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0.03 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% from fat) or to a high-fat diet (HF: 45 kcal% from fat) during 6, 14 or 32 weeks. Reactivity of resistance vessels was characterized by perfusion clamp technique. After 32 weeks of diet, HF animals exhibited increased plasma leptin levels, but reduced plasma adiponectin levels. Endothelial function, assessed by ACh (10⁻⁹–10⁻⁴ M), was significantly impaired in HF animals (Emax 90.7 ± 4.1 % vs LF 60.3 ± 1.7 %, p<0.005). Furthermore, NO production was observed in mesenteric arteries of LF animals, but was reduced in HF animals. In group-1 (0.062 ± 0.04) as compared with animal group-2 (0.122 ± 0.03). 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.

0.04 Skeletal myoblasts transfected with liposoma 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-gf-g) gene transfer into skeletal muscle (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 (eGFP) and assessed by flow cytometry. After optimization, SkMs were transfected with CD lipoparticles carrying plasmid-VEGF-gf-g (CD-pVEGF-gf-g) and transplanted into rabbit ischemic limbs. Animals were randomized to receive intra muscular injection of either Medium (n=199; group-1), non-transfected SkM (group-2), CD-pVEGF-gf-g transfected SkM (group-3). Flow cytometry revealed that up to 16% rabbit SkMs were successfully transfected with gEFP and inserts. Dosing to the optimized transfection condition, transfected rabbit SkMs expressed VEGF-gf-g up to day-18 with peak at day-4. SkMs were observed in all cell-transplanted groups, as visualized with DAPI and BrdU. Angiographic blood vessel score revealed increased collateral vessel development in group-3 (39.7 ± 2.0; p<0.001) compared with group-2 (21.6; 1.1%). Immunostaining for CD31 showed significantly increased capillary density in group-3 (14.88 ± 0.9; p<0.001) compared with group-2 (6.5 ± 0.49) and group-1 (0.69 ± 0.3). Improved blood flow (m³/min/g) was achieved in animal group-3 (0.173 ± 0.04) as compared with animal group-2 (0.127 ± 0.016; p=0.047) and group-1 (0.062 ± 0.012; p<0.001). In conclusion, CD liposome mediated VEGF-gf-g gene transfer with SkMs effectively induced neovascularization in the ischemic hind limb and may serve as a safe and new therapeutic modality for the repair of acute ischemic limb disease.

0.03 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-blind, placebo-controlled, parallel group study in 50 non-diabetic patients with resistant hypertension. All patients at enrolment received ACE inhibitors or angiotensin receptor blocker drugs and diuretics together with a third drug. We additionally treated for six months with eplerenone 50 mg or tried 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 Mean±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) at 24-hour BP: 18±13/9±6 vs 13±8±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.

0.03 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 for men and women were 0.82 (0.62–1.11) and 0.77 (0.66–0.99); for those aged 80–85 or over 85, 0.76 (0.60–0.96) and 0.87 (0.64–1.20). The corresponding values for CV events were 0.69 (0.50–0.96), 0.65 (0.49–0.88), 0.63 (0.49–0.82) and 0.75 (0.51–1.20). Other subgroups are below. The interaction terms between active treatment and the various subgroups were not significant for total mortality (0.30–<1.00) or CV events (0.42–<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 treatment of very elderly hypertensives.

TABLE

<table>
<thead>
<tr>
<th>Total Mortality</th>
<th>CV events</th>
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<tr>
<td>History of CVD</td>
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<tr>
<td>No history of CVD</td>
<td>0.76 (0.48–1.20)</td>
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<tr>
<td>SBP 160–169 mmHg</td>
<td>0.89 (0.56–1.10)</td>
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<tr>
<td>SBP 170–179 mmHg</td>
<td>0.82 (0.61–1.06)</td>
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<td>SBP ≥ 180 mmHg</td>
<td>0.69 (0.45–1.04)</td>
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0.03 Subjects with premature cardiovascular disease have a diminished glyocalyx volume as compared to healthy controls

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Introduction: The inner surface of the vessel wall consists of a glycoprotein layer, which is called the glyocalyx. This surface protects the vessel wall 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 glyocalyx volume is still unknown. We therefore investigated glyocalyx volumes in subjects with premature CVD and compared this with healthy controls.

Hypothesis: Compared to controls, subjects with premature CVD have a diminished glyocalyx volume. Methods: We investigated 13 subjects with a premature CVD before the age of 40 and a positive family history for CVD, and 12 control subjects. We measured the systemic glyocalyx volume, the level of classic risk factors, pulse wave velocity (PWV) and IMT. Results: Subjects with premature CVD had a diminished glyocalyx volume as compared to controls (0.47 ± 0.26l vs 0.81 ± 0.39l, p<0.05). Further, they had increased clearance of Dextran-40 (0.4 ± 0.1 min⁻¹ vs 0.2 ± 0.1 min⁻¹, p<0.02), independent of creatinine clearance. Also, they had a higher PWV (10.8 ± 1.7m/s vs 9.6 ± 0.9m/s, p<0.04) and an increased IMT (596.9 ± 71.4 mm vs 543 ± 90.0 mm). Conclusion: Subjects with premature CVD have a diminished glyocalyx volume, in a loss of transfer function. This suggests that they are less protected against the influence of classic risk factors. When these findings are confirmed
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 evaluated 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±25/104±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, 0.044; 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=0.01), and increased LV fractional shortening (FS, untreated: 9.8±0.9; 20.5±1.3%); P<0.01) and cardiac output (CO; untreated: 18.8±1.5; 28.0±2.0 liter/min; P<0.05). In PTP1B−/− mice with CHF (n=13), LVEDD and LVESD were reduced (LVEDD: 5.2±0.2 cm; P<0.01; LVESD: 3.9±0.3 mm, P<0.01), while FS and CO were increased (FS: 22.7±2.5%: P<0.01; CO: 22.2±1.1 liter/min; P<0.05).

Vascular studies showed that chronic AS279 increased FMD in WT CHF mice (untreated 7±1; n=5; AS279 24±6%; n=7; P<0.01). Compared to WT, FMD was also increased in PTP1B−/− mice (16±6%; n=6; P<0.05).

Thus, chronic pharmacological inhibition or genetic disruption of PTP1B both restores endothelial function and improves cardiac dysfunction and remodeling, suggesting that this enzyme may be a new target for the treatment of CHF.

Adipose cannabinoid CB1 receptor expression and adiponectin correlate with microvascular damage in human kidney

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Background: Overactivity of the peripheral endocannabinoid system (ECS) is present in human visceral adipose tissue (VAT). Blockade of the cannabinoid CB1 receptor withrimonibastatin plasma level, with "protective" effect on the cardiovascular system. The aim of this study was to study the expression of CB1 receptor and adiponectin in human VAT in relation with renal microvascular damage.

Methods: Kidney and VAT samples were obtained from 35 consecutive non-diabetic patients (mean age 64.8±12.5 years) undergoing renal surgery for renal cell carcinoma (UT2T; N=40). CB1 and adiponectin gene expression was studied by RealTime Taq-Man assay and mRNA levels were normalized by 18SrRNA. Histological grading of microvascular and glomerular lesions, blinded to clinical and gene expression results, was carried over in the renal cortex at least 3 cm away from the tumour. Total and HMW adiponectin levels were measured by ELISA. Results: After correction for multiple variables, the group (n=11) with more advanced microvascular damage (characterized by the presence of arteriolar sclerosis) had a significant increase (P=0.041) of VAT CB1 receptor and a decrease in adiponectin expression levels (P=0.030). Moreover, the HMW adiponectin levels inversely correlated with increase glomerular sclerosis (r=-0.467, P=0.034).

Conclusion: VAT overexpression of CB1 receptors together with lower adiponectin expression and lower HMW adiponectin correlate with the presence of more advanced renal microvascular and glomerular damage. Dysregulation of VAT ECS may cause microvascular damage through lower adiponectin production in humans.

Pharmacological inhibition or genetic disruption of protein tyrosine phosphatase 1B attenuates both myocardial and endothelial dysfunction in mice with heart failure

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We have shown previously that acute, in vitro inhibition of protein tyrosine phosphatase 1B (PTP1B) restores endothelial function and especially flow-mediated, NO-dependent vasodilation (FMD) in peripheral resistance arteries with chronic heart failure (CHF). The present study evaluates the impact of chronic pharmacological inhibition or genetic disruption of PTP1B on cardiac and endothelial dysfunction in CHF mice.

Chf mice fed high salt (14508±824 mg/day) for 12 months. Echocardiographic analysis of left ventricular (LV) function and evaluation of FMD were performed.

In WT mice, echocardiography showed that AS279 decreased LV end-diastolic diameter (LVEDD: untreated: 6.1±0.2, n=13; AS279: 5.0±0.2 cm, n=8; P<0.05) and end-systolic diameters (LVESD, untreated: 5.5±0.2; AS279: 4.0±0.1 mm; P<0.01), and increased LV fractional shortening (FS, untreated: 9.8±0.9; AS279: 20.5±1.3%; P<0.01) and cardiac output (CO; untreated: 18.8±1.5; AS279: 28.0±2.0 liter/min; P<0.05). In PTP1B−/− mice with CHF (n=13), LVEDD and LVESD were reduced (LVEDD: 5.2±0.2 cm; P<0.01; LVESD: 3.9±0.3 mm; P<0.01), while FS and CO were increased (FS: 22.7±2.5%: P<0.01; CO: 22.2±1.1 liter/min; P<0.05).

Vascular studies showed that chronic AS279 increased FMD in WT CHF mice (untreated 7±1; n=5; AS279 24±6%; n=7; P<0.01). Compared to WT, FMD was also increased in PTP1B−/− mice (16±6%; n=6; P<0.05).

Thus, chronic pharmacological inhibition or genetic disruption of PTP1B both restores endothelial function and improves cardiac dysfunction and remodeling, suggesting that this enzyme may be a new target for the treatment of CHF.

