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

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ECCR Oral Presentations

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 8, 14 or 32 weeks. Reactivity of resistance vessels was characterized by pertussis toxin (PTX) and were compared to control mice (CT). After 32 weeks of diet, HF animals exhibited increased plasma leptin levels together with an improved relaxant response to acetylcholine (ACh, 10−9−10−4 M, 8.7±1.6 % vs 94.1±2.0 %; p<0.05) compared to LF animals. As well, a higher NO production was observed in mesenteric adipose tissue, determined by confocal microscopy with DAF-2 DA. After 32 weeks of diet, HF animals also exhibited increased 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 Emax=60.1±1.7 %; p<0.005). Furthermore, NO production in mesenteric arteries was significantly reduced in this group. Accordingly, contractions to noradrenaline (10−7 to 10−6 M) were significantly higher in this group. No differences in vascular function between groups were observed after 14 weeks of diet. These results suggest an improvement of endothelial function of resistance vessels as an early adaptation to DIO followed by a development of endothelial dysfunction in obese mice. (Supported by SAF 2006−02456, SAF 2005–05180, FUSP-CEU, SESCAMET. MG-O).

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 (0.63-1.11) 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 (0.51−1.2). Other subgroups are below. The interaction terms between active treatment and the various subgroups were not significant for total mortality (0.30<p<1.00) or CV events (0.42<p<0.80). Conclusions: For both total mortality and CV events benefits were seen across sex, age, previous CV disease and initial SBP. This adds support for the treatment of very elderly hypertensives.

0.03 Subjects with premature cardiovascular disease have a diminished glycoalyx 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 glycoalyx. 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 glycoalyx volume is still unknown. We therefore investigated glycoalyx volumes in subjects with premature CVD and compared this with healthy controls. Hypothesis: Compared to controls, subjects with premature CVD have a diminished glycoalyx 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 glycoalyx volume, the level of classic risk factors, pulse wave velocity (PWV) and IMT. Results: Subjects with premature CVD had a diminished glycoalyx volume as compared to controls (0.47 ± 0.26 vs 0.81 ± 0.38, 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 ± 1.7 m/s vs 9.6 ± 0.9 m/s, p<0.04) and an increased IMT (59.6 ± 7.14 μm vs 54.3 ± 90.0 μm). Conclusion: Subjects with premature CVD have a diminished glycoalyx volume and a loss of transfer function. This suggests that they are less protected against the influence of classic risk factors. When these findings are confirmed

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-blinded, placebo-controlled, parallel group study in 50 non-diabetic patients with resistant hypertension. All patients at enrolment received ACE inhibitors or angiotensin receptor blockers and diuretics together with a third drug. We additionally treated for six months with eplerenone 50 mg or to reach BP control without the use of MR antagonists by optimizing antihypertensive treatment (placebo group). Primary endpoint of the study was reduction of LV mass as assessed by MRI. Comparisons of groups were done by t-test. All values are given as M±SD. Eplerenone treatment reduced casual BP by 35±20/15±11 mmHg, in the placebo group BP was lowered by 30±18/13±7 mmHg (p=0.40 and 0.57, respectively)24-hour BP: 18±13/9±8 vs. 13±8±7; p=0.13 and p<0.033. 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. hyperkaemia) 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.
Aortic stiffness is associated with silent cerebrovascular disease in hypertensive patients

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Aortic stiffness predicts an excess risk of stroke, supposedly via cerebral small-vessel disease. Therefore, we assessed whether aortic stiffness is related to white matter hyperintensities (WMHs), silent lacunar infarcts (LACs) and brain microbleeds (BMBs). In 167 hypertensive patients (85 males) without a history of cardio- or cerebrovascular disease, a mean age of 51.8±13.1 years and untreated office blood pressure levels of 169±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, 0.041; 95% CI, 0.005–0.078; P=0.05) and the presence of LACs (odds ratio [per SD increase in aPWV], 1.78; 95% CI, 1.06–2.99; P=0.05), but not with microbleeds (odds ratio, 1.13; 95% CI, 0.67–1.91). The models for pulse pressure failed to reach statistical significance in multivariate analyses. In conclusion, aortic stiffness is independently associated with cerebral small-vessel disease in hypertensive patients without a history of cardio- or cerebrovascular disease.

Exacerbated cardiomyopathy in pressure-overload in mice lacking thrombospondin-4

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Background: Myocardial expression of the ECM- molecule thrombospondin (TSP)-4 rises markedly in hypertrophic and failing hearts, and generally observed with increased fibrogenesis. The protein is thought to link collagen, though its underlying role in such pathophysiology is unknown. Methods: Mice lacking TSP4 (TSP4-/-) (n=47) and WT controls (n=24) underwent transverse aortic constriction (TAC) for 30min up to 3wks. Results: 10 min after the onset of TAC, WT hearts displayed a positive inotropic effect (AP/Dp/Dtmax: WT: +19±4%, Anrep-effect) at reduced preload and normalized stroke volume despite high afterload. In contrast, TSP4-/- had reduced function (ΔAP/Dtmax, -75±6% p<0.001) and dilated. Scanning EM showed less mature collagen ultrastructure and decreased myofibrillar alignment. However, matrix fibrosis genes (i.e. CTGF, TGF-B2, collagen type I and III) already started to rise (all: p<0.001), and with 3wks TAC, were markedly increased accompanied by a pronounced fibrotic response (~400% more than in WT, p<0.001), worsened LV dilation (p=0.017) and decreased ejection fraction (p=0.04). Conclusion: Myocardial TSP4 plays a key role in transducing acute pressure-overload stress to enhance myocardial contractile responses. Rather than being a primary contributor to fibrosis, its matrix-linker role likely negatively regulates matrix synthesis function by influencing the ultrastructure of these proteins in the tissue.

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) restored endothelial function and especially flow-mediated, NO-dependent vasodilatation (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 was induced by coronary ligation. Mice deficient (PTP1B-/-) BALB/c mice. WT CHF mice were untreated or treated with the PTP1B inhibitor AS279 for 2 months. Echocardiographic analysis of left ventricular (LV) function and evaluation of FMD were performed. In WT with CHF, echocardiography showed that AS279 decreased LV end-diastolic (LVEDD: untreated: 6.1±0.2, n=13; AS279: 5.0±0.2 mm, 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 ml/min; p<0.05). In PTP1B-/- mice with CHF (n=13), LVEDD and LVESD were reduced (LVEDD: 5.2±0.2cm, p<0.01; LVESD: 3.9±0.3mm, p<0.01), while FS and CO were increased (FS: 22.7±2.5%, p<0.01; CO: 22.2±1.1 mm/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.
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 NODAX oxidative activity and contributes to endothelial dependent vasodilation in distal artery segments

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We investigated the contribution of the NODAX-oxidative (NOX) activity to superoxide anion formation in human intrarenal arteries and the modulation of agonist-induced vasoconstriction as well as endothelium-dependent vasodilation by superoxide anions. Interlobar, arcuate and interlobular artery segments were dissected from non-cancerous regions of kidneys obtained from thirteen patients who underwent nephrectomy because of a renal tumor. Using inhibitors of the major oxygen radical forming systems (xanthine oxidase, NO-synthase, mitochondrial respiratory chain) we found that in human intrarenal arteries 75% of the superoxide anion formation was NOX-dependent. NOX activity was two-fold higher in distal (interlobular) than in proximal (interlobar) artery segments. 

W3.04 Angiotensin-(1–7) improves renal endothelial dysfunction by inhibiting nitric oxide bioavailability in apoE deficient mice

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ApoE-deficient mice (-/-) are associated with endothelial dysfunction caused by a reduced NO bioavailability. Ang-(1–7) in apoE(-/-) mice fed with a lipid rich Western diet were divided into 3 group and treated via osmotic minipump either with saline, Ang-(1–7) or additional with D-Ala-Ang-(1–7) (100 mg/kg/day). In isolated perfused kidneys of Ang-(1–7)-treated apoE(-/-) mice Carbachol induced an improved endothelial dependent vasorelaxation mediated via the AT-1 receptor. Tempol (1mM) ameliorated carbachol induced vasorelaxation in untreated mice whereas no effect was observed in Ang-(1–7) treated mice. Additional treatment with D-Ala-Ang-(1–7), a specific MAS-receptor antagonist attenuated the improved renal endothelial relaxation in Ang-(1–7) treated mice. Endothelium-independent vasorelaxation to GSN showed no differences in kidneys of treated and untreated mice. Hydrogen peroxidase production as well as gp91phox and p47phox expression was reduced in isolated preganglionic arterioles of Ang-(1–7) treated compared to untreated mice, whereas eNOS- and catalse expression was increased. Our data showed that chronic infusion of Ang-(1–7) improves renal endothelial function via the MAS in a model of atherosclerosis.

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 Nito-nitro-L-arginine methyl ester (L-NAME) to induce hypertension. The tail-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 tail-to-lumen ratio with L-NAME treatment (from 0.104 ± 0.002 to 0.130 ± 0.003), 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.

W3.06 Lack of circulating serotonin increases blood pressure in mice

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Serotonin (5-HT) has generally been considered to be a vasoconstrictive and hypertensive factor. Cardiovascular hypersensitivity to 5-HT is a hallmark of hypertension, and plasma levels of free 5-HT are elevated in hypertension. The circulating levels of 5-HT are 90–95% reduced in mice deficient in tryptophan hydroxylase 1 (TPH1), since they are devoid of platelet serotonin. In this study, we investigated basic mean arterial pressure (MAP) and heart rate (HR) and the response to 5-HT, 5-HT receptor agonists and antagonists in TPH1-/- deficient mice on a C57BL/6 background.

MAP in TPH1-/- (n = 21) mice was significantly (p < 0.005) higher under baseline conditions than in wild-type (n = 18) mice (116.1 ± 1.3 vs. 108.2 ± 1.1 mmHg). One minute after injection of the 5-HT receptor agonist, 8-OH-DPAT, MAP decreased in TPH1 knockout mice but not in C57BL/6 mice (-10.3 ± 1.4 vs. -22.2 ± 1.5 mmHg). In the same time, HR fell more strongly in TPH-/- mice (-180 ± 6.16 vs. -49.0 ± 19.6 beats/min, p = 0.002), which may explain the difference in blood pressure reduction. No significant differences in the response to the 5-HT3 receptor antagonist, WAY-106653, were observed. Alpha-methylserotonin, a nonselective 5-HT1 receptors antagonist, induces a dose dependent decrease in MAP in all mice, but TPH-/- animals showed a more pronounced fall. On the other hand, only in C57BL/6 mice, cyproheptadine, a 5-HT4 receptor antagonist, increased MAP.

Our data suggest that the unexpectedly elevated blood pressure in TPH deficient mice is caused by the chronic absence of the ligand for 5-HT, and especially for 5-HT1 receptors, which both show antihypertensive properties.

