediately after an overnight fast. A strain gauge cuff was inflated to a pressure of 50 mmHg above systolic blood pressure and released to blood pressure. This was followed by an ultrasound scan to measure brachial artery diameter. An occlusion of a total of 5 minutes was performed to induce hyperaemia and another ultrasound scan of the brachial artery was obtained immediately after release of the strain gauge cuff. The changes in ultrasound diameter of the brachial artery were measured from baseline values using pulsed Doppler and analysed using the resistive index and wavelet transform technique.

Results: FMD (measured as % increase from baseline) was significantly impaired in IGT subjects (mean 2.09% +/- 1.36%, control mean 3.92% +/- 1.43%, p < 0.001) and DSS (dyn/cm²) was also significantly reduced in IGT mean 23.5 +/- 11.82 control mean 32.58 +/- 15.63, p = 0.042. There was a significant difference in frequency band 7 of the reactive hyperaemia velocity waveform (IGT mean 11.49 +/- 3.22; control mean 9.68 +/- 2.84, p < 0.05). Resistive index was not significantly different between the two groups. There was a significant correlation between DSS and FMD (R² = 0.501, p < 0.001).

Conclusion: These results suggest that microcirculatory dysfunction is present in IGT, represented by reduced shear stress stimulus and impaired FMD.

PC.09

Ethnic differences in sympathovagal balance and baroreceptor function are explained by dysglycaemia
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Background: People of Indian Asian descent have elevated risks of both cardiovascular disease (CVD) and diabetes compared with Europeans. We hypothesised that Indian Asians with type 2 diabetes have diminished cardiac autonomic function (CAF) compared with Europeans and that this is due to dysglycaemia.

Methods: 149 Europeans and 151 Indian Asians were recruited from the general population, 66% were male (aged 35–75yrs) and 34% female (aged 55–75yrs). Metabolic haemodynamics in impaired glucose tolerance (IGT).

Results: FMD (measured as % increase from baseline) was significantly impaired in IGT subjects (mean 2.09% +/- 1.36%, control mean 3.92% +/- 1.43%, p < 0.001) and DSS (dyn/cm²) was also significantly reduced in IGT mean 23.5 +/- 11.82 control mean 32.58 +/- 15.63, p = 0.042. There was a significant difference in frequency band 7 of the reactive hyperaemia velocity waveform (IGT mean 11.49 +/- 3.22; control mean 9.68 +/- 2.84, p < 0.05). Resistive index was not significantly different between the two groups. There was a significant correlation between DSS and FMD (R² = 0.501, p < 0.001).

Conclusion: These results suggest that microcirculatory dysfunction is present in IGT, represented by reduced shear stress stimulus and impaired FMD.

PC.10

Relationship between TNF-alpha and vasomotor dysfunction in metabolic syndrome patients with insulin resistance
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Aim: to evaluate the relationship between tumor necrosis factor (TNF-alpha), insulin resistance and cutaneous vasomotor responses in metabolic syndrome (MS) patients with insulin resistance. Material and Methods: MS patients with insulin resistance were divided into two groups: 18 patients with type-2 diabetes mellitus (without insulin therapy and pronounced diabetic complications) (DM) and 18 patients without DM. Both groups were divided into further subgroups: 18 insulin resistant patients with MS and one treated (n=18), and one untreated group (n=18). The matching controls (n=6), one untreated (n=6), and one treated (n=6)).

Insulin resistance was measured by Homeostasis Model Assessment (HOMA) IR method (1) and TNF-alpha was measured by enzyme-linked immunosorbent assay (ELISA) method.

Results: The mean age of the whole cohort was 58.2 years, with 52% women. Mean blood pressures (mmHg): untreated cases-169/104, treated cases-155/94, and controls-123/78. Mean BMI (kg/m²): untreated cases-25.4, treated cases-27.6, and controls-25.2. Models comparing untreated hypertensives v. controls, and treated hypertensives v. controls, obtained

inspiration on the pulp of the toe; and heat (+4 4c,PeriTemp 4005) induced hyperemia
(m2-LDF) on the dorsum of the foot. Results: b-LDF and local skin temperature did not differ between the study groups (p > 0.05). Only the patient group with diabetes demonstrated a significant diminution in v-LDF compared to the group of healthy subjects (p < 0.05). m1-LDF was decreased in both patient groups in comparison with the group of controls (p < 0.05), but only in diabetics the decrease of m2-LDF was significant (p < 0.05). TNF-alpha level was elevated in both patient groups. Conclusions: Our findings show that MS patients with insulin resistance have significant cutaneous vasomotor dysfunction and elevated TNF-alpha level.

PC.11

Microparticles from patients with metabolic syndrome induce in vivo vascular hypo-reactivity via Fas/Fas-Ligand pathway by increasing oxidative and nitrosative stresses in mouse aorta
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Microparticles (MPs) are membrane vesicles with procoagulant properties. We studied effects of in vivo treatment of circulating MPs from healthy subjects and patients with metabolic syndrome (MS) on macrocircu

PC.12

High glucose and low lactate: a metabolic signature of hypertension in human serum?
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Introduction: Hypertension is a critical health issue. Despite contributing to more deaths globally than any other condition, and over 200 years of research, the exact cause is unknown in most patients. New approaches, therefore, are required. We used 1H NMR spectroscopy, in concert with modern multivariate methods, to investigate the metabolic profile of hypertension in human serum. Methods: We analysed serum from two hypertensive populations, one untreated (n = 36), and one treated (n = 115), and their matched controls (n = 151), using 600 MHz 1H NMR spectroscopy. Following baseline and phase correction, unsupervised and supervised chemometric techniques (principal component analysis and orthogonal partial least squares discriminate analysis respectively) were applied to build models to explore whether biochemical profiles differed between groups. Model stability was tested using a 1,000 resample bootstrapping procedure. Results: The mean age of the whole cohort was 58.2 years, with 52% women. Mean blood pressures (mmHg): untreated cases-169/104, treated cases-155/94, and controls-123/78. Mean BMI (kg/m²): untreated cases-25.4, treated cases-27.6, and controls-25.2. Models comparing both untreated hypertensives v. controls, and treated hypertensives v. controls, obtained

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reasonable separation between groups (RX=0.29, QY=0.62 and RX=0.27, QY=0.55 respectively). Interestingly, in both models the metabolites contributing most were found to be glucose (higher in cases) and lactate (higher in controls), results verified by bootstrapping, and retrospective conventional biochemical analysis. Conclusion: This exploratory study shows that hypertensive subjects have higher glucose and lower lactate in serum compared to normotensive counterparts, and this difference appears to be independent of treatment effects.

PD.01
Eplerenone survival benefits in heart failure patients post myocardial infarction are independent of their diuretic and potassium-sparing properties: Insight from the EPHESUS Data
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Background: EPHESUS showed that the addition of the aldosterone antagonist eplerenone (E) to optimal therapy in patients with acute myocardial infarction, heart failure, and low ejection fraction improved survival and cardiovascular outcomes. Aims: To determine whether a diuretic effect may be detectable in E-treated patients as compared to placebo (P) in EPHESUS (n = 6632) and whether this was associated with the beneficial effects of E on myocardial infarction outcomes. Methods: A diuretic effect was indirectly defined as a 1 month vs baseline body weight decrease ≥ 20% median change in the P group (-0.05 kg), AND a 1 month vs baseline blood pressure increase ≥ median change in the P group (+4 g/dL). A potassium (K) sparing effect was defined as a serum K increase ≥ median change in the P group (+0.11 mmol/L). ACR results: In the E group, median weight decreased (p<0.0001), whereas blood protein (p<0.0001) and serum K increased (p<0.0001) as compared to P. A K-sparing was independently associated with lower all-cause mortality [HR 0.83 (0.71–0.96); p=0.012] as well as lower CV death or CV hospitalization [0.76 (0.67–0.87); p<0.0001]. A diuretic effect [1.15 (1.02–1.30); p=0.025], was independently associated with a worse CV outcome. There was no statistically significant interaction between the beneficial effects of E on CV outcomes and K-sparing or diuretic effect. Conclusions: Although a diuretic effect is associated with worse CV outcome, beneficial effects of E on survival and CV outcome are independent from its K-sparing and diuretic effects. This suggests that aldosterone antagonism provides a cardiovascular protection beyond its diuretic and K-sparing properties.

PD.02
Albumin:creatinine ratio at presentation predicts mortality and functional outcomes after stroke
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Background: Elevated albumin:creatinine ratio (ACR), a proxy for systemic microvascular function on, predicts stroke outcomes and post-myocardial infarction mortality. We explored associations between ACR and recovery post-stroke. Methods: ACR was measured in 57 consecutive stroke unit admissions. Barthel Index (BI), a measure of patients’ function in activities of daily living, at days 0, 7, and blood pressure at presentation, day 3, 5 & consecutive stroke unit admissions. Regression co-efficient ACR was the only independent predictor of mortality (p<0.008). In the supine group (n=663), was independently associated with a 0.96); p=0.11 mmol/L). ACR results: In the E group, median weight decreased (p<0.0001), whereas blood protein (p<0.0001) and serum K increased (p<0.0001) as compared to P. A K-sparing was independently associated with lower all-cause mortality [HR 0.83 (0.71–0.96); p=0.012] as well as lower CV death or CV hospitalization [0.76 (0.67–0.87); p<0.0001]. A diuretic effect [1.15 (1.02–1.30); p=0.025], was independently associated with a worse CV outcome. There was no statistically significant interaction between the beneficial effects of E on CV outcomes and K-sparing or diuretic effect. Conclusions: Although a diuretic effect is associated with worse CV outcome, beneficial effects of E on survival and CV outcome are independent from its K-sparing and diuretic effects. This suggests that aldosterone antagonism provides a cardiovascular protection beyond its diuretic and K-sparing properties.

PD.03
Adrenal vein sampling for identification of surgically curable primary aldosteronism: impact of accessory hepatic veins on the selectivity index
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Background: Primary aldosteronism (PA), the most common endocrine cause of hypertension, is surgically curable in most cases. Adrenal vein sampling (AVS) is the ‘gold standard’ for their identification, but its accuracy might be hindered by blood dilution from accessory vein blood. Hence, we investigated the impact of accessory veins on AVS results. Methods: We prospectively assessed at phlebography the presence of accessory veins draining in adrenal veins in 62 consecutive PA patients undergoing AVS in whom we calculated the selectivity index (SI), as the ratio of cortisol levels in the right or left adrenal vein and the infrarenal inferior vena cava. The diagnosis of aldosterone-producing (APA) was based on pathology and follow-up data. Results: On the right side we detected no accessory veins in 44% and hepatic and capsular accessory veins in 11% and 45%, respectively. On the left side capsular and phrenic accessory veins were seen in 11% and 69%, respectively. No effect of capsular and phrenic accessory veins on the SI on either side was found. By contrast, the presence of hepatic accessory veins resulted in four-fold lower SI values (2.61±0.89 vs 11.03±2.38, p<0.05), even when adrenal catheterization was selective. Conclusion: Hepatic accessory veins draining into the right adrenal vein, which is feasible by volumetric angiography, can predict a low SI even despite selective right adrenal vein catheterization. As bilaterally selective AVS data are required to determine lateralization of the excess aldosterone secretion to the APA side these results are crucial for a proper interpretation of AVS data.

PD.04
Are sphygmomanometer sitting and standing blood pressure readings adequate for the diagnosis of Orthostatic Hypotension?
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Introduction: Anecdotally, we know that sitting and standing blood pressures are used increasingly for the diagnosis of Orthostatic Hypotension (OH) in the setting of Outpatient clinics. The aim of this paper was to assess the sensitivity and specificity of this practice for the diagnosis of OH through comparison with the current Gold Standard investigation. Methods: 731 consecutive patients with suspected OH on the basis of clinical history attended for Head-Up-Tilt testing. Prior to testing, each patient underwent sitting and standing BP measurement employing a Semi-Automatic Sphygmomanometer. Continuous BP monitoring during tilt studies was provided by a TNO Finometer. Results: Using HUT with Finometer monitoring as the “Gold Standard” for the diagnosis of OH we determined that sitting and standing blood pressure measurements have a sensitivity of 15.7%, specificity of 89.85%, positive predictive value of 62.1%, negative predictive value of 50.15% and a likelihood ratio of 1.55. Conclusions: Standard protocols for sitting and standing BP measurements demonstrate very low sensitivity for the identification of OH. The current practice of sitting and standing blood pressure measurements for the diagnosis of OH should be revised. If a diagnosis of OH is suspected, a more definitive investigation should be sought.

PD.05
Symptomatic orthostatic hypotension: is it how far you fall or how low you go?
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Introduction: There are little data available to date which help us in the prediction of symptoms associated with Orthostatic Hypotension (OH). Methods: Head-Up Tilt (HUT) tests were performed using a standard three minute protocol following five minutes at rest in the supine position. Continuous haemodynamic monitoring was provided using a TNO Finometer. Symptoms during orthostasis were recorded by a specialist nurse. Results: The results of 1,451 consecutive HUT tests in patients suspected of having OH were analysed. P = 0.001).