High salt intake in pregnancy programs altered kidney morphology in the offspring

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The intrauterine environment may influence kidney development of the offspring. The present study was designed to explore whether dietary salt intake during pregnancy modulates kidney development in the offspring. Sprague-Dawley rats were fed low (0.15%), medium (1.3%), or high (8.0%) salt diet during pregnancy and weaning. The offspring were weaned at 4 weeks of age and maintained on the same diet. Kidney morphology was assessed at 1 and 7 weeks postnatal. Systolic blood pressure was controlled by intraaortic measurement at week 7. At the age of 1 week the number of S-shape bodies was significantly lower and the number of layers of developing glomeruli was higher in the high-salt offspring compared with medium- and low-salt. In parallel the expression of Pax-2, FG2, and VEGF was significantly lower and WT-1 higher in kidneys of high-salt offspring compared with medium- and low-salt. The final number of glomeruli at the age of 7 weeks was still significantly (p<0.001) lower in the offspring of mothers fed high salt (14508±3125) compared with medium (1885±6201) and low salt (1752±69). No difference between male and female offspring was observed. No difference between the groups in birth-weight was observed. At week 7 no significant
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 interlobar artery segments were dissected from non-cancerous regions of kidneys obtained from thirteen patients who underwent nephrectomy because of 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 (interlobar) than in proximal (interlobar) artery segments. mRNA expression levels of the isoenzymes NOX2 and NOX4 and the small subunits p22phox and p47phox where similar in interlobar, arcuate and interlobar arteries. Phosphorylase (PE) and endothelin-1 (ET-1) induced similar maximum tension in arcuate and interlobar arteries with a p20 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 iron. 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 NOX-dependent activity. Superoxide formation contributes to endothelium-dependent vasodilation in distal intrarenal arteries of human kidneys.

W3.04

**Angiotensin-(1–7) improves renal endothelial dysfunction by influencing 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 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+ CD68+ 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+ CD68+ leukocytes in the vessel wall.

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 N2-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+ CD68+ 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+ CD68+ leukocytes in the vessel wall.
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 vivo 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 adipose and kidney tissue were obtained 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 (-175GA and -163GA) in strong linkage disequilibrium (LD = 0.90) 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 (-6AG; -217GA) 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 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 non-essential RNA induced its tyrosine phosphorylation in a Src dependent manner. Furthermore, NA induced an interaction of Hic-5 with paxillin rich tyrosine kinase (PKY2) but not Src or p125focal adhesion kinase. This interaction was Src dependent suggesting that Hic-5 was a substrate for PKY2 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 p38MAPK activation. Inhibition of either p38MAPK or Src inhibited the 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 serinprotease inhibitor) in epicardial fat during the development of obesity-mediated cardiac hypertrophy and the effect of vaspin on cardiac fibroblasts. In male C57Bl/Jm mice 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 6.4+/-0.66g/g to 6.88+/-0.61g/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 fibroblast proliferation by BrdU-assay which shows a potent induction by vaspin (1.8-fold, vaspin 1ug/g, p<0.05 vs. 0.5%FBS, and 2.0-fold 0.1ug/g vaspin p<0.005 vs. 0.5%FBS) compared to a 1.9-fold induction by angiotensin II (10uM).

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 PP aggregator-dependent gene transcription in vascular smooth muscle cells

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The study aimed to identify new nuclear cofactors for PP aggregator (peroxisome proliferator-activator receptor gamma)-dependent gene transcription in human aortic smooth muscle cells (HASMC) in order to develop new PP aggregator-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 A1 protein (HMGA1) protein expressed in unstimulated HASMC.

PP Aggregator-dependent gene regulation was studied by analysis of PMA-induced Mmp-9 (matrix metalloproteinase 9) expression ± pioglitazone (pio 10uM). PMA (50ng/ml) stimulated Mmp-9 mRNA expression by 46.3+/-22.3-fold (p<0.05 vs. vehicle) which was markedly blocked by pio (10uM). HMGA1 mRNA was 17.4+/-4.8-fold upregulated by PMA alone (p<0.05). PMA induced HMGA1-pioglitazone promoter activity by 45% in transactivation assays in Hek293 using a p3.3L-Mmp-9 construct.

To evaluate the role of HMGA1, gene-silencing experiments with siRNA for HMGA1 were performed (0.1% in HASMC and 80.2% in Hek293 reduction of HMGA1 protein expression). HMGA1 siRNA completely abolished PP Aggregator-mediated Mmp9-mRNA repression (control siRNA: pio-mediated Mmp-9 regulation vs. PMA: -66.8 % in HASMC and -59.3% in Hek293 p<0.01; HMGA1 siRNA; pio-mediated Mmp-9 regulation vs. PMA: -10.7 % in HASMC and +14.7% in Hek293 vs. PMA p=n.s.).

Using ChIP assay we could demonstrate that pioglitazone-induced PP Aggregator activation leads to a potent recruitment of PP Aggregator (3.0 fold vs.1.5 fold PMA) and HMGA1 complexes (1.24 fold vs. 0.0 fold PMA) to the Mmp9 promoter in HASMC.

In conclusion, HMGA1 is required for PP Aggregator-mediated repression of Mmp-9 gene transcription. Ligand-induced HMGA1-PP Aggregator 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 assess the involvement of the p190A Rho GTPase-activating-protein (GAP) in this process. Small interfering RNA (siRNA-mediated) p190A silencing in VSMC increased basal RhoA activity (9.33±0.4% of control, n=3) and abolished its activation by 5 min of Ang II stimulation (26±5% of control, n=4). We then measured p190A tyrosine phosphorylation known to reflect its activity. In resting VSMC, p190A was basally phosphorylated. Activation of AT1R induced p190A dephosphorylation that was maximal at 5 min of Ang II stimulation (26±5% of control, n=4). Using siRNA, we have shown that the tyrosine phosphatase SHP2 was necessary to maintain p190A phosphorylation by Abi kinase. Using SHP2- mutants we have demonstrated that SHP2 activity was necessary for Ang II-mediated p190A dephosphorylation and RhoA activation. Our work demonstrates that the tight regulation of p190A-activity by SHP2 is required to maintain a RhoA activity low, as well as to induce RhoA activation by Ang II in VSMC.
ECRR Poster Presentations

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 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 transmisral 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 < 0.0075). 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 = 0.015) after adjustment for age, gender, type of infarction (STEMI vs. Non-STEMI), history of hypertension and diabetes. Conclusions: The study suggest the existence of an independent association between CFVR and LV filling pressure in patients with AMI. The pathophysiological 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 reflectance spectroscopy (ORS) and near infrared spectroscopy (NIRS). Mean blood saturation (SmbO2) and tissue oxygen index (TbO2) 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 an increase 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 guarantee adequate microcirculatory function or effective tissue oxygenation. Aim: To examine the correlation between two traditional macrocirculatory resuscitation targets (cardiac index (CI), Oxygen delivery (DO2), central venous pressure (CVP) and mean arterial pressure (MAP)) and downstream markers of microcirculatory 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 (DeltaL), 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.

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 macula) thickness in individuals without diabetes or overt cardiovascular disease. Fovea thickness was determined in the right eye of 28 subjects (age range 26–74 years, 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. Systolic 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 skin 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 dependent (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 (∆D) were evaluated on each image sequence. Variabilities were presented as the coefficients of variation. Results: The intraobserver inassassiation variability was 7%–6% for IMT,
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 endothelium dependent vasodilatation significantly when compared to placebo (mean: SEM: 93: 8% Vs 145± 11%, p<.001). FMD (mean: SD: 4.1± 1.8%, Rs: 5.4± 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 atherosclerotic events in coronary artery disease over and above their current therapies.

Glomerular 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 dilatation (FMD), the carotid intima-media thickness (CIMT) and the carotid arterial distension (COST). 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 FFMD has slope 0.99 and intercept 0.001. 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 0.36% 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 CDIST.

PA.07

PA.08

PA.09

PA.10

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 ultrasonic 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 ultrasonography 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 naive state to Atorvastatin 10mg once daily (1.1% to 4.06%, Z = 2.803, p<0.001). 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.

PA.11

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, micro-albuminuria, and lipids were also measured. Mean age was 28 ± 2.54 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 metabolite production.

PA.12

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
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 flow 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 myograph 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 kept at 35 mmHg. W/L ratio significantly increased in WKY cultured at 60 mmHg from day 0 to day 3 (day 0: 0.06±0.007; day 3: 0.07±0.002; p<0.05), but not in WKY cultured at 35 mmHg and SHR. Conclusion: Increase in wall stress per se induces small arteries remodelling, at least in WKY kept at 60 mmHg, possibly due to sustained increase in myogenic tone.

PB.02

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 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 hypercontractility in HF arteries without affecting diameter enlargement, reduced hypercontractility and improved endothelium-dependent dilation. Superoxide 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.04

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 enhancing NO/cGMP signalling on aortic remodelling in a model of experimental hypertensive nephropathy. 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 ultrasound 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 nm, 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 <25.5 nm). 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

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 ultrasound 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 nm, 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 <25.5 nm). 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.

PA.13

Pressure-induced remodelling in a blood pressure-independent manner.

This study indicates that gender regulates hypertensive-uremic aortic wall changes and that enhancing NO/cGMP signalling by Bay 41-4134 significantly ameliorates aortic remodelling in a blood pressure-independent manner.

PB.03

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 weeks old were included in the present study. First order mesenteric arteries were isolated and mounted in a culture myograph 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 (Ctr: 0.08768±0.00253; Ang: 0.08799±0.00763; p=NS) and in stress/strain 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.
Thus, in resistance arteries hypertrophy associated with a chronic rise in blood flow depended on angiotension II production and ERK1/2 activation. These findings might be of importance in the treatment of ischemic diseases and hypertension.