05.01 Adenosine increases VEGF and decreases soluble VEGF receptor-1 production

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Angiogenesis is largely controlled by Vascular Endothelial Growth Factor (VEGF). The soluble form of the VEGF receptor-1 (sVEGFR-1) neutralizes free VEGF, thereby preventing angiogenesis. This study aimed to determine the effects of adenosine on the production of angiogenic factors. Macrophages obtained from blood monocytes of healthy volunteers were pre-incubated for 15 minutes with 10 μM adenosine before activation for 24 hours with Toll-Like Receptor-4 (TLR4) ligands: heparan sulfate, hyaluronan, IL-1β or LPS. Specific agonists of adenosine receptors (A2a: C141, A1: IB-MECA) and mice over-expressing A2a or A1 adenosine receptor in a cardiac-specific and inducible manner were used. Levels of VEGF, sVEGFR-1, Hypoxia Inducible Factor (HIF)-1α, and peroxisome-proliferator-activated receptor-γ coactivator -1α (PGC-1α) were measured by ELISA and quantitative PCR

Adenosine robustly amplified the secretion of VEGF induced by LPS and hyaluronan (22-fold increase after 24 hours of LPS, P < 0.0001). At the same time, adenosine inhibited the production of sVEGFR-1 induced by LPS. heparan sulfate and IL-1β (43% decrease after 24 hours of LPS, P < 0.008). The effect of adenosine was reproduced by the A2a agonist and in mice over-expressing A2a or A1 adenosine receptor in a cardiac-specific and inducible manner were used. Levels of VEGF, sVEGFR-1, Hypoxia Inducible Factor (HIF)-1α, and peroxisome-proliferator-activated receptor-γ coactivator -1α (PGC-1α) were measured by ELISA and quantitative PCR

In conclusion, adenosine increases VEGF production and antagonizes sVEGFR-1 production through its A2a receptor, confering these cells with a pro-angiogenic phenotype. This could emerge as new therapy of angiogenesis.
Novel and known Angiotensinogen promoter variants and tissue specific Angiotensinogen expression in human kidney and visceral adipose tissue

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Objective: Angiotensinogen (AGT), is involved in essential hypertension especially when obesity-related. Human AGT gene promoter polymorphism has been associated with altered AGT transcription in vitro and in 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 (-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 (-9A/ -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 they might destabilize the binding of factors with specific locations. In contrast, 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 neotestosterone (NT) induced its tyrosine phosphorylation in a Src dependent manner. Furthermore, NA induced an interaction of Hic-5 with paxillin in smooth muscle cells suggesting a potential role of Hic-5 in vasorelaxation. Hsp27 was also shown to interact with Hic-5, and Src which suggest the possibility of interaction between Hsp27 and Hic-5 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. This shows 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 serineprotease inhibitor) in epicardial fat during the development of obesity-mediated cardiac hypertrophy and the effect of vaspin on cardiac fibroblasts. In male C57BL/6J mice significant differences in cardiac hypertrophy were detected after 15 weeks of high fat diet (HFD) and in low fat diet (LFD) control mice. However, heart weight/lean bodyweight ratio significantly increased from 6.42±0.66 g/g to 6.88±0.61 g/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, H9C2 cardiomyoblasts were stimulated with vaspin and leucine uptake was analyzed. Vaspin did not regulate leucine uptake. Next we investigated murine primary cardiac fibroblasts proliferation by BrdU-assay which shows a potent induction by vaspin (1.8-fold, vaspin 1µg/l, p<0.05 vs. 0.5%FBS, and 2.0-fold 0.1µg/l vaspin p<0.005 vs. 0.5%FBS) compared to a 1.9-fold induction by angiotensin II (10µM). The present study identifies 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 PPARalpha-dependent gene transcription in vascular smooth muscle cells

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The study aimed to identify new nuclear cofactors for PPARalpha (peroxisome proliferator-activated receptor gamma)-dependent gene transcription in human aortic smooth muscle cells (HASMC) in order to develop new PPARalpha-ligands with improved clinical safety. Using an Oligo GEArray® Human Nuclear Receptors and Coregulators Microarray, we identified the transcriptional regulator and coactivator modifying High Mobility Group (HMG) A1 protein expressed in unstimulated HASMC. PPARalpha-dependent gene regulation was studied by analysis of PMA-induced MMP-9 (matrix metalloproteinase 9) expression ± pioglitazone (pio) 10µM. PMA (50ng/ml) stimulated MMP-9 mRNA expression by 46.3±22.3-fold (p<0.05 vs. vehicle) which was markedly blocked by pio (10µM). PMA stimulated PPARalpha-dependent MMP-9 promoter activity by 45% in transactivation assays in HEK293 using a pGL3-MMP-9 construct. To evaluate the role of HMG1A, gene-silencing experiments with siRNA for HMG1A were performed (0.1% in HASMC and 80.2% in HEK293 reduction of HMG1A protein expression). HMG1A siRNA completely abolished PPARalpha-mediated MMP9 mRNA repression (control siRNA: pio-mediated MMP9 regulation vs. PMA: -66.8% in HASMC and -59.3% in HEK293 p<0.01; HMG1A siRNA: pio-mediated MMP9 regulation vs. PMA: +10.7% in HASMC and +14.7% in HEK293 vs. PMA p=n.s.). Using ChIP assay we could demonstrate that pi-included PPARalpha activation leads to a potent recruitment of PPARalpha (3.0 fold vs 1.5 fold PMA) and HMG1A complexes (1.24 fold vs 0.61 fold PMA) to the MMP9 promoter in HASMC. In conclusion, HMG1A is required for PPARalpha-mediated repression of MMP-9 gene transcription. Ligand-induced HMG1A-PPARalpha 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


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 GTase-activating-protein (GAP) in this process. Small interfering RNA(s)mediated p190A silencing in VSMC increased basal RhoA activity (9.3±3%; 0.4% of control, n=3) and abolished its activation by 5 min of Ang II stimulation (26.5±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±5% of control, n=4). Using siRNA, we have shown that the tyrosine phosphatase SHP2 was necessary to maintain p190A phosphorylation by Ang II. Using SHP2- mutants we have demonstrated that SHP2 activity decreased the basal activity of RhoA, and in contrast expression of catalytically inactive or phosphorylation-deficient p190A mutants increased the basal activity of RhoA and inhibited its activation by Ang II. Moreover, using siRNA, we have shown that the tyrosine phosphatase SHP2 was necessary to maintain p190A phosphorylation by Akt 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 of left ventricular (LV) filling pressure in patients with a recent acute myocardial infarction (AMI). Materials and Methods: A median of 4 days (IQR: 2–7 days) after admission for AMI, echocardiograms were obtained in 102 consecutive patients. We excluded patients with prior myocardial infarction, significant valvular disease and a known stenosis of >70% in the left anterior descending coronary artery (LAD). LV filling pressure was estimated using the ratio of early transmural flow velocity (E) to early diastolic mitral annulus velocity (E/e’). Transthoracic echocardiographic Doppler recordings of coronary flow in the distal LAD were performed at rest and during Adenosine infusion (140 μg/kg/min). CFVR was calculated as the hyperemic-to-resting coronary diastolic peak velocity ratio. Results: In patients with CFVR below 2.0, an accepted limit for dysfunctional CFVR, E/e’ was 11.9 and in patients with CFVR above 2.0 E/e’ was 9.6 (P = 0.0075). In patients with E/e’ below and above 12, CFVR was 1.65 and 2.0 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.

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.02
Is mean blood saturation (SmbO2) a useful marker of tissue oxygenation?
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Increasingly we are monitoring the distribution of oxygen through the microcirculation using optical techniques such as optical reflectance spectroscopy (ORS) and near infrared spectroscopy (NIRS). Mean blood saturation (SmbO2) and tissue oxygen index (TOI) derived using optical techniques such as optical reflectance spectroscopy (ORS) and near infrared spectroscopy (NIRS) respectively, evoke a concept that we can measure oxygen delivery to tissue to normalising macrocirculatory parameters which does not ensure adequate microcirculation.

Conclusion: Diabetes retinopathy is the leading cause of blindness in Europe’s working age population. Assessment of macular oedema (thickness), an important sequelae of diabetic retinopathy, has recently been revolutionised by Optical Coherence Tomography (OCT) enabling the detection of subclinical thickness changes in the macula. This study aims to examine whether systemic or capillary blood pressures are associated with fovea (central region of the macular) thickness in individuals without diabetes or overt cardiovascular disease. Fovea thickness was determined in the right eye of 28 subjects (age range 26–74 (mean 48) years, 12males) by OCT. Finger nailfold capillaries were canvased 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 16.0confidence 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 1.72μm increase in fovea thickness in the right eye. Systemic blood pressure was not associated with fovea thickness. This study demonstrates that finger nailfold capillary pressure is independently associated with fovea thickness in individuals without diabetes or overt cardiovascular disease. Further research needs to explore whether lowering capillary pressure reduces fovea thickness and thus delay the progression of macular oedema.

PA.03
Upstream and downstream cardiovascular variables are “disconnected” in patients with severe sepsis
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The lethality of severe sepsis is not declining. This may be due to over-emphasis on normalising macrocirculatory parameters which does not ensure adequate microcirculatory function or effective tissue oxygenation. Aim: To examine the correlation of traditional macrovascular recirculation targets (cardiac index (CI), Oxygen delivery (DO2), central venous pressure (CVP) and mean arterial pressure (MAP)) and downstream markers of microvascular or tissue function (lactate, arterial pH and urine output). Methods: Macro and microvascular data were concurrently collected from 22 intensive care patients with severe sepsis on admission, at 6 and 12 hours, using seosaphagel Doppler cardiac output monitoring (Delteix), 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 analysis. 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
Peninsula Medical School, Exeter, United Kingdom

The lethality of severe sepsis is not declining. This may be due to over-emphasis on normalising macrocirculatory parameters which does not ensure adequate microcirculation.

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.05
The effect of glibenclamide on acetylcholine and sodium nitroprusside induced vasodilation 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 response in 14 healthy male volunteers on the skin of the forearm. In the case of endothelium dependent (acetylcholine (ACH) induced) vasodilation, saline solution (control) or solution of glibenclamide (KATP channel blocker) were randomly injected each into distinct forearm following the iotophoresis of ACH. We tested endothelium independent (sodium nitroprusside (SNP) induced) vasodilation by random microinjection of glibenclamide or saline solution each into distinct forearm, followed by the iotophoresis 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 vasodilation 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 (ΔD) were evaluated on each image sequence. Variabilities were presented as the coefficients of variation. Results: The intraobserver intrasession variability was 7%–8% for IMT,

TABLE 1. (6 hr results)

<table>
<thead>
<tr>
<th>Downstream markers</th>
<th>Macrohemodynamic variables</th>
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<td>Fovea thickness</td>
<td>Cardiac Index</td>
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<td>Mean arterial BP</td>
<td>P = 0.4</td>
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<tr>
<td>Lactate</td>
<td>P = 0.9</td>
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<tr>
<td>Urine output</td>
<td>P = 0.4</td>
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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. Intima arterial C and acetylcholine co-infusion was used to assess vascular OS. Allopurinol improved endothelial dependent vasodilatation significantly when compared to placebo (mean: SEM: 93: 8% Vs 145±11%, p<0.006). FMD (mean: SD: 4.1± 1.8% Vs 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 atherothrombotic events in coronary artery disease over and above their current therapies.

Gluomeric 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 uric acid was also measured. Wave reflection 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<0.001). 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 (CDIST).

The FMD method was tested on a total of 20 examinations. An expert analyzed twice the dataset, both manually and automatically. The correlation analysis between automatic and manual FMD has slope 0.98 and intercept 0.01. The coefficient of variation of the automatic system is 3.0%. CIMT measurements were carried out on 150 ultrasound images. The comparison between the automatic and gold-standard measurements shows a bias of 0.001 mm with a SD of the differences of 0.035 mm. As regards the technical reproducibility, the intra-observer variability was 0.36% 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 lower (p=0.0001) correlated with the parameter (r=0.77) evaluated by our system.

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

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 naïve state to Atorvastatin 10mg once daily (1.1% to 4.6%, Z=−2.803, p<0.005). There was no further increase in FMD at higher doses. There was a significant increase in urinary nitric oxide metabolites measured at atorvastatin 80mg. There was no significant change in hsCRP, microalbuminuria, adhesion molecules or selectins.

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

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

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

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

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

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In order to evaluate the level of cardiovascular risk (CVR) and its relation to inflammatory activity in rheumatoid arthritis (RA) 71 young RA patients aged 18 to 44 years were...
screened for CVR, according to European Society of Cardiology Guidelines, 2007. Results: 60.56% of patients were normotensive, 39.44% had mild/moderate arterial hypertension. Patients had no history of myocardium infarction, stroke or diabetes mellitus. 30.98% of RA patients had moderate and 60.02% – high RA activity (DSAS28). 26.76% RA patients had no risk factors (RF). 39.44% – 1 RF, 14.08% – 2 RF and 19.72% – 3 and more RF. Most frequent RF were: hypercholesterolemia, family history of CV disease, abdominal obesity, smoking, hypertension, hyperuricemia, 71.15% of patients had subclinical organ damage (OD): 25.0% – left-ventricular hypertrophy (LVH), 17.3% - increased intima-media thickness (IMT), 26.92% - combination of LVH and increased IMT, 7.69% - slight increase in plasma creatinine. Ankle–brachial index never was < 0.9. 61.29% of normotensive RA patients had subclinical OD and higher DSAS28 (P < 0.05) than normotensives with DSAS28 0.128% of patients had average CVR, 25.35% – low, 2.82% – moderate, 53.52% – high and 5.64% – very high CVR. It was found that age and DSAS28 were significantly associated with CVR in RA patients in a multiple regression model (adj. R² = 0.53). Thus, RA patients had lower favourable CVR profile with high frequency of traditional RF and frequent subclinical OD. Inflammation evidently influences CVR level in RA. Therefore, control of inflammation is essential in RA patients in order to prevent cardiovascular disease onset and progression.