PD.06
Orthostatic hypotension: a new classification system
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Introduction: Orthostatic hypotension (OH) is a common cause of syncope, falls and dizziness. It is defined as a reduction in systolic blood pressure of ≥ 20 mmHg or diastolic
blood pressure of ≥10 mmHg within 3 minutes of orthostatic stress. We suggest a new classification system for OH which should result in more focused treatment. Methods: Utilising total peripheral resistance (TPR) and cardiac output (CO) measurements obtained during tilt-table testing (TNO Finometer, Modelflow method), we analysed haemodynamic parameters of 110 patients with OH. We applied our proposed classification system and categorized them as arteriolar, venular, or mixed. In arteriolar OH, absence of the normal compensatory increase in TPR after orthostatic stress would reflect impaired peripheral vasoconstriction. In venular OH, a reduction in CO after orthostatic stress often despite marked tachycardia, suggests that the predominant defect is an excessive reduction in cardiac output. Our primary outcome was the difference in 12-hour ambulatory systolic BP change from baseline to 6 months (∆SBP) in both groups. TAU at 7 days, 1, 2, and 4 months included patient-focused education and goal setting using motivational interviewing, promoting patient-led management of risk factors and lifestyle. Mean baseline BP was 145/83 (SD 21/14). There was no significant difference in ∆SBP over 6 months with TAU. Median ∆SBP was 0 mmHg (IQR 20) in the TAU group and 3.0 mmHg (20) in the control group (P = 0.29). More patients in both groups were taking statins at follow-up (p < 0.02) and cholesterol was significantly lower at 6 months (mean reduction 0.95 mmol/L; P < 0.001). There were no differences between groups in number of antihypertensive agents taken, level of exercise, quality of life or total cholesterol at 6 months. Our study found that TAU that promotes patient-led management of risk factors was ineffective in improving BP control, or in increasing the number of antihypertensive agents taken, over six months follow-up in primary care after Stroke/TIA.

Conclusion: This is a practical classification tool and when validated physiologically, this system could be useful in directing treatment of OH.

Ethnic and sex differences in circulating endothelin levels: a novel marker of atherosclerotic and cardiovascular risk in a British multi-ethnic population

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Background: Circulating endothelin levels are associated with atherosclerosis. Moreover, ethnic differences in pro-inflammatory markers may be associated with ethnic differences in atherosclerotic and cardiovascular (CVD) and coronary heart disease (CHD) risk.

Objective and Methods: To investigate ethnic differences in circulating plasma endothelin levels, its soluble receptor (sET-1), and high-sensitivity CRP (hs-CRP). 193 individuals, aged 40–59 years, 62 white (30 women), 66 of African origin (33 women) and 63 South Asians (33 women), free from coronary heart disease (CHD), stroke, CVD and diabetes were randomly selected from the UK ‘Wandsworth Heart and Stroke Study’. Results: Age-adjusted endothelin levels were lower in women than in men (p < 0.002) and were highest in South Asians (13.3 EU/mL; [95% CI 12.0 to 14.7]) and lowest in individuals of African origin (10.1 EU/mL; [9.1 to 11.1]) (p < 0.017 and p = 0.008 women). Endothelin levels were positively associated with waist, waist-to-hip ratio, total cholesterol, serum triglycerides and serum insulin levels and negatively associated with serum HDL-cholesterol. Serum hs-CRP and plasma sET-1 varied by ethnic group (p = 0.001) but was not associated with endothelin.

Conclusions: This study is the first to demonstrate a significant trend for increasing endothelin levels from black Africans to whites to South Asians, which is consistent with the ethnic difference in CHD risk. Whilst these findings support the concept that the innate immune system (IS) may contribute significantly to the metabolic component underlying the development of CVD and CHD risk, further studies are required to see if endothelin levels are causally related to the development of CHD.

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PD.07

Does telephone follow up improve blood pressure after stroke/TIA?

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Hypertension is a common risk factor for stroke/TIA and there is good evidence that blood pressure (BP) control prevents recurrent stroke. Our aim was to investigate whether regular Telephone Follow-Up (TFU) improved risk factor management in hypertensive patients after a single visit to a Stroke Clinic. We conducted a RCT and assigned hypertensive patients within 1 month of stroke or TIA to receive TFU (n = 29) or no TFU (n = 27). Our primary outcome was the difference in 12-hour ambulatory systolic BP change from baseline to 6 months (∆SBP) in both groups. TFU at 7 days, 1, 2, and 4 months included patient-focused education and goal setting using motivational interviewing, promoting patient-led management of risk factors and lifestyle. Mean baseline BP was 145/83 (SD 21/14). There was no significant difference in ∆SBP over 6 months with TFU. Median ∆SBP was 0 mmHg (IQR 20) in the TFU group and 3.0 mmHg (20) in the control group (P = 0.29). More patients in both groups were taking statins at follow-up (P < 0.02) and cholesterol was significantly lower at 6 months (mean reduction 0.95 mmol/L; P < 0.001). There were no differences between groups in number of antihypertensive agents taken, level of exercise, quality of life or total cholesterol at 6 months. Our study found that TFU that promotes patient-led management of risk factors was ineffective in improving BP control, or in increasing the number of antihypertensive agents taken, over six months follow-up in primary care after Stroke/TIA.

Conclusion: This is a practical classification tool and when validated physiologically, this system could be useful in directing treatment of OH.

PD.09

Ethnic variation in levels of circulating IgG autoantibodies to oxidised low-density lipoprotein

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Background: Oxidized low-density lipoprotein (Ox-LDL) plays a key role in atherosclerosis. Our aim was to determine whether serum autoantibodies against Ox-LDL (Ab Ox-LDL) differ by ethnic group. Design and Methods: Soluble serum Ab Ox-LDL levels were measured in 250 white (113 females), 169 African origin (91 females) and 196 South Asian (92 females) individuals from the Wandsworth Heart and Stroke Study (WHSS) population. All were free from coronary heart disease (CHD), stroke, other cardiovascular disease, diabetes, drug therapy for hypertension or high lipids, hormone replacement therapy or oral contraceptive pill. Results: There were no sex differences in levels of Ab Ox-LDL, but levels were higher in non-smokers (430 U/L [95% CI 471 to 596]) than in smokers (384 U/L [316 to 468]) (p = 0.009). Age- and sex-adjusted levels of Ab Ox-LDL were higher in people of African origin and South Asians compared to whites. This difference was maintained in South Asian women following adjustment for multiple risk factors (82% [21 to 175]; p = 0.004). Ab Ox-LDL levels were negatively associated with serum triglycerides and positively associated with sVCAM-1. Conclusions: Higher IgG Ab to Ox-LDL are associated with higher levels of sVCAM-1 and, are elevated in female South Asian individuals who have an increased risk of atherosclerosis compared to whites.

PD.08

Gender differences in the cross-sectional relationships between sleep duration, interleukin 6 and high sensitive C-reactive protein the Whitallite Study

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Background: Emerging evidence suggests that sleep disturbances play a role in the morbidity of chronic conditions, including the development of hypertension and cardiovascular disease for which an underlying inflammatory component has been proposed. Methods and Results: The relationships between sleep duration and two markers of inflammation, interleukin-6 (IL-6) and high-sensitivity C-reactive protein (hs-CRP) were examined in white-collar British civil servants (all white individuals) from the Whitehall II study (n = 4642 for IL-6; n = 4677 for hs-CRP). Following multiple adjustments for demographic characteristics and cardiovascular risk factors including blood pressure, there were no overall linear or non-linear trends between sleep duration and IL-6. However, shorter sleep (men not reported) was significantly lower in individuals who slept 8hrs (11% [95%CI 4 to 17]) as compared to 7hrs. With hs-CRP there was no association between hs-CRP and sleep duration in men. However, both IL-6 and hs-CRP levels vary with sleep duration in women. The observed pattern of variation was different according to the inflammatory marker observed. Further longitudinal studies are required to fully investigate possible temporal relationships between short sleep and markers of inflammation.

Conclusions: No significant variation in inflammatory markers with sleep duration was observed in men. By contrast, both IL-6 and hs-CRP levels vary with sleep duration in women. The observed pattern of variation was different according to the inflammatory marker observed. Further longitudinal studies are required to fully investigate possible temporal relationships between short sleep and markers of inflammation.

PD.11

Neopterin levels in coronary artery disease subsets

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Inflammation plays an important role in the pathogenesis of atherosclerosis. In coronary artery disease (CAD) the release of different cytokines activates cellular defense. Activated macrophages synthesize metalloproteinases and neopterin; a plerin derivative which can be used as a marker of macrophage activation. This study is designed to evaluate the neopterin levels in the spectrum of ischemic syndromes. Consecutive patients admitted with a diagnosis of acute coronary syndrome (ACS) to coronary care unit and patients with stable angina pectoris evaluated as outpatient basis between September 1, 2008 to December 1, 2008 built the study group. Control group constitute of healthy volunteers. ACS patients were categorized into three subgroups according to ECG findings and cardiac enzymes (markers): Unstable angina pectoris (USAP), ST elevation myocardial infarction (STEMI) and ST elevation myocardial infarction (NSTEMI). In the ACS group blood samples for determination of neopterin levels was done at the 72nd hour of hospitalization. 72nd hour neopterin levels in ACS subgroups showed no significant difference. But neopterin levels of ACS patients were significantly higher compared to stable angina pectoris patients. Stable angina pectoris patients showed similar neopterin levels with controls, a finding which can be attributed to chronic intensive medical therapy of these patients. In conclusion high neopterin levels is a hallmark of ACS.
consistent with ongoing inflammatory process. The prognostic significance of this marker should be evaluated in larger patient populations.

**PD.12 Impact of depression on mortality and cardiovascular morbidity in very elderly hypertensives: data from The Hypertension In The Very Elderly Trial [HIVET]**

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**Introduction:** Depression is prevalent in the elderly and has been linked to an increased risk of cardiovascular (CV) disease. Active treatment in HIVET resulted in a reduction in CV events and total mortality. We have assessed whether depression at baseline influenced mortality or CV morbidity. **Method:** HIVET was a randomised double-blind placebo-controlled trial in subjects aged 80 or more. Entry criteria included a systolic blood pressure (SBP) of 160mmHg or more. Active treatment was indapamide (SR) 1.5mg with the optional addition of perindopril 2–4mg. Depression scores were collected via the 15-item Geriatric Depression Scale (GDS) at baseline and annually. Completion was voluntary (69% of patients completed the GDS at baseline). Results: 2656 completed questionnaires were received with GDS information. Mean age was 83.5 years, 60.6% female and mean SBP 173mmHg. Hazard ratios (HR) with 95% confidence intervals adjusted for age, gender, treatment allocation, country area, educational level, living alone, number of comorbidities, previous CV disease, previous treatment and previously diagnosed hypertension for total mortality was 1.07(1.04–1.10, HRp=0.001) for 1 unit increase in GDS. The results for CV mortality and CV events were 1.09(1.05–1.13, HRp=0.001) and 1.07(1.04–1.10, HRp=0.001) respectively. **Discussion:** The results suggest that depression increases the risk of CV events in the very elderly, even after adjustment for several variables. This additional finding suggests the use of antidepressives with antidepressants in the very elderly may be beneficial and further research is required.

**PD.13 Markers of inflammation, blood pressure, and other hemodynamic variables in obese subjects from the general population**

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**Purpose:** To compare the RAS blockers PK-PD on 2 contrasted salt diets.

Low-grade chronic inflammation has been proposed to play a major role in the pathogenesis of hypertension. Low-grade chronic inflammation is also closely associated with obesity, an established causative factor in the development of hypertension. The purpose of this study was to investigate the relationship between two markers of inflammation, C-reactive protein (CRP) and fibrinogen, and blood pressure (BP) and other hemodynamic variables in obese subjects. From a large cardiovascular study based in the general population, we selected subjects with a body mass index > 30 kg/m², free of major cardiovascular diseases, not taking BP-lowering or lipid-lowering drugs, and with CRP <10 mg/L. Men, >45 years of age, and women, >45 years of age, with 5%–95% percentiles age > 62 years (36–80). The cardiovascular study included measurements of traditional and new risk factors and measures of subclinical atherosclerosis, such as ankle brachial index. CRP was determined by a high-sensitive assay. In partial Spearman rank correlation analysis, adjusted for age and sex, we found no significant relationships between CRP or fibrinogen and systolic BP or diastolic BP or pulse pressure or ankle brachial index (rho: 0.058 to 0.077, P<0.10). However, fibrinogen and CRP were found to be significantly related to heart rate (rho: 0.134 to 0.143, P<0.005). In this study of generally healthy obese subjects from the general population, we found no significant relationships between markers of inflammation and systolic BP or diastolic BP, showing that obese subjects with higher levels of inflammatory markers do not have higher BP levels than their obese counterparts with lower levels of inflammatory markers.