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 (LF) 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 LF 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 (p47phox and p67phox) expression as well as a rise in superoxide production in LF and ZDF rats. Acetylcholine-induced dilation in NF and HF arteries from ZDF rats was improved by an acute antioxidant (tempol). In ZDF rats superoxide production in LF and ZDF rats. Acetylcholine-induced dilation in NF and HF arteries from ZDF rats was improved by an acute antioxidant (tempol). In ZDF rats superoxide production in LF and ZDF rats. Acetylcholine-induced dilation in NF and HF arteries from ZDF rats was improved by an acute antioxidant (tempol). In ZDF rats superoxide production in LF and ZDF rats. Acetylcholine-induced dilation in NF and HF arteries from ZDF rats was improved by an acute antioxidant (tempol). In ZDF rats superoxide production in LF and ZDF rats. Acetylcholine-induced dilation in NF and HF arteries from ZDF rats was improved by an acute antioxidant (tempol). In ZDF rats superoxide production in LF and ZDF rats. Acetylcholine-induced dilation in NF and HF arteries from ZDF rats was improved by an acute antioxidant (tempol). In ZDF rats superoxide production in LF and ZDF rats. Acetylcholine-induced dilation in NF and HF arteries from ZDF rats was improved by an acute antioxidant (tempol). In ZDF rats superoxide production in LF and ZDF rats. Acetylcholine-induced dilation in NF and HF arteries from ZDF rats was improved by an acute antioxidant (tempol). In ZDF rats superoxide production in LF and ZDF rats. Acetylcholine-induced dilation in NF and HF arteries from ZDF rats was improved by an acute antioxidant (tempol). In ZDF rats superoxide production in LF and ZDF rats. Acetylcholine-induced dilation in NF and HF arteries from ZDF rats was improved by an acute antioxidant (tempol). In ZDF rats superoxide production in LF and ZDF rats. Acetylcholine-induced dilation in NF and HF arteries from ZDF rats was improved by an acute antioxidant (tempol). In ZDF rats superoxide production in LF and ZDF rats. Acetylcholine-induced dilation in NF and HF arteries from ZDF rats was improved by an acute antioxidant (tempol).

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 (0.15%, LS), medium (1.3%, 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 as compared to LS and MS animals. In LS animals, 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 found in smooth muscle cells (VSMC). Rat aorta VSMC were stimulated with vehicle or CT-1 for 18 to 48 hours, without and with antibodies against CT-1 receptors. In addition, the effects of aldosterone (10-8 M) and angiotensin II (10-10 M) on CT-1 expression were also evaluated. Cell proliferation was assayed 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). CT-1 treatment led to an increased expression of collagen type I (p<0.01) and fibronectin (p<0.05), with a parallel 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 spontaneously 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. Upregulation of CT-1 expression by angiotensin II and aldosterone in VSMC suggests a mediator role for this cytokine inalterations 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 NO-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), phosphosensitive (S188A), phosphomimetic (S188E) and double-mutants Q63L-S188A and Q63L-188E could regulate VSMC migration, we used a scratch-wound repair assay in VSMC monolayers. Expression of phosphosensitive mutants reduced the wound-closure, while, in contrast, phosphomimetic mutants greatly accelerated it. The NO donor sodium nitroprusside (100 μM) or 8pCPT-cGMP (100 μM) accelerated the wound-closure in VSMC expressing the WT RhoA but not in VSMC expressing the phosphosensitive mutant S188A. 8pCPT-cGMP induces Rac1 localisation at the plasma membrane and phosphorylation of the Rac1 effector PAK 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 NO 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 isoform of Na+ pump regulates vascular gap junctions via interaction with the Na+/Ca2+/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 isoform of the Na+-pump and the Na+/Ca2+/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+/Ca2+ exchanger with 1 μM SEAO400 also uncoupled the SMCs. Depletion of [Na+]i and clamping [Ca2+]i at low levels prevented the uncoupling. 10 μM ouabain evoked spatially restricted [Ca2+]i 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+/Ca2+ exchanger and connexin-43. The α2 Na+-pump subunit was not associated with these proteins but co-immunoprecipitated with connexin-43. Based on these experiments we suggest that α2 Na+-pump subunit is involved in regulation of the intercellular communication via interaction with the Na+/Ca2+/Ca2+ exchanger-1 leading to local [Ca2+]i 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) pathway. Rat cultured SMCs between passages 2 and 18 or platelet microvesicles (PMV) were incubated with recalcified human platelet-free citrated plasma and recombinant human tissue factor at 0.25 µM. Thrombin formation and its deactivation by APC were assessed by the endogenous thrombin potential without APC (ETP0) and APC concentration reducing ETP0 by 50% (IC50-APC) using thrombography. Procoagulant phospholipids on SMCs and PMV were quantified by phospholipid-related procoagulant activity (phosphatidylinerine 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 2516 ± 484 nM phosphatidylinerine 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 ETP0. Similar ETP0 were observed with SMCs and PMV whereas IC50-APC values were higher with SMCs. In conclusion, SMCs provide a membrane binding sites on which all of the plasma-derived procoagulant and anticoagulant complexes can be assembled. However, inhibition of thrombin by APC was less efficient on SMCs than on PMV. Thus, SMCs may act as additional contributors to thrombin formation and represent potential targets for new antithrombotic developments.

Cyclic 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 SMCs differentiation in response to cyclic mechanical stretch. Rat SMCs were plated on silicone elastomer–bottomed culture plates precoated with collagen type I (Falcon), and subjected to cyclic stretch with a Cyclic Stress Unit (FX4000 AFC-CTL, Falcon). Deformation (1 Hz) and 10% elongation were applied from 1 to 5 days. At day 2 cyclic stretch induced a significant increase in contractile differentiation markers: 1.8 fold for SM-myosin heavy chain, 1.5 fold for SM-α-actin and a 1.8 fold for heavy-caldesmon, which was maintain up to day 5. Under basal conditions SMCs express β1, β3, αv, α5 and αv integrins at the protein level. There was a significant time-dependent increase for β1, β3, α5 and αv integrin but not for α1, with a maximum at day 5. Augmentation of integrins’ expression was accompanied by increased phosphorylation of Fak-Tyr 576/577 and increased immunostaining of focal contact determined by vinculin labeling. In parallel, there was a significant increase of metalloproteinase 2 and 9 activity.

Our study demonstrates that stretch-dependent SMC differentiation involved overexpression and activation of integrins β1, β3, β1, αv and αv organized in focal contacts, coupled with the activation of MMP2 and 9.

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 progression 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 aimed to investigate the effects of Telmisartan on the release of MMP-2 and MMP-9 by aortic vascular smooth muscle cells (VSMCs). VSMCs were cultured from the abdominal aorta of Wistar rats. The cells were stimulated for 48h with Interleukin-1α (IL-1α, 10 ng/mL). Simultaneously, the IL-1α stimulation was performed together with Telmisartan (100 µM, 10 µM, 1 µM). Expression of MMP-2 and MMP-9 was analyzed by quantitative real-time RT-PCR and gelatin zymography. IL-1α stimulation increased MMP-2 expression (1.6 fold vs. control) and induced MMP-9 expression. Telmisartan (10 µM) significantly reduced (43 %) the cytokine-induced MMP-9 release. Moreover, mRNA analysis revealed that Telmisartan (10 µM) reduced the MMP-9 mRNA expression (49 %). MMP-2 was regulated only at a high concentration (100 µM) of Telmisartan. Taken together, these data demonstrate that the AT1 receptors are involved in the regulation of MMP-9. Furthermore, the increased release of MMP-9 by aortic VSMCs could be prevented by the AT1 receptor antagonist Telmisartan, thus, contributing to vascular protection.

Tissue Engineering and simulated microgravity: A new technology for development of blood vessels
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Tissue engineering might help to deliver surgeons with tiny blood vessels, which are needed in hand, plastic and reconstructive surgery. Culturing endothelial cells (EC) on a random positioning machine (RPM) has the advantage that growing vessels are floating in a fluid. Subconfluent monolayers of EC that are exposed to microgravity simulated by a RPM form structures resemble the intima of blood vessels. After two days of exposure, some EC start to loose contact from the culture dish surface with contact to surrounding cells and start to form rod-shaped aggregates. On the fifth day, precursors of the intima-like structures become visible, elongate and form thin threads when cultured under microgravity for another two week. The walls of these structures consist of EC that are attached to each other to form a cylinder that contains laminin, fibronectin, osteopontin, collagen type VIII and F-actin filaments. The luminal surface of the walls is smooth, whereas the outer surface is rough and often binds single or aggregated EC. Our observations suggest that culturing EC on an RPM is a way to accelerate the process and to generalize intima-like structures, which will be useful in blood vessel engineering and basic research.

Leptin interacts with RAS: A new mechanism for hypertension in obesity?
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Leptin is a hormone with effects on energy balance. It also influences blood pressure (BP), but the involved mechanisms aren’t yet elucidated. We hypothesized that leptin may be a link between hypertension and obesity acting through the renin-angiotensin system (RAS). Ob/ob mice, a model lacking leptin, are described as obese and diabetic, characteristics of the metabolic syndrome, which is normally associated with increased BP. However, intra-arterial as well as telemetric measurement showed that these animals are normotensive (ob/ob = 112 ± 2 mmHg, n=8; WT = 117 ± 2 mmHg, n=8). Using telemetry we studied the long-term effects of leptin (minipumps - 50µg/leptin/day for one month) on BP in ob/ob and wild-type mice. BP did not change after leptin infusion in both strains, but the heart rate (HR) increased in ob/ob mice (ob/ob = 538 ± 12 to 624 ± 29 bpm; WT = 574 ± 18 to 563 ± 12 bpm; p<0.05). Additional treatment with the ACE inhibitor, captopril, for one week elicited a more pronounced decrease in BP (but not HR) in ob/ob mice than in WT (MMP: ob/ob = 79 ± 2 mmHg; WT = 93 ± 1 mmHg; p<0.01). When captopril was given without leptin, BP and HR changed equally in both groups. The low-frequency variability of the HR increased in WT and ob/ob mice under leptin treatment. This effect was blocked by captopril. Based on these data, we suggest that leptin interacts with the RAS and thereby influences the autonomic nervous system and BP regulation.

In vitro and in vivo induction of adhesion molecules and leucocyte recruitment: interaction between high glucose and inflammation
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The study was to analyze the effect of high glucose and IL-1β, either alone or in combination, on adhesion molecules induction and leucocyte recruitment in endothelial cells. Cultured
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 in 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 was measured by an echocardiographic system. Thrombin formation and decay were assessed using plasma recalcification 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 glyceremia were increased in fa/fa rats. Carotid arterial diameter and arterial thickness were not significantly different between both groups. Elastin 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 nM/mn). This occurred independently of platelet activation, was associated with an increase in fibrinogen (4.9±0.2 vs 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 causative role of thrombin generation in delayed cell-wall modifications and thrombosis in the metabolic syndrome.
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 endothelium-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).

Methods: Brachial artery FMD was performed on 40 IGT patients and 24 controls using real time ultrasound analysis (VIA). Velocity waveforms during the first 15 seconds of reactive hyperaemia were acquired 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 (dyne/cm²) was also significantly reduced (IGT mean 23.1±18.52 control mean 32.58 (+/- 15.83), p=0.04). 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). Resitive 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 stimuli and impaired FMD.

