Abstracts From the 10th Annual Meeting of the European Council for Cardiovascular Research (ECCR)

La Colle sur Loup
Nice, France
October 14–16, 2005
1.4 LOSS OF SPARC RESULTS IN FATAL CARDIAC RUPTURE OR HEART FAILURE AFTER ACUTE MYOCARDIAL INFARCTION.

Schellings MWM1, van Loon M2, Sage EH3, Pinto YM4, Heymans S5
1 Experimental & Molecular Cardiology, CARM, Maastricht University, Maastricht, Netherlands. 2 Hope Heart Program, Benaroya Research Institute at Virginia Mason, Seattle, United States

The matricellular protein SPARC (secreted protein acidic and rich in cysteine, also known as osteonectin) mediates cell-cell interaction during matrix remodeling after tissue injury. Expression of SPARC is low in the normal heart, but reappears after myocardial infarction (MI). However, whether SPARC expression after MI is important for cardiac integrity is not yet determined. We induced MI in SPARC wild-type (WT, n=7) and SPARC knock-out (KO, n=7) mice by permanent ligation of the left coronary artery. After 2 weeks or at premature death, hearts were sampled for further analysis. Infarct size did not differ between the WT and KO group (WT: 46.4±11.5% vs. KO: 50.6±8.2%). However, mortality within 14 days after MI was significantly higher in SPARC KO compared to WT mice (% mortality, WT: 22% vs. KO: 66%, p<0.05). Only one WT mouse died due to fatal cardiac rupture at 4 days (10%), whereas 5 out of 7 KO mice (71%) succumbed after rupture at 3–5 days after MI. The surviving SPARC deficient mice displayed pronounced tachycardia, associated with severe systolic dysfunction and left ventricular dilatation at echocardiography. Detailed histological analysis revealed increased inflammation in osteonectin KO myocardium compared with WT injured myocardium at 4 days. Rupture of KO infarcts was associated with increased transmural fibrosis of red blood cells all over the infarct area. This latter finding suggests a lack of matrix integrity in absence of SPARC. Together, these data indicate that increased expression of SPARC as observed after MI is essential to maintain cardiac integrity. Absence of SPARC in mice resulted in increased mortality due to fatal cardiac rupture and failure after MI in mice.

1.5 CELLULAR CARDIOMYOPLASTY USING GENETICALLY MODIFIED SKELETAL MYOBLASTS FOR CARDIAC REPAIR

Ye Li1, Haider Kh H2, Jiang SJ2, Tan RS2, Ge RW2, Law PK2, Aziz S2, Sim EKW2, 3, 4, 5, 6
1 National University Medical Institute, Singapore, 2 Department of Pathology and Laboratory Medicine, University of Cincinnati, Ohio, Cincinnati, United States, 3 National Heart Centre, Singapore, 4 Department of Biological Sciences, National University of Singapore, Singapore, 5 Cell Transplants Singapore Pte Ltd, Singapore, 6 Washington Hospital Centre, Washington, United States. 7 Department of Surgery, National University of Singapore, Singapore & Gleneagles JPMC Cardiac Center, Brunei Darussalam.

Introduction: Angiopoietin-1 (Ang-1) has emerged as a new and promising angiogenic factor for therapeutic angiogenesis. We compared the effectiveness of Ang-1 with Ang-1 + VEGF165 delivery for therapeutic angiogenesis through skeletal myoblasts. Methods: Human myoblasts carrying Lac-Z gene were transduced with adenoviral bicistronic vectors carrying Ang-1 (Ad-Ang-1) or Ang-1 + VEGF165 (Ad-Bic). Myocardial infarction was created in pigs by coronary artery ligation and grouped as DMEM injected/group-1 n=6), adenoviral-null vector transduced myoblast transplanted/group-2 n=5), myoblast carrying Ang-1 + VEGF165 transplanted/group-3 n=6) and myoblasts carrying Ang-1 + VEGF165 transplanted/group-4 n=7). After 3 weeks, SMI dme with or without 3T3C10 cells were intramyocardially transplanted. Animals were immunosuppressed for 6 weeks. Pigs were euthanized and hearts were harvested at 6-weeks post-treatment and processed for histological studies. Results: Transduced myoblasts secreted VEGF165 and/or Ang-1 as revealed by dual fluorescent immunostaining and RT-PCR. Extensive survival of the Lac-Z expression skeletal myoblasts was observed in pig heart. Average vascular density at low power field (x100) by double immunofluorescent staining for VWF-Factor VIII and smooth muscle actin in group-4 (45.2±6.2%, 36.6%: 2.13) and group-3 (39.3±3.09, 34.7±2.52) was significantly higher than group-1 (16.18±0.91, p<0.05; 7.88±0.52, p<0.08) and group-2 (26.57±2.09, p<0.05; 20.14±1.68, p<0.05) at 6-weeks post-treatment. Mature blood vessel count and regional blood flow in group-4 was the highest (86.33% and 2.91 ml/min/g), followed by group-3 (95.8±2.8%, 2.66%, 2.73±0.33ml/min/g) as compared with other two groups. Significantly improved EF was achieved by group-3 (34.9±2.2%, 3.92%, p<0.007) and group-4 (50.5%, p<0.019) as compared with group-1. There was no significant difference was achieved between group-3 and group-4. Conclusion: Ang-1 delivered by myoblasts achieved the same effectiveness for therapeutic angiogenesis as simultaneous delivery of Ang-1 + VEGF165 via myoblasts for cardiac repair.

888

Oral Presentations

1.1 INCREASED SUSCEPTIBILITY TO HEART FAILURE IN RESPONSE TO VOLUME OVERLOAD IN TRANSGENIC MICE WITH INCREASED ALDOSTERONE IN HEART.

Nehme J1, Milieoz P1, Favre J2, Bonnin P1, Ambroisine ML3, Marotte F1, Loyer X1, Samuel JL1, Richard V1, Delacay C1
1 CRCL-U689, Paris, France, 2 Inserm U644, Rouen, France

Male transgenic (TG) mice with cardiac-specific overexpression of aldosterone-synthesase have increased cardiac aldosterone (x 1.7), unchanged cardiac function, but abolished coronary reserve. We aimed to determine if this altered coronary reserve may alter the cardiac response to work overload. An aortic-caval fistula (ACF) was imposed to 3 month-old wild-type (WT) and TG male mice. ACF increased 7.5-fold aortic flow rate (aortic echo-Doppler). Blood pressure was unchanged. Echocardiography was performed one day before (day-1), and 7 days after ACF (day7). Coronary function was explored on left coronary artery segments mounted in a wire myograph. In WT-ACF mice LVEDD and fractional shortening (FS%) were unchanged at day7 relative to day-1. Treatment with spironolactone from day-21 until day7 totally decreased FS% (-19.6%; p<0.05) in TG-spironolactone mice.

1.2 IMPACT OF THE RENIN-ANGIOTENSIN SYSTEM (RAS) ON APOPTOSIS IN ISCHEMIA AND REPERFUSION

Charite University Medicine, Berlin, Germany

We examined the influence of the RAS on different regulators of apoptosis using an isolated hemoperfused working porcine heart model of acute ischemia (2 h), followed by reperfusion (4 h). 23 porcine hearts were randomized to 5 groups: hemoperfused non-infarcted hearts (C), infarcted hearts treated with angiotensin-2, infarcted hearts treated with angiotensin-1 and angiotensin-1 (2A), TUNEL-staining and electron microscopy revealed an increase in cardiomyocyte apoptosis (4-fold) in the infarct area of injured hearts. Quinapril significantly reduced the rate of apoptosis (~75%), p<0.05, C vs. 2A and further decreased by Ang I (C: 2-fold, 2A: 1.4-fold, bcl-2: 2.18-fold, p53: 1.2-fold vs. 2A). Quinapril reduced bcl-2 (-27%) and p53 (-50%). Bcl-2 was elevated in 0 hearts and reduced in the QA group. An early upregulation of caspase-3 gene (5-fold) and protein expression (6-fold/12-fold) was detected in QA and Ang I hearts compared to C. Reduced caspase-3 gene expression (~40%), but had no effect on caspase-3 and Fas protein. 85-kDa apoptosis-related cleavage fragments resulting from enhanced 116-kDa poly(ADPribose) polymerase activity was detected in MI and Ang I hearts compared to C. Q reduced caspase-3 gene expression (5-fold) and protein expression (6-fold/12-fold) was detected in QA and Ang I hearts compared to C. Reduced caspase-3 gene expression (~40%), but had no effect on caspase-3 and Fas protein. 85-kDa apoptosis-related cleavage fragments resulting from enhanced 116-kDa poly(ADPribose) polymerase activity was detected in MI and Ang I hearts compared to C. Q reduced caspase-3 gene expression (5-fold) and protein expression (6-fold/12-fold) was detected in QA and Ang I hearts compared to C. Reduced caspase-3 gene expression (~40%)

Downloaded from http://hyper.ahajournals.org/ by guest on November 11, 2017
1.6

**COMBINED TREATMENT OF ACUTE ISCHEMIC STROKE WITH ERYTHROPOIETIN AND OLMESARTAN IMPROVES SURVIVAL AND PREVENTS POST-STROKE MEMORY DYSFUNCTION IN THE GERBIL**

Faure S, Oudart N, Javelaud J, Fournier A, Achard JM
Faculté de Médecine, Limoges, France

Erythropoietin (EPO) is markedly protective in experimental stroke and emerges as promising in pilot clinical studies. Stimulation of angiogenesis production by AT1 blockade promotes non-AT1 dependent neuroprotective effect, whereas angiostatin/AT4 have enhancing effects on cognitive function and memory. We therefore examined the synergetic potential of EPO and olmesartan combination therapy in the single carotid ligation stroke model in the gerbil (n = 50 per exp. group). EPO was administered (200, 500 U/kg 2 48 hours after the stroke. At 36 hours, olmesartan (10 mg/kg), ramipril (3 mg/kg) or nothing, was added to the tap water of EPO-treated survivors, and survivors were submitted at day 30 to immediate (Object Recognition Test (ORT) and spatial (Morris Water Maze (MWM)) memory function tests. EPO significantly increased survival at day 30 (38 vs 14% for controls; p < 0.002). For a comparable BP lowering effect olmesartan further increased survival rate to 58% whereas ramipril decreased it to 24 % (p < 0.001, EPO/olm vs EPO/Ram). EPO-treated survivors had markedly altered performances in both ORT (p < 0.001) and MWM (p < 0.0001) tests compared to normal gerbils. Combined therapy with ramipril further deteriorated immediate and spatial memory, whereas EPO/olmesartan treatment fully restored normal response to the memory tests. Conclusion: delayed treatment with olmesartan combined to early EPO therapy has a BP independent additive protective effect on survival, and completely prevents long term memory dysfunction in the gerbil stroke model.