**Effect of dietary salt intake on the pharmacokinetics (PK) and the pharmacodynamic (PD) effects of different blockers of the renin-angiotensin system (RAS) in normotensive subjects**

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**Background:** Low salt (LS) intake increases systemic availability of oral verapamil and quinidine affecting their PD profiles. Whether such phenomenon exists with RAS blockers is unknown. **Objective:** To compare the RAS blockers PK-PD on 2 contrasted salt diets.

Methods: 64 normotensive male subjects (n=16/drug) were randomly assigned to LS (NaCl ≤50mmol/d) or high salt diet (NaCl ≥250mmol/d) in a double-blind, placebo-controlled, crossover study. They were given a single oral dose of Candesartan 8mg, Valsartan 160mg, Ramipril 10mg, or Atenolol 50 mg at each period. Plasma drug concentration and cumulative urine drug excretion (Ue), plasma renin concentration (PRC) and PR interval (Atenolol) were measured for 24h. Results: LS diet increased significantly the Cmax, and the AUC(t) for Candesartan (+41% and +45 %) and Atenolol (+38 % and +26 %). LS diet increased significantly Ue of Atenolol (+30 %). The increase in PR after Candesartan, Valsartan or Ramipril was significantly larger with the LS diet. Using PK-PD modeling, we estimated that ~30% of the changes in PR with Candesartan on the LS diet was due to the increase in drug exposure. The Atenolol induced-increase in PR interval was larger with the LS vs. the HS diet + 7.7 ms (95%CI 1.9–13.4, P<0.05).

**Conclusions:** LS diet increases Candesartan and Atenolol plasma concentration affecting their PD profiles, but does not affect Valsartan and Ramipril PK. Increased Ue of Atenolol suggests that its bioavailability is increased by a LS diet. Further study is needed to explain the differential effect of LS diet on the PK of RAS blockers with different chemical characteristics.

**PE.02 Eprosartan modulates reflex activation of the sympathetic nervous system in sodium restricted patients with essential hypertension**

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**Purpose:** AT1 receptor antagonists possess a psycho-social inhibitory effect in vitro and in animal experiments, but in humans conflicting results exist regarding the presence of this effect. We tested the hypothesis that very short-term treatment with the AT1 receptor antagonist eprosartan inhibits reflex activation of the sympathetic nervous system (SNS) in sodium restricted patients with essential hypertension. **Methods:** The effect of eprosartan on renal tubular function, systemic hemodynamics and vasoactive hormones was measured before, during and after a cold pressor test (CPT) and sodium nitroprusside (SNP) infusion in a randomized, placebo controlled, double blinded, crossover study in fourteen patients with essential hypertension. **Results:** After eprosartan treatment, in contrast to placebo treatment, a SNP induced reduction in mean arterial blood pressure of 10 mmHg increased plasma levels of angiotensin II (mean: ± SD) (7.2 ± 10.0 pmol/l, P<0.05) and decreased fractional excretions of sodium (0.23 ± 0.22 %, P<0.01) and lithium (21.2 ± 17.7 %, P<0.01). The increases in HR and plasma levels of noradrenaline during the SNP infusion were similar after both treatments. Eprosartan compared to placebo had no impact on the activation of the SNS during the CPT. **Conclusion:** These findings do not support a direct sympatho-inhibitory effect of eprosartan. In fact, these results suggest that eprosartan treatment increased renal sympathetic nerve activity during arterial baroreflex mediated activation of the SNS – the likely result of an increased sensitivity of the arterial baroreflex in the control of renal sympathetic nerve activity.

**PE.03 Anti-inflammatory effect of ACEI in smokers with cardiovascular disease**

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**Aim:** According to the current inflammatory concepts of atherosclerosis many investigators focus on markers of inflammation, which may become independent risk indicators for cardiovascular events. The aim of this study was to determine the link between inflammatory cytokines and the potential benefit of angiotensin converting enzyme inhibitors (ACEIs) use in smokers with cardiovascular disease (CAD). **Methods:** 60 smokers and 10 nonsmokers (control) suffering from CAD enrolled our study. Patients were divided into subgroups: smoking less than 15 cigarettes per day, more than 15 cigarettes per day, and smoking more than 10 years. Blood samples were taken twice: before and after 12 weeks of quinapril therapy (20mg/day). Serum concentrations of macrophage colony stimulating factor (M-CSF), C-reactive protein (CRP) and interleukin 6 (IL-6) were measured. The effect of quinapril administration was assessed under placebo-controlled conditions. **Results:** The baseline values of cytokines were higher in smokers than in control (p<0.001). 12-week treatment with quinapril reduced M-CSF (p<0.050), IL-6 (p<0.001) and CRP (p<0.05) in patients smoking less than 15 cigarettes/day. In patients smoking more than 15 cigarettes/day only IL-6 value was reduced (p<0.010). The baseline values of cytokines varied in patients smoking more than 10 years (higher level) comparing with those who smoked less than 10 years (lower level). **Conclusion:** The effect of quinapril therapy reducing cytokine level depends on daily nicotine dosage and is more evident in patients smoking less than 15 cigarettes per day.
Changes in baroreflex sensitivity with home orthostatic training in vasovagal syncope - a randomized, placebo controlled pilot study

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Introduction: The benefits and physiological changes attributed to orthostatic training in vasovagal syncope (VVS) have never been evaluated against placebo. We studied the changes in baroreflex sensitivity (BRS) in response to home orthostatic training (HOT) in a randomized, placebo-controlled pilot study. Methods: 22 subjects, aged 18 to 85 years, with recurrent WS were randomized to daily HOT (n = 12) or sham training (n = 10) for 6 months. BRS was determined using the sequence method during 10 minutes' supine rest at enrolment, week 1, week 4 and week 24. Sympathetic reactivity was assessed with event diaries. Results: 6 (50%) subjects in the intervention group and 2 (20%) subjects in the placebo group were syncope free at week 24. Down slope and total BRS were significantly increased from baseline following one week of HOT therapy compared to placebo (median change = 3.13 vs. -1.46 ms/mmHg, p < 0.05; 1.21 vs. -1.26 ms/mmHg, p < 0.05). Multivariate analysis showed an effect on week 4 (n = 12). Conclusion: Our pilot study demonstrated positive trends in symptom benefit and significant improvements in BRS with HOT in patients with VVS.

Risk profile and lipid peroxidation in young patients with coronary artery disease

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In this study we aimed to determine the risk profile of young patients with coronary artery disease (CAD), including new emerging risk factors as lipoprotein (a), uric-acid, abdominal obesity and whether premature onset of coronary atherosclerosis is associated with increased levels of lipid peroxidation expressed as thiobarbituric acid reactive substances (TBARS) and antioxidant enzymes (GS). This study included 55 patients, <40 years of age (mean age 35.7 ± 4.1) with angiographically proven CAD and 31 age and gender matched healthy controls (mean age 38.9 ± 5.2), Lp(a) (34.1 ± 31 vs 20.5 ± 3.8 mg/dl, p < 0.05), uric acid level (5.3 ± 4.1 vs 4.4 ± 0.05 mg/dl, p < 0.05) and increased lipid peroxidation expressed as TBARS (6.35 ± 2.9 vs. 4.67 ± 0.7 mmol/l) were significantly higher in patient than in control. The activities of antioxidant enzymes (GS vs. SOD) were significantly lower in patient than in control. Conclusion lipid peroxidation is significantly elevated in young patients with stable CAD and can be used as a risk marker. Increased lipid peroxidation despite antioxidant therapy and similar modifiable risk profile with controls may be an indicator of underlying genetic tendency and warrants a more aggressive therapy in this young patient population.

Differences in coronary artery haemodynamics due to changes in flow and vascular geometry after percutaneous coronary intervention

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The effects of changes in flow velocity waveform and arterial geometry before and after percutaneous coronary intervention (PCI) in a right coronary artery (RCA) were investigated using computational fluid dynamics. An RCA from a patient with a stenosis was reconstructed based on multislice CT images. A non-stenosed model, simulating the same RCA after PCI, was also constructed. Blood flows in the RCA models were simulated using pulsatile flow waveforms acquired with an intravascular ultrasound Doppler probe in the RCA of a patient undergoing PCI. It was found that differences in the waveforms before and after PCI did not affect time-averaged wall shear stress (TAWSS) and oscillatory shear index (OSI), but the phase-angle between pressure and wall shear stress on the endothelium (stress phase-angle: SPA), differed markedly. The median SPA was -63.9 deg (range -204 to -10.0 deg) for pre-PCI state whereas it was 10.4 deg (range -71.1 to 25.4 deg) in the post-PCI state; i.e. more asynchronous in the pre-PCI state. SPA has been proposed to be pro-atherogenic.1 Our results suggest that the haemodynamics in the RCA was improved markedly after PCI in terms of SPA, and differences in pulsatile flow waveform may have an important influence on atherosclerosis, although associated with only minor changes in TAWSS and OSI. SPA may be a useful indicator in predicting sites prone to atherosclerosis.


Left ventricular hypertrophy in ‘normotensive’ individuals: would further reduction in blood pressure enhance regression of LHV?

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Background: Patients with normal blood pressure (‘normotensive’) and left ventricular hypertrophy (LHV) are common. It is unknown whether extra BP reduction in them would regress their LHV. Objective: The aim of the study was to assess whether lowering systolic blood pressure already in the normal range by approximately 10mmHg would lead to a reduction in LHV. Methodology: Fifty-hypertensive patients with echocardiographic LHV were randomly assigned to either active treatment arm (extra antihypertensive medica-
tion); or placebo in a ratio of 2:1. Cardiac magnetic resonance imaging (CMRI) was used to establish changes in left ventricular mass index (LMVI) over the 12 months’ study period. Thirty-five subjects completed the study (active 23, placebo 12). Results: Average baseline systolic blood pressure was 122.10mmHg in the active group and 123.83mmHg in the placebo group (p = 0.646). The mean baseline CMRI LMVI was 59.16g/m² in the placebo group and 65.89g/m² in the active group (p = 0.114). The mean difference between baseline and end of study blood pressure was −9.33mmHg in the active group and −6.08mmHg in the placebo group (p = 0.007). This is a much greater BP fall than, for example, the HOT study. The mean change in CMRI LMVI was −4.68g/m² in the active group and −1.97g/m² in the placebo group (p = 0.014). Conclusion: It is possible to cause LHV regression if BP is reduced further, even when baseline BP begins below target BP. This may translate into a reduction in cardiovascular events in those with normotensive LHV.

Left ventricular global function assessment by automated function imaging (AFI) reveals reduced overall systolic strain in patients with hypertensive hypertrophy

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Background: The novel speckle tracking echocardiography allows measuring left ventricular segmental strain parameters from 2D gray scale images. On board integration of this method (AFI) into echocardiography machines enables quick, automated evaluation of left ventricular function. Aim: In our study, we aimed to evaluate LV longitudinal deformation properties in patients with untreated hypertension and compared them with age and sex matched healthy subjects. Methods: 29 patients with newly diagnosed untreated hypertension [Group HT (10 females,19 males)] and 27 healthy subjects [Group N (9 females, 8 males)] underwent transthoracic echocardiographic examination. Global longitudinal strain (GLS) was obtained from the analysis of A2C, A2C and APLAX 2D images. GLS and its relationship to LV mass index(LMVI) was compared between groups.

Results: There was no difference between the two groups regarding demographic data. LMVI in Group HT (M = 147.48 gr/m²; SD = 12.08) was significantly higher relative to Group N (M = 91.85 gr/m²; SD = 9.19)(F4 = −19.27, p < 0.001). LV global longitudinal strain in Group HT (M = −19.30%; SD = 0.72) was remarkably lower comparative to Group N (M = −22.66%; SD = 0.89)(F4 = −15.48, p < 0.001). A linear regression analysis revealed that increased LMVI was a highly significant predictor of reduced GLS (B = 0.96; p < 0.001).Y = −0.061*X − 28.31). Mean calculation time of AFI was 82 seconds(SD=4.45).

Conclusion: LV concentric hypertrophy due to hypertension reduces left ventricular systolic performance as assessed via GLS, even though conventional systolic performance (like EF) is normal or even higher in these patients.

Peak velocity is quicker and less variable than VTI ratio in the assessment of aortic valve area

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Aims: The aortic valve area (AVA), in aortic stenosis (AS), is estimated by Doppler echocardiography, by the application of the continuity equation. VTI ratio is commonly recommended for its calculation with the ratios of peak velocity as an acceptable short-cut alternative. We investigated if the increased time required calculating the VTI is justified through reduced variability, achieved through it’s inherent averaging by the use of the continuity equation. VTI ratio is commonly recommended for its calculation with the ratios of peak velocity as an acceptable short-cut alternative.

We investigated if the increased time required calculating the VTI is justified through reduced variability, achieved through it’s inherent averaging by the use of the continuity equation. VTI ratio is commonly recommended for its calculation with the ratios of peak velocity as an acceptable short-cut alternative.