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 would have altered sympathovagal function in comparison to Europeans. 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 profiling and sympathovagal balance assessment, using heart rate variability (HRV) and baroreceptor sensitivity (BRS), was performed. Results: Indian Asians had shorter mean RR intervals than Europeans (970 ± 148 vs 1021 ± 148 ms, p<0.004), and attenuated total, low and high frequency components of RR intervals (p=0.016, 0.004 and 0.029 respectively). These HRV markers were inversely related to measures of dysglycaemia (Mean RR interval v HbA1c beta coefficient = -0.220, p<0.001). The ethnic difference in mean RR interval persisted after adjustment for age, systolic BP and medications, but would also have altered sympathovagal function and impaired baroreceptor sensitivity.

Conclusion: Indian Asians have lower mean RR intervals than Europeans and adverse HRV and BRS measures explained by dysglycaemia. Poorer sympathovagal balance and impaired baroreceptor function in Indian Asians may contribute to their increased CVD risk.

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. 18 healthy subjects were selected as controls (C). The study groups were matched for age and sex. The MS patients were divided into two groups: 18 patients with type-2 diabetes mellitus (without insulin therapy) and 18 patients without DM. 18 healthy subjects were selected as controls (C). The study groups were matched for age and sex.

Methods: DSS, FMD and forearm microcirculatory haemodynamics in impaired glucose tolerance (IGT). We performed on 40 IGT patients and 24 controls using real time ultrasound analysis (VIA). Velocity waveforms during the first 15 seconds of reactive hyperaemia were acquired 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 (dyne/cm²) was also significantly reduced (IGT mean 23.1±18.52 control mean 32.58 (+/- 15.83), p=0.04). 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). Resitive 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 stimuli and impaired FMD.

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 vasomotoricity. MPs obtained from whole blood either from patients (MSMPs) or healthy subjects (HSMPs) or vehicle were injected i.v. to mice. MSMPs injection induced vascular hypo-reactivity to serotonin (5HT) in aorta compared to vehicle or HSMPs. Besides MSMP treatment was associated with an increase of NO production accompanied with enhanced expression of iNOS-synthese. Interestingly, the NO-synthases inhibitor completely reversed the hypo-reactivity induced by MSMPs. Also, MSMPs induced ROS production via enhanced expression of the NADPH oxidase subunits, gp91phox and p47phox. The non selective COX inhibitor significantly reduced contraction to 5HT in aortas taken from the three groups of mice. Interestingly, the selective COX-2 inhibitor reduced contraction to 5HT in vessels from vehicle- and HSMP- but not from MSMP-treated mice. These results are in favour of hypothesis that the equilibrium of the COX metabolite release is shifted toward the increase of vasodilator substance. Indeed, MSMPs increased prostacyclin production in aortas. Importantly, pre-incubation of MSMPs with anti-Fas-L, antibody, before being injected to mice, completely prevented the vascular hypo-reactivity suggesting the involvement of Fas/Fasl pathway. We provide evidence that MSMPs induce in vivo vascular hypo-reactivity in aortas by increasing both oxidative and nitrosative stresses and by altering the release of COX metabolites. We underscore a critical role of MPs as a vector of biological message leading to vascular dysfunction in MS triggered by Fas/Fasl pathway.

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=31), and their matched controls (n=151), using 600 MHz 1H NMR spectroscopy. Following baseline and phase correction, unsupervised and supervised chemometric techniques (principal components and orthogonal partial least squares discriminant 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 vs. controls, and treated hypertensives vs. controls, obtained
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 from its 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 = 6,632) and whether this was associated with the beneficial effects of E on myocardial infarction outcomes. Methods: A diuretic effect was indirectly defined as a mean 1 month vs baseline body weight decrease ≥ 1% median change in the P group (-0.05 kg), AND a 1 month vs baseline blood protein increase ≥ median change in the P group (+ 4 g/l). A potassium (K) sparing effect was defined as a serum K increase ≥ median change in the P group (+ 0.11 mmol/l). Results: In the E group, body weight decreased (-0.0001), whereas blood protein (p < 0.001) and serum K increased (p < 0.001) as compared to P. K-sparing was independently associated with lower all-cause mortality (HR 0.83(0.71–0.96)); p = 0.015) as well as lower CV death or CV hospitalization (0.76 (0.67–0.87); p = 0.0001). A diuretic effect (1.151.02–1.30–0.023), 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 dysfunction, predicts stroke severity and post-myocardial infarction mortality. We explore 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 & 30 and blood pressure at presentation, day 3, 5 & 7, were recorded. Results could not be obtained within 72 hours for 4 patients and 4 patients subsequently had non-stroke diagnoses. Mean age was 66 years (± 17). Age was inversely associated with BI at day 0, 7 & 30 (p = 0.007, 0.01 & 0.003 respectively). ACR was inversely associated with age-adjusted BI such that those with higher ACR had poorer function (β regression co-efficient SE –1.65 ± 0.61, p = 0.01; -2.0 ± 0.8; p = 0.02; -2.1 ± 0.8, p = 0.02 for day 0, 7 & 30 respectively). There were no associations between blood pressure at any time and BI. In multivariate analysis, ACR was the only independent predictor (p after adjustment = 0.015). Nine of the 36 patients died in the first 30 days. ACR was higher in those that died compared to survivors (13.8(0.05) CI, 5.8–32.8) vs. 2.8(1.5–5.2) μg/mmol; p = 0.004), independent of other risk factors. In multivariate analysis ACR was the only independent predictor of mortality (p = 0.008).
Conclusions: Presentation ACR, as a proxy of microvascular function, predicts mortality and functional recovery after stroke. Further work should be performed to determine mechanisms of changes ACR and if early pharmacological intervention could improve rehabilitation outcomes.

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 capsulary accessory veins in 11% and 45%, respectively. On the left side capsulary 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 ± 2.28, p = 0.005), 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 if selective right adrenal vein catheterization. A 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 NIN 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. Conclusion: 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. In the E group haemodynamic monitoring was being used during monitoring. In the S group blood pressure was being used. 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. Conclusion: 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.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 (Tennine, 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 was reflected impaired venous return. In venular OH, a reduction in CO after orthostatic stress often despite marked tachycardia, suggests that the predominant defect is an excessive reduction in venous return. Mixed OH is due to a combination of both these mechanisms. Results: Significant differences between the groups for the magnitude and time to reach the nadir of systolic blood pressure post-head-up tilt. The mixed OH category had the largest systolic blood pressure reduction (42.5, 31.9, 53.3 mmHg, P < 0.001) and took the longest time to reach nadir (18.6, 20, 30.7 s, P = 0.002). Conclusion: This is a practical classification tool and when validated physiologically, this system could be useful in directing treatment of OH.

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 clinic 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 promoted 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.

Gender differences in the cross-sectional relationships between sleep duration, interleukin 6 and high sensitive C-reactive protein the Whitelaw II 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, among women 80 years and over (interaction p = 0.04) there was a trend for IL-6 to be lower in women who slept 8 hours (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, there was a significant non-linear U-shaped association in women, the level of hs-CRP being significantly lower in women who slept 8 hours (11% [95% CI 4 to 17]) as compared to 7hrs. Conclusion: There was a significant non-linear U-shaped association in women, the level of hs-CRP was 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, there was a significant non-linear U-shaped association in women, the level of hs-CRP being significantly lower in women who slept 8 hours (11% [95% CI 4 to 17]) as compared to 7hrs. 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 endotoxin levels: a novel marker of atherosclerotic and cardiovascular risk in a British multi-ethnic population

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Background: Circulating endotoxin 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 endotoxin levels, its soluble receptor (sCD14), 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 endotoxin 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 = 0.07 (men) and p = 0.008 (women)). Endotoxin levels were positively associated with waist, waist-hip-ratio, total cholesterol, serum triglycerides and serum insulin levels and negatively associated with serum HDL-cholesterol. Serum hs-CRP and plasma sCD14 varied by ethnic group (p < 0.001) but was not associated with endotoxin. Conclusions: This study is the first to demonstrate a significant trend for increasing endotoxin 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 endotoxin levels are causally related to the development of CHD.

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 Asian origin 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 sCD4. Conclusions: Higher IgG Ab to Ox-LDL are associated with higher levels of sCD14-1 and are elevated in female South Asian individuals who have an increased risk of atherosclerosis compared to whites.

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 pleridin 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, 2009 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), Non ST elevation myocardial infarction (NSTMI) and ST elevation myocardial infarction (STEMI). 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 [HYVET]

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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 HYVET resulted in a reduction in CV events and total mortality. We have assessed whether depression at baseline influenced mortality or CV morbidity. Method: HYVET 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 (SIr) 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 85.5 years, 60.6% female and mean SBP 173mmHg. Hazard rates (HR) with 95% confidence intervals adjusted for age, gender, treatment allocation, country area, educational level, living alone, number of co-morbidities, previous CV disease, previous treatment and previously diagnosed hypertension for total mortality was 1.07(1.04–1.10p<0.001) for 1 unit increase in GDS. For CV mortality and CV events were 1.09(1.05–1.13p<0.001) and 1.07(1.04–1.10p<0.001) respectively Discussion: These 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 antidepressive therapies 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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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, n=451; women 49.5%; median (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.

PE.01
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 effects. 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 (HS) (NaCl=250mmol/d) for 24h. Results: PK: LS diet significantly increased the Cmax, and the AUC0–24 for Candesartan (+41% and +45 %) and Atorvastatin (+38% and +26%). LS diet increased significantly Ut of Atorvastatin (+30%). The increase in PRC after Candesartan, Valsartan or Ramipril was significantly larger with the LS diet. Using PK-PD modeling, we estimated that ~30% of the changes in PRC with Candesartan on the LS diet was due to the increase in drug exposure. The Atorvastatin induced-increase in PRC 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 Atorvastatin plasma concentration affecting their PD profiles, but does not affect Valsartan and Ramipril PK. Increased Ut of Atorvastatin 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 sympatho-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 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 %, 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 the 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/less 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<0.05), 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<0.01). 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 homeostatic 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 homeostatic training (HT) in a randomized, placebo-controlled pilot study. Methods: 22 subjects, aged 18 to 85 years, with recurrent WSM 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 enrollment, week 1, week 4 and week 24. Syncope recurrence 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). Conclusion: Our pilot study demonstrated promising trends in symptom benefit and significant improvements in BRS with HOT in patients with VVS.