2.1

**SIGNAL TRANSDUCTION OF THE RENIN/ PRORENIN RECEPTOR**

Funke-Kaiser H, Scheife JH, Unger T.
Center for Cardiovascular Research (CCR) Inst. PharmacoL and Toxicol., Charite, Berlin, Germany

A human renin/prorenin receptor (RER), which can specifically bind renin and prorenin, has recently been cloned. This receptor, which is highly expressed in heart, brain, and other tissues, may serve as a specific target for drugs. The RER binds both renin and prorenin, and the interaction is regulated by the renin proconvertase, a complex of two disulfide-linked subunits, K(ERK) and K(ER2). Little is known about the receptor, the complex, or the binding properties of the RER. We have now identified the RER and signal transduction cascade. Initially, using RT/PCR and Northern blotting, we found evidence for an ubiquitous expression pattern in the human and mouse genome. Consistently, we were able to identify several transcriptional start sites by 5'-RACE and a high promoter activity utilizing luciferase assays. In addition, overexpression targeted the receptor, we demonstrated an intracellular expression pattern in addition to a published cell-surface localization. RER activation by recombinant (but not extracted) renin in HEK-293 cells caused a strong ERK1/2 activation (1-314% at 5 min). Interestingly, we could identify PLZF (promyelozytic zinc finger protein) as direct protein interaction partner of the RER by yeast two-hybrid screening. Our experiments indicate that the RER exhibits intracellular functions, and might - besides the activation of MAPK - transduce signals to the nucleus with PLZF as adaptor protein.

2.2

**GPCR AGONIST SIGNALING OF VASCULAR SMOOTH MUSCLE CELLS INVOLVES EGFR RECEPTOR TRANSDUCTION THROUGH ADAM12 ACTIVATION**

Youssi S, Polidano E, Faverdin C, Hadjadj M, Marche P
CNRS UMR 7131 and Univ P. & M. Cune, Paris, France

In VSMC, GPCR agonist signaling which results in p42/44 MAP kinase (ERK1/2) activation, hence cell differentiation, migration and proliferation, usually requires the transactivation of EGFR receptor (EGF/R). Although HB-EGF like growth factor and metalloproteases have been described to participate in EGFR transactivation, little is known on the mechanisms involved in this phenomenon. The finding that cardiac hypertrophy could be efficiently inhibited HB-EGF like growth factor shedding and EGFR phosphorylation but did not affect neither ERK1/2 phosphorylation nor DNA synthesis. By contrast, the ADAM12 inhibitor KB-R7785 attenuated, in a dose-dependent manner, both EGFR and ERK1/2 activation as well as DNA synthesis. MMP-2 and MMP-9 inhibitors produced similar results, as did the addition of HB-EGF like growth factor neutralizing antibody. These results indicated that HB-EGF like growth factor is involved in GPCR agonist signaling via ERK1/2 and that its shedding could result from ADAM12 activation. The participation of MMP-2 and MMP-9 in these events cannot nevertheless be ruled out.

2.3

**THE AT1-RECEPTOR BLOCKER TELMISARTAN IS A PARTIAL PPARALPHA AGONIST**

Clementz M., Frost N., Schupp M., Unger T., Staeli B., Kintscher U.
1 Center for Cardiovascular Research CCR, Charité (CCM-Universitätsmedizin Berlin, Berlin, Germany, 2 Institut Pasteur de Lille, Université Lille, Lille, France

Clinical studies have shown that telmisartan (Teilim) significantly improves diabetogenic hyperglycemia and hypercholesterolemia by unknown mechanisms. Telim is an AT1-receptor blocker (ARB), and a partial agonist of the anti-diabetic nuclear hormone receptor PPARalpha. To identify new mechanisms of the beneficial effects of telim on dyslipidemia, we analyzed whether it is capable of activating PPARalpha, a major regulator of lipid metabolism. PPARalpha activity was measured with a chimeric Gal4-DNA-binding domain–PPARalpha-ligand-binding domain (LBD) fusion protein on a Gal4–dependent luciferase reporter system. Telim significantly induced the activation of the PPARalpha-LBD. Maximum activation by telim at 100 μM was reached 22% of the maximum response induced by the full PPARalpha agonist WY-14643 (WY), identifying Teilim as a partial PPARalpha agonist. EC50 values were calculated for PPARalpha-LBD activity: WY EC50: 6.4 μM/L, teilim EC50: 21.8 μM/L. The present study identifies the ARB telmisartan as a partial PPARalpha agonist potently inducing target gene expression. PPARalpha activation by telmisartan may explain some of the dyslipidemic actions of this ARB.

2.4

**INVolvement of α1β1 Integrin in ARTerial stiffening and Hypertrophy in response to angiotensin II**

Louis H1, Li Z2, Kakou A1, Mercier N1, Daniel Lamazière J-M1, Labat C1, Glukhova M1, Gardiner H1, Safar M1, Lacolley P1
1 INSERM U684, Vandoeuvre les Nancy, France, 2 Laboratoire de biologie moleculaire de la differentiation, Paris, France, 3 INSERM U441, Pessac, France, 4 UM144 CNRS, Paris, France, 5 Biogen Incorporated, Cambridge, Massachusetts, United States, 6 Centre de diagnostic, Hotel Dieu, Paris, France

The role of α1 integrin, a major receptor for collagen within the vascular wall has not been addressed in large arteries. We have studied the in vivo mechanical properties of the same degree as acetylcholine (0.1–10 μM). Inactivation of integrin had no effect for the absence of integrin (α1KO) was shifted rightwards compared with WT, indicating a decreased arterial stiffness. Under basal condition ERK 1/2 phosphorylation was higher in α1KO than in WT. mRNA analysis of α1KO mice showed an increase of contractile proteins; and of α2α, αβ and β1 integrin that may partly compensate for the absence of α1. In α1KO the Angiotension-did not increase ERK 1/2 phosphorylation as in WT mice. Inactivation of α1 integrin results in important changes of signalling including up-regulation of c-fos and Grp-1. In conclusion, suppression of α1 integrin i) modifies VSMCs phenotype and signalling ii) inhibits hypertrophic response to AngII independently of AP. This is the first study showing that α1 integrin plays a role in arterial stiffening and in vascular effects of AngII in vivo.

2.5

**OLEANOLIC ACID, A COMPONENT OF OLIVE OIL RESIDUES, CAUSES CALCIUM-INDEPENDENT RELEASE OF ENDOTHELium-DERIVED NITRIC oxide**

Rodriguez-Rodriguez R1, Stankevicius E1, Herrera MD2, Petersen LB3, Anderson MR2, Ruiz Gutierrez V2, Simonsen U1
1 Department of Pharmacology, University of Aarhus, Aarhus, Denmark, 2 Instituto de la Grasa, Consejo Superior de Investigaciones Científicas, Sevilla, Spain

Olive oil plays a key role as part of the Mediterranean diet, which has beneficial effects on endothelial function and hence in atherogenesis. **Objective**: the aim of the present study was to investigate the mechanisms by which oleanolic acid, a component of olive oil residues, increases release of nitric oxide (NO). **Methods**: Rat superior mesenteric artery rings were mounted in myographs and a NO-sensitive microsensor introduced for simultaneous measurements of isometric tension and NO concentration. Moreover, calcium measurements and immunoblotting were performed in cultured human umbilical ven endothelial cells. **Results**: In normoxaline (0.5 μM)-contracted arteries, oleanolic acid (3–30 μM) induced endothelium-dependent increases in NO concentration to the same degree as acetylcholine (0.1–10 μM), while relaxation per nanomolar NO were less than for acetylcholine. These responses were inhibited by an inhibitor of NO synthase, asymmetric dimethylarginine (300 μM) and cyclooxygenase. The NO increases induced by oleanolic acid were unaltered in Ca2+–free physiological solution and in the presence of the
sarcoplasmic reticulum calcium-ATPase inhibitor thapsigargin (1μM). In contrast to histamine, oleic acid did not increase intracellular calcium in human endothelial cells, but increased phosphorylation of endothelial NO synthase at Ser1177. Conclusion: The present results suggest that oleic acid increases NO and causes vasodilatation by calcium-independent phosphorylation of endothelial NO synthase. The larger luminal increase in NO compared with conventional endothelium-dependent agonists may afford protection against atherosclerosis.

2.6 LOWER LEVELS OF ASYMMETRIC DIMETHYLARGININE AND INCREASED ENDOTHELIAL-DEPENDENT NITRIC OXIDE-MEDIATED RELAXATION OF UTERINE SMALL ARTERIES FROM PREGNANT WOMEN

Andersen MR1, Simonsen U2, Hedegaard M3, Stender S3, Aalikjær C4
1 Department of Obstetrics and Gynecology, Aarhus University, Skejby Hospital, Aarhus, Denmark, 2 Institute of Pharmacology, Aarhus University, Aarhus, Denmark, 3 Department of Clinical Biochemistry, Gentofte University Hospital, Copenhagen, Denmark, 4 The Water and Salt Research Center, Institute of Physiology, Aarhus University, Aarhus, Denmark

We investigated the endothelium-dependent nitric oxide- (NO) mediated vasodilatation of uterine small arteries from pregnant and nonpregnant women. Included: 21 pregnant undergoing caesarean section and 19 nonpregnant (<45 years) undergoing hysterectomy. Serum estradiol and plasma L-arginine (NO synthase (NOS) substrate), asymmetric dimethylarginine (ADMA, NOS inhibitor), and symmetric dimethylarginine (SDMA, inactive ADMA stereoisomer) were determined. Myosin phosphorylation was determined from uterine incision and isometric responses assessed in small vessel myographs after normalization of the artery diameter to a transmural pressure of 100 mmHg. Pregnant had higher estradiol (mmol/l 140 vs. 140, P<0.001) and lower L-arginine (μmol/l 34.3 vs. 62.8, P<0.001) and ADMA (0.59 vs. 0.01, 0.37 vs. 0.05) levels, than nonpregnant, but SDMA levels were similar. Normalized lumen diameters of arteries from pregnant were larger than from nonpregnant (μmol/l 409±16 vs. 254±13, P<0.001), but the concentration-dependent tensions to U66169 (thromboxaneA2, analog) was similar. Arteries from pregnant demonstrated enhanced concentration- and endothelium-dependent relaxation to bradykinin compared with those from nonpregnant (%Kmax 322±25 vs. 423±19, P<0.01). The bradykinin-induced relaxations were attenuated in the presence of N-nitro-L-arginine (NOS inhibitor), so that it became similar, and was unaffected by indomethacin (cyclooxygenase inhibitor, %Kmax 344±21 vs. 412±20, P<0.05). Endothelium-independent relaxations induced by sodium nitroprusside (NO donor) were similar. The findings suggest that during pregnancy higher estradiol and lower levels of asymmetric dimethylarginine and increased NO compared with conventional endothelium-dependent agonists may afford some cardiovascular benefit for patients with EH.