Methods: 1008 Doppler echocardiographic images from 24 patients with mild to severe AS were obtained from sequential clinical echocardiography studies. 21 operators across 6 central London centres performed analysis of Doppler traces for VTI and peak velocity. Dimensionless Indices were calculated and calculated for both techniques along with the average time taken and compared statistically by paired Student’s t-tests. Results: Inter-operator variability of VTI Dimensionless Index was 7.5 times greater than peak velocity (17% ± 0.05 versus 2% ± 0.01, p < 0.001). VTI-based Dimensionless Index variability was 4.1 times greater than peak-based measurement (5% ± 0.04, 1% ± 0.04, p < 0.001). The average time taken for VTI was 5.3 times greater than peak maximal velocities (23.7 ± 3.5 vs. 4.5 ± 1.2 s, p < 0.001).

Conclusions: The measure-

ment of velocity-time integral is a markedly more variable and more time consuming
measure than peak velocity ratio in the assessment of aortic stenosis. Peak velocity Dimensionless indices should be recommended as the preferred method (and not a short-cut) for quantification of aortic valve area.

**Accounting for the biphasic change in blood pressure that occurs following cardiac pacemaker optimisation**


**Background:** Non-invasive blood pressure monitoring by continuous finger photoplethysmography (Finometer) is valuable in the optimisation of biventricular pacemakers. However, the immediate increment in blood pressure (BP) diminishes after a few seconds and it is unclear whether this is due to a fall in stroke volume or instead a (desirable) compensatory reduction in peripheral resistance. This study explores this question by measuring stroke volume using Doppler echocardiography as a gold standard, and BP using Finometer during and after (atrio)ventricular AV delay adjustment. **Design:** Cardiac output and BP were measured using a Finometer whilst AV delay was changed from 40ms to 120ms in 19 subjects with pacemakers. Simultaneously the velocity time integral of left ventricular outflow tract Doppler was used to calculate stroke volume and hence cardiac output (Qecho). **Results:** The immediate effect of an AV delay change on mean arterial pressure (MAP) and Qecho correlated well across all patients (r

**Conclusions:** During optimization of AV delay, the abrupt rise in cardiac output is followed by a compensatory fall in peripheral resistance which causes cardiac output to fall, whilst stroke volume is maintained. Optimisation of AV delay using a Finometer should be undertaken immediately following changes in pacing configuration to minimise information loss from vascular compensation.

**Real-time ventilatory manipulation by cardiac pacemaker: minimising blood pressure changes**


**Aims:** Step changes of heart rate between two values, using a cardiac pacemaker, generates periodic oscillations in end-tidal carbon dioxide (et-CO2) and ventilation. This could be developed to treat periodic breathing in heart failure. We studied whether gradual variation of cardiac output can achieve comparable ventilatory effects with less sudden changes in blood pressure (BP). **Methods:** We applied fluctuations in heart rate (by 30 bpm) or AV delay (between 30–120ms) or both, with period of 60 seconds, in 19 heart failure patients (age

**Conclusions:** When developing therapeutic pacemaker algorithms, a sine wave pattern is preferable to a square wave, because it minimizes the BP fluctuation yet achieves comparable ventilatory effects.

**Why does ejection fraction not fall with age? Discriminating ventricular and atrial contributions to ejection fraction using 2D echo**

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**Introduction:** It appears paradoxical that ventricular systolic function, measured by 2D Ejection Fraction (EF), stays stable with age, while ventricular diastolic function, as assessed by Tissue Doppler velocities, declines. This could be because EF is calculated from a baseline ventricular volume at end-diastole, which includes the blood delivered by atrial contraction, rather than from the true resting volume just prior to atrial contraction i.e. diastasis. **Methods:** 29 healthy volunteers (16 men, aged 15–94 years) underwent measurement of left ventricular volume by Simpson’s method of discs at three time points: End Systole, End Diastole and at Diastasis (immediately prior to atrial contraction). From these were calculated conventional ejection fraction (EF) and its two components: ventricular contribution to ejection fraction (VEF) and atrial contribution to ejection fraction (ACEF). **Results:** There was a clear age-related decline in VCEF (r = –0.561, p ≤ 0.01), and age-related increase in ACEF (r = –0.769, p < 0.01). Conventional EF did not change with age (p ns). In parallel, peak E wave velocity decreased (r = –0.48, p ≤ 0.01) and A wave increased (r = –0.644, p < 0.01). **Conclusion:** The ventricle contributes 4 times more to EF than the atrium at age 15, however both contribute equally by age 90. The apparent preservation of ventricular function with age on 2D imaging results from measuring volumes only at end-diastole and end-systole. If volume is also measured at the resting state (diastasis), we see opposing changes in atrial and ventricular contributions concealed by conventional EF.

**A system for monitoring of systolic and diastolic duration in exercise stress test**

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The duration of systole and diastole as a function of heart rate provides important information on heart functionality. In this study we assessed the feasibility of the cardiologic systole and diastole times measurement by a precordial cutaneous accelerometer during exercise stress tests. The sensor was positioned in the precordial region to assess heart sound vibrations. The acceleration signal was recorded and processed by a laptop PC, together with an ECG signal. Systole and diastole duration were computed for each cardiac beat. The system was tested in 103 patients which performed semi-aerobic bicycle exercise in stress echo lab. Patients were 71M/32F, age 57±14 years, 17 healthy people 86 patients with cardiovascular diseases. Consistent first and second heart sound signals were obtained in 88% patients at rest and during stress. The diastolic time decreased from 541±143msc to 250±59msc, the systolic/diastolic time ratio increased from 0.64±0.15 to 1.00±0.23. At higher heart rates (100bpm), systolic/diastolic time ratio was lower in the 17 control subjects (0.74±0.12) than in the patients with systemic hypertension (0.94±0.12), coronary (0.98±0.11), valvular (0.93±0.14) or dilated heart (0.86±0.10) disease. In conclusion, cardiologic systole and diastole duration can be monitored in exercise stress test by using an acceleration sensor that measures first and second heart sound vibrations. The same accelerometer sensor and the same arrangement were used, in a previous work, to assess the cardiac force-frequency relation, which can then be assessed simultaneously.
WKIN – functional analyses of variants associated with blood pressure and essential hypertension

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Gain of expression mutations in WKIN cause Gordon’s Syndrome, a rare disorder characterised by hypertension and hyperkalemia. We have previously reported association between WKIN and BP in the BRIGHT Study and 24-hour ambulatory BP in the GRAPHIC study. The associated SNPs map to the promoter and regulatory regions in intron 1, suggesting that changes in WKIN expression may contribute to BP and risk in the general population. This study was performed to investigate whether associated variants affect gene expression.

Two SNPs were selected for functional analysis, one promoter variant (rs1468326 C/A) and one in intron 1 (rs765250 A/G). Carriers of allele A for rs1468326 have on average lower SBP (-5.05 mmHg 95%CI (-9.21,-0.66), p = 0.015). For each SNP, multiple copies of each allele were cloned into pGL3 and transfected into HEK293 cells, followed by luciferase assay. Reporter assays demonstrated that rs1468326C had lower activity than rs1468326A, ~3.36 fold decrease in luciferase activity 95%CI (-3.93,-2.80), p = 3.6 × 10^-10. The intronic SNP rs765250A showed a ~1.50 fold increase compared to rs765250G, 95%CI (1.10,1.90), p = 1.14 × 10^-4.

These results correlate well with the reported genetic associations. The SNPs associated with increased (decreased) BP also demonstrate increased (decreased) reporter activity, suggesting that these are functional variants that could alter WKIN expression. These new data lend further support to the hypothesis that variation in WKIN expression contributes to BP and EH.

Heritability of Plasma Lipoprotein-associated Phospholipase A2 (LP-PLA2), a new marker of cardiovascular risk

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Background: Lipoprotein-associated phospholipase A2 (LP-PLA2), is involved in degrada- tion of platelet-activating factor (PAF) and phospholipids associated with LDL, and in production of lysophosphatidylcholine (lysoPC) and oxidized non-esterified fatty acids (NEFA). Aim: To verify whether the LP-PLA2 plasma levels are genetically determined. Materials and Methods: 54 healthy twins were enrolled. The levels (mass) and activity of LP-PLA2, were measured. We estimated genetic variance and heritability of LP-PLA2 mass and activity with variance and path analyses. Twins were genotyped at PLAC07 gene functional single nucleotide polymorphisms (SNPs): Thr189ile (exon 7), His92arg (exon 4) and Ala379Val (exon 9).

Zygosity was assessed with highly discriminating VNTR (variable number of tandem repeats) micro- and minisatellites systems were analyzed, by PCR and gel electrophoresis. Results: 26 twin pairs were monzygotic (M2) and 28 dizygotic (D2). The mean LP-PLA2 mass and activity were correlated (r = 0.87, p < 0.001) and similar in M2 and D2. ICC estimates of heritability for LP-PLA2 were 0.27 (mass) and 0.28 (activity). ACE model-based estimates of heritability were 0.37 (mass) and 0.54 (activity). Heritability estimates were significant for activity, but not for LP-PLA2 mass. The within pairs differences of LP-PLA2 mass and activity showed significant differences of LP-PLA2 activity between Hispanic discordant twins and between the former and the latter MZ twins.

Conclusions: These results suggest heritability for activity, but not for mass, and indicate an effect of the His92Arg SNP on LP-PLA2 activity in healthy Caucasians.

A functional LDL receptor-related protein 6 gene variant is an independent risk factor for early carotid artery atherosclerosis in hypertensive patients

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Introduction: A rare LRPR gene mutation causes a monogenic form of hypertension, hypercholesterolemia and early coronary artery disease because of reduced Wnt7b/catenin signalling. We investigated whether a common Val-1062 LRPR variant with similar functional consequences (about 5-fold lower signalling) was related to carotid artery atherosclerosis (CAA) in hypertensive patients.

Methods: Cross-sectional study of 336 hypertensive patients (age <65 years) undergoing carotid artery ultrasonography. Hypertension, type 2 diabetes, dyslipidemia, chronic kidney damage (CKD), and smoking habit were evaluated. Genotyping was carried out using real time PCR. CAA was defined by the presence of atherosclerotic plaques (intima-media thickness >1.3 mm) at the level of common, bifurcation and/or internal carotid arteries. Logistic regression models were used to estimate the independent effect of V1062 allele controlling for CAA established risk factors.

Results: In our hypertensive patients, age, gender, dyslipidemia, smoking habit, pulse pressure and CKD confirmed as risk factors for CAA. The V1062 LRPR variant was a strong risk factor for CAA in both unadjusted (OR 2.12, 95%CI 1.10–4.08; p = 0.030) and adjusted models (OR 2.09, 95%CI 1.17–3.74; p = 0.013). When a more strict criterion to define CAA (atherosclerotic plaques with >15% lumen reduction, class C and above following 3Tayor and Strandness) was considered, the results were also stronger (unadjusted OR 2.78, 95%CI 1.65–4.87, p = 0.001; adjusted OR 2.67, 95%CI 1.49–4.77, p = 0.001).

Conclusions: Beside the role established risk factors, V1062 LRPR variant and CAA are strongly associated in hypertensive patients, making LRPR a novel interesting candidate gene for early coronary and carotid artery atherosclerosis.

A human fatty acid amide hydrolase (FAAH) functional gene variant is associated with lower blood pressure in young males

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Background: Fatty acid amide hydrolase (FAAH) inhibitors, preventing endocannabinoid (EC) degradation, reduce blood pressure (BP) and heart rate in young male (YM) hypertensive rodents. The functional human FAAH 123T gene variant results in reduced protein level and enzymatic activity but its relationship with BP is unknown. This study investigates the relationships among FAAH P129F alleles and cardiovascular features in YM at baseline and after 5-year follow-up, and in older male obese hypertensive (OH) patients, in whom the EC system is overactive.

Methods: Genotype analysis was performed in 215 Caucasian male students (24 (0.2) years old) and in 185 older OH patients (50 (0.2) years old). YM were also followed up for 9 years. Clinical and anthropometric variables, BP, cardiac and carotid artery echographic measurements were evaluated.

Results: YMs with the FAAH 129T allele had lower systolic (P = 0.042) and mean BP (P = 0.022), and a trend toward lower diastolic BP (P = 0.06). Such significant association was maintained at follow-up. In contrast, the same allele was not associated with BP in older OH. No association was found with other cardiac and vascular variables.

Conclusion: An FAAH defective gene variant results in lower BP in YMs, similar to the findings in young rodents. This effect is lost in older OH patients. Because cannabinoid CB1 receptor blockade is associated with BP reduction in OH patients, EC effects and the use of ECS-interfering drugs is likely to be age and clinical-condition dependent.