PA.13

Association between LDL subclasses and carotid intima-media thickness in coronary artery disease

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Background: Small LDL subclasses (sLDL) are known to play a causative role in atherosclerosis and its clinical manifestation-coronary artery disease (CAD). Previous studies show increased prevalence of sLDL in CAD patients with normal levels of traditional lipid risk factors. Carotid intima media thickness (IMT) is considered as a marker of atherosclerosis and in prediction of clinical coronary events. With increasing interest in the role of non-traditional lipid risk factors in CAD, we undertook this study to relate LDL subclass profile and carotid intima-media thickness in patients with CAD. 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 mm, and the mean IMT in all patients was 0.89 ± 0.13 mm (0.6–1.2 mm). LDL size was inversely correlated with IMT (r = −0.36; p < 0.01). Prevalence of subjects with increased IMT was higher among subjects with small LDL subclasses (d ≤ 25.5 mm). Conclusion: LDL size shows a strong association with carotid IMT in CAD patients with normal levels of traditional lipid risk factors and may play an important role in assessment of coronary risk in addition to traditional coronary risk factors.

PB.01

Impact of gender and enhanced NO/cGMP signalling on aortic remodelling in experimental hypertensive nephropathy

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Cardiovascular complications represent the most important comorbidity in renal patients, with impaired NO production as key pathway. Estrogens seem to be cardioprotective in premenopause. This study investigates the effects of both gender and enhancing NO/cGMP signalling on aortic remodelling in a model of hypertensive nephropathy. Male (M) and female (F) Wistar rats were each assigned for 18 weeks into following groups: 1) subtotally nephrectomised (SNx); 2) SNx Bay 41–8543-treated, 3) SNx hyalurazil-nitrazide-treated, 4) untreated. Bay 41–8543 is a stimulator of soluble guanylate cyclase. SNx led to an increase in systolic blood pressure, with lower levels in F rats (F-SNx 120 ± 13 mmHg vs. M-SNx 140 ± 11 mmHg). Bay 41–8543 and hyalurazil reduced blood pressure to control levels. Aortic remodelling was characterized by marked media thickening, reduced intima-media ratio and increased media-to-lumen ratio (the latter solely significant in SNx males as indicator of aortic hypertrophy). Bay 41–8543 significantly ameliorated media thickness (111 ± 10 vs. 127 ± 9% of control in M; 101 ± 7 vs. 105 ± 8% of control in F). In media and media-to-lumen ratio (101 ± 13 ± 9% of control in M). Despite the similar reductions in blood pressure, hyalurazil did not alter aortic remodelling. This study indicates that gender regulates hypertensive-uremic aortic wall changes and that enhancing NO/cGMP signalling by Bay 41–8543 significantly ameliorates aortic remodelling in a blood pressure-independent manner.

PB.02

Pressure-induced remodelling in resistance sized arteries cultured for three days in a culture myograph system

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Objectives: Culture myography is an in-vitro ex-vivo technique that allows to study the effects of different stimuli on isolated resistance arteries. It is well known that sustained hypertension determines small arteries remodelling; to date, different roles of wall stress and fluid shear stress in this process are not completely elucidated. Aim of this study was to assess whether different culture pressures can cause different degrees of remodelling in a 3-day-experiment. Methods: Eighteen rats, 6 Spontaneously Hypertensive Rats (SHR) and 12 Wistar-Kyoto rats (WKY), 12-weeks-old were used for the present study. First order mesenteric arteries were mounted in a culture myography system. Vessels were incubated at different pressures (SHR 60 mmHg, WKY 60 mmHg and WKY 35 mmHg) for 3 days. Every day pressure-diameter (P/D) curves (10–140 mmHg) were recorded in the absence of smooth muscle tone. Vessel viability was assessed by norepinephrine-induced constriction. Results: SHR did not show any remodelling during the three days of culture as well as WKY kept at 35 mmHg, WKY cultured at 60 mmHg showed a significant remodelling (p < 0.05) from day 0 and day 3 as compared to WKY kept at 35 mmHg. W/L ratio significantly increased in WKY cultured at 60 mmHg from day 0 to day 3 (day 0: 0.061 ± 0.007; day 3: 0.073 ± 0.002; p < 0.05), but not in WKY cultured at 35 mmHg and SHR. Conclusion: Increase in wall stress per se induces small resistance arteries remodelling, at least in WKY kept at 60 mmHg, possibly due to sustained increase in myogenic tone.

PB.03

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 myograph 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 (Crt: 0.08768 ± 0.00230; 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.

PB.04

Role of angiotensin II in the remodelling induced by chronic changes in blood flow in rat mesenteric resistance arteries in vivo

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6University of Angers, Angers, France, 1CNRS, Angers, France, 2INSERM, Angers, France, 3CHU of Angers, Angers, France

Flow (shear stress)-induced remodelling of resistance arteries occurs in physiological (growth, pregnancy, exercise) and pathological processes such as ischemic diseases, atherosclerosis or hypertension. Angiotensin II has trophic properties and flow activates its production. We investigated the effect of angiotensin I converting enzyme inhibition (perindopril) and angiotensin II type 1 receptor blockade (candesartan) on outward hypertrophic remodelling induced by high blood flow in mesenteric resistance arteries. Arteries were ligated in vivo in order to generate high (HF) flow arteries and analyzed after 1 week. HF arteries diameter increased in association with medial hypertrophy, eNOS overexpression, hypercontractility to angiotensin II and superoxide production. ERK1/2 phosphorylation increased in HF arteries. Perindopril and candesartan, not hyalurazil, prevented hypertrophy 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 (0126) prevented HF remodelling.
Thus, in resistance arteries hypertrophy associated with a chronic rise in blood flow depended on angiotensin 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 (LZ) rats. Mesenteric arteries, alternatively ligated in vivo, were submitted to a high flow (HF) or normal flow (NF) for 21 days and isolated for structural and functional analysis in vitro. By opposition with LZ rats, diameter and endothelium (flow- and acetylcholine)-dependent dilation decreased in HF arteries from ZDF rats. The chronic increase in flow induced in a resistant artery in eNOS and NADPH-oxidase subunits (gp91 an gp7) expression as well as a rise in superoxide production in LZ and ZDF rats. Acetylcholine-induced dilation in NF and HF arteries from ZDF rats was improved by an acute antioxidant (tempol). In ZDF rats, diameter and endothelium-dependent dilation was restored to a level similar to that observed in LZ rats HF arteries. In conclusion, acute and chronic (tempol) antioxidant treatment improves endothelial function in diabetes.}

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 rat fetuses were fed low (0.15%), medium (1.3%), and high (8.0%) salt diets 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. There were no differences in blood pressure 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 offspring of HS mothers irrespective of the diet post-weaning (HS, LS), the same was true for muscular arteries in the systemic (mesenteric) and pulmonary circulation. Serum ADMA was significantly higher in offspring of HS mothers irrespective of the diet post-weaning (HS, LS), and in offspring of LS mothers switched to HS compared with offspring of MS and LS mothers maintained on LS post-weaning. High salt intake in pregnant rats has long-lasting effects on modeling of central and muscular arteries independent of postnatal salt intake and blood pressure.

PB.07
Regulation and actions of Cardiotrophin-1 in cultured rat vascular smooth muscle cells
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Cardiotrophin-1 (CT-1) is a cytokine belonging to the interleukin-6 family that exhibits trophic and survival properties in a number of cell types. CT-1 protein expression has been identified within the media of ath erosclerotic arteries, but its role in the vessel is still unknown. This study aims to characterize the actions and regulation of CT-1 in vascular smooth muscle cells (VSMC). Rat aorta VSMC were stimulated with vehicle or CT-1 (10^{-11}-10^{-8} M) for up to 48 hours, without and with antibodies against CT-1 receptors. In addition, the effects of aldosterone (10^{-10}-10^{-8} M) and angiotensin II (10^{-10}-10^{-8} M) on CT-1 expression were also evaluated. Cell proliferation was assayed by MTT assay. The expression of CT-1, collagen type I and fibronec tin was quantified by Western blot. Matrix metalloproteinases (MMPs) activities were assessed by gelsatin and casein zymographies. A 48-hour treatment with CT-1 induced VSMC proliferation in a dose-dependent manner (p<_0.01), 24-hour incubation with CT-1 led to an increased expression of collagen type I (p<_0.01) and fibronectin (p<_0.05), with a parallel and dose-dependent increase in active MMP-2 (p<_0.01), MMP-3 (p<_0.05) and MMP-9 (p<_0.01), all of these effects being reversed in the presence of antibodies against CT-1 receptors. Whereas VSMC 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 in alterations of these cells caused by the RAAS in vascular diseases.

PB.08
Nitric oxide activates the Vav3-Rac1 pathway via RhoA phosphorylation to promote aortic smooth muscle cell migration
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Nitric oxide (NO) is well known for its ability to induce angiogenesis and arteriogenesis. Although numerous studies described its effects on endothelial cells, very little is known about its role on vascular smooth muscle cells (VSMC) migration. We previously shown that in VSMC, RhoA is phosphorylated on Ser188 by 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), phosphomimetic (S188E) and double-mutants Q63L-S188A and Q63L-S188E regulated 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) accelerated the wound-closure in VSMC expressing the WT RhoA but not in VSMC expressing the phosphosensitive mutant S188A. RhoA phosphorylation induces Rac1 localization 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 RhoA exchange factor Vav3 is necessary for this activation.

PB.09
The ouabain-sensitive isoform of Na+ pump regulates vascular gap junctions via interaction with the Na+ /Ca2+ exchanger in macrophage 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 the ouabain-sensitive isoform of the Na+–pump and the Na+/Ca2+exchanger leading to increases in [Ca2+]i in discrete areas near the plasma membrane. This suggests close association of these transport proteins in microdomains. Using PCR and co-immunoprecipitation we aimed to test this hypothesis in SMCs from mesenteric small arteries and in AT5 cell line. Intercellular electrical coupling was evaluated in functional studies. SMCs were electrically uncoupled when the ouabain-sensitive Na+–pump was inhibited by 10 μM ouabain. Inhibition of the Na+/Ca2+exchanger with 1 μM SEADO400 also uncoupled the SMCs. Depletion of [Na+]i and clamping [Ca2+]i at low levels prevented the uncoupling. Ten μM ouabain evoked spatially restricted [Ca2+]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+ exchange-1 and connexin-43. The α3 Na+/pump subunit was not associated with these proteins but co-immunoprecipitated with caveolin-1. 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+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 from passages 2 and 18 or platelet microvesicles (PMV) were incubated with recombinant human platelet-free citrated plasma and recombinant human tissue factor at 0, 25, 250, and 1000 pM. Thrombin formation and its delimitation by APC were assessed by the endogenous thrombin potential without APC (ETP) and APC concentration reducing ETP by 50% (IC50-APC) using thrombography. Procoagulant phospholipids on SMCs and PMV were quantified by phospholipid-related procoagulant activity (phosphatidylserine equivalents). Both thrombin generation and inhibition were supported by SMCs. Mean ETP, 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 phosphatidylserine equivalents for passages 2–14 and 561 ± 55 nM for passages 15–18, suggesting a role of cell differentiation. No significant correlation was observed between this amount and ETP. Similar ETP, were observed with SMCs and PMV where 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.

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

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We have recently shown that α1 integrin plays a major role in hypertrophic response to angiotensin II in smooth muscle cells (SMCs) and in mechanical properties of the vascular wall (Louis H et al, Am J Physiol Heart Circ Physiol. 2007). In this study, the objective was to investigate the expression of various integrins during SMCs differentiation in response to cyclical mechanical stretch. Rat SMCs were plated on silicone elastomer–bottomed culture plates precoated with collagen type I (Falcone), and subjected to cyclical stretch with a Cyclic Stress Unit (VR4000, AFC-CTL, France). Deformation (1 Hz) and 10% elongation were applied from 1 up to 5 days. At day 2 cyclical 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 1.8 fold for heavy-caldesmon, which was maintain up to day 5. Under basal conditions SMCs express β1, β3, α1, 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 detected 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, α1, α5 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 atherosclerosis in rats by preventing proinflammatory 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 48 h 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 (1.8 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 surgeries with tiny blood vessels, which are needed in hand, plastic and reconstructive surgery. Culturing endothelial cells (EC) on a random positioning machine (RPM) with 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 model the intima and to generate intima-like structures, which will be useful in blood vessel engineering and basic science.