2.7 A NOVEL MUTATION IN THE STEROIDGENIC ACUTE REGULATORY (STAR) PROTEIN GENE PROMOTER LEADING TO REDUCED PROMOTER ACTIVITY

Casal A1, Sinclair V2, Ferrari P3, Capponi A4
1 University Hospital, Geneva, Switzerland, 2 Fremantle Hospital, Perth, Bahamas

The Steroidogenic Acute Regulatory (STAR) protein plays a crucial role in intramitochondrial cholesterol supply for mineralcorticoid biosynthesis. We have identified a novel C→T polymorphism of the human STAR gene promoter located 59bp downstream of the SFI binding site and 99bp upstream of the TATA box. Carriers of this mutation have a high prevalence of primary aldosteronism. In transfection experiments, basal STAR promoter activity was unaltered by the mutation in murine Y1 cells and human H295R cells. In Y1 cells, forskolin (25 μM, 6h) significantly increased wild-type promoter activity to 230±33% (P<0.05, n=4). In contrast, forskolin increased mutated promoter activity only to 150±27%, with a significant 35 % reduction compared to wild type (P<0.05, n=3). In H295R cells, AngII (10ng/ml) increased wild type STAR promoter activity to 265±22% (P=0.01, n=3), while mutated STAR promoter activity in response to AngII only reached 190±29% of controls (P=0.01, n=3). Moreover, overexpression of DAX-1 led to a significantly greater concentration-dependent reduction of mutated promoter activity in response to AngII as compared to wild-type promoter activity. Gel mobility shift assays showed the formation of two additional complexes with the mutated promoter, one with the transcription repressor DAX-1 and another with a yet unidentified factor, which strongly binds the SFI response element. Thus, this novel mutation in the human STAR promoter is critically involved in the regulation of STAR gene expression and is associated with reduced promoter activity, a finding relevant for adrenal steroid response to physiological stimulants.

4.1 FLAVANOL-RICH DARK CHOCOLATE DECREASES BLOOD PRESSURE, IMPROVES ENDOTHELium-DEPENDENT VASORELAXATION, AND AMELIORATES INSULIN SENSITIVITY IN PATIENTS WITH ESSENTIAL HYPERTENSION

Grassi D1, Neccione S2, Lippi G3, Croce G1, Valeri L4, Pasqualetti P2, Desideri G2, Blumberg J.B3, Ferri C1
1 Department of Internal Medicine and Public Health, University of L’Aquila, L’Aquila, Italy, 2 Jean Mayer USDA Human Nutrition Research Center on Aging, Tufts University, Boston, United States

Dark chocolate (DC) has been shown to decrease blood pressure (BP) and insulin resistance in healthy subjects, suggesting similar benefits in patients with essential hypertension (EH). Therefore, we tested the effect of DC on 24-h ambulatory BP (ABP), flow-mediated dilation (FMD), and oral glucose tolerance tests (O GTT) in patients with EH. After a 7-d chocolate-free run-in phase, 20 never-treated, grade I patients with EH (10 M; 43.7±7.8 y) were randomised to receive either 100 g/d DC (88 mg flavanols) or 90 g/d flavanol-free white chocolate (WC) in an isocaloric manner for 15 d. After a second 7-d chocolate-free period, patients were crossed over to the other treatment. 24-h ABP, FMD, OGTT, serum cholesterol and markers of vascular inflammation were evaluated at the end of each treatment. The homeostasis model assessment of insulin resistance (HOMA), quantitative insulin sensitivity check index (QUICKI), and insulin sensitivity index (ISI) were calculated from OGTT values. ABP decreased after DC (24-h SBP: −11.9±7.7 mmHg, p<0.0001; 24-h DBP: −8.4±5.0 mmHg, p<0.0001) but not WC. DC but not WC decreased HOMA (p<0.0001), while improving QUICKI, ISI, and FMD. DC also decreased serum LDL cholesterol (from 3.4±0.5 to 3.0±0.6 mmol/L, p<0.05), decreased BP, insulin resistance and serum LDL cholesterol and improved FMD in hypertensives. These results suggest that, balancing total calorie intake, flavanols from cocoa products may provide some cardiovascular benefit for patients with EH.

4.2 HIV INFECTION AS AN INDEPENDENT RISK FACTOR FOR PREMATURE ATHEROSCLEROSIS

Lorenz M1, Harmantje A2, Staszewski S2, Buehler A1, von Kegler S1, Steckel D3, Sitzor M3, 1 J.W. Goethe-Universität Frankfurt, Department for Neurology, Frankfurt, Germany, 2 J.W. Goethe-Universität Frankfurt, Department for Internal Medicine III, Frankfurt, Germany

Premature atherosclerosis is overrepresented in HIV patients. In a case control design we investigated the association of HIV infection with ultrasonic signs of atherosclerosis in the carotid artery system. 292 consecutive HIV patients were examined with B-mode ultrasound of the carotids arteries, including intima media thickness (IMT). 116 controls were randomly selected from the Carotid Atherosclerosis Progression Study (CAPS) population (matched for age and sex). HIV patients had a 5.0% (95% confidence interval: [2.3, 7.6%], p=0.0002) or 0.036mm [0.012, 0.060mm] (p=0.0034) higher common carotid IMT (adjusted for multiple risk factors). In the carotid bifurcation IMT values were 19% [14, 24%] or 0.180mm [0.123, 0.238mm] higher in HIV patients (p<0.0001, respectively). This effect was not significant for internal carotid IMT, but showed a trend (IMT 4.3% [7.9, 0.3] or 0.037mm [0.016, 0.069] higher, p=0.005 or 0.1271). Plaques in the internal carotid arteries of HIV patients were significantly more frequent (12.3% vs. 7.8%, p=0.0312) in HIV patients in unadjusted models, but this effect did not prove independent. The present data show that long term HIV infection is an independent risk factor for early atherosclerosis. Assuming a risk ratio like found in large population-based trials, the observed IMT elevation suggests that vascular risk is 4–14% higher in HIV-positive subjects, or that their ‘vascular age’ is 4–5 years higher. Future research has to be done to find an approach to specific pathophysiology and to evaluate the role of HIV-specific medication.

4.3 IGG ANTIBODIES TO MALONDIALDEHYDE-LYSEINE LOW-DENSITY LIPOPROTEINS PREDICT MORTALITY IN HIGH RISK CORONARY ARTERY DISEASE PATIENTS

Rossi G.P1, Maiolino G.2, De Toni R.3, Cesari M.2, Zanchetta M.1, Sticchi D.2, Pedon L.1, Maiolino P.1, Pesina A.C.2
1 Servizio di Emotimica & Divisione di Cardiologia Ospedale di Cittadella, Cittadella, Italy, 2 DMCS-Internal Medicine 4 University of Padua, Padova, Italy

Background. Antibodies to oxidized low-density lipoprotein (oxLDL) or oxLDLAbs are detectable in serum of patients with atherosclerosis but their role in atherogenesis remains controversial. We therefore tested the hypothesis that oxLDLAbs predict cardiovascular (CV) death in a prospective cohort study. Methods. In 475 consecutive patients of the GENICA Study, who underwent coronary angiography, we measured by ELISA the titer of IgG antibodies to malondialdehyde-lyseine LDL, which serve as a model of oxLDL epitope. We then determined the incidence of CV death and events at follow-up. Based on the cutoff value of 15 EU/mL oxLDLAbs, that best differentiated the survivors from the deceased patients at ROC curve analysis and roughly corresponds to the 75th percentile of the distribution in healthy controls, patients were divided in a high and a low oxLDLAbs titer group. Results. Follow-up data were obtained in 95% of patients. After a median follow-up of 1408 days (range 4–2053 days) we observed 61 all-cause deaths, of which 36 (59%)
were CV deaths. Kaplan-Meier analysis demonstrated a worse CV death (p = 0.0017) and event-free survival (p = 0.0047) in the high oxLDLabs group. Cox regression analysis confirmed the impact of high oxLDLabs on CV death (p = 0.020) and events (p = 0.043), independent on the coronary atherosclerotic burden and the major CV risk factors. **Conclusions.** These findings favor the hypothesis that a high oxLDLabs titer plays an independent pathogenic role in high-risk Caucasian patients referred for coronary angiography.

### 4.4 EARLY LIFE FACTORS AND BLOOD LIPID CONCENTRATIONS AT AGE 31 IN THE 1966 NORTHERN FINLAND BIRTH COHORT

Jarvelin MR,1,2 Lauren L,1,2 Lens M,1 Sovio U,1 Canoy D,1 Posta A,1 Hartikainen A-L,1 Ruokokoski R,1 Elliott P

1 Imperial College London, London, United Kingdom, 2 University of Oulu, Oulu, Finland

**Background:** Findings from studies investigating the association between foetal growth and adult lipid concentrations have been inconsistent. Aim and methods: We examined the shape and size of association between determinants of foetal growth, size at birth (birth weight, birth length, ponderal index), and adult blood lipid levels at 31 years in the prospective northern Finnish 1966 birth cohort followed from early pregnancy (n = 5877). **Results:** Size at birth showed a reverse relation with total cholesterol, low-density lipoprotein and triglycerides (TG), whereas the relation with HDL was direct. The associations were consistent and statistically significant for TG, and less so for other lipids. Adjusted (gestational age and maternal factors) regression coefficients showed 7.3% lower TG per kg 1 higher birth weight (95% CI -11.0, -3.4; p < 0.001) in men and 3.9% (95% CI -7.4, -0.3; p = 0.038) in women. Adjusting additionally for BMI resulted in stronger regression estimates in both sexes. **Conclusions and discussion:** Our study, the largest to date, with extensive pre/perinatal and lifecourse data supports the concept that early growth is an important determinant of blood lipid levels in later life, especially TG. Furthermore, a clear inverse association between birth weight and adult blood pressure was found in the same population. Research is needed to elucidate foetal programming of metabolic diseases. Integrins are glycoproteins transmitting signals from the extracellular matrix to the cytoskeleton. Several integrins might serve as mechanotransducer in metabolic diseases. Integrins are glycoproteins transmitting signals from the extracellular matrix to the cytoskeleton. Several integrins might serve as mechanotransducer in microcirculation, by transmitting the signal to the NO-synthase through activation of Akt.