A functional systemic analysis of 123 candidate genes reveals two novel genes for hypertension

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Discovering the genes causing hypertension is proving a challenging task. Recently, we performed a genomewide association scan (GWAS) for susceptibility genes for HT as part of the Wellcome Trust Case Control Consortium (WTCCC). This analysis revealed no SNPs with significance below 1x10^-8. We are currently performing further GWAS and meta-analy- sis, however a distinct and complementary approach is to interrogate candidate genes. We therefore compiled a comprehensive list of 123 candidate genes from 10 functional pathways known to regulate blood pressure (BP) and then used Tagger (http://www.broad.mit.edu/mpg/tagger/) to select tag SNPs, aiming to tag all SNPs with minor allele frequency >0.05 with r2>0.8. 1358 BP, 733 SNP genes were thus identified.

We found four SNPs to reach statistical significance (p<4.33x10^-4) after adjusting for multiple SNPs and traits. The strongest associations were with SNPs in two solute carrier genes; SLC6A2 (norepinephrine transporter) and SLC34A3 (sodium / hydrogen exchanger isoform 7 and 8) and systolic and diastolic blood pressure, adjusting for effect of age, BMI.

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Functional and structural profiling of the human thrombopoietin gene promoter

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Human thrombopoietin (TPo) is involved in cardiovascular disease (CVD) as it regulates megakaryocyte development and enhances platelet adhesion/aggregation. As TPo promoter structure is still controversial, using RT-PCR, we evidenced that TPo promoter is differentially regulated by complex genetic constellations.

We herein redefined the transcriptional organisation of TPo and conclude that the P1 promoter is differentially regulated by complex genetic constellations.

Exercise induced arrhythmias within a population of genetically proven carriers of hypertrophic cardiomyopathy

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Hypertrophic cardiomyopathy (HCM) is a genetic disease of sarcomeric contractile proteins characterized by left ventricular hypertrophy. HCM often presents with exercise-induced arrhythmias or even sudden death. However, the relation between arrhythmias seen on exercise testing and structural changes in genotypic HCM is not known. Methods: From a population of 109 genetically proven carriers of a HCM mutation, 33 patients (mean age 33 [16, 65] years, 64% male) underwent exercise-eCG-test and cardiovascular magnetic resonance imaging study (CMR). We used the Mann-Whitney test, the Students-t-test and Fisher’s Exact Test to analyse the data in SPSS.RESULTS: Arrhythmias such as sustained and non-sustained ventricular tachycardia did not occur. Nine patients (27%) demonstrated ventricular premature beats (VPBs) during exercise or during the recovery period. Patients with VPBs tended to have a higher maximal wall thickness on CMR than patients without VPBs (24mm ± 6.0 vs 19mm ± 6.6, p = 0.10). All patients with VPBs showed evidence of fibrosis on CMR, whereas VPBs were not seen in patients without fibrosis (Pearson Chi-Square p = 0.023 and Fisher’s Exact Test p = 0.032). Among the subjects with fibrosis, patients with VPBs had a significant higher fibrosis score on CMR as compared to patients without VPBs (7.4 ± 3.9 vs 3.4 ± 3.6, p = 0.02). Conclusions: On exercise testing in a population of 31 HCM genotyped patients, no life-threatening arrhythmias occurred. VPBs were only observed in subjects with myocardial fibrosis on CMR; thus exercise induced VPBs are related to myocardial fibrosis rather than to hypertrophy per se.

Integrated network and microarray analysis to identify new biomarkers of heart failure after myocardial infarction

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An approach combining the power of biological information networks and the precision of microarray analysis was undertaken to identify new early biomarkers of heart failure (HF) after myocardial infarction (MI). Since angiogenesis may be related to MI and HF, a protein-protein interaction (PPI) network was constructed by first extracting from the Entrez-Gene database a set of genes relevant to angiogenesis and MI, and second retrieving annotated interactions from the Human Protein Reference Database. Whole genome expression profiles of blood cells taken at the time of MI in two groups of 16 patients (high ejection fraction (EF) at 1 month, EF>45% and low EF at 1 month, EF$<$40%) obtained by microarrays were compared by Statistical Analysis of Microarrays (SAM). Prediction models based on machine learning classified low and high EF patients. The PPI network included 556 nodes (proteins) and 666 edges (interactions), among which 38 proteins were found differentially expressed by SAM. Further filtering reported 3 genes as the optimal biomarker set: area under the receiver operating characteristic curve (AUC) of 0.82. These were: Vascular Endothelial Growth Factor B (VEGFB), Placental Growth Factor (PGF), both pro-angiogenic, and the anti-angiogenic protein Thrombospondin-1 (THBS1). In conclusion, our approach allowed to identify a set of 3 powerful biomarkers, which could not be identified by applying standard gene expression data analysis only. Therefore, combined network and microarray analysis allows a systematic and less biased approach to biomarker discovery.

The estrogen receptor α gene A-351G and T-397C polymorphisms are associated with early myocardial infarction

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Background: Estrogens exert their cardioprotective function both on systemic level (e.g. regulation of lipid profile, coagulation, fibrinolysis) and directly on blood vessels, through either non- genomic action or regulation of transcription of essential genes. Estrogens regulate gene expression by interaction with specific nuclear receptors, known as estrogen receptors (ERs). Aim of the Study: The purpose of the study was to assess potential association of two intrinsic single nucleotide polymorphisms (SNPs) in the ERα with early myocardial infarction (MI) in Polish population. Materials and Methods: 188 young patients (aged under 55) suffering from MI and 414 healthy controls were genotyped for T-397C and A-351G SNPs in ERα using PCR-RFLP method. Results: The A-351AA genotype was significantly more frequent in MI group than in healthy controls (p = 0.002, 51.3% vs. 37.9%, OR = 1.7), whereas genotypes of the T-397C SNP were equally distributed in studied groups. The analyzed polymorphisms were in linkage disequilibrium and constructed haplotypes. Haplotype “TA” occurred more frequently in affected group compared to healthy controls (p = 0.042, 55.0% vs. 48.6%). Conclusions: In our study “AA” homozygotes of the A-351G ERα SNP were at higher risk of early MI than the carriers of other genotypes. Moreover, allele A together with allele T of T-397C ERα SNP constructed a haplotype, which occurred more frequently in MI patients. Our results are consistent with other reports, which correlated this “TA” haplotype with higher risk of MI and fatal ischemic heart disease.
Phosphoproteome analysis of left ventricular remodeling in an experimental model of heart failure

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Protein phosphorylation is known to play an important role in various cellular processes, whereas abnormal phosphorylation is a cause or consequence of numerous diseases. However, there is until now no data related to a global phosphoproteomic study of left ventricular (LV) remodeling after acute myocardial infarction (MI). The purpose of our work is to study the phosphorylation variations on LV proteins in an experimental rat model of congestive heart failure (CHF). The comparison of LV phosphoproteome between sham and CHF group was performed using two dimensional gel electrophoresis. Phosphoproteins detection was performed after Coomassie staining while the phosphorylation level of the proteome was performed on the same gel using Sypro®Ruby. Numerised images of phosphoproteome and LV proteome from sham and CHF-rats were compared using bioinformatic analysis. This technique allowed us to demonstrate a higher percentage of phosphorylated proteins in CHF (19%) than in sham group (11%). This bioinformatic analysis revealed 79 spots presenting variation of their phosphorylation level in remodelled LV from CHF rats. We identified using mass spectrometry 29 proteins, classified in different functional groups as being heat shock, oxidative stress, contractile proteins and glycolytic enzymes. Data were validated using immunoprecipitation and western blot analysis. This work allowed us to identify phosphoproteins involved in CHF presenting a variation of their phosphorylation level and a better understanding of cellular mechanisms involved in LV remodeling after acute MI.

Cardiostatin-1 (CT-1) is a cytokine that promotes longitudinal cardiomyocyte growth in vitro. Plasma concentration and myocardial expression of CT-1 are increased in patients with heart failure. We aimed to investigate the effect of chronic CT-1 administration on cardiac function and morphology in vivo. Vehicle or recombinant rat CT-1 (20 μg/Kg) was daily administrated to male Wistar rats along 21 days. Blood pressure and heart rate were continuously recorded by telemetry. Left ventricle (LV) dimensions and cardiac function were analyzed by M-mode echocardiography and Doppler assessment. At the end of treatment, hearts were processed for histological studies. Neither vehicle nor CT-1 treatment modified systolic hemodynamics. Whereas non significant echocardiographic changes were observed in rats receiving vehicle, CT-1 administration resulted in increased (P<0.05) LV systolic and diastolic diameters, increased (P<0.01) LV/A and decreased (P<0.01) fractional shortening and ejection fraction. Histological analysis confirmed that hearts from CT-1-treated rats exhibited larger (p<0.01) LV chamber dimensions and thinner (P<0.05) LV free wall than vehicle-treated rats. Finally, cardiomyocyte cross-sectional area was larger in CT-1 than in vehicle-treated rats. Cardiomyocyte length was associated with cardiac ejection fraction (R²=0.604, p=0.005) and with E/A ratio (R²=0.551, p=0.009). These findings indicate that chronic CT-1 overloading results in LV dilatation and cardiac function alteration. We suggest that cardiomyocyte elongation may be a mechanism by which CT-1 participates in cardiac remodelling associated with heart failure.

Compensatory role of tissue transglutaminase in development of right ventricular hypertrophy in pulmonary hypertension

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Right ventricular hypertrophy (RVH) induced by pulmonary hypertension may progress to right heart failure. In systemic hypertension tissue transglutaminase (Tg) play an important role in cardiovascular remodelling, but it has not been addressed whether the same is the case in pulmonary hypertension. Therefore, we investigated whether inhibition of Tg by cysteamine alone or combined with the phosphodiesterase type 5 inhibitor, sildenafil, lowers pulmonary pressure and inhibits development of RVH in chronic hypoxic rats. Nine weeks old Wistar rats were divided into five groups and exposed to normoxia or chronic, hypobaric hypoxia and treated with vehicle, cysteamine 40 mg/kg/day, sildenafil 25 mg/kg/day, or the combination. Right ventricular pressure and systemic pressures were measured and protein and mRNA levels for tTG evaluated. Hypoxia increased Tg expression at mRNA and protein levels in the right ventricle compared to normoxia. Right ventricular systolic pressure (RVSP) was significantly increased in hypoxic vs. normoxic group. Sildenafil significantly lowered RVSP. Cystamine tended to raise RVSP and blunted the hypoxic effect of sildenafil. Tg and right ventricular end-diastolic pressure (RVEDP) compared to normoxia. Sildenafil lowered dP/dt compared to hypoxia. Cystamine blunted the effect of sildenafil. Hypoxia raised right ventricle to left ventricle+ septum weight ratio (RV/LV+S) significantly compared to normoxia. Cystamine and sildenafil tended to lower RV/LV+S. The present investigation suggests that increased expression of tTG counteracts the development of right ventricular hypertrophy.

Inotropic effects of chronic CT-1 overloading in vivo

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Cardiostatin-1 (CT-1) is a cytokine that promotes longitudinal cardiomyocyte growth in vitro. Plasma concentration and myocardial expression of CT-1 are increased in patients with heart failure. We aimed to investigate the effect of chronic CT-1 administration on cardiac function and morphology in vivo. Vehicle or recombinant rat CT-1 (20 μg/Kg) was daily administrated to male Wistar rats along 21 days. Blood pressure and heart rate were continuously recorded by telemetry. Left ventricle (LV) dimensions and cardiac function were analyzed by M-mode echocardiography and Doppler assessment. At the end of treatment, hearts were processed for histological studies. Neither vehicle nor CT-1 treatment modified systolic hemodynamics. Whereas non significant echocardiographic changes were observed in rats receiving vehicle, CT-1 administration resulted in increased (P<0.05) LV systolic and diastolic diameters, increased (P<0.01) LV/A and decreased (P<0.01) fractional shortening and ejection fraction. Histological analysis confirmed that hearts from CT-1-treated rats exhibited larger (p<0.01) LV chamber dimensions and thinner (P<0.05) LV free wall than vehicle-treated rats. Finally, cardiomyocyte cross-sectional area was larger in CT-1 than in vehicle-treated rats. Cardiomyocyte length was associated with cardiac ejection fraction (R²=0.604, p=0.005) and with E/A ratio (R²=0.551, p=0.009). These findings indicate that chronic CT-1 overloading results in LV dilatation and cardiac function alteration. We suggest that cardiomyocyte elongation may be a mechanism by which CT-1 participates in cardiac remodelling associated with heart failure.