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 (normotensives) 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-five consecutive patients with echocardiographic LHV were randomly assigned to either active treatment arm (extra antihypertensive medica-

ion) or placebo in a ratio of 2:1. Cardiac magnetic resonance imaging (CMRI) was used to establish changes in left ventricular mass index (LVMi) 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±8mmHg in the placebo group (p = 0.646). The mean baseline CMRI LVMi was 59.16±7m² in the placebo group and 65.89±8m² in the active group (p = 0.114). The mean difference between baseline and end of study blood pressure was −9.33±9mHg in the active group and −5.89±7mHg 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 LVMi was −4.68±7m² in the active group and −1.97±9m² 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. BP may translate into a reduction in cardiovascular events in those without normal LHV.

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

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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 A4C, A2C and APLAX 2D images. GLS and its reliability 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 mm²; SD = 12.08) was significantly higher relative to Group N (M = 91.85 mm²; SD = 9.19) [F(1,45) = -19.27, p < 0.01]. 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)[F(1,45) = 15.48, p < 0.001]. A linear regression analysis revealed that increased LMVI was a highly significant predictor of reduced GLS (R² = 0.966; p < 0.001, R² = 0.061; R² = 0.28,31). 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.

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 geometry after percutaneous coronary intervention and vascular geometry after percutaneous coronary intervention was also analyzed 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 at baseline and post-PCI. We investigated if the increased time required calculating the VTI is justified as an alternative. We investigated if the increased time required calculating the VTI is justified as an alternative. We investigated if the increased time required calculating the VTI is justified as an alternative. We investigated if the increased time required calculating the VTI is justified as an alternative.

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 its inherent averaging by the use of the complete flow profile. Method: 1000 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 calibrated 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%; p<0.001). VTI-based Dimensionless Index variability was 4.1 times greater than peak-based measurement (%: p<0.01). The average time taken for VTI was 5.3 times greater than peak maximal velocities (23.7±3.5 s; 4.5±1.2 s, p<0.001).

Conclusions: The measurement of velocity-time integral is a markedly more variable and more time consuming
**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 (atrioventricular) 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 = 0.74, p < 0.0001). However, after a few seconds, MAP gradually falls (0.65mmHg/beat, r = 0.98, p < 0.0001), whilst Qecho remains elevated (r = 0.046, p = NS). The signal-to-noise ratio was significantly better for measurements of MAP than Qecho (6.3 ± 3.6 vs 2.1 ± 1.4, p < 0.0001). **Conclusions:** During optimisation 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.

**PE.11 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 73 ± 11y, EF 29% ± 12%) with biventricular pacemakers. These fluctuations were made either as a step "square wave" or more gradually, "sine wave". **Results:** Each manipulation successfully generated oscillations in et-CO2, which were sinusoidal even when the cardiac output manipulation was square wave (better fit to sinusoidal profile r² = 0.77, than to square wave profile, r² = 0.22, p < 0.001). Peak-to-trough BP swing was almost twice as large with square wave than a sine wave (22.4 ± 11.7 mmHg versus 13.6 ± 4.45 mmHg, p < 0.01). The fastest 5-second slope was 2.5 times steeper with square wave oscillation than sine wave (19.8 ± 10.0mmHg) versus 7.6 ± 3.2 mmHg, p < 0.01). **Conclusion:** 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.

**PE.12 Long-term changes of left ventricular (LV) structure and function after adrenalectomy or medical treatment for primary aldosteronism**

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**Background:** As the long-term effects of adrenalectomy or medical treatment for primary aldosteronism (PA), the most common curable endocrine cause of hypertension, on the left ventricle (LV) are unknown, we evaluated in PA patients. **Methods:** After a baseline assessment of LV wall thickness and dimensions and transluminal LV filling flow velocity indexes, 55 PA patients were prospectively followed-up with echocardiography for 6 years (range 4.5 to 9 years). We excluded adrenalectomy (n = 41) or medical treatment (n = 14). Aldosterone producing adenoma (APA) and idiopathic hyperaldosteronism (IHA) were diagnosed based on adrenal vein sampling and the PAPY study criteria (JACC 2006). **Results:** 47 patients had APA and 8 IHA. At baseline both groups showed excess LV hypertrophy (LHV) and concentric remodeling: altered LV diastolic filling indexes were seen only in the medically-treated patients. At follow-up both adrenalectomy and medical treatment lowered blood pressure (by 34 ± 5/20 ± 5 mmHg, p = 0.001), LV end-diastolic diameters and LV mass index (LVMi) from 115 ± 22 to 106 ± 18 g/m², p = 0.02, in adrenalectomized patients and from 118 ± 26 to 103 ± 21, NS, in medically-treated patients. The altered LV diastolic filling indexes normalized in the medically-treated patients. **Conclusion:** 1) Both treatments reduce BP, LV mass index and the prevalence of LHV; 2) With a similar fall of blood pressure and despite a greater reduction of antihypertensive drugs, these changes were more prominent in adrenalectomized patients; 3) An improvement of LV diastolic filling occurs also in the medically-treated PA patients when it is altered at baseline.

**PE.13 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 (VCEF) 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.

**PE.14 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 precardial 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-supine bicycle exercise in stress echo lab. Patients were 71±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±143 msec to 250±59msec, 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 (0.98±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.
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Gain of expression mutations in WNK1 cause Gordon’s Syndrome, a rare disorder characterised by hypertension and hyperkalemia. We have previously reported association between WNK1 and BP in the BRIGHT Study and 24-hour ambulatory BP in the GENIUS study. The associated SNPs map to the promoter and regulatory regions in intron 1, suggesting that changes in WNK1 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 (rs14683236 C/A) and one in intron 1 (rs765250 A/G). Carriers of allele A for rs14683236 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 pG3 and transfected into HEK293 cells, followed by luciferase assay. Reporter assays demonstrated that rs1468326C had lower activity than rs1468326A, with 3.36 fold decrease in luciferase activity 95%CI (-3.93,-2.80), p = 3.6 x 10^{-10}. The intrinsic SNP rs765250A showed a 1.50 fold increase compared to rs765250G, 95%CI (1.10,1.90), p = 1.4 x 10^{-10}. 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 WNK1 expression. These new data lend further support to the hypothesis that variation in WNK1 expression contributes to BP and EH.

\section*{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 degradation of platelet-activating factor (PAF) and phospholipids associated with LDL and in production of lysophosphatidylcholine (lysoPC) and oxidized non-esterified fatty acids (NEFA).

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 PLAC2G1 gene functional single nucleotide polymorphisms (SNPs): Thr198ile (exon 7), His92Arg (exon 4) and Ala379Val (exon 9).

Results: Although plasma Lp-PLA2 levels were associated with BMI (P = 0.042) and PF.05, and PF.02, 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 York, 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.

\section*{Systematic analysis of 123 candidate genes reveals two novel hypertension genes}

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Discovering the genes causing hypertension is proving a challenging task. Recently, we performed a genome-wide 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 5x10^{-7}. We are currently performing further GWAS and meta-analyses, 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. 1536 SNPs were selected; these were genotyped using the Illumina Goldgene array (Illumina) in 1700 hypertensive cases and 1700 normotensive controls. We are currently performing further GWAS and meta-analyses of these SNPs, and will use the results to refine our model for hypertension risk.

\section*{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 LRPL6 gene mutation causes a monogenic form of hypertension, hypercholesterolemia and early coronary artery disease because of reduced Wnt/β-catenin signalling. We investigated whether a common Val1062 LRPL6 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 >0.5 mm) at the level of common, bifurcation and/or internal carotid arteries. Logistic regression models were used to estimate the independent effect of Val1062 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 Val1062 LRPL6 variant was a strong risk factor for CAA in both unadjusted (OR 2.13, 95%CI 1.30–3.49; p = 0.003) 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 the American Stroke Association guidelines) was considered, the results were also stronger (unadjusted OR 2.78, 95%CI 1.66–4.87; p <0.001; adjusted OR 2.67, 95%CI 1.49–4.77, p <0.001). Conclusions: Beside the role of established risk factors, Val1062 LRPL6 variant and CAA are strongly associated in hypertensive patients, making LRPL6 a novel interesting candidate gene for early coronary and carotid artery atherosclerosis.
both loci are important BP genes; we are currently performing replication studies in large replication cohorts to confirm these findings.

**PF.06** 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 THPO promoter structure is still controversial, using RT-PCR, we evidenced that THPO transcription is cell-line dependently initiated at two alternative promoters, which we newly designated P1a and P1. We subsequently electrophoretically scanned and resequenced these portions in 95 and 57 patients with CVD, respectively, and identified eight variants (-1456/del58bp, C-920T, A-622G, C-413T, C +5A, C -102A, G +115A, and C +135T). After subcloning of 1032 bp fragments of THPO P1 in pGL3-basic vectors, five molecular haplotypes (MolHaps -5) were, respectively, observed: [A-622-C-413-C-G-115G], wildtype (wt), [A-622-G-413-C-G-115G], [G-622-A-413-G-C-115G], [A-622-G-413-A-C-115G] and [A-622-C-413-C-A-115G], and analysed in reporter gene assays in HEK293T and HepG2 cells. While MolHaps 2, 4, 5 were significantly more active than wt (at all P-values < 0.01), MolHap3 exerted a substantial loss of promoter activity (P < 0.0001 in HEK293T; P = 0.001 in HepG2, compared to wt). EMSAs revealed that A-622G and C-413T in single assays differed from MolHaps in their DNA-protein interaction patterns and Supershift assays identified CEBPs as binding protein exclusively for the -622A allelic portion. We herein redefined the transcriptional organisation of THPO and conclude that the P1 promoter is differently regulated by complex genetic constellations.

**PF.07** 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 proven 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%) 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.

**PF.08** 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 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 686 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 Thrombomodulin-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.