PC.01

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-aortal 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 - 5 μg/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= from 538 ± 12 to 624 ± 29 bpm; WT = from 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 in WT) 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.

PC.02

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

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

PC.03 Telmisartan attenuates outward aortic remodeling associated with diet-induced obesity

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We investigated the impact of the AT1 receptor antagonist Telmisartan on vascular outward remodeling in a diet-induced obesity-model in C57/Black6J mice. Mice were fed with a high-fat diet (HF, 5% kcal from fat) for 14 weeks. One group received additional treatment with telmisartan (3mg/kg/d) for the last 4 weeks of diet. Animals with a low-fat diet (LFD, 10% kcal from fat) served as controls (n=10 in each group). Aortic diameter was measured by ultrasound biomicroscopy. Aortic tissues were studied using morphometric histology, immunohistology, quantitative real-time RT-PCR and immune-blotting. In the HF group, the aortas were diluted compared to controls (1.1±0.04mm vs. 0.9±0.03mm, p<0.001). MMP2 mRNA expression was strongly up-regulated in the media (4-fold, p<0.05). MMP9 was increased in media and adventitia (4- and 10-fold, respectively, p<0.05). TNF-α was strongly up-regulated in the adventitia of the fat-diet group as compared to LFD-treated animals (10-fold, p<0.05). In addition, TNF-α was co-localised with adipocytes in the adventitia, suggesting that mainly these cells contribute to inflammatory processes during vascular remodelling. Telmisartan abolished aortic dilatation (0.68±0.04mm vs. 1.1±0.04mm, p<0.001), prevented cystic media degeneration, preserved elastin content and significantly decreased elastin fragmentation.

PC.04 High prevalence of prolonged QTc interval in Type 2 Diabetes in the South Asian population in UK: The UK Asian Diabetic Study

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Objective: Corrected QT (QTc) prolongation is a promising predictive value of cardiovascular mortality in Type1 diabetic. As part of the UKADS Prospective Study, we have assessed the prevalence of prolonged QTc in South Asian people cohort with type2 diabetes in a local population attending diabetic services at a teaching hospital setting. Research Design and Methods: Of the 393 type 2 diabetic South Asian subjects, a total of 345 patients were included in the study. As a baseline, demographic data, HbA1c, duration of diabetes, blood pressure and ECG were done. All the anterior chest leads of the ECG were independently assessed by five different clinicians. QTc intervals were obtained using Bazett’s equation, QTc >0.44 s was considered abnormally prolonged. The data was analysed using SPSS. Results: Cumulative prevalence of prolonged QTc in the South-Asian cohort was 35.01%, which is significantly higher than Caucasians. Interestingly there was significant female predilection (women vs. men: 42.44 vs. 27.74 %, P < 0.004). Conclusions: In type 2 diabetic subjects from the UKADS cohort, there is strong correlation between female sex and QTc interval. There was also direct correlation between duration of diabetes and hypertension. However, there is no demonstrable correlation between QTc and age.

PC.05 In vitro hypercoagulability in an early phase of the metabolic syndrome in Zucker rats

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We investigated whether an increased reactivity of the clotting system was correlated with changes 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 and the elastic modulus were measured by an echotomography 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±2 versus 158±2 mmHg). Cholesterolemia and glycemia were increased in fa/fa rats. Carotid arterial diameter and arterial thickness were not significantly different between both groups. Elastic content in the aorta was significantly reduced in fa/fa but this was not associated with changes in distensibility, elastic modulus and PWV. In contrast the total thrombin activity was significantly different between fa/fa and FA/FA rats (420±29 versus 252±43 mIU.min). This occurred independently of platelet activation, was associated with an increase in fibrinogen (4.9±0.2 versus 3.2±0.3 g/l) but was not paralleled by a modification of traditional plasma markers of thrombin generation in vivo.

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

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Background: Dietary sodium intake is involved in the pathogenesis of left ventricular hypertrophy (LVH), also independently of blood pressure. Nonetheless, it has not been investigated yet whether obesity can influence the relationship between salt intake and left ventricular mass (LVM). Obesity may influence LVH through inappropriately “normal” aldosterone levels. Methods: 150 overweight-obese essential hypertensives (<65-year-old, glomerular filtration rate >55 ml/min) were studied. Anthropometric and echocardiographic parameters, plasma aldosterone, 24h urinary sodium (NaU) and aldosterone were collected. Patients were classified as overweight (OW), grade 1 obesity (OB), and grade 2+ (OB+). LVM was indexed by body surface area or height2/3, and LVH was defined by 3 different criteria. NaU was considered as continuous or categorical cut-off: median value 114 mEq/24h variable. Logistic and linear regression analyses were used to estimate the effect of 24h NaU on LVM, adjusting for covariates and separately for BMI classes. Results: With each LVM definition, 24h NaU was a significant and independent factor of LVH. A NaU above 114 mEq/24h conferred an independent risk of LVH (OR 2.41, 95%IC 1.14-5.11, p<0.02). The linear relationship between 24h NaU and indexed LVM increased with increasing BMI (r=0.1 in OW, r=0.33 in OB, and r=0.37 in OB+). Plasma and urinary aldosterone levels were not reduced in the patients with higher NaU. Conclusions: Dietary sodium intake appears a significant and independent factor of LVH. The effect of LVM is increased by adiposity, most likely because plasma and urinary aldosterone levels were not adequately reduced in OW/OB patients with higher NaU.

Diabetes-accelerated elastocalcinosis is dependent on advanced glycation endproducts accumulation

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Duration and severity of diabetes is associated with advanced-glycation endproducts (AGEs) accumulation and an acceleration of elastocalcinosis. We propose that the acceleration of elastocalcinosis is related to AGEs deposition and AGEs receptor (RAGE) activation. Male Wistar rats received a high fat diet during 2 months and then a low dose injection of streptozotocin (30mg/kg i.p.) to induce diabetes (D). Elastocalcinosis was induced by warfarin and vitamin K (WK). We started the WK treatment 28 days after the injection of streptozotocin for 3 or 7 weeks. Pyridoxamine or ALT711 were administrated after the streptozotocin or 3 or 7 weeks after the beginning of WK treatment, respectively. Ex vivo, femoral arteries from normal Wistar or streptozotocin-induced diabetic rats were incubated in normal media or a calcifying media (CM). N-methylpyridoxin, an agonist of RAGE, was added to normal media and CM. Inhibitors of several signaling pathways of RAGE were added.In this animal model, femoral arteries presented simplified calcification and AGEs deposition on collagen. These two parameters were significantly reduced by pyridoxamine and with ALT711. Ex vivo, calcification was enhanced by N-methylpyridoxin only in arteries of diabetic rats. Only inhibitors of ERK1/2, JAK2 and Ras were able to limit the augmentation of elastocalcinosis. Diabetes-accelerated elastocalcinosis was prevented with pyridoxamine and limited by ALT711. Moreover, the stimulation of RAGE enhanced calcification ex vivo. It suggests that, in diabetes, elastocalcinosis is linked to AGEs formation and to its interaction with RAGE.
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). Method: Brachial artery FMD was performed on 40 IGT patients and 24 controls using real time ultrasound analysis (SonoSite, X-51B). We also performed sympathetic activation of the first 15 second postocclusive vasoconstriction measured using pulsed Doppler and analysing 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 (dynes/cm²) was also significantly reduced in IGT mean 23.15 +/- 18.92 control mean 32.58 +/- 15.63, p = 0.042. There was a significant difference in frequency band 7 of the reactive hyperemia velocity waveform (IGT mean 11.49 +/- 3.22; control mean 9.68 +/- 2.84; p < 0.05). Resistive index was not significantly different between the two groups. There was a significant correlation between DSS and FMD (R²= 0.501, p<0.001). Conclusion: These results suggest that microcirculatory dysfunction is present in IGT, represented by reduced shear stress stimulus and impaired FMD.

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 also have altered sympathovagal function. 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 syndrome was defined by the NCEP-ATPIII criteria. Methods: 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 frequencies 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) and DSS (Dyne/cm²) was also significantly reduced in Indian Asians (control mean 23.15 +/- 18.92 control mean 32.58 +/- 15.63, p = 0.042). There was a significant difference in frequency band 7 of the reactive hyperemia velocity waveform (IGT mean 11.49 +/- 3.22; control mean 9.68 +/- 2.84; p < 0.05). Resistive index was not significantly different between the two groups. There was a significant correlation between DSS and FMD (R²= 0.501, p<0.001). Conclusion: These results suggest that microcirculatory dysfunction is present in IGT, represented by reduced shear stress stimulus and impaired FMD.

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. Methods: MS patients were studied for the following variables: basal Doppler flux (LDF; PeriFlux 4001) in the foot. The following variables were measured: basal LDF (b-LDF), postocclusive hyperemia (m1-LDF), vasoconstrictor response (v-LDF) to deep inspiration on the pulp of the toe, and heat (44 oC; PeriTemp 4005) induced hyperemia (m2-LDF) on the dorsum of the foot. Results: b-LDF and local skin temperature did not differ between the study groups (p>0.05). Only the patient group with diabetes demonstrated a significant diminution in v-LDF compared to the group of healthy subjects (p<0.05). m1-LDF was decreased in both patient groups in comparison with the group of controls (p<0.05), but only in diabetics the decrease of m2-LDF was significant (p<0.05). TNF-alpha level was elevated in both patient groups. Conclusions: Our findings show that MS patients with insulin resistance have significant cutaneous vasomotor dysfunction and elevated TNF-alpha level.

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 MPs in vivo treatment of circulating MPs from healthy subjects and patients with metabolic syndrome (MS) on vasomotoricity. Patients with MS were selected as controls (C). The study groups were matched for age and sex. MPs obtained from whole blood either from patients (MSMPs) or healthy subjects (HSMPs) or vehicle were injected i.v. to mice. MSMPs injected 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-synthase. Interestingly, the NO-synthases inhibitor completely reversed the hypo-reactivity induced by MSMPs. Also, MSMPs increased 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 aorta. Interestingly, pre-incubation of MSMPs with anti-FasL-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=115), and their matched controls (n=151), using 600 MHz 1H NMR spectroscopy. Following baseline and phase correction, unsupervised and supervised chemometric techniques (principal component analysis and orthogonal partial least squares discriminate analysis respectively) were applied to build models to explore whether biochemical profiles differed between groups. Model stability was tested using a 1.000 resample bootstrapping procedure. Results: The mean age of the whole cohort was 58.2 years, with 52% women. Mean blood pressures (mmHg): untreated cases-169/104, treated cases-155/94, and controls-123/78. Mean BMI (kg/m²): untreated cases-25.4, treated cases-27.6, and controls-25.2. Models comparing both untreated hypertensives vs. controls, and treated hypertensives v. controls, obtained
reasonable separation between groups (RFX=0.29, QY=0.62 and RFX=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 = 6632) and whether this was associated with the beneficial effects of E across cardiovascular outcomes. Methods: A diuretic effect was indirectly defined as a 1 month vs baseline weight decrease ≥ 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, weight decreased (-0.001), whereas blood protein increased (p=0.0001) and serum K increased (p=0.0001) as compared to P. K-sparing was independently associated with lower all-cause mortality [HR 0.83 (0.71–0.96); p=0.012] as well as lower CV death or CV hospitalization [0.76 (0.67–0.87); p<0.0001]. A diuretic effect [1.15 (1.02–1.30); p=0.025], was independently associated with a worse CV outcome. There was no statistically significant interaction between the beneficial effects of E on CV outcomes and K-sparing or diuretic effect. Conclusions: Although a diuretic effect is associated with worse CV outcome, beneficial effects of E on survival and CV outcome are independent from its K-sparing and diuretic effects. This suggests that aldosterone antagonism provides a cardiovascular protection beyond its diuretic and K-sparing properties.

PD.02
Albumin:creatinine ratio at presentation predicts mortality and functional outcomes after stroke
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Background: Elevated albumin:creatinine ratio (ACR), a proxy for systemic microvascular functional deterioration, predicts stroke re-infarction and post-myocardial infarction mortality. We explored associations between ACR and recovery post-stroke. Methods: ACR was measured in 57 consecutive stroke unit admissions. Barthel Index (BI), a measure of patients’ function in rehabilitation outcomes. Conclusions: Presentation ACR, as a proxy of microvascular function, predicts stroke incidence and post-myocardial infarction mortality. We explored associations between ACR and recovery post-stroke.