### W1.3 INVOLVEMENT OF α1-β1 INTEGRINS IN THE ACTIVATION OF AKT AND NO-SYNTHESIS AFTER FLOW (SHEAR STRESS) ACTIVATION OF VASODILATION IN RESISTANCE ARTERIES.

Launffari L, Dumont O, Lacolley P, Henrotin D

1 UMR CNRS 6188, Angers, France, 2 INSERM U884, Nancy, France

Resistance arteries are the site of the earliest manifestations of many cardiovascular and metabolic diseases. Integrins are glycoproteins transmitting signals from the extracellular matrix to the cytoskeleton. Several integrins might serve as mechanotransducer in endothelial cells migration and in acute endothelial-dependent vasodilation. The α1-β1 integrin plays a key role in angiogenesis. Flow (shear stress) is the main physiological stimulus for the vascular endothelium and its role in angiogenesis is well established. We assessed the diatolic response to acute changes in flow in mesenteric resistance arteries (MRA) isolated in arteriograms. In MRA of α1-β1 integrin deficient mice (floxed-integrin flox/−−) flow-mediated dilatation (FMD) was lower than in control mice (23.8 ± 1 versus 11.4 ± 0.6 μm maximal dilatation). Pressure- (myogenic tone) and agonist-induced contractions, as well as acetycholine- and sodium nitropusside-induced dilatations, were not affected by the absence of α1-integrin. In control mice, α1-integrin was present in MRA endothelial cells. In MRA from α1-β1−/− mice, endothelial NO-synthase expression was not affected but phosphorylated-Akt was lower, compared to α1+/− mice. Thus, α1-β1 integrin might have a key role in flow (shear stress)-dependent vasodilation in the microcirculation, by transmitting the signal to the NO-synthase through activation of Akt. Due to the central role of flow (shear stress) activation of the endothelium in vascular disorders this finding opens new perspectives in the pathophysiology of the microcirculation.

### W1.4 CHANGES IN THE EXTRACELLULAR MATRIX IN SUBCUTANEOUS SMALL RESISTANCE ARTERIES OF PATIENTS WITH PRIMARY ALDOSTERONISM

Rizzoni D,1 Porteri E,1 De Cucieis C,1 Paiardi S,1 Rodella L,2 Rezzani R,2 Boari G.E.M,1 Zani F,1 Miclini M,1 Tiberio G.A.M,1 Giulinì S.M,1 Bianchi R,1 Agabiti Rosei E.2

1 Chair of Internal Medicine, University of Brescia, Brescia, Italy, 2 Chair of Human Anatomy, University of Brescia, Brescia, Italy

**Objectives:** To evaluate the aldosterone profibrotic action in the human microcirculation. **Design and Methods:** Thirteen patients with primary aldosteronism, 7 hypertensives and 10 normotensives were submitted to a biopsy of subcutaneous tissue. Small resistance arteries were dissected and mounted on an isometric myograph, and the tunica media to internal luminal ratio (M/L) was measured. In addition, the total collagen content within the tunica media was detected (Sirius red staining and image analysis). Collagen subtypes were evaluated using polarized light microscopy; under this condition thicker and denser type I collagen fibers appear orange or red, while thinner type III collagen fibers are yellow or green. Results: The M/L was significantly increased in primary aldosteronism and hypertensives compared with normotensives. Clinic blood pressure values were similar in primary aldosteronism and in essential hypertension, and greater than in normotensives. Total collagen and type III vascular collagen was significantly greater in primary aldosteronism (7.8 ± 2.91% and 5.92 ± 1.73%, p < 0.01) than in essential hypertension (7.0 ± 0.46% and 5.5 ± 0.16%, respectively). Moreover, controls had less total and type III collagen in respect with the two hypertensive groups (3.2 ± 1.63% and 1.6 ± 0.64%, p < 0.01). Type I collagen was less in primary aldosteronism (2.21 ± 1.07%, p < 0.05) than in normotensive controls (2.51 ± 1.03%). **Conclusions:** Our results indicate that, in small resistance arteries of patients with primary aldosteronism, a pronounced fibrosis may be detected, even more evident in blood-pressure matched patients with essential hypertension.
Early and transient blockade of the renin-angiotensin system abates the development of hypertension in SHR. We investigated the degree and extent of cardiac protection by brief angiotensin antagonism in young SHR. Male and female SHR (n = 18) and WKY (n = 9) aged 4 weeks were used. Three SHR with losartan (SHRLos; 20mg/kg/day sc) for 4 weeks. In half of each group telemetry devices were implanted. Blood pressure measurements (at 2-week intervals) and echocardiography (3-monthly intervals) were performed till an age of 60 weeks. Data are given as means ± SE in the order WKY, SHR, SHRLos. Blood pressure development was abated in SHRLos till week 36. Echocardiography revealed from week 24 onward significantly increased enddiastolic and end systolic volume (EDV; ESV) in untreated SHR compared to WKY and SHRLos (week 60: EDV: 0.29 ± 0.06, 0.54 ± 0.03, 0.32 ± 0.04 cm³; ESV: 0.11 ± 0.02, 0.33 ± 0.03, 0.16 ± 0.01 cm³; P < 0.001). SHRLos were not significantly different from WKY at any time points. Similar results were obtained with respect to ventricular wall volume (VW; week 60: 0.55 ± 0.001, 0.86 ± 0.003, 0.58 ± 0.003 cm³; P < 0.001). Septal, lateral and inferior A’ increased with male gender (0.0001 tricular mass. A total of 55 healthy volunteers, aged 20 –57 years (24 men), underwent standard echocardiography and velocity measurements. We searched for possible covariates of the tissue Doppler velocities using stepwise multiple regression. The lateral and inferior A’ velocities exceeded the septal velocities for S’ (11.5 cm/s and 9.8 cm/s versus 8.6 cm/s, respectively) and E’ (16.2 cm/s and 14.3 cm/s versus 11.9 cm/s), but not A’ (9.1 cm/s and 10.5 cm/s versus 9.4 cm/s). Stepwise multiple regression demonstrated that septal E’ (P < 0.002), age (P < 0.0001) and body mass index (P < 0.001) predicted septal E’. Similarly, lateral and inferior E’ significantly decreased with age (P < 0.0001) and inferior E’ also independently decreased with body mass index (P < 0.0008). Septal, lateral and inferior A’ increased with male gender (0.0001) and inferior A’ increased with age (P < 0.002), age (P < 0.0001), and body mass index (P < 0.001). Thus, the inhibition of ubiquitin-proteasome activity in atherosclerotic lesions of diabetic patients by rosiglitazone therapy is associated with plaque stabilization, possibly by suppression of inflammation NFκB-induced.

W.3.3
LONG-TERM ECHOCARDIOGRAPHIC FOLLOW UP OF BRIEF AND EARLY LOSARTAN TREATED SHR REVEALS SUSTAINED CARDIAC PROTECTION

Baumann M, Smits JFM, Struijkij Boudier HAJ
Dept. of Pharmacology, CARIM, University Maastricht, Maastricht, Netherlands

We evaluated ubiquitin-proteasome activity in carotid plaques of asymptomatic diabetic and nondiabetic patients, and the effect of rosiglitazone, a peroxisome proliferator–acti- vated receptor-γ (PPAR-γ) agonist, in diabetic plaques. Plaques were obtained from 33 type 2 diabetic and 30 nondiabetic patients undergoing carotid endarterectomy. Diabetic patients received 8 mg rosiglitazone (n = 23) or placebo (n = 10) for 4 months scheduled endarterectomy. Plaques were analysed for macrophages (CD68), T-lymphocytes, and HLA-DR+ cells (P < 0.0001), more ubiquitin, proteasome 20S, NFκB, phospho-κB–α, tumor necrosis factor–α (TNF–α), nitrotyrosine, matrix metalloproteinase-9 (MMP–9) and collagen content (by immunohistochemistry and ELISA) and compared with nondiabetic plaques. Diabetic plaques had more macrophages, T-lymphocytes, and HLA-DR+ cells (P < 0.0001), more ubiquitin, proteasome 20S, NFκB, phospho-κB–α, TNF–α, nitrotyrosine, MMP–9 (P < 0.0001) and O2– production along a lesser collagen content and κB–α (P < 0.0001) compared with nondiabetic plaques. Compared with rosiglitazone-treated diabetic plaques, placebo-treated diabetic plaques group had more macrophages, T-lymphocytes, and HLA-DR+ cells (P < 0.01), more ubiquitin, proteasome 20S, NFκB, phospho-κB–α, TNF–α, nitrotyrosine, MMP–9 (P < 0.0001) and O2– production along a lesser collagen content and κB–α (P < 0.0001). Thus, the role of complement and C-reactive protein (CRP) in Ang II-induced vasculopathy is not elucidated.

W.4.3
ANTHROPOMETRIC AND HEMODYNAMIC DETERMINANTS OF MITRAL ANNUAR VELOCITIES IN HEALTHY ADULTS

Kuznetsova T, Staessen JA, Pluimun M
University of Leuven, Leuven, Belgium

In 2003, the MRC funded BRIGHT study reported a ‘first pass’ genome scan for hypertension genes using 2010 severely hypertensive sib-pairs from 1599 white European

5.3
VCAFl, a NOVEL GENE ASSOCIATED WITH VASCULAR CALCIFIED LESIONS

University of Manchester, Manchester, United Kingdom

Vascular calcification is associated with an adverse prognostic risk and presents an important and unresolved dilemma in the clinic. We aim to identify molecules involved in this process in order to develop strategies for treatment. Recent evidence suggests that vascular progenitor cells, also known as pericyte-like cells exist in the vessel wall and can differentiate into osteogenic cells. Previously we have reported the identification of a novel gene (C15), which is expressed in mineralised vascular pericytes and differentiate into osteogenic cells. Previously we have reported the identification of a novel early marker for vascular progenitor cells which, if proved correct, could have implications for the prevention or treatment of this clinically important, multifaceted vasculopathy.