**PF.09** Integration of genetic polymorphisms to predict ventricular function after myocardial infarction

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Since the severity of heart failure is subjected to inter-individual variability, we hypothesized that gene polymorphisms in myocardial infarction (MI) patients could govern the severity of remodeling. A matched sex and age population of 22 patients developing remodeling post-MI (ejection fraction (EF) < 40%) and 22 patients with a favorable outcome post-MI (EF > 45%) was used. Associations between 50 polymorphisms in 36 genes involved in inflammation and remodeling and the severity of heart failure were investigated. Prognostic models used to assess the predictive value of the polymorphisms included an artificial neural network trained with the back-propagation algorithm. Significant associations between 3 polymorphisms and the EF were identified: a mutation in the promoter region of the leukotriene C4 synthetase (P < 0.0015), a synonymous mutation in the coding sequence of nitric oxide synthase 2 (P = 0.005) and a non-synonymous mutation in the coding sequence of matrix metalloproteinase 9 (P = 0.036). When the prognostic models were built based on the integration of these 3 polymorphisms, a significant predictive performance was observed (area under the receiver operating curve (AUC) of 0.79). The artificial neural network model accurately classified patients prone to have a favorable outcome after MI (21/22, 95%). The integration of 3 polymorphisms shows significant potential for the classification of patients with different remodeling severity after MI. This model may help to tailor health care to the individual patient.

**PF.10** 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 nongenomic 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 intronic single nucleotide polymorphisms (SNPs) in the estrogen receptor alpha (ERα) gene in Polish population. **Materials and Methods:** 188 young patients (aged under 55) suffering from MI and 414 healthy controls aged 18-45 were genotyped for T-397C and A-351G SNPs in ERα using PCR-RFLP method. **Results:** The A-351G (A:A) genotype was significantly more frequent in MI group than in healthy controls (p = 0.002, 51.3% vs. 37.9%, OR = 1.34). Moreover, genotypes of the T-397C SNP were equally distributed in studied groups. The analyzed polymorphisms were linked 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" homoygotes 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 phosphorylation between sham and CHF group was performed using two dimensional gel electrophoresis. Phosphoproteins detection was performed using Progenesis software. Phosphoproteome 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 phosphoprotein in CHF (19%) than in sham group (11%). This bioinformatic analysis revealed 79 spots presenting variation of their phosphorylation level in remodeled 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.

Cardiophosphin-1 (CP-1) is a cytokine that promotes longitudinal cardiomyocyte growth in vitro. Plasma concentration and myocardial expression of CP-1 is increased in patients with heart failure. We aimed to investigate the effect of chronic CP-1 administration on cardiac function and morphology in vivo. Vehicle or recombinant rat CP-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 CP-1 treatment modified systemic hemodynamics. Whereas non significant echocardiographic changes were observed in rats receiving vehicle, CP-1 administration resulted in increased (P<0.05) LV systolic and diastolic diameters, increased (P<0.01) LVEF and decreased (P<0.01) fractional shortening and ejection fraction. Histological analysis confirmed that hearts from CP-1-treated rats exhibited larger (p<0.01) LV chamber dimensions and thinner (P<0.05) LV free-wall than vehicle-treated rats. Finally, cardiophosphin-1 length was larger in CP-1 than in vehicle-treated rats. Cardiophosphin-1 expression was associated with collagen accumulation and fibrosis. These findings indicate that chronic CP-1 overloading results in LV dilatation and cardiac function alteration. We suggest that cardiophosphin-1 may affect LV remodeling associated with heart failure.

Transient prehypertensive renin-inhibition

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Background: Transient prehypertensive treatment (TPT) in young SHR results in prolonged blood pressure lowering and target organ protection. The mechanisms of this treatment remain uncertain. We investigated whether prehypertensive renin-inhibition in young SHR protects against L-NNAME induced cardiac damage at adulthood, which is characterized by a reduction in end-diastolic LV dimensions and systolic function. Methods: Four weeks old SHR were treated with enalapril (100 mg/kg/day) or placebo for 4 weeks with NaCl 0.9%, aliskiren 1 and 10mg/kg/day and enalapril 10mg/kg/day (n=8 each group). At 8 weeks of age blood pressure was measured intra-arterially under isoflurane anesthesia (1.5%). After drug withdrawal animals were kept 4 weeks under control conditions. At 12 weeks oral L-NAME (25mg/kg/day) treatment was started. At 16 weeks of age blood pressure was measured intra-arterially and echocardiography was performed. Results: TPT with high-dose aliskiren and enalapril significantly reduced mean arterial pressure at 8 weeks of age (mean±SEM: 76±3.3±9.75±4.68 mmHg) compared to low-dose aliskiren and placebo treatment (89.8± 4.7 and 91.8± 4.9 mmHg; P<0.05). At 12 weeks of age blood pressure was reduced in all TPT groups (low and high aliskiren: 112.3±6.8, 117.3±8.7 and enalapril 127.7±14.3 mmHg) compared to placebo-treated SHR (128.1±6.4 mmHg, P<0.01). End-diastolic volume was significantly increased in both initially aliskiren treated groups (262±13 and 277±41 cm³), as compared to placebo and initially enalapril treated SHR (172±18 and 207±27 cm³). Conclusion: Prehypertensive renin-inhibition protects better against L-NNAME induced cardiac dysfunction as ACE-inhibition. Moreover, transient low-dose aliskiren treatment - without initial blood pressure lowering - leads under L-NNAME to a reduced blood pressure and cardioprotection. This suggests that successful TPT is dependent of initial blood pressure lowering.

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) plays 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 cistamino acid 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, cistamino 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 TG 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. Cistamino tended to raise RVSP and blunted the effect of sildenafil on RvH, whereas right ventricle end-diastolic pressure (RVEDP) compared to normoxia. Sildenafil lowered dP/dt compared to hypoxia. Cistamino blunted the effect of sildenafil. Hypoxia raised right ventricle to left ventricle+ septum weight ratio (RV/LV+S) significantly compared to normoxia. Cistamino and sildenafil tended to lower RV/LV+S. The present investigation suggests that increased expression of TG contributes to the development of right ventricular hypertrophy in pulmonary hypertension.

Cardioprotective effect of insulin (Ins) on diabetic cardiomyopathy in vivo

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The cardio-protective effect of insulin (Ins) on diabetic cardiomyopathy in vivo. Plasma concentration and myocardial expression of CT-1 is 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 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 systemic 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) E/A ratio 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, cardiophosphin-1 length was larger in CT-1 than in vehicle-treated rats. Cardiophosphin-1 expression was associated with collagen accumulation and fibrosis. These findings indicate that chronic CT-1 overloading results in LV dilatation and cardiac function alteration. We suggest that cardiophosphin-1 may affect LV remodeling associated with heart failure.

Immune binds specifically to α1 subunit of cardiac Na/K ATPase pump and prevents acute digoxin toxicity on neonatal rat cardiomyocytes

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The cardio-protective effect of insulin (Ins) on diabetic cardiomyopathy in vivo. Plasma concentration and myocardial expression of CT-1 is 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 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 systemic 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) E/A ratio 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, cardiophosphin-1 length was larger in CT-1 than in vehicle-treated rats. Cardiophosphin-1 expression was associated with collagen accumulation and fibrosis. These findings indicate that chronic CT-1 overloading results in LV dilatation and cardiac function alteration. We suggest that cardiophosphin-1 may affect LV remodeling associated with heart failure.
inorganic phosphate by the isolated enzyme and decreased inhibitory effect ofDig in a non
competitive manner. Biaxore experiments performed on the isolated enzyme in presence
and absence of Ins (Dig),Dig enzyme showed a direct interaction of both Dig and Ins with
enzyme’s ε1 subunit but not on 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 1g/mg) protected both adult and neonatal
rat cardiomyocytes from Dig (10-5 to 10-2 M) toxicity but not from that of ouabain (10-5 and
10-3 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 ε1 subunit
IR. This amelioration was observed only in presence of Dig but not with ouabain. Ins
probably due to its specific binding to Na/K ATPase 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

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We previously documented a clear-cut antihypertensive effect of green tea extract (GTE), 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
μg/kg/d, n=6, via osmotic minipumps). Ang II plus GTE (8 g/L), dissolved in the drinking
water, n=6), or vehicle, was infused to serve 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
through immunoprecipitation and Western Blot analysis with specific antibodies. NAD(P)H
oxidase enzymatic activity was measured by cytochrome c reduction assay.GTE blunted
Ang II-induced blood pressure increase and cardiac hypertrophy. In Ang II treated rats GTE decreased the expression of NAD(P)H oxidase subunit gp91phox and the translocation
of Rac-1, as well as NAD(P)H oxidase enzymatic activity. Furthermore, it specifically
reduced Ang II induced Src, EGFR, and Akt phosphorylation. These results show that GTE
blunts Ang II induced cardiac hypertrophy specifically by regulating ROS production and
the Src/EGFR/Akt signalling pathway activated by Ang II.

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

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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 agonist WIN 55,212 (WIN) in body weight and cardiovascular function
during and after two 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
administration.

Our results show, for the first time, that cardiac side effects could come into sight with a chronic administration of cannabinoid treatment in rats. The effects are due to a lack of desensitization and occur 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.


Exacerbated NOS uncoupling and adverse ventricular remodeling 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 myocardial
response to pressure-overload. Methods: Mice lacking β3-AR (β3- / -) and wild type
(β3, n=25) 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-AR ablation did not change
wall thickness (p=0.05), but reduced WT mice were more likely to die with TAC
(p=0.001). By 3 weeks of TAC, left ventricular (LV) wall thickness (p<0.05) and mass (p<0.05) increased 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<0.01), and
total (p<0.001) and NOS-dependent superoxide (p=0.05) rose, indicating heightened NOS
coupling. GTPCH-1 expression was reduced (p<0.05) rose, indicating heightened NOS
uncoupling. GTPCH-1 expression was reduced (p<0.05), and total
3-AR signalling exacerbates cardiac pressure-overload remodeling coupled with enhanced NOS uncoupling and consequent oxident 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: Tetrahydrobiopterin (BH4) is the rate limiting enzyme of BH4 synthesis, was evaluated in this TAC model (n=20). BH4 treatment in TAC mice increased eNOS
in the aortic media (p<0.05). By 3 weeks of TAC, left ventricular (LV)
wall thickness (p<0.05) and mass (p<0.05) increased 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<0.01), and
total (p<0.001) and NOS-dependent superoxide (p=0.05) rose, indicating heightened NOS
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3-AR signalling exacerbates cardiac pressure-overload remodeling coupled with enhanced NOS uncoupling and consequent oxident stress. Selective stimulation of these receptors may provide a novel approach to reducing pathologic hypertrophy in the failing heart.