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 infra renal inferior vena cava. The diagnosis of aldosterone-producing (APA) was based on pathology and follow-up data. Results: On the right side we detected no accessory veins in 44% and hepatic and capsular accessory veins in 11% and 45%, respectively. On the left side capsular and phrenic accessory veins were seen in 11% and 69%, respectively. No effect of capillary 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<0.05), even when adrenal catheterization was selective. Conclusion: Hepatic accessory veins draining into the right adrenal vein, which is feasible by volumetric angiography, can predict a low SI even in selectived rejective 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?
Cooke JP1, Carew SB1, O’Connor M, Moilef C, Sheeby TA, Costelloe CA, Lyons DJ1
Mid-Western Regional Hospital, Limerick, Ireland

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

PD.05
Symptomatic orthostatic hypotension: is it how far you fall or how low you go?
Coike JP1, Carew SB1, O’Connor M, Costelloe CA, Sheeby TA, Lyons DJ1
Mid-Western Regional Hospital, Limerick, Ireland

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 EPHESUS (n=6632) and whether this was associated with 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.

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blood pressure of ≥10 mmHg within 3 minutes of orthostatic stress. We suggest a new classification system for OH which should result in more focussed treatment. **Methods:** Utilising total peripheral resistance (TPR) and cardiac output (CO) measurements obtained during tilt-table testing (TNT: Finometer, Modelflow method), we analysed haemodynamic parameters of 110 patients with OH. We applied our proposed classification system and categorized them as arteriolar, venular, or mixed. In arteriolar OH, absence of the normal compensatory increase in TPR after orthostatic stress would reflect impaired peripheral vasoconstriction. In venular OH, a reduction in CO after orthostatic stress often despite marked tachycardia, suggests that the predominant defect is an excessive reduction in venous return. Mixed OH is due to a combination of both these mechanisms. **Results:** Significant differences between the groups were found 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.

**PD.07**

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

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Hypertension is a common risk factor for stroke/TIA and there is good evidence that blood pressure (BP) control prevents recurrent stroke. Our aim was to investigate whether regular Telephone Follow-Up (TFU) improved risk factor management in hypertensive patients after a single visit to a Stroke Clinic. We conducted a RCT and assigned hypertensive patients within 1 month of stroke or TIA to receive TFU (n=29) or no TFU (n=27). Our primary outcome was the difference in 12-month 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 promotes patient-led management of risk factors was ineffective in improving BP control, or in increasing the number of antihypertensive agents taken, over six months follow-up in primary care after Stroke/TIA.

**PD.08**

Gender differences in the cross-sectional relationships between sleep duration, interleukin 6 and high sensitive C-reactive protein the Whitehall 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, a gender interaction was noted (interaction p=0.04) (interaction p=0.05). **Conclusions:** There were no significant differences in inflammatory markers with sleep duration was observed in men. By contrast, both IL-6 and hs-CRP levels vary with sleep duration in women. The observed pattern of variation was different according to the inflammatory marker observed. Further longitudinal studies are required to fully investigate possible temporal relationships between short sleep and markers of inflammation.

**PD.09**

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

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1Warwick Medical School, Coventry, United Kingdom, 2Federico II University, Naples, Italy, 3Imperial College London, London, United Kingdom

**Background:** Circulating endothelin levels are associated with atherosclerosis. Moreover, ethnic differences in pro-inflammatory markers may be associated with ethnic differences in atherosclerotic and cardiovascular (CVD) and coronary heart disease (CHD) risk. **Objective and Methods:** To investigate ethnic differences in circulating plasma endothelin levels, its soluble receptor (sEDR), and high-sensitivity CRP (hs-CRP). 193 individuals, aged 40–59 years, 62 white (30 women), 66 of African origin (33 women) and 63 South Asians (33 women), free from coronary heart disease (CHD), stroke, CVD and diabetes were randomly selected from the UK ‘Wandsworth Heart and Stroke Study’. **Results:** Age-adjusted endothelin levels were higher in women than men in 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)). Endothelin 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 sEdr were varied by ethnic group (p=0.001) but was not associated with endothelin. **Conclusions:** This study is the first to demonstrate a significant trend for increasing endothelin levels from black Africans to whites to South Asians, which is consistent with the ethnic difference in CHD risk. Whilst these findings support the concept that the innate immune system (IS) may contribute significantly to the metabolic component underlying the development of CVD and CHD risk, further studies are required to see if endothelin levels are causally related to the development of CVD.

**PD.10**

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

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

**PD.11**

Neopterin levels in coronary artery disease subsets

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Inflammation plays an important role in the pathogenesis of atherosclerosis. In coronary artery disease (CAD) the release of different cytokines activates cellular defense. Activated macrophages synthesize metalloproteinases and nopterin; a plasminogen 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 2006 to December 1, 2008 built the study group. Control group constitute of healthy volunteers. ACS patients were categorized into three subgroups according to CFI findings and cardiac enzymes (markers ); Unstable angina pectoris (USAP), NSTMI and 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 theraphy 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 (SR) 1.5mg with the optional addition of perindopril 2–4mg. Depression scores were collected via the 15-item Geriatric Depression Scale (GDS) at baseline and annually. Completion was voluntary (69% of patients completed the GDS at baseline). *Results:* 2656 completed questionnaires were received with GDS information. Mean age was 83.5 years, 60.6% female and mean SBP 173mmHg. Hazard rates (HR) with 95% confidence intervals adjusted for age, gender, treatment allocation, country area, educational level, living alone, number of comorbidities, previous CV disease, previous treatment and previously diagnosed hypertension for total mortality was 1.01 (0.95–1.07) and 0.99 (0.92–1.06) respectively for the placebo and active treatment groups. *Conclusion:* Placebo had no impact on the activation of the SNS during the CPT. 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.

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

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Purpose: The purpose of this study was to investigate the relationship between two markers of inflammation (CRP, fibrinogen) and blood pressure with obesity, an established causative factor in the development of hypertension. The aim was to determine whether markers of inflammation are related to obesity and whether these variables are linked to blood pressure variables. This additional finding suggests the use of antihypertensives with antidepres- sants in the very elderly may be beneficial and further research is required.

**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 posses 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 effect of eprosartan on renal tubular function, systemic hemodynamics and vasoactive hormones was measured before, during and after a cold pressor test (CPT) and sodium nitroprusside (SNP) infusion in a randomised, 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 excretion of sodium (0.23 ± 0.22 %, P < 0.01) and lithium (21.2 ± 17.7 %, P < 0.01). The increases in HR and plasma levels of norepinephrine during the SNP infusion were similar after both treatments. Eprosartan compared to placebo had no impact on the activation of the SNS during the CPT. *Conclusion:* These findings do not support a direct sympatho-inhibitory effect of eprosartan. In fact, these results suggest that eprosartan treatment increased renal sympathetic nerve activity during arterial baroreflex mediated activation of the SNS – the likely result of an increased sensitivity of the arterial baroreflex in the control of renal sympathetic nerve activity.

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

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

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Introduction: The benefits and physiological changes attributed to orthostatic training in vasovagal syncope (VVS) have never been evaluated against placebo. We studied the changes in baroreflex sensitivity (BRS) in response to home orthostatic training (HOT) in a randomized, placebo-controlled pilot study. Methods: 22 subjects, aged 18 to 65 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 enrolment, week 1, week 4 and week 24. Sympathetic recurrency was assessed with event diary, while diastolic flow waveforms in the intervention group and 2 (20%) subjects in the placebo group were syncpe 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<0.03; 1.21 vs. -1.26 mmHg/mmHg, p<0.05). Maximum improvements were seen at week 4 and sustained up to week 24.

Conclusion: Our pilot study demonstrated positive trends in symptom benefit and significant improvements in BRS with HOT in patients with VVS.

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


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In this study we aimed to determine the risk profile of young patients with coronary artery disease (CAD), including new emerging risk factors as lipoprotein (a), uric-acid, abdominal obesity and whether premature onset of coronary atherosclerosis is associated with increased levels of lipid peroxidation expressed as thiobarbituric acid reactive substances (TBARS) and antioxidant enzymes as glutathione peroxidise (GPx), superoxide dismutase (SOD). This study included 55 patients, < 40 years of age (mean age 35.7±4.1) with angiographically proven CAD and 31 age and gender matched healthy controls (mean age 39.8±5.2). Lipid profiles (34±3 vs 20±19 mg/dl, p<0.03), uric acid level (5.3±4.1 mg/dl vs 4.15±0.005) and increased lipid peroxidation expressed as TBARS (6.35±2.9 vs. 4.67±0.7 mmol/l) were significantly higher in patient than in control. The activities of antioxidant enzymes (GPx, SOD) were significantly lower in patient than in control. Conclusion lipid peroxidation is significantly elevated in young patients with stable CAD and can be used as a risk marker. Increased lipid peroxidation despite antilipid therapy and similar modifiable risk profile with controls may be an indicator of underlying genetic factors or increased oxidative stress in young patients with CAD.

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


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The effects of changes in flow velocity waveform and arterial geometry before and after percutaneous coronary intervention (PCI) in a right coronary artery (RCA) were investigated using computational fluid dynamics. An RCA from a patient with a stenosis was reconstructed based on multislice CT images. A non-stenosed model, simulating the same RCA after PCI, was also constructed. Blood flows in the RCA models were simulated using pulsatile flow waveforms acquired with an intravascular ultrasound Doppler probe in the RCA of a patient undergoing PCI. It was found that differences in the waveforms before and after PCI did not affect average-waved wall shear stress (TAWSS) and oscillatory shear index (OSI), but the phase-angle between pressure and wall shear stress on the endothelium (stress phase-angle: SPA), differed markedly. The median SPA was -53.9 deg (range -204 to -10.0 deg) for pre-PCI state whereas it was 10.4 deg (range -71.1 to 25.4 deg) in the post-PCI state; i.e. more asynchronous in the pre-PCI state. SPA has been reported to regulate secretion of vasoactive molecules (e.g. NO, PGF, and ET-1) and asynchronous SPA (~190 deg) is proposed to be pro-atherogenic.1 Our results suggest that the haemodynamics in the RCA was improved markedly after PCI in terms of SPA, and differences in pulsatile flow waveform may have an important influence on atherogenesis, although associated with only minor changes in TAWSS and OSI. SPA may be a useful indicator in predicting sites prone to atheroerosion.


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-one patients with echocardiographic LHV were randomly assigned to active treatment arm (extra antihypertensive medica- tion) or placebo in a ratio of 2:1. Cardiac magnetic resonance imaging (CMR) 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 122.9±15.6mmHg in the active group and 123.8±18.2mmHg in the placebo group (p = 0.646). The mean baseline CMR LVMi was 59.16±18.5mg/m² in the placebo group and 65.89±9.4mg/m² in the active group (p = 0.1). The mean difference between baseline and end of study blood pressure was – 9.3±3mmHg in the active group and – 0.08±4mmHg in the placebo group (p = 0.007). This is a much greater BP fall than, for example, the HOT study. The mean change in CMR LVMi was – 4.68±1.2mg/m² in the active group and +1.97±2mg/m² in the placebo group (p = 0.014). Conclusion: It is possible to cause LHV regression if BP is reduced further, even when baseline BP begins below target BP. It may translate into a reduction in cardiovascular events in those with normotensive LHV.

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

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Background: The novel speckle tracking echocardiography allows measuring left ventricular segmental strain parameters from 2D gray scale images. On board integration of this method (AFI) into echocardiography machines enables quick, automated evaluation of left ventricular function. Aim: In our study, we aimed to evaluate LV longitudinal deformation properties in patients with untreated hypertension and compared them with age and sex matched healthy subjects. Method: 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 AIC, A2C and APLAX 2D images. GlS and its relationship to LV mass indexed (LVMi) was compared between groups. Results: There was no difference between the two groups regarding demographic data. LVMi in Group HT (M=147.48 gr/m²; SD=12.08) was significantly higher relative to Group N (M=91.85 gr/m²; SD=9.19 (F45) ~19.27, p<0.001). LV global longitudinal strain in Group HT (M=−19.30% SD=0.72) was remarkably lower comparative to Group N (M=−22.66%, SD=0.89% (F45) ~15.48, p<0.001). A linear regression analysis revealed that increased LVMi was a highly significant predictor of reduced GLS (R² = 0.966, p<0.001, Y = −0.061*X +28.31). Mean calculation time of AFI was 82 seconds (SD=4.45) Conclusion: LV concentric hypertrophy due to hypertension reduces left ventricular systolic performance as assessed via GLS, even though conventional systolic performance (like EF) is normal or even higher in these patients.