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inorganic phosphate by the isolated enzyme and decreased inhibitory effect of Dig in a non
competitive manner. Bicarbonate experiments performed on the isolated enzyme in presence
and absence of Ins (D2/Ins) showed a direct interaction of both Dig and Ins with
enzyme’s α1 subunit but not in the same site. Western blot analysis confirmed the latter
when pre-incubation of enzyme with Dig or ouabain both decreased enzyme’s immuno-
reactivity (IR) while enzyme’s IR was restored and increased in presence of Ins, (only in presence of Dig), but not with ouabain. Ins (10-5 mg/ml) protected both adult and neonatal
cardiac myocytes from Dig (10-5 to 10-1 M) toxicity but not from that of ouabain (10-4 and 10-5 M). In immunohistological studies, neonatal cardiomyocytes treated with Dig or
ouabain decreased significantly the enzyme’s IR while their co-incubation with Ins in
different time period (0.25, 0.5, 1 and 24 h) restored and increased enzyme’s α1 subunit
IR. This amelioration was observed only in presence of Dig but not with ouabain. Ins
due to its specific binding to Na/K ATPase α1 subunit increase enzyme’s activity and
prevent Dig’s interaction. This is of major interest for elaboration of new therapeutics for
Dig-cardio toxicity in particular and cardiac arrhythmia in general.

Green tea attenuates angiotensin II- induced cardiac hypertrophy in rats by modulating reactive oxygen species production and the Src/EGFR/Akt signalling pathway

We previously documented a clear-cut antihypertensive effect of green tea extract (GTE), which was associated with correction of endothelial dysfunction and prevention of left
ventricular hypertrophy in an Angiotsenin II (Ang II)-dependent model of hypertension, but
the molecular mechanisms remain to be defined. As several effects of Ang II involve
production of reactive oxygen species (ROS) and activation of second messengers, such as
MAPKs and Akt, we investigated the effect of GTE on these signal transduction
pathways in Ang II treated rats. Rats were treated for 2 wk with Ang II infusion (700
mg/kg/1, n=6, via osmotic minipump), Ang II plus GTE (6 g/L), dissolved in the drinking
water, (n=6), or vehicle, GTE as controls. Blood pressure was monitored by
telemetry throughout the study. The activation and expression of NAD(P)H oxidase subunits, PKC isoforms, Src, EGFR, Akt and MAPKs were determined in the heart
in vitro

Cardiac and vascular effects in two different chronic cannabinoid treatments in rats

Cannabinoids are proposed for the treatment of an increasing number of pathologies, but the side effects of their chronic administration are not well-known. In the rat, acute
administration of cannabinoids induces cardiovascular alterations. We studied the effects of the cannabinoid against WIN 55,212 (WIN) in body weight and cardiovascular function
during and after different patterns of chronic administration. Male Wistar rats received saline, vehicle or WIN (0.5 or 5 mg/kg, i.p.), either once a day for 14 days or once a week for 4 weeks. Cardiac, aorta and mesenteric functionality were evaluated right after the first dose (acute) and after the last dose (chronic) of either chronic treatment. Changes in body weight gain were also recorded. Acute administration of WIN did not cause cardiovascular alterations in the animals. Daily or weekly chronic administration of WIN did not also induce any significant vascular effect. However, a dose-dependent alteration on the left ventricular functionality was observed, but only after weekly administration. Body weight gain was significantly reduced after daily
WIN administration, but no modification was observed in this parameter after weekly
treatment. Our results show, for the first time, that cardiac side effects could come into sight with a chronic administration of the treatment in rats. The effects seem to be dose-dependent and occurs with low frequency of administration. Conversely, body weight modifications come out with high frequency of administration. More research is needed to determine the mechanism by which these alterations are induced.


Hypoxia Regulated vascular endothelial growth factor gene expression system for ischemic heart disease

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Objective: To investigate the feasibility and efficiency of a hypoxia regulated vascular endothelial growth factor (VEGF) gene delivery system in ischemic myocardium as well as restoring the contractility of the ischemically weakened heart. Background: VEGF has been studied widely for its angiogenic activity. With encouraging data from animal studies, clinical trials of VEGF gene therapy have been carried out. Skeletal myoblast transplanta-
tion to augment the number of functioning myocyte in a failing heart may be more effective if combined with improvement in angiogenesis. Thus, transplantation of skeletal
myoblasts modulated to over-express angiogenic factor is a novel ex-vivo method to repopulate the myocardium. Methods: We have already shown that myoblasts are
carrier of human VEGF for angiomyogenesis in infarcted heart. Considering that myocardial ischemia is a recurring and progressive disease, a regulable gene delivery
system (pHRE-VEGF) is expected to overcome this obstacle.

Results: VEGF over-expression in hypoxia condition. These myoblasts carrying pHRE-Luc have shown increased luciferase activity under hypoxia. VEGF expression under hypoxia is upregulated at both RNA and protein levels. This system is being evaluated in vivo by injection of autologous skeletal myoblasts into an established rabbit heart model of myocardial infarction. Conclusion: This study will envision a new, realistic and safe approach for cardiac therapeutic angiogenesis and may be applied to the treatment of human ischemic heart diseases.

Exacerbated NOS uncoupling and adverse ventricular remodelling from pressure-overload in mice lacking Beta3-Adrenoreceptor

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Background: The β3-adrenoreceptor (AR) is thought to couple to the inhibitory G-protein G, with downstream signalling through nitric oxide, though its role in the heart remains controversial. In this study, we tested whether lack of β3-AR influences the myocariac response to pressure-overload. Methods: Mice lacking β3-AR (β3−/−) and wild type (WT, n=25) controls underwent mild transverse aortic constriction (TAC) or sham surgery and were followed for 9 weeks. Results: Baseline cardiac morphology and function by echocardiography were similar in WT and β3−/− mice. β3−/− mice had greater mortality after TAC than WT controls (p<0.001). By 3 weeks of TAC, left ventricular (LV) wall thickness (p<0.05) and mass (p<0.05) increased far more in β3−/− hearts than WT, and systolic function was worse. In addition, after 9 weeks of TAC, β3−/− mice had greater LV dilation (p<0.01), myocyte hypertrophy (p<0.001) and enhanced fibrosis (p<0.01). NOS activity declined in β3−/− TAC animals more than in controls (p<0.001), and total (p<0.001) and NOS-dependent superoxide (p<0.05) rose, indicating heightened NOS uncoupling. GTPCH-1 expression was reduced (p<0.001), as was the level of eNOS phosphorylation (p<0.001) and enhanced fibrosis (p<0.01). Conclusion: Lack of β3-AR signalling exacerbates cardiac pressure-overload remodeling coupled with enhanced NOS uncoupling and consequent oxidant stress. Selective stimulation of these receptors may provide a novel approach to reducing pathologic hypertrophy in the failing heart.

Tetrahydrobiopterin reverses established heart failure by re-coupling of uncoupled eNOS

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Background: Pressure overload triggers eNOS as a prominent source of myocardial ROS that contribute to diastolic remodeling and cardiac dysfunction. Administration of
tetrahydrobiopterin (BH4) can prevent pressure-load remodeling. The aim of this study
was to investigate that BH4 can reverse established non-decompensated heart failure by interacting with uncoupled eNOS, and on this way prevent the evolution to end-stage heart failure. Methods: Compensated cardiac remodeling was induced in 60 mice by transverse aortic constriction (TAC). After 4wks, mice were randomized to receive BH4 (250 mg/kg/d, n=30) or placebo (n=30) for the following 5wks. Overexpression of endothelial GTPCH, the rate limiting enzyme of BH4 synthesis, was evaluated in this TAC model (n=15 mice). Results: BH4 significantly reversed cardiac hypertrophy (heart weight: p<0.001, idem for myocyte dimensions, wall thickness and calculated LV mass) and diminished fibrosis (p<0.05). BH4 prevented the evolution towards cardiac decompensation (p<0.001,

PG.07

PG.08

PG.09

PG.10

PG.11
confirmed by MRI and PV loop analysis. BH4 increased the already uncoupled eNOS and increased its activity back to the normal level. Superoxide generation (total and NOS-dependent) was markedly reduced by BH4. BH4 improved fractional shortening and calcium-kinetics in isolated myocytes. Endothelial upregulation of BH4 by GTPβS-Tg had no beneficial effect on remodeling. Conclusion: BH4 can reverse established cardiac remodelling by re-coupling uncoupled eNOS and as a consequence less NOS dependent ROS is generated, leading to less hypertrophy and fibrosis and an amelioration of cardiac function.

Ph.02
Endothelial dysfunction caused by chronic cisplatin treatment is improved by the synthetic cannabinoid, Win55,212-2

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Cisplatin is an alkylating agent with a wide spectrum of antineoplastic activity. Cisplatin toxicity has been associated to cisplatin treatments, although the responsible mechanisms are unknown. Cannabinoids have been proposed as therapeutic agents in cardiovascular disease.

The aim of this study was to evaluate in rat aorta the vascular alterations induced by a chronic cisplatin treatment and the possible improvement of these alterations by the cannabinoid, Win55,212-2.

Male Wistar rats received cisplatin 2mg/kg i.p. once a week for 4 weeks. Afterwards, aorta rings from the animals were mounted in a tissue bath for tension measurements. In one experimental group, vascular constriction and relaxation was assessed by phenylephrine (Phe, 10^-6 - 10^-4 M) and by carbachol (10^-9M-10^-4M) concentration-response curves, respectively. In a second group of experiments, Phe-precontracted rings were treated with Win55,212-2 (1 μM), just before the carbachol concentration-response curve. Data are given as the mean ± s.e.m (6 –12 rings). Vasorelaxation was expressed as % of relaxation of Phe-induced tone. A two-way ANOVA (Bonferroni Dunn post hoc test) was used (*P < 0.05).

A slight, but not significant, increase in Phe-vasoconstriction, and a significant inhibition of the endothelium dependent vasorelaxation in the aorta of cisplatin treated-animals was observed. The treatment with Win55,212-2 significantly improved the endothelium-dependent vasorelaxation of aortic rings from cisplatin treated-animals, obtaining values similar to control animals. Our results show that Win55,212-2 could restore the endothelial dysfunction caused by chronic cisplatin treatment in rats. The responsible mechanism involved is not definitely established.


Ph.03
Attenuation of endothelium-dependent relaxation in rat mesenteric arteries by ApoB protein of low density lipoprotein, but not cholesterol

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Aims: Vascular endothelium is a primary target for many of the cardiovascular risk factors. The present study has investigated the effects of the risk factors: low density lipoprotein (LDL), ApoB protein of LDL or cholesterol, respectively. The endothelium-dependent relaxation induced by acetylcholine following pre-constriction was studied in a sensitive myograph system. Nitric oxide (NO), cyclooxygenase (COX) and endothelium-derived hyperpolarizing factor (EDHF)-pathways were characterized by using their specific inhibitors. LDL oxidation was monitored by TBARS assay during the organ culture.

Results: i) Organ culture of the mesenteric arteries in the presence of LDL for 24 hrs reduced the endothelium-dependent vasorelaxation in a concentration-dependent manner. However, 6 hrs of incubation with LDL had no effects. ii) The reduced relaxation was mainly via decreasing in NO- and EDHF-mediated vasoactivities. iii) ApoB protein of LDL but not cholesterol, was responsible for the reduced relaxation. iv) The TBARS assay revealed that LDL oxidation took place during the organ culture process. Conclusion: ApoB protein of LDL, but not cholesterol, attenuated the NO- and EDHF-mediated endothelium-dependent relaxation. LDL protein oxidation may cause the damage to the endothelial functions and thus contributes to the development of cardiovascular disease.
Deletion of estrogen receptor-alpha abolishes endothelial response to wine polyphenols without affecting the main cardiovascular parameters in mice
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We tested the hypothesis that the effect of the red wine polyphenol compounds, Provinols®️, on the endothelium is mediated by estrogen receptor-alpha (ER-alpha) using overexpressed ER-alpha in aorta knockout mice. Deletion of ER-alpha did not affect echocardiographic measurements in terms of left ventricular end-systolic and -diastolic diameters and systolic function. Deletion of ER-alpha induced hyperreactivity of the aorta in presence but not in absence of functional endothelium and did not alter relaxation to acetylcholine. Both basal N0 and superoxide anion productions, assayed by electron paramagnetic resonance technique, were not significantly different in mesenteric arteries from the two strains. Interestingly, the endothelium-dependent relaxation to Provinols®️ and to delphinidin, an anthocyanin with similar pharmacological properties than the original extract, was completely blunted in aorta from ER-alpha null mice. The capacity of the two compounds in stimulating the NO pathway linked to se, eNOS and cGMP in endothelial cells was abolished after silencing ER-alpha. In summary, deletion of ER-alpha was not associated with abnormalities of the main cardiovascular parameters except a release of unknown endothelial vasoconstrictor metabolites in the aorta. They provide the first evidence that Provinols®️ and delphinidin mediate endothelial-dependent relaxation via activation of ER-alpha and NO pathway. This study underlines a direct involvement of ER-alpha on therapeutic benefit of wine polyphenols in cardiovascular diseases associated with endothelial dysfunction.