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 transplan-
tation 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 excellent
carriers of human VEGF for angiogenesis in infarcted heart. Considering that
myocardial ischemia is a recurring and progressive disease, a regulable gene delivery
system (pHRE-VEGF) is designed. In this system, VEGF over-expression will be switched on
by binding of hypoxia inducible factor alpha (HIF) to 5 copies of hypoxia response element
(S0XRES) in hypoxia condition. Results: Rabbit keletal myoblasts have been isolated and
purified. These myoblasts carrying pHR-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.
confirmed by MRI and PV loop analysis. BH4 recovered the already uncoupled eNOS and increased its activity back to the normal level. Superoxide generation (total and NO-dependent) was markedly reduced by BH4. BH4 improved fractional shortening and calcium-kinetics in isolated myocytes. Endothelial upregulation of BH4 by GTPyS had no beneficial effect on remodeling. Conclusion: BH4 can reverse established cardiac remodelling by re-coupling uncoupled eNOS and as a consequence less NO dependent ROS is generated, leading to less hypertrophy and fibrosis and an amelioration of cardiac function.

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Mitral regurgitation (MR) due to myxomatous mitral valve disease is a common cause of heart failure in dogs. Cavalier King Charles Spaniels (CKCS) are predisposed to the disease which is similar to mitral valve prolapse in man. The CKCS have therefore been suggested as a spontaneous animal model.

Identifying endothelial and platelet activation markers that are affected in asymptomatic stages of MR could be beneficial regarding future diagnostics, prognostics and treatment. The aim of the study was to measure a panel of different endothelial and platelet activation markers and furthermore investigate the effect of an exercise challenge in dogs with asymptomatic MR.

Three dog groups consisting of CKCS with minimal MR, CKCS with moderate to severe MR and Cairn Terriers with minimal MR went through a clinical examination, blood sampling, echocardiography and an exercise challenge. Plasma cyclic guanosine monophosphate (cGMP) concentration was significantly increased in the CKCS with moderate to severe MR. Plasma von Willebrand factor (vWF) concentration was decreased in the CKCS with moderate to severe MR. The plasma concentration of nitrate and nitrite (NOx) as well as maximal platelet aggregation and platelet surface P-selectin expression were similar in the groups. There were no significant differences in plasma NOx and vWF concentrations following the exercise challenge.

The major new finding in this study was that cGMP increases with increasing MR. This may be associated with an increase in natriuretic peptides which have previously been shown to increase in severe asymptomatic stages of MR in dogs.

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Progression of renal disease and its cardiovascular complications seems to be slower in subtotally nephrectomized rats.

Male (M) and female (F) Wistar rats were assigned into SNX, SNX hydralazine-treated and control groups for 18 weeks. Renal ablation induced hypertension, with lower values of blood pressure in F than in M (114 ± 14 vs. 140 ± 11 mmHg, p < 0.05). Contrasting the degree of proteinuria indicating renal injury was higher in F (445 ± 197 vs. 191 ± 106 mg/day, p < 0.01). SNX led to left ventricular hypertrophy and diastolic dysfunction in both groups, with significant higher relative values of left ventricular mass in M (19.3 ± 3 vs. 16.1 ± 3 g/m^2 tibia length in females, p < 0.05), without impairment in cardiac function. Renal compensatory hypertrophy occurred in all SNX groups, with significant differences (42 ± 3 vs. 27 ± 3 g/m^2 tibia length in F vs. 67 ± 10 mg/m^2 tibia length in M, p < 0.01). SNX animals developed glomerulosclerosis and renal functional impairment, with no further influence of gender. Hydralazine treatment reduced blood pressure to control levels, without altering cardiac or renal disease severity.

In a model of mild uremia, female gender was found to exert beneficial blood pressure to control levels, without altering cardiac or renal disease severity.

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. Cardiovascular 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^-5 M - 10^-3 M) and by carbachol (10^-6 M - 10^-4 M) 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 (8–12 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 definitively 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) and 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 relaxation 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 vasodilations. 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 endothelium 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 ovariectomized female ER-alpha knockout mice. Deletion of ER-alpha did not affect echocardiographic measurements in female mice, but did affect the systolic function. Deletion of ER-alpha induced hyperactivity of the aorta in presence but not in absence of functional endothelium and did not alter relaxation to acetylcholine. Both basal NO 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 cGK, eNOS and caveolin-1 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 vasococontractor 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.

The effect of melatonin on endothelial function and in L-NAME-induced hypertensive rats
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Melatonin reduced experimental 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-NAME, melatonin and L-NAME + 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-preconstricted femoral and mesenteric arteries. Endothelin-derived constricting factor (EDCF)-mediated component of ACh-induced responses and inner diameter were determined in femoral arteries. L-NAME treatment caused hypertension, impaired ACh-induced relaxations, decreased NO-component assisted EDCF-component and reduced inner diameter. L-NAME also inhibited NOS activity in the brain and the aorta, in which the endothelial NOS expression was not altered, and COX-2 expression was enhanced. Concomitant treatment with melatonin decreased BP by 15%, failed to improve NOS activity, NO or COX-2 expression, vascular structure or function. We conclude that BP reduction after was less pronounced in NO-deficient 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. (GUK 14/3/2008, VEGA 1/3429/06, 2/B148/26)

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 arteriotomy. 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 5x10⁶ 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 carotid after arteriotomy. We conclude that MSC administration limits the arteriopathy-induced stenosis in rat carotids presumably through an immunomodulatory action.

Phospholipase C and α1D adrenoceptors in rat mesenteric small arteries
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Activation of phospholipase C and α1D adrenoceptors in rat mesenteric small arteries
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Stimulation of α1D-adrenergic receptors (α1D-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 α1D-ARs to PLC activity in intact tissues are unclear. In expression studies using cultured cell lines, α1A and α1D-ARs coupled to PLCα1 via the atypical G protein Goα. 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 PLCα1 and Goα, and on PKC activation, were determined by western blot and co-immunoprecipitation. Contractility was measured by pressure myography and PLC activity by [H]-PPI hydrolysis in vitro. BMC 7378 (100M), a specific inhibitor of α1D-AR, reduced NA-induced contraction, and both PLC and PKC activity. PLCα1 and Goα were detected in RMSA with a similar subcellular distribution, which was not altered by NA. Neither PLCα1, nor PLC activity were detected in Goα immunoprecipitates from either control or stimulated arteries. These results show that in intact RMSA, NA signals via α1D-AR, leading to PKC activation and contraction. However, there was no interaction detected between PLCα1 and Goα, which suggests this is not a major mechanism for regulating PLCα1 activity in vascular tissue. 1. Kang et al (2002). Biochem.Biophys.Res.Commun. 293:383–390

Phospholipase Cδ1 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 (PIP2) hydrolysis, increased intracellular calcium and contraction. In rat mesenteric small arteries (RMSA) PIP2 hydrolysis in response to noradrenaline (NA) or endothelin (ET-1) occurs solely in caveolae/rafts. Here we have investigated whether PLCδ1, a PLC isoform implicated in α1D-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, PIP2 or inositol phosphates (InsPs) were measured with 33pH or [3H]-inositol, PLCδ1 was localised by immunoblot and neutralised by delivery of PLCδ1 antibody. Contractility was measured by pressure myography. PLCδ1 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. PLCδ1 was detected in caveolae/rafts and NA but not ET-1 stimulated a rapid calcium dependent increase in PLCδ1 levels in these domains. NA-induced PIP2 hydrolysis and InsP3 formation was also calcium dependent whereas ET-1-induced PIP2 hydrolysis was not. Delivery of PLCδ1 antibody to RMSA prevented NA-induced PLCδ1 association with caveolae/rafts and attenuated the sustained phase of the contractile response to NA when compared to control antibodies. In contrast, PLCδ1 antibody had no effect on ET-1 or potassium induced contraction. These data show a novel and selective role for PLCδ1 in NA-induced sustained contraction in intact vascular tissue.

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 (NIOX) 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·d⁻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 vasodilatation depends on...
NADPH oxidase inhibition at all. Apocynin potently inhibited human granulocyte NOX but not NOX-dependent oxygen radical formation in rat aortic rings and small intestinal arteries, when compared with negative control groups. Apocynin inhibited the release of superoxide anion in rat aortic tissue in vitro. Apocynin dilated 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 dilatation than wild-type aortic rings. Apocynin-induced vasodilation was not significantly affected by NOS, PKA, or PKG inhibition, but did not depend on extracellular Ca²⁺ but was sensitive to Rho-kinase inhibition. Apocynin per se does not inhibit vascular NOX-dependent superoxide formation and requires high peroxidase activities for efficient NOS 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 Cushings 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) in SRA in segments preconstricted with U46619. Two-way ANOVA was used as statistical test. NE exhibited a biphasic response with initial contractions followed by relaxations at concentrations higher than 10⁻⁴M. 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 pathophysiologically concentrations induces desensitization of vasodilator responses. This effect may participate in the increased risk of cardiovascular complications in patients with Cushings 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 pathways 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 demonstrate that cardiovascular risk factor DMSO-soluble smoke particles (DSP) enhance the ET-system activity through up-regulation of vascular endothelin type A (ET₁) receptors in vasculature and to investigate the molecular mechanisms behind the DSP-induced activation of the 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 ET₁ receptor up-regulation with enhanced ET₁ receptor-mediated contraction (myograph), increased ET₁ receptor mRNA (real-time PCR) and protein (immunohistochemistry with confocal microscopy) expressions in the SMC. Inhibition of transcription or translation abolished DSP-enhanced ET₁ receptor-mediated contraction. Post-transcriptional mechanisms were suggested by that DSP accelerated ET₁ receptor mRNA degradation but enhanced ET₁ receptor-mediated contraction. Inhibition of translation, ERK1/2 or PKCε activities attenuated the DSP effects. Conclusion: Up-regulation of ET₁ receptors by DSP involves transcriptional mechanisms and enhanced translation of ET₁ receptors in the vascular SMC via activation of intracellular PKCε and ERK1/2. The ET₁ receptor up-regulation by DSP in the SMC may contribute to the development of smoking-associated cardiovascular diseases.