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


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Aims: The aortic valve area (AVA), in aortic stenosis (AS), is estimated by Doppler echocardiography, by the application of the continuity equation. VTI ratio is commonly recommended for its calculation with the ratios of peak velocity as an acceptable short-cut alternative. We investigated if the increased time required calculating the VTI is justified for example, the HOT study. The mean change in CMR LVMi was – 4.68±1.2mg/m² in the active group and +1.97±2mg/m² in the placebo group (p = 0.014). Conclusion: It is possible to cause LHV regression if BP is reduced further, even when baseline BP begins below target BP. It may translate into a reduction in cardiovascular events in those with normotensive LHV.

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

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


**Background:** Non-invasive blood pressure monitoring by continuous finger photoplethysmography (Finometer) is valuable in the optimisation of biventricular pacemakers. However, the immediate increment in blood pressure (BP) diminishes after a few seconds and it is unclear whether this is due to a fall in stroke volume or instead a (desirable) compensatory reduction in peripheral resistance. This study explores this question by measuring stroke volume using Doppler echocardiography as a gold standard, and BP using Finometer during and after (atrio)ventricular AV delay adjustment. **Design:** Cardiac output and BP were measured using a Finometer whilst AV delay was changed from 40ms to 120ms in 19 subjects with pacemakers. Simultaneously the velocity time integral of left ventricular outflow tract Doppler was used to calculate stroke volume and hence cardiac output (Qecho). **Results:** The immediate effect of an AV delay change on mean arterial pressure (MAP) and Qecho correlated well across all patients (r = 0.74, p < 0.0001), however after a few seconds, MAP gradually falls (0.65mmHg/beat, r = 0.12). 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 optimization of AV delay, the abrupt rise in cardiac output is followed by a compensatory fall in peripheral resistance which causes cardiac output to fall, whilst stroke volume is maintained. Optimisation of AV delay using a Finometer should be undertaken immediately following changes in pacing configuration to minimise information loss from vascular compensation.

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


**Aims:** Step changes of heart rate between two values, using a cardiac pacemaker, generates periodic oscillations in end-tidal carbon dioxide (et-CO2) and ventilation. This could be developed to treat periodic breathing in heart failure. We studied whether gradual variation of cardiac output can achieve comparable ventilatory effects with less sudden changes in blood pressure (BP). **Methods:** We applied fluctuations in heart rate (by 30 bpm) or AV delay (between 30–120ms) or both, with period of 60 seconds, in 19 heart failure patients (age 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-peak BP swing was almost twice as large with square wave than a sine wave (22.4 ± 11.7 mmHg versus 13.6 ± 4.5 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 over 5s versus 7.9 ± 3.2mmHg over 5s, p < 0.01). **Conclusions:** When developing therapeutic pacemaker algorithms, a sine wave pattern is preferable to a square wave, because it minimizes the BP fluctuation yet achieves comparable ventilatory effects.

**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 6.4 years (range: 4.5 to 8 years). **Results:** 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 PAP 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 (LMI) 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.

**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 concealing by conventional EF.

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

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The duration of systole and diastole as a function of heart rate provides important information on heart functionality. In this study we assessed the feasibility of the cardiologic systole and diastole times measurement by a precordialcutaneous 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 71M/32F, age 57 ± 14 years, 17 healthy people 86 patients with cardiovascular diseases. Consistent first and second heart sound signals were obtained in 86% patients at rest and during stress. The diastolic time decreased from 541 ± 143msc to 250 ± 53msc, 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 (0.88 ± 0.11), valvular (0.83 ± 0.14) or dilated heart (0.68 ± 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.
WNK1 – functional analyses of variants associated with blood pressure and essential hypertension

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Gain of expression mutations in WNK1 cause Gordon’s Syndrome, a rare disorder characterised by hypertension and hyperkalaemia. We have previously reported association between WNK1 and BP in the BRIGHT Study and 24-hour ambulatory BP in the GRAPHIC Study. The associated SNPs map to the promoter and regulatory regions in intron 1, suggesting that changes in WNK1 expression may contribute to BP and risk for the general population. This study was performed to investigate whether associated variants affect gene expression. Two SNPs were selected for functional analysis, one promoter variant (rs1468326 C/A) and an intron variant (rs765250A/G). Carriers of allele A for rs1468326 have on average lower SBP (-5.05 mmHg 95%CI (-9.21,-0.66), p=0.015) and are at increased risk for hypertension (OR 1.34 95%CI(1.05,1.70),p=0.013). For each SNP, multiple copies of each allele were cloned into pGL3 and transfected into HEK293 cells, followed by luciferase assay. Reporter assays demonstrated that rs1468326C had lower activity than rs1468326A, ~3.36 fold decrease in luciferase activity 95%CI (-3.83,-2.80), p= 3.6 x 10^-10. The intronic SNP, rs765250A showed a ~1.50 fold increase compared to rs765250G, 95%CI (1.10,1.90), p= 1.14 x 10^-4. These results correlate well with the reported genetic associations. The SNPs associated with increased (decreased) BP also demonstrate increased (decreased) reporter activity, suggesting that these are functional variants that could alter WNK1 expression. These new data lend further support to the hypothesis that variation in WNK1 expression contributes to BP and EH.

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

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Background: Lipoprotein-associated phospholipase A2 (LP-PLA2), is involved in degrada- tion of platelet-activating factor (PAF) and phospholipids associated with LDL and in production of lysophosphatidyicholine (lysoPC) and oxidized non-esterified fatty acids (NEFA). Aim: To verify whether the LP-PLA2 plasma levels are genetically determined. Materials and Methods: 54 healthy twins were enrolled. The levels (mass) and activity of LP-PLA2, were measured. We estimated genetic variance and heritability of LP-PLA2 mass and activity with variance and path analyses. Twins were genotyped at PLAP2G7 gene functional single nucleotide polymorphisms (SNPs): Thr198Ile (exon 7), Pro147Arg (exon 4) and Ala379Val (exon 9).

Zygosity was assessed with highly discriminating VNTR (variable number of tandem repeats) micro- and minisatellites systems were analyzed, by PCR and gel electrophoresis. Results: 26 twin pairs were monozygotic (M2) and 28 dizygotic (D2). The mean LP-PLA2 mass and activity were correlated (r = 0.87, p<0.001) and similar in M2 and D2. ICC estimates of heritability for LP-PLA2 were 0.27 (mass) and 0.28 (activity). ACE model- based estimates of heritability were 0.37 (mass) and 0.54 (activity). Heritability estimates were significant for activity, but not for LP-PLA2 mass. The within pair differences of LP-PLA2 mass and activity showed significant differences of LP-PLA2 activity between Hispanic and discordant pairs even between the former and the latter M2 twins. Conclusions: These results suggest heritability for activity, but not for mass, and indicate an effect of the His92Arg SNP on LP-PLA2 activity in healthy Caucasians.

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

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Introduction: A rare LRPR6 gene mutation causes a monogenic form of hypertension, hypercholesterolemia and early coronary artery disease because of reduced Wnt/j- catenin signalling. We investigated whether a common Val-1062 LRPR6 variant with similar functional consequences (about 5-fold lower signalling) was related to carotid artery atherosclerosis (CAA) in hypertensive patients. Methods: Cross-sectional study of 336 hypertensive patients (age <65 years) undergoing carotid artery ultrasonography. Hypertension, type 2 diabetes, dyslipidemia, chronic kidney damage (CKD), and smoking habit were evaluated. Genotyping was carried out using real time PCR. CAA was defined by the presence of atherosclerotic plaques (intima-media thickness >1.3 mm) at the level of common, bifurcation and/or internal carotid arteries. Logistic regression models were used to estimate the independent effect of V1062 allele controlling for CAA established risk factors. Results: In our hypertensive patients, age, gender, dyslipidemia, smoking habit, pulse pressure and CKD confirmed as risk factors for CAA. The V1062 LRPR6 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 followed by Taylor and Strandness) was considered, the results were also stronger (unadjusted OR 2.78, 95%CI 1.66–4.87; p<0.0001; adjusted OR 2.67, 95%CI 1.49–4.77, p<0.001). Conclusions: Beside the role of established risk factors, V1062 LRPR6 variant and CAA are strongly associated in hypertensive patients, making LRPR6 a novel interesting candidate gene for early coronary and carotid artery atherosclerosis.

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

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Background: Fatty acid amide hydrolase (FAAH) inhibitors, preventing endocannabinoid (EC) degradation, reduce blood pressure (BP) and heart rate in young male (YM) hypertensive rodents. The functional human FAAH 1237G variant results in reduced protein level and enzymatic activity but its relationship with BP is unknown. This study investigates the relationship among FAAH PF219 alleles and cardiovascular features in YM at baseline and after 5-year follow-up, and in older male obese hypertensive (OH) patients, in whom the EC system (ECS) is overactive. Methods: Genotype analysis was performed in 215 Caucasian male students (24 (0.2) years old) and in 185 older OH patients (50 (0.2) years old). YMs were also followed up for 5 years. Clinical and anthropometric variables, BP, cardiac and carotid artery echographic measurements were evaluated. Results: YMs with the FAAH 1237G allele had lower systolic (P = 0.042) and mean BP (P = 0.022), and a trend toward lower diastolic BP (P = 0.06). Such significant association was maintained at follow-up. In contrast, the same allele was not associated with BP in older OH. No association was found with other cardiac and vascular variables. Conclusion: An FAAH defective gene variant results in lower BP in YMs, similar to the findings in young rodents. This effect is lost in older OH patients. Because cannabinoid CB1 receptor blockade is associated with BP reduction in OH patients, EC effects and the use of ECS-interfering drugs is likely to be age and clinical-condition dependent.

Systematic analysis of 123 candidate genes reveals two novel genes for hypertension

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Discovering the genes causing hypertension is proving a challenging task. Recently, we performed a genomewide association scan (GWAS) for susceptibility genes for HT as part of the Wellcome Trust Case Control Consortium (WTCCC). This analysis revealed no SNPs with significance below 5x10^-8. We are currently performing further GWAS and meta-anal- ysis; 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 Human1M-Duo BeadChip. 764 SNPs were genotyped using the Illumina Human610-Quad BeadChip. Genotyping was performed using the Fluidigm Instrument and analyzed with the Fluidigm genotyping software. We found four SNPs to reach statistical significance (p<4.33e-4) after adjusting for multiple SNPs and traits. The strongest associations were with SNPs in two solute carrier genes; SLC6A2 (norepinephrine transporter) and SLC9A3 (sodium / hydrogen exchanger isoform 3) and systolic and diastolic BP, see table 1. Both genes have been implicated in BP regulation in animal models, but there are limited human studies. Our results indicate
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 TPO promoter structure is still controversial, using RT-PCR, we evidenced that TPO 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 (-1450/del58bp, C-920T, C-5+5A, C-1102A, G+115A, and C+135T). After subcloning of 1032 bp fragments of TPO P1 in pGL3-basic vectors, five molecular haplotypes (MolHaps1–5) were respectively observed: [A-622-C-413-C

**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 sarcometric 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 and cardiovascular magnetic resonance imaging study (CMR). We used the Mann-Whitney test, the Students-t-test and Fisher’s Exact Test to analyse the data in SPSS.RESULTS: Arrhythmias such as sustained and non-sustained ventricular tachycardia did not occur. Nine patients (27%) demonstrated ventricular premature beats (VPBs) during exercise or during the recovery period. Patients with VPBs tended to have a higher maximal wall thickness on CMR than patients without VPBs (24mm ± 6.0 vs 19mm ± 6.6, p = 0.10). All patients with VPBs showed evidence of fibrosis on CMR, whereas VPBs were not seen in patients without fibrosis (Pearson Chi-Square p = 0.023 and Fisher’s Exact Test p = 0.032). Among the subjects with fibrosis, patients with VPBs had a significant higher fibrosis score on CMR as compared to patients without VPBs (7.4% ± 3.9 vs 3.4% ± 3.6, p = 0.02). Conclusions: On exercise testing in a population of 31 HCM genotyped patients, no life-threatening arrhythmias occurred. VPBs were only observed in subjects with myocardial fibrosis on CMR; thus, exercise induced VPBs are related to myocardial fibrosis rather than to hypertrophy per se.