PH.04

Activation of phospholipase C and α1D adrenoceptors in rat mesenteric small arteries
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Stimulation of α1-adrenergic receptors (α1-AR) by noradrenaline (NA) regulates vascular smooth muscle contraction through activation of phospholipase C (PLC), leading to Ca2+-mobilisation and protein kinase C (PKC) activation. This response is important for regulation of peripheral vascular resistance and blood pressure. However, the mechanisms coupling α1-ARs to PLC activity in intact tissues are unclear. In expression studies using cultured cell lines, α1α and α1β-ARs coupled to PLCβ1 via the atypical G-protein Gα11. Our aim was to investigate whether such a mechanism was important in vascular tissue. Using rat mesenteric small arteries (RMSA), the effects of NA stimulation on the subcellular localisation and interaction of PLCβ1 and Gα11, and on PKC activation, were determined by western blot and co-immunoprecipitation. Contractility was measured by pressure myography and PLC activity by H-PIPs hydrolysis in vitro. BMY 7378 (100µM), a specific inhibitor of α1α-AR, reduced NA-induced contraction, and both PLC and PKC activity. PLCβ1 and Gα11 were detected in RMSA with a similar subcellular distribution, which was not altered by NA. Neither PLCβ1 nor PLC activity were detected in Gα11 immunoprecipitates, from either control or stimulated arteries. These results show that in intact RMSA, NA signals via α1α-AR, leading to PKC activation and contraction. However, there was no interaction detected between PLCβ1 and Gα11, which suggests this is not a major mechanism for regulating PLCβ1 activity in vascular tissue.

PH.05

The effect of melatonin on endothelial function and in L-NNAME-induced hypertensive rats
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Melatonin reduced experimental and clinical hypertension, but the mechanisms of its blood pressure (BP) lowering effect are not completely understood. We elucidated the role of NO in the antihypertensive effects of melatonin. Four groups (n=10 each) of male Wistar®️ rats were investigated: controls, L-NNAME, melatonin and L-NNAME + melatonin for 5 weeks. BP was measured non-invasively each week. NOS activity and RNA expression of NOS and COX were determined in the aortas. Acetylcholine(ACh)-induced responses and their NO-mediated component were evaluated in phenylephrine-precontracted femoral and mesenteric arteries. Endothelium-derived constricting factor (EDCF)-mediated component of ACh-induced responses and inner diameter were determined in femoral arteries. L-NNAME treatment caused hypertension, impaired ACh-induced relaxations, decreased NO-component, augmented EDCF-component and reduced inner diameter. L-NNAME also inhibited NO activity in the brain and the aorta, in which the endothelial NO expression was not altered, and COX-2 expression was increased. Concomitant treatment with melatonin decreased BP by 15%, failed to improve NOS activity, NOS or COX-2 expression, vascular structure or function. We conclude that BP reduction after was less pronounced in NA-induced hypertensive rats than previously reported on spontaneously hypertensive rats. Thus, the enhancement of NO pathway might represent a major mechanism of the antihypertensive effect of melatonin. However other NO-independent mechanisms may be involved in the residual BP lowering effect of melatonin.

PH.06

Mesenchymal stem cells effectively reduce surgically-induced stenosis in rat carotids
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Objective: Restenosis following vascular injury remains a pressing clinical problem. The possible therapeutic role of Mesenchymal Stem Cells (MSCs) in vascular stenosis has been poorly investigated. We tested the effectiveness of MSCs in reduction of stenosis in a model of rat carotid arteriopathy. Methods: Rat MSCs were isolated from bone marrow and tested for their capacity of pluripotential differentiation, expression of specific surface antigens, proliferative activity and senescence after propagation in vitro. Wistar male rats were submitted to carotid arteriotomy and to venous administration with 5x10⁵ MSCs (n=16) or control DMEM (n=16). Homing of MSCs at the injury site and differential analysis of gene expression were verified at 3 and 7 days and morphometric evaluation of treatment at 30 days after arteriotomy. Results: MSCs in vitro were able to differentiate into mesenchymal lineage cells, retained antigens CD73, CD90 and CD105, were mainly in proliferative phase of cell cycle and showed limited senescence. MSCs in vivo homed in the adventitia of injured carotids since 3 days after arteriotomy but not in contralateral uninjured carotids. Lumen area in MSC-treated carotids was 37% greater than in control arteries (p<0.016) and inward remodelling was limited in MSC-treated carotids (p<0.030) 30 days after arteriotomy. Differential expression analysis revealed that MSC treatment affected the expression level of inflammation-related genes IL-1β, TGF-β and MCP-1 in carotids after arteriotomy. We conclude that MSC administration limits the arteriopathy-induced stenosis in rat carotids presumably through an immunomodulatory action.

PH.07

Phospholipase Cò1 modulates sustained contraction of rat mesenteric small arteries in response to noradrenaline, but not endothelin-1
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Activation of phospholipase C (PLC) leads to phosphatidylinositol 4,5-bisphosphate (PI(2)P) hydrolysis, increased intracellular calcium and contraction. In rat mesenteric small arteries (RMSA) PI(2)P hydrolysis in response to noradrenaline (NA) or endothelin (ET-1) occurs solely in caveolae/rafts. Here we have investigated whether PLCò1, a PLC isoform implicated in α1-adrenoceptor signalling and the pathogenesis of hypertension is involved in NA and ET-1 induced contraction. Caveolae/rafts were prepared from RMSA by sucrose density centrifugation, PI(2)P or inositol phosphates (InsPs) were measured with 33P or [3H]-inositol. PLCò1 was localised by immunoblot and neutralised by delivery of PLCò1 antibody. Contractility was measured by pressure myography. PLC inhibition with U73122 (3µM), but not the negative control U73342, inhibited NA and ET-1 contraction without affecting potassium or phorbol ester responses, implicating PLC activity in receptor-mediated contraction. PLCò1 was detected in caveolae/rafts and NA but not ET-1 stimulated a rapid calcium dependent increase in PIP2 levels in these domains. NA-induced PI(2)P hydrolysis and InsP formation was also calcium dependent whereas ET-1-induced PI(2)P hydrolysis was not. Delivery of PLCò1 antibody to RMSA prevented NA-induced PLCò1 association with caveolae/rafts and attenuated the sustained phase of the contractile response to NA when compared to control antibodies. In contrast, PLCò1 antibody had no effect on ET-1 or potassium induced contraction. These data show a novel and selective role for PLCò1 in NA-induced sustained contraction in intact vascular tissue.

PH.08

Apocynin does not lower arterial pressure in spontaneously hypertensive rats (SHR) and its acute vasodilator action is not due to NADPH oxidase inhibition
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We tested the hypothesis that chronic NADPH oxidase (NOX) inhibition with apocynin would lower arterial pressure and improve endothelial function in SHR. While apocynin effectively dilated arterial segments in vitro, it failed to lower blood pressure or to improve endothelial function in SHR. In addition, apocynin did not reduce arterial pressure acutely in young adult SHR when given at 50, 100, or 150 mg·kg⁻¹ orally over one-week-intervals or when given i.v. Further experiments in normotensive rats and in NADPH oxidase subunit knock-out mice were performed to test if apocynin-induced vasodilation depends on

PH.09
NADPH oxidase inhibition at all. Apocynin potently inhibited human granulocyte NOX but not NOX-dependent oxygen radical formation in rat aortic rings and small intrarenal arteries. This effect was also observed when using rat aortic tissue in vitro. Apocynin diluted rat intrarenal and coronary arteries independently of pharmacological interventions that reduce vascular superoxide radical abundance and actions. Aortic rings from p47phox-/- mice were more sensitive to apocynin-induced dilation than wild type aortic rings. Apocynin-induced vasodilation was not significantly affected by NOS, PKA, or PKG inhibition, did not depend on extracellular Ca²⁺ but was sensitive to Rhö-kinase inhibition. Apocynin per se does not inhibit vascular NOX-dependent superoxide formation and requires high peroxide activities for efficient NOX inhibition. Its use as a pharmacological tool to investigate vascular NOX and the role of NOX activity for arterial pressure regulation and hypertension should be discontinued.

**PH.10** Short-term hydrocortisone incubation reduces vasodilator responses in human resistance arteries

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Patients with Cushing’s Syndrome exhibit cortisol oversecretion. Since the major cause of morbidity and mortality in these patients is cardiovascular disease, we hypothesized that elevated circulating cortisol might have adverse effects on vascular function. Vascular function was assessed with wire myography using human subcutaneous resistance arteries (SRA) from abdominal fat biopsies obtained from 5 healthy subjects. We analyzed the effect of preincubation at 4°C during 24h with physiological (300nm) or high (1200 nM) HC concentrations, or preincubation with 1200 nM HC plus addition to the bath during the experiment, on concentration-response curves (CRC) to Norepinephrine (NE) and acetylcholine (ACh). NE exhibited the biphasic response with initial contractions followed by relaxations at concentrations higher than 10^{-11} M. Segments with high HC preincubation plus addition to the bath lost the vasodilator component of the CRC. ACh induced vasodilator responses, which were significantly reduced in segments preincubated with high HC. In addition, HC preincubation (with or without addition to the bath) significantly reduced the second ACh-CRC with respect to the first one. In conclusion, short-term cortisol incubation at pathophysiological concentrations induces desensitization of vasodilator responses. This effect may participate in the increased risk of cardiovascular complications in patients with Cushing’s syndrome.

**PH.11** The cardiovascular risk factor DMSO-soluble smoke particles enhance transcription and translation of endothelin type A receptors via activation of PKCε and ERK1/2 pathways in vascular smooth muscle cells

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Aims: Enhanced endothelin (ET)-system activity plays an important role in cardiovascular disease pathogenesis. The present study was the first designed to show that cardiovascular risk factor DMSO-soluble smoke particles (DSP) enhance the ET-system activity through up-regulation of vascular endothelin type A (ETα) receptors in vascular and to investigate the molecular mechanisms behind the DSP-induced activation of ET-system. Methods and results: In organ culture of rat mesenteric arteries, DSP could activate protein kinase Cε (PKCε) and extracellular regulated protein kinase 1 and 2 (ERK1/2) in the smooth muscle cells (SMC). This resulted in ETα receptor up-regulation with enhanced ETα, receptor-mediated contraction (myograph), increased ETα receptor mRNA (real-time PCR) and protein (immunohistochemistry with confocal microscopy) expressions in the SMC. Inhibition of transcription or translation abolished DSP-enhanced ETα receptor-mediated contraction. Post-transcriptional mechanisms were suggested by that DSP accelerated ETα receptor mRNA degradation but enhanced ETα receptor-mediated contraction. Inhibition of translation, ERK1/2 or PKCε activities attenuated the DSP effects. Conclusion: Up-regulation of ETα receptors by DSP involves transcriptional mechanisms and enhanced translation of ETα receptors in the vascular SMC via activation of intracellular PKCε and ERK1/2. The ETα receptor up-regulation by DSP in the SMC may contribute to the development of smoking-associated cardiovascular disease.

**PH.12** Vascular smooth muscle relaxation in soluble guanylyl cyclase β1 His 105 PHE mutant mice

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The binding of nitric oxide (NO) on the heme group of soluble guanylyl cyclase (sGC) induces important changes such as vascular smooth muscle relaxation, thereby controlling blood pressure, blood flow and erection. The sGCβ1,β2 and sGCα2β2 are the physiologically active heterodimers, in which the histidine residue at position 105 of the β1 subunit functions as an axial ligand for the heme prosthetic group. Substitution of this histidine by phenylalanine abolishes the heme-dependent activation of sGC. This is the case in the sGCβ1,β2-mice from which an aortic, femoral artery and corpora cavernosa (CC) segments were mounted for isometric tension recording. In comparison with the preparations isolated from the wild type littermatoes, the responses to endogenous NO released from the endothelium in response to acetylcholine (ACH) and exogenous NO (from the NO donor sodium nitroprusside (SNP)) were completely abolished in the aorta from the sGCβ1,β2-mice, but not in the femoral arteries. In CC the relaxation response to Ach (releasing endothelial NO) and electrical field stimulation (releasing neuronal NO) was abolished, while SNP response was only reduced. The response to the NO-independent sGC-stimulator (BAY 41–2272) was also significantly reduced in the different preparations of sGCβ1,β2-mice, indicating that the heme group plays a role in the BAY 41–2272-induced activation of sGC. Our results demonstrate the importance of sGC as the sole target for NO in regulating vasodilatation in mice aorta but not in femoral artery. Furthermore, the remaining relaxing effect of BAY 41–2272 in the sGCβ1,β2-mice suggests that the heme-binding pocket is very important but not indispensable for the interaction of BAY 41–2272 with sGC.