5.4
C-REACTIVE PROTEIN AND COMPENSATION IN ANGIOTENSIN II-INDUCED ORGAN DAMAGE

1 HELIOS, Franz-Volhard Clinic, Berlin, Germany, 2 Dept. Medicine-Nephrology, Hannover Medical School, Hannover, Germany, 3 Max-Delbrueck-Center, Berlin-Buch, Germany

The role of complement and C-reactive protein (CRP) in Ang II-induced vasculopathy is not defined. We studied complement in an Ang II model and tested whether complement activation in the kidneys precedes or is the consequence of albuminuria. We also examined whether complement C3 is induced differently in smooth muscle cells (VSMC) with a synthetic compared with the ‘contractile’ phenotype. We used double transgenic rats harboring human renin and angiotensinogen genes (STGR) with or without losartan (LOS).
INHIBITOR OF DIFFERENTIATION ID2 IS INVOLVED IN THE PATHOGENESIS OF ANGIOTENSIN II-INDUCED END-ORGAN DAMAGE


1 HELIOS, Franz-Volhard Clinic, Berlin, Germany, 2 Dept. Medicine-Nephrology, Hannover Medical School, Hannover, Germany, 3 Humboldt University, Berlin, Germany, 4 Max-Delbrueck-Center, Berlin-Buch, Germany, 5 Uni. Med. Sch.Rheinisch-Westfälische Technische Hochschule Aachen, Aachen, Germany

The helix-loop-helix transcription factor inhibitor of differentiation (id) 2 has a pivotal role in determining cell lineage choice and differentiation. Id2 deficient mice (id2-/-) lack angiotensin (Ang) II-induced cardiac hypertrophy and blood pressure increases. We measured id2-/- 0.5mg/kg, with TGM. We compared TGMid2-/- with TGMid2+/- and the non-transgenic strains (WTid2-/- and WTid2+/-). Transgenic TGMid2+/+ showed significantly increased heart weight, cardiac hypertrophy index (5.4±2 mg/g), cardiac fibrosis (60%; p<0.05) and left ventricular brain natriuretic peptide (BNP) expression. Albinouima (235±40 pg/ml), renal fibrosis, and cell infiltration were also significantly elevated. TGMid2-/- showed normal heart weight, extracellular matrix deposition, and tubular changes in the outer medulla. In contrast, cardiac hypertrophy (4.3±0.2 mg/g), albuminuria (235±10 pg/ml), renal and cardiac fibrosis, macrophage and T-cell accumulation in the kidney and heart, were all significantly reduced in TGMid2+/-, Albuminuria in WTid2-/- and WTid2+/- was 10±2 and 22±5 pg/ml, respectively. No histopathological changes were observed in TGMid2+/- and the non-transgenic groups. Our results demonstrate that id2 plays a major role in the pathogenesis of Ang II-induced renal and cardiac damage.

REDUCED INFLAMMATION UNDER CANDESA TAN TREATMENT IN TRANSIENT FOCAL ISCHEMIA IN RATS AND IN ASTROCYTE PRIMARY CULTURE

Schmerbach K1, Krikov M1, Schiefl E1, Neumann C1, Mueller S1, Vlirringer A1, Unger T1, Thoeni-Reineke C1

1 Center for Cardiovascular Research, Charité-Universitätsmedizin Berlin, Berlin, Germany, 2 Clinic and Polyclinic for Neurology, Charité-Universitätsmedizin Berlin, Berlin, Germany

In recent studies, the AT1 receptor blocker (ARB), candesartan (C), was shown to reduce infarct size and improve neurological outcome after stroke. Since C also exerts anti-inflammatory actions and since stroke is associated with an inflammatory response, we hypothesized that candesartan ischaemia-induced brain injury by an anti-inflammatory action. Normotensive wistar rats were pretreated (p.o.; twice daily) for 5 days with 0.1 mg/kg C or vehicle (0.9% NaCl). Middle cerebral artery occlusion (MCAO) was performed for 90 min with reperfusion. Infarct volume was measured by MRI 48h after stroke. The results of our in vivo and in vitro studies identify anti-inflammatory mechanisms of candesartan which may contribute to the known beneficial effects of this ARB in cerebral ischemia.

LEPTIN-INDUCED VASODILATATION IS IMPAIRED IN SPONTANEOUSLY HYPERTENSIVE RATS

Gonzalez MC1, Abderrahim F1, Calvez-Prieto B2, Sommoza B1, Fernández-Alfonso MS2

1 Departamento de Fisiología, Facultad de Medicina UAM, Madrid, Spain, 2 Unidad de Cartografía Cerebral, Instituto Plusdisciplinar, UCM, Madrid, Spain, 3 Dep Farmacología, Facultad de Ciencias Experimentales, USP, Madrid, Spain

Leptin is produced by adipose tissue and participates in blood pressure regulation by inducing a direct relaxation of blood vessels (Lembo et al, 2000). The aim of this study is to analyze if there are differences in leptin-induced relaxation in aorta of 3-month-old Wistar Kyoto (WKY) and aged-matched spontaneously hypertensive rats (SHR). Contraction to 75 mM KCl and endothelium-dependent relaxation to acetylcholine (10^-4 to 10^-6 M) were not different between groups (area under curve (AUC-WKY) = 280.7 ± 19; AUC-SHR = 290 ± 10). Vasodilatation induced by leptin (10^-10 M) in aortic rings preconstricted with 10^-4 M phenylephrine was significantly lower in SHR compared to WKY (25.9 ± 5.5% in WKY; 9.4 ± 6% in SHR). In presence of leptin, relaxation to acetylcholine (10^-10 to 10^-5 M) was significantly reduced in both strains (AUC-WKY = 88 ± 10; AUC-SHR = 86 ± 10; p<0.05). However, relaxation was more significantly reduced in SHR (74.4%) than in WKY (60%; p<0.05). We have determined the expression of both leptin receptors in the aorta, the short form (Ob-Ra) and the long form (Ob-Rb). Ob-Ra was lower in aortic homogenates from SHR when compared to WKY rats (aorta: WKY = 21 ± 1.6 arbitrary units; SHR = 11.1 ± 0.8 arbitrary units; p<0.05). No differences were detected in the expression of Ob-Rb receptor between strains. These results suggest that leptin-induced relaxation is reduced in SHR and that leptin decreases endothelium-dependent relaxation. These effects might contribute to the increased blood pressure in this strain. (Supported by Comunidad Autónoma de Madrid GR/SAL/0558/2004).

GLUTATHIONE DEPLETION ACCELERATES TELOMERE ATTENTION IN SPECIFIC MOUSE TISSUES

Cattan V1, Labat C2, Bezje Y, Benetoes A3, Gardiner JP, Avis A1, Lacolley P1

1 Inserm U684, Vandoeuvre les Nancy, France, 2 Hopital Saint Joseph, Paris, France, 3 CIC Inserm CHU, Nancy, France, 4 Hypertension Research Center, Newark, United States

Accumulation of oxidative damage has long been regarded as a risk factor for age-related disorders, including cardiovascular disease. Telomere attension in vivo appears to register not only the replicative history but also the cumulative burden of oxidative stress in somatic cells. We previously reported that age-adjusted telomere length in human leukocytes was inversely correlated with pulse pressure and pulse wave velocity, indices of arterial aging. We hypothesized that oxidative stress may accelerate arterial aging and telomere attrition. To this end, we subjected CAST/Ei mice (n=14) to glutathione depletion with the glutathione synthase inhibitor buthionine sulfoximine (BSO, 30mg/kg drinking water) for 6 weeks vs. placebo. The BSO-treated mice showed a 2-fold decrease in tissue glutathione content and an increase in the systemic oxidative stress, expressed by increased tissue carboxyl content and plasma advanced oxidative protein products levels (45%, p<0.05). Glutathione depletion did not induce significant elevation of arterial pressure in CAST/Ei mice. TRF length significantly decreased in the white fat, brown fat, skin and testis of BSO-treated mice. However, TRF length was not different in the heart, kidney, lungs, liver, spleen, muscle and large intestine between BSO-treated and control mice. In conclusion, we showed that an increase in oxidative stress, induced by glutathione depletion in vivo in the CAST/Ei mice, could accelerate telomere attrition in specific tissues. However, we did not observe an effect of glutathione depletion on cardiovascular phenotypes, possibly due to the magnitude of oxidative stress and/or the duration of BSO treatment.

PHARMACOLOGICAL ACTIVATION OF ENOS IMPROVES VASCULAR FUNCTION IN STREPTOZOTOCIN-INDUCED DIABETES MELLITUS TYPE 1 RATS IN VIVO

Rad A1, von Linscheid S1, Mohr Z2, Rutten H2, Tschop C1

1 Charité, Berlin, Germany, 2 Aventis Pharma, Frankfurt, Germany

Introduction: Diabetes mellitus leads to endothelial dysfunction associated with a reduced bioavailability of NO. In the present study we investigated the influence of pharmacological activation of the endothelial NO synthase (eNOS) on endothelial function under diabetic conditions in vivo. Methods: Streptozotocin- induced diabetic Sprague Dawley rats were treated with the eNOS enhancer S803 for 48 days and compared with untreated STZ-rats. The non-endothelial-dependent dilatation was analyzed after application of sodium nitroprusside. Vascular response to the stimuli was analyzed after calculation of the integral (I, mmHg*g) of the pressure decrease during the vasodilatation. ENOS protein expression in hindlimb muscles was determined by Western Blot. Results: Both, endothelial-dependent and endothelial-independent vasodilatation responses were signific-
incidentally reduced in untreated STZ compared to normoglycemic Co (p<0.05). This finding was associated with a 1.5-fold reduced (p<0.03) expression of eNOS in STZ rats vs. Co. Endothelial-dependent vasodilatation of the STZ/+/+ group was significantly improved (p<0.05). The endothelial-independent dilatation was not influenced. Improved endothelial-dependent vasodilatation of the STZ/−/− group was correlated with a 2.2-fold (p<0.05) increase in eNOS expression compared to the STZ group. Conclusion: These data suggest that improved eNOS expression caused by Si803 has beneficial effects on endothelial dysfunction under diabetic conditions. This could be a new therapeutic strategy against the endothelial dysfunction under diabetic conditions.