**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 early biomarkers of heart failure (HF) after myocardial infarction (MI). Since angiogenesis may be related to MI and HF, a protein-protein interaction (PPI) network was constructed by first extracting from the Entrez-Genome 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 Thrombospondin-1 (TBS1).

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 synthase (P<0.015), 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<0.04). 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 intrinsic single nucleotide polymorphisms (SNPs) in the ERα with early myocardial infarction (MI) in Polish population. Materials and Methods: 188 young patients (aged under 55) suffering from MI and 414 healthy controls (aged 18–45) were genotyped for T-397C and A-351G SNPs in ERα using PCR-RFLP method. Results: The -351“AA” genotype was significantly more frequent in MI group than in healthy controls (p<0.002, 51.3% vs. 37.9%, OR = 1.7), whereas genotypes of the T-397C SNP were equally distributed in studied groups. The analyzed polymorphisms were in linkage disequilibrium and constructed haplotypes. Haplotype “TA” occurred more frequently in affected group compared to healthy controls (p=0.042, 55.0% vs. 48.6%). Conclusions: In our study “AA” homozygotes of the A-351G ERα SNP were at higher risk of early MI than the carriers of other genotypes. Moreover, allele A together with allele T of T-397C ERα SNP constructed a haplotype, which occurred more frequently in MI patients. Our results are consistent with other reports, which correlated this “TA” haplotype with higher risk of MI and fatal ischemic heart disease.
Phosphoproteome analysis of left ventricular remodeling in an experimental model of heart failure

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

Cardiac effects of chronic CT-1 overloading in vivo

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

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

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Right ventricular hypertrophy (RvH) induced by pulmonary hypertension may progress to right heart failure. In systemic hypertension tissue transglutaminase (tTG) play an important role in cardiovascular remodelling, but it has not been addressed whether the same is the case in pulmonary hypertension. Therefore, we investigated whether inhibition of tTG by cystamine alone or combined with the phosphodiesterase type 5 inhibitor, sildenafil, lowers pulmonary pressure and inhibits development of RVH in chronically hypertensive rats. Nine weeks old Wistar rats were divided into five groups and exposed to normoxia or chronic, hypobaric hypoxia and treated with vehicle, cystamine 40 mg/kg/day, sildenafil 25 mg/kg/day, or the combination. Right ventricular pressure and systemic pressures were measured and protein and mRNA levels for tTG evaluated. Hypoxia increased tTG expression at mRNA and protein levels in the right ventricle compared to normoxia. Right ventricular systolic pressure (RVSP) was significantly increased in hypoxic vs. normoxic group. Sildenafil significantly lowered RVSP. Cystamine tended to raise RVSP and blunted the protective effect of sildenafil regarding RVSP. Right ventricular end-diastolic pressure (RVEDP) compared to normoxia. Sildenafil lowered RVEDP compared to hypoxia. Cystamine blunted the effect of sildenafil. Hypoxia raised right ventricle to left ventricle + septum weight ratio (RV/LV+S) significantly compared to normoxia. Cystamine and sildenafil tended to lower RV/LV+S. The present investigation suggests that increased expression of tTG contributes the development of right ventricular hypertrophy.
inorganic phosphate by the isolated enzyme and decreased inhibitory effect of Dig in a non-competitive manner. Bicarbonate experiments performed on the isolated enzyme in presence and absence of Ins showed a direct interaction of both Dig and Ins with enzyme’s α1 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 (10-6-10-9 M) protected both adult and neonatal rat cardiomyocytes from Dig (10-9 to 10-6 M) toxicity but not from that of ouabain (10-9 and 10-7 M). In immunohistological studies, neonatal cardiomyocytes treated with Dig or ouabain decreased significantly the enzyme’s IR while their co-incubation with Ins in different time period (0.25, 0.5, 1 and 24 h) restored and increased enzyme’s α1 subunit IR. This amelioration was observed only in presence of Dig but not with ouabain. Ins probably due to its specific binding to Na/K ATPase α1 subunit increase enzyme’s activity and prevent Dig’s interaction. This is of major interest for elaboration of new therapeutics for Dig cardio-toxicity in particular and cardiac arrhythmia in general.

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

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We previously documented a clear-cut antihypertensive effect of green tea extract (GTE), or vehicle (n/H9251, via osmotic minipumps), Ang II plus GTE (6 g/L), dissolved in the drinking water, n=6, via osmotic minipumps, Ins, Ins, with or without ouabain.) . Ang II and GTE concentration were determined in the heart of Ang II treated rats. Ang II-induced cardiac hypertrophy was associated with increased production of reactive oxygen species (ROS) and activation of second messengers, such as MAPKs and Akt. Ins was investigated to determine 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 g.Kg-1.d-1, n=6, via osmotic minipumps), Ang II plus GTE (6 g/L), dissolved in the drinking water, n=6, or vehicle, hypoxia was used to serve as controls. Blood pressure was monitored by telemetry throughout the study. The activation and expression of NADPH oxidase subunits, PKC isoforms, Src, EGFR, Akt and MAPKs were determined in the heart of Ang II treated rats. Akt and MAPKs were activated by Ang II. Our results show, for the first time, that cardiac side effects could come into sight with a high frequency of administration. More research is needed to determine the mechanism by which these alterations are induced.

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 cannabinoids against 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 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 high frequency of administration. More research is needed to determine the mechanism by which these alterations are induced.

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

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Objective: To investigate the feasibility and efficiency of a hypoxia regulated vascular endothelial growth factor (VEGF) gene delivery system in ischemic myocardium as well as restoring the contractility of the ischemically weakened heart. Background: VEGF has been studied widely for its angiogenic activity. With encouraging data from animal studies, clinical trials of VEGF gene therapy have been carried out. Skeletal myoblast transplantation 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 mycardium. Methods: We have already shown that myoblasts are excellent carriers of human VEGF for angiomyogenesis in infarcted heart. Considering that myocardial ischemia is a recurring and progressive disease, a regulatable gene delivery system (pHRE-VEGF) is designed. In this system, VEGF over-expression will be switched on or off by binding of hypoxia inducible factor1 alpha (HIF) to 5 copies of hypoxia response element (5XHRE) in hypoxia condition. Results: Rabbit skeletal myoblasts have been isolated and purified. These myoblasts carrying pHRE-Luc have shown increased luciferase activity under hypoxia. VEGF expression under hypoxia is upregulated at both RNA and protein levels. This system is being evaluated in vivo by injection of autologous skeletal myoblasts into an established rabbit heart model of myocardial infarction. Conclusion: This study will envision a new, realistic and safe approach for cardiac therapeutic angiogenesis and may be applied to the treatment of human ischemic heart diseases.

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

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

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

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Background: Pressure overload triggers eNOS as a prominent source of myocardial ROS that contribute to dilatory remodeling and cardiac dysfunction. Administration of tetrahydrobiopterin (BH4) can prevent pressure-load remodeling. The aim of this study was to investigate that BH4 can reverse established non-decompensated heart failure by interacting with uncoupled eNOS, and on this way prevent the evolution to end-stage heart failure. Methods: Compensated cardiac remodeling was induced in 60 mice by transverse aortic constriction (TAC). After 4wks, mice were randomized to receive BH4 (250mg/kg, n=30) or placebo (n=30) for the following 5wks. Overexpression of endothelial GTPCH, the rate limiting enzyme of BH4 synthesis, was evaluated in this TAC model (n=15 mice). Results: BH4 significantly reversed cardiac hypertrophy (heart weight: p<0.001, idem for myocyte dimensions, wall thickness and calculated LV mass) and diminished fibrosis (p<0.05). BH4 prevented the evolution towards cardiac decompensation (p<0.001).
confirmed by MRI and PV loop analysis. BH4 rescued the already uncoupled eNOS and increased its activity back to the normal level. Superoxide generation (total and NOS-dependent) was markedly reduced by BH4. BH4 improved fractional shortening and calcium-kinetics in isolated myocytes. Endothelial upregulation of BH4 by GTP-Ch,Tg had no beneficial effect on remodeling. Conclusion: BH4 can reverse established cardiac remodelling by re-coupling uncoupled eNOS and as a consequence less NOS dependent ROS is generated, leading to less hypertrophy and fibrosis and an amelioration of cardiac function.

PG.12 Endothelial and platelet activation markers in dogs with asymptomatic mitral regurgitation

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Mitril 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 controls with minimal MR, CKCS with moderate to severe MR and Cairn Terriers with minimal MR were included in the study. A clinical examination, blood sampling, echocardiography and an exercise challenge were performed. A significant difference 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.

PG.13 Beneficial effects of female gender on cardiovascular but not on renal disease severity in subtotally nephrectomized rats

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Progression of renal disease and its cardiovascular complications seems to be slower in premenopausal women. This study characterizes the impact of gender on cardiovascular and renal changes in subtotally nephrectomized rats (SNX).

Male (M) and female (F) Wistar rats were assigned into SNX, SNX hydralazine-treated and control groups for 18 weeks.

Male Wistar rats received hydralazine 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 phenylphrine (Ph, 10^-3 - 10^-5 M) and by carbachol (10^-3 - 10^-4 M) concentration-response curves. Data are given as the mean ± s.e.m (n=8–12 rings). Vasorelaxation was expressed as % of relaxation of Pre-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 Win52,212-2 could restore the endothelial dysfunction caused by chronic cisplatin treatment in rats. The responsible mechanism involved is not definitively established.


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

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

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

Male Wistar rats received cisplatin 2mg/kg i.p. once a week for 4 weeks. Afterwards, aorta rings from the animals were mounted in a tissue bath for tension measurements. In one experimental group, vascular constriction and relaxation was assessed by phenylphrine (Ph, 10^-3 - 10^-5 M) and by carbachol (10^-3 - 10^-4 M) concentration-response curves, respectively. A second group of experiments, Phe-precontracted rings were treated with Win55,212-2 (1µM), just before the carbachol concentration-response curve. Data are given as the mean ± s.e.m (n=8–12 rings). Vasorelaxation was expressed as % of relaxation of Pre-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 Win52,212-2 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)-, 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 vasodilatations. 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 ER-alpha knockout mice. Deletion of ER-alpha did not affect echocardiographic measurements in terms of left ventricular end-systolic and -diastolic diameters and systolic function. Deletion of ER-alpha induced hyperreactivity of the aorta in presence but not in absence of functional endothelium and did not alter relaxation to acetylcholine. Both basal 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 eNOS was not significantly different in rat carotids. Thus, the enhancement of NO pathway might represent a major mechanism of the effect of red wine on vascular function in NO-deficient hypertensive rats than previously reported on spontaneously hypertensive rats. The effect of melatonin on endothelial function and in L-NAME-induced hypertensive rats was not altered, and COX-2 expression was enhanced. Concomitant treatment with melatonin for 5 weeks. 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 hypertensive 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-precontracted femoral and mesenteric arteries. Endothelium-derived constricting factor (EDCF)-mediated component of ACh-induced responses and inner diameter were determined in femoral arteries. L-NAME treatment caused hypertension, impaired ACh-induced relaxations, decreased NO-component, augmented 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, NOS or COX-2 expression, vascular structure or function. We conclude that BP reduction after was less pronounced in 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 N0-independent mechanisms may be involved in the residual BP lowering effect of melatonin. (GUK 143/2008, VEGA 1/3429/06, Bratislava, Slovakia, Institute of Physiology and CRC, Academy of Sciences of the Czech Republic, Bratislava, Slovakia)

Melatonin reduced experimental and clinical hypertension, but the mechanisms of its blood pressure (BP) lowering effect are not completely understood. We elucidated the role of NO in the antihypertensive effects of melatonin. Four groups (n=10 each) of male Wistar hypertensive 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-precontracted femoral and mesenteric arteries. Endothelium-derived constricting factor (EDCF)-mediated component of ACh-induced responses and inner diameter were determined in femoral arteries. L-NAME treatment caused hypertension, impaired ACh-induced relaxations, decreased NO-component, augmented 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, NOS or COX-2 expression, vascular structure or function. We conclude that BP reduction after was less pronounced in 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 N0-independent mechanisms may be involved in the residual BP lowering effect of melatonin. (GUK 143/2008, VEGA 1/3429/06, Bratislava, Slovakia, Institute of Physiology and CRC, Academy of Sciences of the Czech Republic, Bratislava, Slovakia)

PH.04
Deletion of estrogen receptor-alpha abolishes endothelial response to wine polyphenols without affecting the main cardiovascular parameters in mice

PH.05
The effect of melatonin on endothelial function and in L-NAME-induced hypertensive rats

PH.06
Mesenchymal stem cells effectively reduce surgically-induced stenosis in rat carotids

PH.07
Activation of phospholipase C and α1D adrenoceptors in rat mesenteric small arteries

PH.08
Phospholipase Cα1 modulates sustained contraction of rat mesenteric small arteries in response to noradrenaline, but not endothelin-1

PH.09
Apocynin does not lower arterial pressure in spontaneously hypertensive rats (SHR) and its acute vasodilator action is not due to NADPH oxidase inhibition
ADP is oxidase inhibition at all. Apocynin potently inhibited human granulocytes NOX but not NOX-dependent oxygen radical formation in rat aortic rings and small intratheral arteries. When added to isolated rat carotid artery rings, Apocynin inhibited vasodilation (middle panels) but not contraction (lower panels). Apocynin-induced vasodilation was not significantly affected by NOS, PKA, or PKG inhibition, did not depend on extracellular Ca 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 NOX inhibition. Its use as a pharmacological tool to investigate vascular NOX and the role of NOX activity for arterial pressure regulation and hypertension should be discontinued.