**PH.13** Antioxidant activity of Liver Growth Factor, a Bilirubin covalently attached to albumin

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Treatment of SHR with Liver Growth Factor (LGF), an albumin-bilirubin complex with a covalent bond, improves nitric oxide (NO)-dependent vasodilation and exerts vascular antifibrotic actions. Bilirubin and albumin-bound bilirubins exert antioxidant properties. Based on these findings and the chemical nature of LGF, we hypothesize that LGF might exert its cardiovascular actions through an antioxidant mechanism. We have tested the capacity of LGF, Trolox, open-form bilirubin and seroalbumin in mg/ml range: 1) to scavenge reactive oxygen species (ROS) in aqueous medium: ABTS (colorimetry), peroxyl radical (ORAC-Fluorescein) and hydroxyl radical (TBARS assay and chemiluminescence) and 2) to protect endothelial NO generated by acetylcholine from degradation by pyrogallol-induced superoxide anion (isometric tension in Sprague Dawley rat carotid arteries). LGF, bilirubin and Trolox exhibited scavenging capacity against all ROS tested, while seroalbumin exerted negligible effect. LGF and Trolox were able to protect NO from superoxide anion degradation, while seroalbumin did not have effect. Taking into account the molecular weight of the molecules tested, LGF exerted its antioxidant effects at much lower molar concentration. The present data suggest that LGF is likely to exert its cardiovascular actions, at least in part, through an antioxidant mechanism, scavening potential harmful ROS and protecting NO from degradation. The potent antioxidant capacity of LGF compared to bilirubin and albumin is possibly linked to a conformational change in the albumin, creating a molecule with higher ROS scavenging capacity than its components alone.

**PH.14** Targeted disruption of kinin B1 or B2 receptor gene in mice alters vascular reactivity and nitric oxide metabolism

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B1 and B2 receptors play an essential role in inflammation and cardiovascular homeostasis. Vascular reactivity and nitric oxide (NO) metabolism was studied in knockout B1 (B1-/-), B2 (B2-/-) and wild type (WT) mice (n=8). Isolated mesenteric arteriolar beds were tested with acetylcholine (ACH), sodium nitroprusside (SNP) and angiotensin II (ang II); responses were expressed as % of noradrenaline (10µM)-induced contraction. Vascular nitric oxide synthase (NOS) activity and plasma NO levels were determined. ACH-vasodilation was significantly reduced in B1-/- (1.5 ± 0.2*, 0.9 ± 0.2, 1.2 ± 1.2, 4.9 ± 1.1) and B2-/- (0.7 ± 0.5, 5.8 ± 1.8, 8.0 ± 1.9) in comparison to WT preparations (6.3 ± 0.6, 12.6 ± 1.5, 14.7 ± 1.3), at doses of 0.1, 1 and 10 nmol, respectively. On the other hand, SNP responses were similar among strains. Constrictr responses to ang II were reduced in B1-/- (1.3 ± 0.9, 1.5 ± 0.8, 2.0 ± 0.1) when compared to B2-/- (6.6 ± 0.5, 7.0 ± 1.0, 7.2 ± 1.2) and WT (5.7 ± 0.7, 6.7 ± 1.3, 6.6 ± 0.9) at doses of 50, 100 and 200 nmol, respectively. Plasma NO levels(µM) were reduced in B1-/- (60 ± 11*) and B2-/- (67 ± 3*) vs WT (142 ± 17), while NOS activity (pmol/mg/min) was higher in the mesenterial arteries B1-/- (1.7 ± 0.7, P<0.05) and B2-/- (1.2 ± 0.3) vs WT (0.4 ± 0.09). The endothelial dysfunction accompanied by decreased circulating NO and augmented NOS activity suggest the exacerbation of NO inactivation in both B1-/- and B2-/- mice. B1-/- gene deletion might affect negatively the ang II mediated signaling in vascular cells. These data may provide new approaches in the field of the interactions among angiotensin, kinin and NO systems.
Pharmacological stimulation of the AT2 receptor ameliorates experimental diabetic nephropathy in a blood pressure-independent manner

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This study analysed the effects of the novel non-peptide AT2 receptor antagonist Compound 21 (C21) in diabetic nephropathy. Diabetes was induced in SHR by a single streptozocin injection (STZ: 60 mg/kg BW i.v.) and was treated for 12 weeks according to the following protocols: 1) non-diabetic controls, 2) STZ (diabetic controls); 3) STZ+Hcan; 4) STZ+Hcan; 5) STZ+21 (0.33 mg/kg/d i.p.); 6) STZ+both (Hcan+21). Systolic BP was only lowered in the STZ+Hcan group (169±24 mmHg) and the STZ+both (188±17 mmHg) but not in the STZ+Lcan (222±10 mmHg) and, remarkably, not in the STZ+21 (251±14 mmHg) groups. Elevated albuminuria in STZ rats (51±43% STZ+Hcan, 25±18% STZ+Lcan, 19±17% STZ+21, 17±12% STZ+both) was significantly lower with Can and 21 (STZ+Hcan -15%, STZ+Lcan -12%, STZ+21 -11%, STZ+both -18%). Increased tubulointerstitial collagen I expression (STZ 70±22% per section) and glomerular collagen IV deposition (STZ 51±1.0%) was reduced in all treatment groups (STZ+Hcan -15%/ -43%, STZ+Lcan -65%/ -27%, STZ+21 -56%/ -43%, STZ+both -33%/ -26%). We conclude that pharmacological AT2 receptor stimulation limits experimental diabetic renal hypertrophy and protects kidneys.

The adipokine visfatin is synthesized by human endothelial cells and promotes inflammation in human smooth muscle cells

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Although initially described as an insulin mimetic, recent evidence supports an inflammatory role for the adipokine visfatin. Its circulating levels are enhanced in metabolic disorders like type 2 diabetes and obesity, characterized by low-grade inflammation and an enhanced cardiovascular risk. This work aims to study the potential inflammatory effect of visfatin on vascular smooth muscle and to assess whether visfatin can be synthesized by vascular cells. In human aortic smooth muscle cells (HASMC) stimulated with increasing concentrations of the adipokine for 18 h, a concentration-dependent increase in iNOS levels and NF-κB activity was observed by Western blot and EMSA respectively. Visfatin triggered ERK 1/2 activation following a biphasic pattern with a transient increase at 10 min followed by a sustained and gradual activation that peaked at 18 h. By using the respective ERK 1/2 and NF-κB inhibitors, PD98059 and pyrrolidine dithiocarbamate (PDTC), we established that iNOS induction by visfatin required the consecutive upstream activation of ERK 1/2 and NF-κB. Furthermore, visfatin was basally detected in human umbilical vein endothelial cells (HUVECs).

Vasoactive peptide signalling and survival of nociceptive neurons

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Signalling through the calcitonin receptor-like receptor (CRLR), the receptor for adenyl cyclase activating peptide and calcitonin gene-related peptide (CGRP) that are on a molar basis the most potent vasodilators known so far, is involved in nociception. Mice overexpressing a smooth muscle a-actin promoter driven CRLR tg showed higher tolerance in tests of pain for hierarchy suggesting reduced nociception. To investigate this observation further, noxious heat hyperalgesia was applied to the left hind paw during Ethamolamide anesthesia with and without pre-treatment with the CCR5 antagonist CCR5(8–37). In parallel, blood pressure and heart rate was measured. Two hours later the mice were perfusion-fixed for counting of foscarnet positive neurons in the dorsal horn of the spinal cord. Compared to wild type (wt) mice, tg mice showed significantly less decrease in blood pressure as well as heart rate during the stimulus. Accordingly, the number of foscarnet positive neurons was significantly lower in tg mice com-pared to wt mice. CCR5(8–37) decreased the cardiovascular response and the number of foscarnet positive neurons in wt mice to that of untreatedtg mice. The CCR5 antagonist had no effect in tg mice. Compared to wt animals CCR5 positive nerve fibres in the dorsal horn and dorsal root ganglia of tg mice were reduced. Moreover the number of CLR positive neurons in lamina I of the dorsal horn of tg mice appears to be reduced. In conclusion, nociception is reduced in our CLR tg mice presumably due to loss of peripheral and maybe also central nociceptive neurons.

Extracellular RNA, a pro-inflammatory factor promoting arteriogenesis

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The growth of pre-existing arteriolar anastomoses to large conductance arteries as compensation for the occlusion of a main artery is described as arteriogenesis. The initial trigger is fluid shear stress activating the endothelium surface, and leads to modulation of processes controlling the recruitment of circulating cells. The inflammatory aspect of this activation is apparent by the increased expression of MCP-1, a chemotaxin protein responsible for the adhesion and migration of monocytes to the endothelium. In order to induce arteriogenesis in mice, the right femoral artery was ligated and the left was sham operated. Arterial growth was measured via Laser Doppler Imaging (LDI) and quantified as relative perfusion recovery (right/left) before and after surgery until day 7. Treatment with RNase (42μg/kg/d), but not with DNase (42μg/kg/d) immediately before occlusion of the artery until day 7 reduced the extent of arteriogenesis, suggesting that extracellular RNA plays a role in this process. Accordingly, in vitro studies confirmed that RNA acts as a chemotaxin for monocytes. In an endothelial cell monolayer the migration of monocytes was increased by RNA to nearly the same extent as by MCP-1. In addition there was an increase in adhesion of monocytes to microvascular endothelial cells by RNA, but not by DNA. The expression of ICAM-1 on endothelial cells was increased by RNA and abolished in the presence of RNAse. Furthermore, RNA led to expression of pro-inflammatory factors like P-Selectin from Weibel-Palade bodies. In summary, our data indicate that proinflammatory effects of extracellular RNA promote arteriogenesis.

Leptin drives cardiac fatty acid metabolism by reducing the sensitivity of carnitine palmitoyltransferase I to malonyl-CoA

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Leptin drives cardiac fatty acid metabolism by reducing the sensitivity of carnitine palmitoyltransferase I to malonyl-CoA.
malonyl-CoA-sensitive component (CPT-II). The aim of this work is to characterize the effect of leptin in regulating cardiac CPT inhibition by malonyl-CoA. We analyzed the effect of 50 μM malonyl-CoA in inhibiting cardiac CPT activity in mice made hyperleptinemic by 1 dietary treatment with a high-fat diet or 2 acute administration of leptin (1 mg/kg). CPT activity was measured in enriched-mitochondria preparation from left ventricle. The inhibitory effect of malonyl-CoA on cardiac CPT was not detected in leptin-treated mice. On the other hand, inhibition of cardiac CPT by malonyl-CoA was reduced in diet-induced hyperleptinemic animals. The positive correlation between plasma leptin concentration and malonyl-CoA-insensitive CPT activity suggests a link between cardiac leptin receptors and CPT regulation. In NF animals we detected an up-regulation of phosphorylated Akt (pAkt). We observed that pAkt positively correlated with plasma leptin concentration as well as with malonyl-CoA-insensitive CPT activity. Because pAkt is a Ser/Thr kinase our data suggest that Akt-mediated phosphorylation of CPT or cytoskeletal components regulating CPT activity might account for the effect of leptin. Supported by Ministerio de Educación y Ciencia (SAF 2006-02456 and SAF 2005-0518), FUSP-CEU, and SESCAMET.

The first selective non-peptide angiotensin AT2-receptor agonist

**Compound 21 attenuates TNFα-induced IL-6 expression through inhibition of NF-κB activity and activation of phosphatases**

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Ang II elicits pro-inflammatory actions via the AT1-receptor (AT1R), e.g. activation of serine/threonine phosphatase, ocidaic acid (10nM). C21 was not effective in fibroblasts with malonyl-CoA-insensitive CPT activity. Because pAkt is a Ser/Thr kinase our data suggest that Akt-mediated phosphorylation of CPT or cytoskeletal components regulating CPT activity might account for the effect of leptin. Supported by Ministerio de Educación y Ciencia (SAF 2006-02456 and SAF 2005-0518), FUSP-CEU, and SESCAMET.

**Sympathetic overactivity in α2-adrenergoreceptor deficient mice**

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The mechanisms of increased renal neurotransmission in renal failure are still unclear. Therefore, effects of experimental renal failure on renal neuro-transmission in α2-adrenergoreceptor deficient mice (KO) were investigated. KO and wild type (WT) mice underwent 5/6 nephrectomy (SNX) or sham operation (SHAM). KO- and WT-mice developed a high blood pressure after SNX. Renal nerve stimulation (RNS) induced NA-release was higher in SHAM KO- compared to WT-mice. α2-receptorblockade increased RNS-induced NA-release in WT, whereas no effect was observed in KO mice indicating no other relevant presynaptic α2-receptor subtypes. 60 days after SNX, RNS induced renal NA-release was significantly increased in WT- but surprisingly not in KO mice. After SNX the effect of α2-receptor blockade on renal NA-release was attenuated in WT-mice. Realtime-PCR revealed that SNX had no influence on mRNA-expression of α2A, α2B and α2C. Angiotensin (Ang) II increased RNS induced NA-release in SHAM WT- but not KO-mice, indicating a pivotal role of α2A-receptors in the regulation of Ang II induced facilitation of renal neurotransmission. Interestingly, the facilitatory effect of Ang II on renal NE-release was abolished already 10 days after SNX operation. The present study verifies, that renal sympathetic neurotransmission is increased in renal failure. As these effects were attenuated in α2A-KO mice, one has to speculate that α2A-receptors play a pivotal role in regulating renal sympathetic neurotransmission. In addition, Ang II seems to be one mechanism triggering sympathetic overactivity.
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