PA.4
THE EFFECT OF CARVEDILOL VERSUS METOPROLOL ON ENDOTHELIAL FUNCTION IN PATIENTS WITH TYPE 2 DIABETES.
Kweilborg B1, Herrmann T1, Major-Petersen A1, Dominguez H3, Ittienne M4, Kober L5, Torp-Petersen C1
1 Bispebjerg University Hospital, Copenhagen, Denmark, 2 Gentofte University Hospital, Copenhagen, Denmark, 3 Rigshospitalet University Hospital, Copenhagen, Denmark, 4 Frederiksberg Hospital, Copenhagen, Denmark

Background: Carvedilol compared to metoprolol improves survival in patients with heart failure. Carvedilol also reduces the rate of new onset diabetes. Patients with type 2 diabetes have endothelial dysfunction and insulin resistance. To elucidate the possible beneficial vascular effect of carvedilol, we studied the effect of metoprolol and carvedilol on endothelial function in a group of patients with type 2 diabetes. Methods: 18 patients with type 2 diabetes were randomized in an open parallel study design to receive a treatment with either carvedilol or metoprolol. Forearm blood-flow was measured by using bioelectric venule occlusion plethysmography. Serotonin was used to assess endothelium-dependent vasodilation and sodium nitropusside to assess endothelium-independent vasodilatation. Insulin-stimulated endothelial function was studied by co-infusion of serotonin after local infusion of insulin for 60 minutes. Forearm blood-flow was studied before and after a 24 month treatment period with either metoprolol or carvedilol. Results: Absolute flow before and after the treatment with carvedilol was 4.35±0.42 ml/min/100 ml tissue and 4.22±0.42 ml/min/100 ml tissue respectively and 3.85±0.32 ml/min/100 ml tissue and 4.44±0.41 ml/min/100 ml tissue before and after treatment with metoprolol respectively. Insulin stimulated vasodilation did not differ before and after treatment with neither carvedilol nor metoprolol. Conclusion: Endothelial function in patients with type 2 diabetes was not improved by either metoprolol nor carvedilol treatment. Neither was insulin dependent endothelial function changed from the beta blocker treatment.

PA.5
UPREGULATION OF PPAR-γ IN AN EXPERIMENTAL MYOCARDIAL INFARCTION RAT MODEL DOES NOT LEAD TO UPREGULATION OF PPAR-γ METABOLIC TARGET GENES
Fleger D1, Becher E1, Riad A2, Schubert C1, Techupe C1, Negitz-Zagrosek V1
1 Charité University Medicine, Berlin, Germany, 2 German Heart Institute, Berlin, Germany

The nuclear receptor PPAR-γ induces metabolic target genes. As myocardial injury leads to changes in the substrate preference of the energy metabolism, we assumed that PPAR-γ expression is a central switch point for cardiac energy metabolism in myocardial injury. We determined the effects of isobutane, since angiotensin receptor blockers interact with PPAR-γ. Male Wistar rats were treated with placebo or isobutane after experimental myocardial infarction. Hemodynamic conductance analysis was preformed with all animals. Western Blot and Real Time RT-PCR (Taq-Man) analysis was done after experimental myocardial infarction. Hemodynamic conductance analysis was preformed with all animals. Western Blot and Real Time RT-PCR (Taq-Man) analysis was done after experimental myocardial infarction. Forearm blood-flow was studied before and after a 24 month treatment period with either metoprolol or carvedilol. Results: Absolute flow before and after the treatment with carvedilol was 4.35±0.42 ml/min/100 ml tissue and 4.22±0.42 ml/min/100 ml tissue respectively and 3.85±0.32 ml/min/100 ml tissue and 4.44±0.41 ml/min/100 ml tissue before and after treatment with metoprolol respectively. Insulin stimulated vasodilation did not differ before and after treatment with neither carvedilol nor metoprolol. Conclusion: Endothelial function in patients with type 2 diabetes was not improved by either metoprolol nor carvedilol treatment. Neither was insulin dependent endothelial function changed from the beta blocker treatment.

PA.6
KININOGEN DEFICIENCY CONTRIBUTES TO FATTY LIVER DEGENERATION PROMOTING PROTEOLYSIS AND LIPAOPTOSIS
Kaschyna E1, Stoll M2, Sommerfeld M3, Kreutz R4, Unger T1
1 Center for Cardiovascular Research, Charité University Medicine Berlin, Germany, 2 Institute for Atherosclerosis Research, University of Munich, Munich, Germany, 3 Department of Clinical Pharmacology, Charité University Medicine Berlin, Germany

Previously, we have shown that Brown Norway (BN) and BN Katholik (BN/Ka) rat strains are both susceptible to develop fatty liver degeneration after exposing them to atherogenic diet. BN/Ka are different from BN by a genetically determined deficiency in kinogen, the latter known to inhibit cystein proteases (cathepsins). Using a model of fatty liver disease, we tested the hypothesis that, by kinogen deficiency, cathepsins may be more easily activated and, therefore, promote cell damage. Both rat strains (BN and BN/Ka) were proven to be inbred. After 12 weeks on high fat diet, plasma liver tests, in situ TUNEL staining, Western Blot and immunostaining for cathepsins B,D,L, apoptotic marker Fas, and measurement of cathepsin B activity in the liver were performed. BN/Ka compared to BN developed more severe liver damage characterized by hepatomegaly (4.2 vs 3.5-fold of liver/brain index), pronounced liver apoptosis and an increase in total plasma bilirubin (4.7±0.3 vs 3.0±0.2 µl, p<0.005). Protein expression of active cathepsins B, D, L and Fas was strongly up-regulated, the activity of cathepsin B was 3-fold higher in the liver of BN/Ka, compared to BN. Fas, was co-localized with cathepsin B. Thus, kinogen deficiency contributes to organ damage in fatty liver disease promoting cathepsin - mediated proteolysis and Fas - mediated lipaoptosis.

PA.7
LOW PLASMA ADIPONECTIN IS A HALLMARK OF THE METABOLIC SYNDROME IN NON DIABETIC HYPERTENSIVE HIGH-RISK PATIENTS
Cesari M1, Zanchetta M1, Maioli G1, Pessina AC2, Rossi GP2
1 Division of Cardiology, Cittadella Hospital, Cittadella, Italy, 2 DMCS - Clinica Medica 4, Padova, Italy

Background: The association between plasma adiponecin levels (pA) with hypertension (HT) and the metabolic syndrome (MS) in non-diabetic Caucasians is unknown. Aim: To investigate the relationship of pA with HT and THE MS in non-diabetic high-risk Caucasian patients. Methods: We investigated 400 non-diabetic HT and NT patients of the GENICA Study, undergoing coronary angiography for suspected CAD. HT was diagnosed according to the ESHEC guidelines or if patients were on antihypertensive treatment. MS was defined according to NCEP guidelines. We measured pA with an ELISA method; insulin resistance was evaluated with the HOMA index. Results: HT was present in 211 (53%) and the MS in 52 (13%). pA, which showed a non-gaussian distribution and therefore required square root transformation, showed no significant differences between NT and HT (9.25±0.5 vs 9.18±0.4 mg/ml, p=NS). By contrast, when patients with and without the MS were compared, significantly lower pA (6.35±0.6 vs 8.67±0.5 mg/ml, p<0.013) and higher HOMA index (3.65±0.7 vs 1.8±0.1, p=0.007) were found in the latter patients. Furthermore, significantly higher pA values (9.28±0.5, p=0.018 vs MS pts) were found in normotenive individuals without the MS. Conclusions: In non-diabetic high-risk Caucasian patients, even though being unrelated to HT, pA was significantly blunted in those with the MS. Therefore, it could be considered as a marker of MS in Caucasian high-risk patients.

PA.8
IDENTIFICATION OF SUBSETS OF ACTH-RESPONSIVE AND NON-RESPONSIVE ALDOSTERONE-PRODUCING-ADENOMA (APA) BY DYNAMIC ADRENAL VEIN SAMPLING (AVS).
Gannazari C1, Miotto D2, De Toni R2, Palumbo G3, Pessina AC2, Rossi GP2
1 DMCS-Clinica Med. 4, University Hospital Padova, Padova, Italy, 2 Radiology, University hospital Padova, Padova, Italy, 3 Internal Medicine, Hospital Legnano, Legnano, Italy

Background. Increased expression of ACTH receptors in APA has been reported, thus suggesting enhanced aldosterone (A) responsiveness to ACTH in these tumours. We prospectively used stimulation with ACTH during AVS to investigate the response of A in APA. Methods. We performed bilateral AVS in 21 consecutive pts with primary aldosteronism and used strict ROC curves-derived criteria for interpretation of results. After 3 hours supine resting, blood was simultaneously obtained from both sides for measurement of Cortisol (C) and ACTH (250 µg, i.v. bolus) was then administrated and AVS was repeated after 30’. Selectivity was assessed by the C side/CIVC ratio. Several historical parameters (Weiss’s criteria: tumour size, nuclear atypia, necrosis, fibrosis, capsule, pushing edges, multiple nodules, adipose cells of fasciculate zona, small cells ofglomerulosa zona, small cells of reticular zona, hybrid cells, oncocytces, and balloon cells) were determined. Results, AVS was selective in 18 pts; APA was diagnosed in 16 and idiopathic hyperaldosteronism in two. In 13 APA pts who had adrenalectomy, the diagnosis was confirmed at histology and follow-up. Only 56% of these APA responded to A. ACTH with an increase of A in 10% in the adrenal vein ipsilateral to APA. When ACTH-responsive and – non-responsive APA were compared, no differences in aforementioned historical features were observed. Conclusions. 1) We found no evidence for an in vivo enhanced response of APA to ACTH; 2) dynamic testing during AVS allows discrimination of ACTH-responsive and non-responsive APA; 3) no differences of histopatology between the APA subtypes was detected.
MOLECULAR INTERACTIONS BETWEEN ESTROGEN RECEPTOR BETA AND PPARGAMMA