**Conclusion:**

- **Up-regulation of ET A receptors by DSP involves transcriptional mechanisms**

**Patients with Cushing’s Syndrome exhibit cortical overscretion. 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 segments precontracted 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 pathophysiological concentrations induces desensitization of vasodilator responses. This effect may participate in the increased risk of cardiovascular complications in patients with Cushing’s syndrome.

**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 (ET A) receptors in vasculature and to investigate the molecular mechanisms behind the DSP-induced activation of ET-system. 

**Methods and results:** 

- In organ culture of rat mesenteric arteries, DSP could activate protein kinase Cε (PKCe) and extracellular regulated protein kinase 1 and 2 (ERK1/2) in the smooth muscle cells (SMC). This resulted in ET A receptor up-regulation with enhanced ET A receptor-mediated contraction (myograph), increased ET A receptor mRNA (real-time PCR) and protein (immunohistochemistry with confocal microscopy) expressions in the SMC. Inhibition of transcription or translation abolished DSP-enhanced ET A receptor-mediated contraction. Post-transcriptional mechanisms were suggested by that DSP accelerated ETA receptor mRNA degradation but enhanced ET A receptor-mediated contraction. Inhibition of translation, ERK1/2 or PKCe/Erk attenuated the DSP effects.

**Conclusion:** 

- Up-regulation of ET A receptors by DSP involves transcriptional mechanisms and enhanced translation of ET A receptors in the vascular SMC via activation of intracellular PKCe and ERK1/2. The ET A receptor up-regulation by DSP in the SMC may contribute to the development of smoking-associated cardiovascular diseases.

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

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The binding of nitric oxide (NO) on the heme group of soluble guanylyl cyclase sGC induces important changes such as vascular smooth muscle relaxation, thereby controlling blood pressure, blood flow and erection. The sGCβ1,β2 and sGCα2β2 are the physiologically active heterodimers, in which the histidine residue at position 105 of the β2 subunit functions as a axial ligand for the heme prosthetic group. Substitution of the histidine by phenylalanine abolishes the heme-dependent activation of sGC. This is the case in the sGCβ1,β2-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 endogenous NO released from the endothelium in response to acetylcholine (ACh) and exogenous N0 from the NO donor sodium nitroprusside (SNP) were completely abolished in the aorta from the sGCβ1,β2-mice, but not in the femoral arteries. In the CC the relaxation response to ACh (releasing endothelial NO) and electrical field stimulation (releasing neuronal NO) was abolished, while SNP response was only reduced. The response to the NO-independent sGC-stimulator (BAY 41–2272) was also significantly reduced in the different preparations of sGCβ1,β2-mice, indicating that the heme group plays a role in the BAY 41–2272-induced activation of sGC. Our results demonstrate the importance of sGC as the sole target for NO in regulating vasodilatation in mice aorta but not in femoral artery. Furthermore, the remaining relaxing effect of BAY 41–2272 in the sGCβ1,β2-mice, suggests that the heme-binding pocket is very important but not indispensable for the interaction of BAY 41–2272 with sGC.
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-SR 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 + 0.2 mg/kg BW/d (p.o.); 4) STZ + C21; low dose Candesartan (0.2mg/kgBW/d p.o.); 5) STZ + C21 (0.3mg/kgBW/d p.o.); 6) STZ + both (C21 + C21). Systolic BP was only lowered in the STZ + C21 (169 ± 24mmHg) and the STZ + both (188 ± 17mmHg), but not in the STZ + C21 (222 ± 10mmHg) and, remarkably, not in the STZ + C21 (251 ± 14mmHg) groups. Elevated albuminuria in STZ rats (51 ± 18 mg/d) was only ameliorated in STZ + C21 (17 ± 7 mg/d). STZ + C21 (32 ± 15 mg/d) and STZ + both (14 ± 7mg/d), but not in C21 (60 ± 21mg/d). Using computer-based histomorphometry, diabetic glomerular hypertrophy (STZ 13.9 ± 13.5% S2 micro) was significantly lower with Can and C21 (STZ + Can -15%, STZ + C21 -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 ± 1.0%) was reduced in all groups -18%). Increased tubulointerstitial collagen I expression (STZ 70 ± 22% per section) and glomerular collagen IV deposition (STZ 5.1 ± 1.0%) was reduced in all groups. Increased tubulointerstitial collagen I expression (STZ 70 ± 22% per section) and glomerular collagen IV deposition (STZ 5.1 ± 1.0%) was reduced in all groups.

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-kB 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-kB inhibitors, PD98059 and pyrroline dihydrocarbarnate (PDT), we established that iNOS induction by visfatin required the consecutive upstream activation of ERK 1/2 and NF-kB. Furthermore, visfatin was basally detected in human umbilical vein endothelial cells (HUVECs). Nevertheless, visfatin levels were enhanced when HUVECs were challenged with increasing concentrations of the cytokine IL-1β for 18 h. This latter effect of IL-1β was prevented by PDT and the enzyme PolyADP-ribose polimerase-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 compensation for the occlusion of a main artery is described as arteriogenesis. The initial trigger is fluid shear stress activating the endothelium surface, and leads to modulation of processes controlling the recruitment of circulating cells. The inflammatory aspect of this activation is apparent by the increased expression of MCP-1, a chemotactic protein responsible for the adhesion and migration of monocytes to the endothelium. In order to induce arteriogenesis in mice, the right femoral artery was ligated and the left was sham operated. Arterial growth was measured via Laser Doppler Imaging (LDI) and quantified as relative perfusion recovery (right/left) before and after surgery until day 7. Treatment with RNase (42U/g kg/d), but not with DNase (42U/g kg/d), immediately before occlusion of the artery until day 7 reduced the extent of arteriogenesis, suggesting that extracellular RNA plays a role in this process. Accordingly, in vitro studies confirmed that RNA acts as a chemotactrant for monocytes. In an endothelial cell monolayer the migration of monocytes was increased by RNA to nearly the same extent as by MCP-1. In addition there was an increase in adhesion of monocytes to microvascular endothelial cells by RNA, but not by DNA. The expression of ICAM-1 on endothelial cells was increased by RNA and abolished in the presence of RNase. Furthermore, RNA led to ectocytosis of pro-inflammatory factors like P-Selectin from Weibel-Palade bodies. In summary, our data indicate that proinflammatory effects of extracellular RNA promote arteriogenesis.

Possible signalling pathways involved in hypoxia-induced endothelial dysfunction in human umbilical vein endothelial cells
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Hypoxia is involved in several pathologies in both the systemic, pulmonary and foetal circulation, such as coronary ischemia and COPD and can lead to pulmonary hypertension. In the circulation the endothelial cells are the first to encounter a lowering in the blood oxygen saturation and therefore many of the responses and the adaptation to hypoxia is conveyed through the endothelium. The chronic response to hypoxia seems to involve changes in the endothelium which can modulate several signalling pathways involving i.e. vascular tone, extracellular matrix dynamics or cell survival. The aim was to elucidate possible novel signalling pathways in human umbilical vein endothelial cells (HUVECs) after hypoxia. This was achieved by using Western blotting, 2D gel electrophoresis/proteomics and confocal microscopy. Solar, the results indicate two possible pathways that have not previously been reported for hypoxic endothelial cells. Up-regulation of eNOS, cofilin and cytoplilamin A point to initiation of angiogenesis pathways. Up-regulation of Grp78 and 94 indicates involvement of ER stress pathways and up-regulation of caspase-12 indicates induction of apoptosis pathways.

Vasoactive peptide signalling and survival of nociceptive neurons
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Signalling through the calcitonin receptor-like receptor (CLR), the receptor for adrenomedullin and calcitonin gene-related peptide (CGRP), and on a molar basis the most potent vasodilators known so far, is involved in nociception. Mice overexpressing a smooth muscle a-actin promoter driven CLR (tg) showed higher tolerance in fights for territoriality and reduced sensitivity to pain. In parallel, blood pressure and heart rate was measured. Two hours later the mice were perfusion-fixed for counting the number of CLR positive neurons in lamina I of the dorsal horn of tg mice. Mice tg mice showed significantly less increase in blood pressure as well as heart rate during the stimulus. Accordingly, the number of CLR positive neurons was significantly lower in tg mice com-pared to wt mice. CGRP (8–37) decreased the cardiovascular response and the number of CGRP positive neurons in wt mice to that of untreated wt 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 lamina I of the dorsal horn of tg mice appears to be reduced. In conclusion, nociception is reduced in our CLR tg mice presumably due to loss of peripheral and maybe also central nociceptive neurons.

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 significantly increases in diet-induced obesity/hyperlipemia (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-II). The aim of this work is to characterize the effect of lepton in regulating cardiac CPT inhibition by malonyl-CoA. We analyzed the effect of 50 μM malonyl-CoA in inhibiting cardiac CPT activity in mice made hyperleptinemic by 1 dietary treatment with a high-fat diet or 2 acute administration of lepton (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 lepton-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 lepton concentration and malonyl-CoA-insensitive CPT activity suggests a link between cardiac lepton receptors and CPT regulation. In NF animals we detected an up-regulation of phosphorylated Akt (pAkt). We observed that pAkt positively correlated with plasma lepton 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 lepton. Supported by Ministerio de Educacion y Ciencia (SAF 2006-02456 and SAF 2005-0518), FUSP-CEDI, 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 NFκB 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 NFκB 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 (10ng/ml) to increase IL-6-expression and NFκB activity, respectively. IL-6 expression was measured by real time RT-PCR. NFκB activity was estimated by immunohistochemical detection of NFκB-p50 nuclear translocation and by a luciferase reporter assay using an expression vector containing the human IL-6 promoter including the NFκB binding site. Stimulation of the AT2R in human and murine primary fibroblasts by C21 (10-6M) led to a significant decrease in TNFα-induced IL-6 expression and NFκB activity. These effects could be inhibited by the specific AT2R-antagonist PD123319 (10-5M) as well as by the inhibitor of protein tyrosine phosphatase, Na-orthovanadate (10nM), or the inhibitor of phosphatases thus leading to inhibition of NFκB activity and activation of phosphatases.

Sympathetic overactivity in α2A-adrenoceptor 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-adrenoceptor 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-receptor blockade increased RNS-induced NA-release in WT, whereas no effect was observed in KO mice indicating no other relevant presynaptic α2-receptor subtype. 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 (10-6 M). Expression of neurotrophins, the apoptosis marker Bax, inflammation markers and toll-like receptors (TLR) 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 (10-6 M). 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 peptstatin A (a renin inhibitor), teprotide (an ACE inhibitor), chymostatin (a chymase inhibitor), amastatin (an 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 involve-ment 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 chomostatin had no significant effects on NR but reduced to a 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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