PH.12
Vascular smooth muscle relaxation in soluble guanylyl cyclase β1 HS 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,β3 and sGCβ2β3 are the physiologically active heterodimers, in which the histidine residue at position 105 of the β3 subunit functions as an axial ligand for the heme prosthetic group. Substitution of histidine by phenylalanine abolishes the heme-dependent activation of sGC. This is the case in the sGCβ1,-β3 mice from which aortic, femoral artery and corpora cavernosa (CC) segments were mounted for isometric tension recording. In comparison with the preparations isolated from the wild type littermates, the responses to endothelium-releasing NO 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,-β3 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,-β3 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 mouse aorta but not in femoral artery. Furthermore, the remaining relaxing effect of BAY 41–2272 in the sGCβ1,-β3 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 vasodilatation and exerts vascular antioxidant 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 hirudin radical (TBARS assay and chemiluminescence) and 2) to protect endothelial NO generated by acetylcholine from degradation by peroxynitrite-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, scavenging 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 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 potential harmful ROS and protecting NO from degradation.

PH.14
Targeted disruption of kinin B1 or B2 receptor gene in mice alters vascular reactivity and nitric oxide metabolism
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Kinin B1 and B2 receptors play an essential role in inflammation and cardiovascular homeostasis. Vascular reactivity and nitric oxide (NO) metabolism were studied in knockout B₁(B₁-/-), B₂(B₂-/-) and wild type (WT) mice (n=8). Isolated mesenteric arterial 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 ± 0.7*, 2.5 ± 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. Constrictor responses to ang II were reduced in B₁(1.3 ± 0.9*, 1.5 ± 0.9*, 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 pmol, respectively. Plasma NO levels[μM] were reduced in B₁(60 ± 11*) and B₂(67 ± 4* vs WT (142 ± 17), while NO activity (pmol/mg/min) was higher in the mesenteric arteries of B₁(1.7 ± 0.8*, 2.5 ± 0.6* 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. B₂ gene deletion might affect negatively the ang II mediated signaling in vascular cells. These data may point to new approaches in the field of the interactions among angiotensin, kinin and NOS 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 (AT2R) agonist Compound 21 (C21) in diabetic nephropathy. Diabetes was induced in SHR-sp by a single streptozotocin (STZ; 60 mg/kg BW i.v.) injection, and animals were treated for 12 weeks according to the following protocols: 1) non-diabetic controls, 2) STZ (diabetic controls); 3) STZ+HCan; high dose Candesartan (10mg/kgBW i.p.); 4) STZ+LCan; low dose Candesartan (0.2mg/kgBW i.p.); 5) STZ+C21 (0.3mg/kgBW i.p.); 6) STZ+both (HCan+C21). Systolic BP was only lowered in the STZ+HCan (169±24mmHg) and the STZ+both (188±17mmHg), but not in the STZ+LCan (222±10mmHg) and, remarkably, not in the STZ+C21 (251±14mmHg) groups. Elevated albuminuria in STZ rats (51.1±18 mg/d) was only ameliorated in STZ+HCan (17.7±7 mg/d), STZ+C21 (32.15±5 mg/d) and STZ+both (14.7±5 mg/d), but not in LCan (60±21 mg/d). Using computer-based histomorphometry, diabetic glomerular hypertrophy (STZ 13.9±4.5; S55 μm2) was significantly lower with Can and C21 (STZ+HCan -15%; STZ+LCan -12%; STZ+C21 -11%, STZ+both -18%). Increased tubulointerstitial collagen I expression (STZ 70±22% per section) and glomerular collagen IV deposition (STZ 5±1.0%) was reduced in all treatment groups (STZ+HCan -15%/-43%; STZ+LCan -65%/-27%; STZ+C21 -56%/- 43%; STZ+both -33%/-26%). We conclude that pharmacological AT2 receptor stimulation by C21 limits experimental diabetic renal hypertrophy and matrix accumulation independently of a reduction in blood pressure.

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 18h. 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 basically detected in human umbilical vein endothelial cells (HUVECs). Nevertheless, visfatin levels were increased when HUVEC were challenged with increasing concentrations of the cytokine IL-1β for 18 h. This latter effect of IL-1β was prevented by PDTC and the enzyme PolyADP-ribose polymersase-1 inhibitor (PJ-34). We conclude that visfatin released either by adipose tissue or locally synthesized by vascular cells, arises as a new agent promoting vascular inflammation.

Extracellular RNA, a pro-inflammatory factor promoting arteriogenesis

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The growth of pre-existing arteriolar anastomoses to large conductance arteries as the main fuel to fulfil cardiac caloric needs. The rate of FA oxidation during the stimulus. Accordingly, the number of cFos positive neurons in the dorsal horn of the spinal cord. Compared to wild type (wt) mice tg mice showed significantly less increase in blood pressure as well as heart rate during the stimulus. Accordingly, the number of cFos positive neurons was significantly lower in tg mice com-pared to wt mice. CGRP(8–37) decreased the cardiovascular response and the number of cFos positive neurons in wt mice to that of untreated tg mice. The CGRP antagonist had no effect in tg mice. Compared to wt animals CGRP positive nerve fibres in the dorsal horn and dorsal root ganglia of tg mice were reduced. Moreover the number of CLR positive neurons in lumina 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.

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

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Fatty acids (FA) are the main fuel to fulfill cardiac caloric needs. The rate of FA oxidation increases in diet-induced obesity/hyperleptinemia (DIO) due to metabolic adaptations aimed at facilitating β-oxidation. A key step for β-oxidation is the mitochondrial uptake of FA by the carnitine palmitoyltransferase (CPT) complex, which includes a
malonyl-CoA sensitive component (CPT-I). The aim of this work is to characterize the effect of leptin in regulating renal 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 HF 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 Educacion y Ciencia (SAF 2006-02456 and SAF 2005-0518), FUSP-CED, 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 NF-κB resulting in increased cytokine expression. There is evidence that the AT2R is anti-inflammatory, although investigation of the role of the AT2R in inflammation (and in general) has always been hampered by the lack of a selective non-peptide AT2R-agonist. In this study, we investigated the effect of AT2R stimulation by the novel non-peptide AT2R agonist Compound 21 (C21) on IL-6 expression and NF-κB activity in human and murine primary dermal fibroblasts. In order to detect a potentially inhibitory effect of AT2R stimulation, human or murine dermal fibroblasts were preincubated with TNFα (10 ng/ml) to increase IL-6 expression and NF-κB activity, respectively. IL-6 expression was measured by real time RT-PCR. NF-κB activity was estimated by immunohistochemical detection of NF-κB p50 nuclear translocation and by a luciferase reporter assay using an expression vector containing the human IL-6 promoter including the NF-κB binding site. Stimulation of the AT2R in human and murine primary fibroblasts by C21 (10 μM) led to a significant decrease in TNFα-induced IL-6 expression and NF-κB activity. These effects could be inhibited by the specific AT2R-antagonist PD123319 (10 μM) as well as by the inhibitor of protein tyrosine phosphatase, Na-orthovanadate (10 μM), or the inhibitor of serine/threonine phosphatase, okadaic acid (10 μM). C21 was not effective in fibroblasts isolated from AT2R-deficient mice. We conclude that direct AT2R stimulation causes activation of phosphatases thus leading to inhibition of NF-κB and reduced IL-6 levels.

Sympathetic overactivity in α2A-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 α2A-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.

The novel non-peptide AT2-R agonist Compound 21 elicits various neuroprotective molecular mechanisms in primary astrocytes and in the neuronal cell line NG108-15

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Objectives: Neuroprotective properties have been attributed to the AT2R, e.g. in the course of pharmacological AT1R-blockade. Recently, a selective, non-peptide AT2R-agonist termed Compound 21 (C21) has been developed. This study aimed to test whether direct AT2R stimulation is able to elicit protective molecular mechanisms in primary astrocytes and neuroprotective activity in NG108-15 cell line. Methods: Primary rat astrocytes were stimulated with LPS (10 μg/ml) and co-incubated with C21 (10 μM). Expression of neurotrophins, the apoptosis marker Bax, inflammation markers and toll-like receptors (TLRs) was determined by qPCR. For determination of neurite outgrowth, the undifferentiated neuronal cell line NG108-15 has been treated with C21 and microscopically examined. Results: LPS stimulation strongly induced expression of TLRs, inflammation markers and Bax in primary astrocytes. Increased levels of these markers were significantly reduced by co-incubation with C21. Expression of neurotrophins (BDNF, TNb) was reduced by LPS and restored by C21. In addition, C21 induced neurite outgrowth in NG108-15 cells. All C21 actions could be inhibited by the well established AT2R antagonist PD123319 (10 μM). Discussion: Our data support the hypothesis, that AT2R stimulation has tissue protective effects in cells derived from the central nervous system. This observation and the availability of a selective, non-peptide AT2R agonist may open new therapeutic options for the treatment of pathologies involving CNS damage.

Activation of vascular renin angiotensin system on pulmonary vessels by antigen sensitization and challenge

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The pulmonary renin angiotensin system (RAS) is activated and participates in allergic airway diseases which could affect pulmonary vessels by an incompletely known mechanism possibly dependent by local RAS. To evaluate if antigen challenge influences the angiotensin synthesis and metabolism on the vessel walls the contractile effects induced by angiotensinogen (the angiotensins precursor) treatment were assessed on pulmonary artery (RPA) and vein (RPV) rings obtained from normal (NR) or OVA sensitised (OSR) rats. Experiments were performed in the presence or in the absence of aminopeptidase inhibitor) or valsartan (a specific AT1 blocker). The contractions were higher on OSR than NR with one third for RPA and up to a half for RPV. Valsartan pre-treatment totally prevented angiotensinogen effects underlying the involvement of AT1 receptors. The enzymes inhibitors had different effects on OSR than NR. On RPA the peptatin inhibitory effects were two times more powerful than NR than OSR. Teprotide had approximately the same inhibitory effects on NR and OSR. On RPV chistimastat had no significant effects on NR but reduced to half the vasoconstriction provoked by angiotensinogen treatment on OSR. The stimulatory effects of amastatin pre-treatment were higher on NR (with one third for RPA and two thirds for RPV) than on OSR. These data suggest the possibility of antigen-induced RAS activation on pulmonary vessel walls which could mediate the vascular impact of airway diseases opening the possibility for new therapeutic approaches.
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