Foryst-Ludwig A, Clemenz M, Hartge M, Sprang C, Unger T, Kintscher U
Center for Cardiovascular Research (CCR), Charite(CCMU)-Universitätsmedizin Berlin, Berlin, Germany

Gender differences in cardiovascular disease are gaining importance in diagnosis and therapy, whereas the molecular mechanisms are widely unknown. The estrogen receptor beta (ERbeta) and PPARGamma belong to the family of nuclear hormone receptors. PPARGamma has been identified to exert multiple cardiovascular protective effects. To elucidate whether ERbeta modulates PPARGamma function, we studied the regulation of PPARGamma activation in murine 3T3-L1 cells by ERbeta. The PPARGamma-ligand pioglitazone (10μM) induced PPARGamma-activity by 14.6±1.8-fold, measured using a Ga4-humanPPARGamma-ligand binding domain (LBD)-Ga5 luciferase reporter assay. This activation was strongly suppressed by co-expression of ERbeta (500ng) (5.6±3.7-fold). Inhibition of PPARGamma-LBD activation by ERbeta (500ng) was not enhanced by the ERbeta ligand estradiol (E2, 100nM) (7±1.5-fold) indicating a ligand-independent mechanism. To address whether this inhibition is due to the competition for nuclear cofactors, we measured PPARGamma-LBD expressing the activator SRC1 together with ERbeta. SRC1 was able to abolish ERbeta's inhibitory effects on PPARGamma activation, identifying SRC1 as an important mediator of PPARGamma/ERbeta interactions. These data indicate that ERbeta inhibits PPARGamma activation in a ligand-dependent manner involving the nuclear coactivator SRC1. Molecular interactions between ERs and PPARs may help to understand gender differences in cardiovascular and metabolic diseases.

The effect of co-supplementation of copper and zinc on the development of atherosclerosis in iron overloaded hypercholesterolemic rabbits

Ettechaid Saeed 1, DiSilvestro Robert A 2, Rashidchadjed Naderneh 3, Hamili Manijeh 3
1 Biochemistry Lab, Drug Applied Research Center, Tabriz University of Medical Sciences, Tabriz, Iran; Human Nutrition, Ohio State University, Columbus, USA; 2 Department of Pathology and Forensic Medicine, Faculty of Medicine, Tabriz University of Medical Sciences, Tabriz, Iran; 3 Department of Animal Science, Faculty of Agriculture, Aazad University of Tabriz, Tabriz, Iran; Human Nutrition Lab, Drug Applied Research Center, Tabriz University of Medical Sciences, Tabriz, Iran

To assess the effect of single and co-supplementation of copper (Cu) and zinc (Zn) on development of thoracic atherosclerosis, 54 juvenile male New Zealand White rabbits were assigned to nine dietary groups including chow fed rabbits without mineral supplement, Cu - Zn, HC - Zn, HC - Cu, Zn - iron overload (FeO), FeO - Cu - Zn, FeO - FeO + Cu, FeO - FeO + Zn, and FeO - FeO + Cu - Zn. Sudan IV, haematoxylin-eosin and immunohistochemistry methods were used for assessment of the effect of various dietary treatments on thoracic atherosclerosis. Immunostaining of the thoracic aorta sections was done with an avidin-biotin complex method using RAM-11 for macrophages and HHF35 for smooth muscle cells (SMCs). A modest increase in aortic sudanophilia was shown in rabbits given HC - FeO (P < 0.001) as compared with other groups except those given HC without mineral supplementation and HC - FeO + Cu. A significant decrease was found in sudanophilia area of rabbits supplemented with single Zn (82.3±3.55%) compared to those given HC - FeO, HC - FeO + Cu, and HC without mineral supplementation, but the differences from animals given HC - Cu, HC - Zn, HC - Cu + Zn, and HC - FeO + Cu - Zn was insignificant (P>0.05). Atherosclerotic lesions in rabbits given HC - Zn were generally fatty streaks, consisting predominantly of intimal macrophage-derived foam cells (17.7±3.58%) and a few demonstrated SMCs (4.8±1.04%). Advanced atherosclerotic lesions and intimal calcification were observed in 40%. 20% and 33.3%, 16.7% of all lesions detected in rabbits given HC - FeO and HC - FeO + Cu, respectively, while transitional lesions were mostly found in rabbits given HC - Cu, HC - FeO + Zn, and HC - FeO + Cu - Zn. The results of this study indicated the inhibitory role of Zn in progression of atherosclerosis.

INCREASING LEFT VENTRICULAR DILATATION AND MORTALITY IN BIGLYCAN KNOCK OUT MICE AFTER INDUCTION OF MYOCARDIAL INFARCTION

Westernman D 1, Petrik C 1, Schlutehus H 1, Unger T 1, Tischho C 1, Fischer J 2
1 Charite, Universitatsmedizin, Berlin, Germany; 2 Charite, CCR, Dept Pharmacology, Berlin, Germany

Introduction: Biglycan is a small leucine-rich proteoglycan that binds and regulates collagen, TGF-β and other matrix molecules and might be involved in extracellular matrix regulation during tissue repair. Therefore, we investigated whether biglycan deficiency alters hemodynamic function and mortality rate after induction myocardial infarction (MI) in mice. Methods: MI was induced in mice with biglycan deficiency (BN-KO) and wildtype mice (BN-WT) by ligation of the left anterior descending coronary artery and were compared to SHAM operated animals. 21 days after induction of MI left ventricular (LV) hemodynamic function was assessed by pressure-volume measurements in vivo to obtain LV systolic pressure (SP), LV enddiastolic pressure (EDP), LV enddiastolic volume (ESV), and LV-endystolic volume (EDV). Furthermore, we analysed mortality rate after induction of MI. Results: LV function was altered in BGN-KO when compared to BGN-WT. This was shown by enlarged dilatation (EVS: 93±4.2 mmHg vs. 75±4.8 mmHg and EDV: 96±4.4 mmHg vs. 111±4.2 mmHg, p<0.05) and elevated EDP (24±2.7 vs. 18±1.8 mmHg; p<0.05) after MI. While SPI was unaltered in BGN-KO compared to BGN-WT. Additionally, mortality rate was significantly increased after myocardial infarction. Conclusion: Biglycan deficiency leads to enhanced dilatation and elevated EDP, surrogate parameters for LV remodelling. Furthermore, mortality rate was increased demonstrating a crucial role for biglycan during myocardial tissue repair in vivo.
effects of ADP and SNP in control and I-R arteries. Therefore, it is suggested that hydrogen peroxide may be a mediator of the cerebral vasodilatation to ADP under normal conditions, and that NO is distinct to hydrogen peroxide may be involved in the decrease of hydrogen peroxide vasodilatation to ADP after ischemia-reperfusion. (This work was supported, in part, by CM (GR/SAL/016/2004) and FMM (2004).

**PB.5**

**APOPTOSIS IS MAINLY INDUCED IN ASTROCYTE BUT NOT MEDIATED VIA ANGIOTENSIN AT1 RECEPTOR AFTER CEREBRAL ISCHEMIA**

Li J, Yihrne-Reineka C, Gerova N, Timm M, Krikov M, Unger T. Center for Cardiovascular Research (CCR), Charité – University Medicine Berlin, Berlin, Germany

Our recent findings suggest that cerebral angiotsin AT2 receptor-mediated neuroprotection is required for the beneficial actions of AT1 receptor antagonist in rat models of brain ischemia. It is unknown whether a direct inhibition of cerebral angiotensin AT1 receptor-mediated actions (i.e. apoptosis) also contributes to these cerebroprotective actions of AT1 receptor antagonists. The present study was designed to examine the in vivo role of AT1 receptors in cerebral ischemia-induced apoptosis. Forty-eight hours after ischemia, Western blot analysis showed an upregulation of p53 in the peri-infarct zone when compared to sham operated controls. Immuno-fluorescence staining further revealed that cerebral ischemia induced the expression of cleaved-caspase-3 in the peri-infarct zone when compared to contralateral side or sham operated controls. By double immuno-fluorescence staining, we could confirm that both AT1 receptor and increased cleaved-caspase-3 were mainly located in GFAP+ astrocytes. To clarify the in vivo role of AT1 receptor in astrocyte apoptosis, an AT1 receptor antagonist (candesartan) was used over a period of 5 days before cerebral ischemia. Candesartan significantly improved neurological outcome and reduced the infarct size forty-eight hours after cerebral ischemia. However, the upregulated p53 and cleaved caspase-3 remained unaltered. Thus, brain AT1 receptors do not seem to be involved in cerebral ischemia-induced astrocyte apoptosis. These findings indicate that a direct inhibition of apoptosis in the brain may not play a role in the neuroprotective effects of the AT1 receptor antagonist after cerebral ischemia.

**PB.6**

**ROLE OF NITRIC OXIDE, PROSTANOIDS AND KCa CHANNELS IN THE RELAXATION OF GOK THERMAL CEREBRAL ARTERIES TO ADP AFTER ISCHEMIA-REPERFUSION**

Sanchez A, Salcedo A, Fernandez N, Monge L, Garcia-Villalon AL, Dieguez G. Universidad Autonoma, Madrid, Spain

The present experiments were performed to examine the role of nitric oxide (NO), prostanoids and KCa channels in the cerebrovascular response to ADP after ischemia-reperfusion (I-R). To this, the left middle cerebral artery was exposed, occluded for 60 min and then reperfused by other 60 min in anesthetized goats. At the end of reperfusion period, the animals were killed, and segments 3 mm in length taken from branches of the middle cerebral artery were preserved for immunohistochemistry. Arterial segments precontracted with U46199 (10^-10 M) showed dose-dependent relaxations to ADP (10^-8 to 10^-6 M) and sodium nitroprusside (SNP, 10^-10 to 10^-7 M), and the relaxation to ADP but not to SNP was lower in I-R arteries. The relaxation to ADP but not to SNP was inhibited by L-NAME (10^-6 M) only in I-R arteries, and this inhibition was comparable to that produced by L-NAME. Therefore, it is suggested that ADP relaxes normal cerebral vessels through the release of NO and activation of KCa channels, and b) this relaxation to ADP is decreased after ischemia-reperfusion, probably related to both the decrease in NO release and the increase in production of vasoconstrictor prostanoids under this condition. (This work was supported, in part, by CM (GR/SAL/016/2004) and FMM (2004).

**PB.7**

**NON INVASIVE, REVERSIBLE AND DOSE DEPENDENT TITRATION OF BLOOD PRESSURE IN CYP1A1TREN-2 TRANSGENIC RATS, A NEW MODEL FOR HYPERTENSION AND ASSOCIATED END ORGAN DAMAGE**

Grisk O, Becher B, Kuttler B, Mullins J, Wanka H, Rettig R, Peters J. 1University of Greifswald, Karlsruhe, Germany, 2 University of Edinburgh, Edinburgh, United Kingdom

A transgenic rat has recently been generated, in which hypertension can be induced by oral uptake of indol-3-carbinol (ISC). In this animal, hepatic expression of a renin gene primarily leads to elevated circulating renin levels. We tested whether or not circulating renin levels as well as blood pressure can be titrated with ISC. A dose dependent increase of plasma renin levels was observed (0.1, 0.3, 1.0, 3.0 and 10.0 fold with 0.03%, 0.08%, 0.1%, 0.5% and 1.5% ISC, respectively). Blood pressure increased dose dependently, starting with a threshold level of 0.08% ISC (+0.55 and +70 mmHg with 0.08%, 0.1% and 0.5%, respectively). All effects were reversible even after 2 weeks of induction. The cyp1a1tren-2 transgenic rat thus is a powerful model for cardiovascular disease, since blood pressure can be titrated non-invasively in a tight dose- and time-dependent manner.

**PB.8**

**PROTECTIVE EFFECT OF ANGIOTENSIN AT4 RECEPTOR IN EXPERIMENTAL ISCHEMIC STROKE IN THE RAT**

Faure S, Chapot R, Tallet D, Javelaud L, Oudart N, Achar JM. Faculté de Médecine, Limoges, France

AngII has a direct protective effect mediated by neuronal AT2 receptors in ischemic stroke. AngIV, acting through its specific AT4 receptor, increases cerebral blood flow. To examine if AngIV contributes to the protective effect of angiotensins, we developed a model of embolic stroke induced by carotid injection of calibrated microspheres (50 μm) in SD rats. Intracerebral injection of high dose of Ang-(1-7) (nM) improved the survival (90 % vs 47 %; p < 0.001) and reduced neurological deficit (p < 0.0001) and cerebral infarct size (185 ± 19 vs 432 ± 26 mm3; p < 0.0001) at 24 hours. The AT4 antagonist Divalinal, or pretreatment with L-NAME both completely abolished the protective effect of AngIV. Sequential cerebral arteriographies evidenced that Ang-(1-7) reduced a redistribution of blood flow to the ischemic areas within minutes. Stimulation of endogenous Ang production by pretreatment with candesartan for five days decreased mortality, neurological deficit and infarct size. Both the AT2 receptor antagonist PD133193 or Divalinal partially abolished the protective effect of AT1 blockade. Inhibition of angiotensin production by 24 hours pretreatment with lisinopril, or compared blockade of AT2 and AT4 in candesartan pretreated rats both had a similar significant deleterious effect. Conclusion : Pharmacological doses of AngIV are protective against acute cerebral ischemia by triggering an AT1-mediated, NO-dependent intracerebral hemodynamic mechanism. AT1 blockade for 5 days induces sufficient endogenous angiotensin production to engage both AT2 and AT4 mediated cerebral protection against acute ischemia.

**PB.9**

**SPONTANEOUS HEART FAILURE IN ACING THROMBOSPONDIN-2 NULL MICE**

Schellings MWLM, Vanhoutte D, Van Loo M, Carmeliet P, Heymans S, Pietro YM. 1Experimental & Molecular Cardiology, CARIM, Maastricht University, Maastricht, Netherlands, 2Center of Transgene Technology & Gene Therapy, Cardiology, KU Leuven, Leuven, Belgium, 3Center of Transgene Technology & Gene Therapy, VB, KU Leuven, Leuven, Belgium

Thrombospondin-2 (TSP2) plays a crucial role as regulator of cardiac matrix integrity, necessary for the myocardium to cope with increased loading. Expression of TSP2 is low in normal young tissue, but reappears with advanced age. Therefore, we examined whether TSP2 is also involved in maintaining cardiac matrix integrity or function in aging hearts. TSP2 wild type (WT) (n=24) and TSP2 deficient (KO) mice (n=20) were followed for 55 weeks, where after cardiac function was evaluated and hearts taken out for further analysis. Survival was significantly reduced in TSP2 KO (survival: 55%) compared with WT mice (survival: 71%). Cardiomyopathology in survival is not significantly increased cardiac dilatation and decreased systolic function in TSP2 KO mice at 55 weeks (end-diastolic diameter, mm; KO 3.05 ± 0.29 vs WT 2.6 ± 0.14, P < 0.05; fractional shortening; KO 35 ± 2.0 vs WT 46 ± 2.7, P < 0.05). Pressure-volume loop analysis confirmed decreased systolic function in TSP2 KO mice (Ees, KO 5.9 ± 2.2 vs WT 9.6 ± 3.5, P < 0.01). Lung/body weight ratio and cardiac collagen content was significantly increased in TSP2 KO compared with WT mice (mg/g: KO 10.7 ± 3.9 vs WT 6.5 ± 1.2, P < 0.01; Sirius red per total area; KO 6.5 ± 3.2 vs WT 0.8 ± 0.4, P < 0.05, respectively). Compared with WT hearts, TSP2 KO mice showed increased immunoreactivity of p16 (aging), ubiquitin (aging, suffering), desmin (suffering) and annexin (apoptosis) in fibroblasts and myocytes, indicating accelerated aging. In conclusion, the spontaneous development of aging related dilated cardiomyopathy suggests a crucial role for TSP2 in the heart to cope with aging related changes.

**PB.10**

**BLOOD PRESSURE-INDEPENDENT NEUROPROTECTIVE EFFECTS OF Candesartan (C) BUT NOT RAMIPRIL (R) AFTER STROKE IN RATS**

Thone-Reinekea C, Krikov M, Muller S, Neumann C, Viltringer A, Unger T. 1Center for Cardiovascular Research/Charité-Universitätsmedizin Berlin, Berlin, Germany, 2Clinic and Polyclinic for Neurology Charité-Universitätsmedizin Berlin, Berlin, Germany

Blood pressure-independent neuroprotective effects of C and R in cerebral ischemia were compared. Normotensive wistar rats were treated with C (2 x 0.1 mg/kg s.c. per day), R (2 x 0.01 mg/kg per day) or vehicle (O; 0.9% NaCl s.c. for 5 days before middle cerebral artery occlusion (MCAO) with reperfusion. Doses were selected on the basis of equal (about 50 %) blockade of pressor responses to i.v. Ang II (C) or Ang I (R), respectively, for 24 h without affecting systemic blood pressure (BP). Neurological deficits were evaluated 24 h and 48 h after MCAO followed by MRI-measurement of infarct volume and quantitative real time PCR of the stress-protein Hsp70. PeriURinary BP, CBF and blood gases were not different between groups. C but not R significantly improved neurological outcome on day 1 and 2 after stroke and reduced infarct volume by about 50 % compared to V. Higher dose of R (2 x 0.1 s.c. per day), which lowered BP during stroke, were also
effective. But not R treatment significantly reduced the expression of Hsp70, in the infract/infarct zone after stroke. Direct comparison of C and R pretreatment at doses, which equivalently inhibited the renin-angiotensin system in vivo but did not lower BP during stroke, revealed neuroprotection after stroke only with C. The reduced post-ische-

COMPARISON OF HEMODYNAMICS DURING HYPERTHERMAL IMMERSION AND EXERCISE TEST IN APPARENTLY HEALTHY FEMALES AGED 50–60 YEARS

Vohnout B1, Vohnout B2, Valent D1, Vachulova A1, Celko J2, Lietava J1
1 2nd Department of Internal Medicine, School of Medicine, Comenius University, Bratislava, Slovakia, 2 Research Laboratories, Catholic University, Campobasso, Italy, 3 Slovak Spa, Trnianske Teplice, Slovakia

Owing to excessive worries regarding cardiac events, hyperbaric balloontherapy for CAD patients is underprescribed. Methods: We therefore compared the effects of hyperthermal immersion (Hi) and bicycle exercise testing (Et) on cardiac hemodynamics in 21 apparently healthy women aged 50–60 years. Hi was carried out in 40°C water and was completed by increasing the core temperature by about 2°C. The left ventricular function was evaluated using continuous measurement of thoracic electric impedance. Results: We further during session. Acupuncture improved significantly symptoms of migraine. Arterial pressure remained higher after the two month treatment course, before the administration of placebo. Conclusion: Hyperthermally immersion induced a lower hemodynamic load in apparently healthy women than standard maximal exercise testing.

IMPROVEMENT OF CAROTID REMODELING AND STIFFENING WITH HIGH DOSE ACE INHIBITION, INDEPENDENT OF BLOOD PRESSURE CHANGES THE DAPHNET STUDY A DOUBLE BLIND, RANDOMIZED TRIAL

Tropeano AI1, Boutourie P1, Pannier B1, Joandres R2, Balkenstein E2, Lalou B1, Laurent S1
1 HOSP, Paris, France

We hypothesized that response of common carotid artery remodeling and stiffness would be improved independently of blood pressure changes by high dose ACEI, in diabetic hypertensive patients. 57 essential hypertensive patients with type 2 diabetes (age 63±7) were randomized to a 6 months double-blind randomized trial, receiving either perindopril 4 mg (Per4) or perindopril 8 mg (Per8). All patients had a preserved renal function (GFR > 60 ml/min). Common carotid diameter, cross-sectional distensibility and elastic modulus were determined with a high resolution echotracking system (Walltrack) and applanation tonometry, respectively. The reduction in casual BP was significantly more important with Per8 (154±188/9±10 mmHg) than Per4 (156±185/7±9 to 148±152/9 mmHg, p0.05). However, ambulatory mean blood pressure did not differ. Carotid diameter decreased with Per8 (P<0.01) but not with Per4 (P>0.05), while aortic stiffness (distensibility and elastic modulus) and aortic stiffness (Pulse wave velocity) were improved with Per8 but not with Per4, with a significant treatment-period interaction (P=0.05). These differences remained significant after adjustment on BP changes. High dose ACEI (Per8) but not low dose ACEI (Per4) improved arterial stiffness (carotid and aortic) and reduce carotid internal diameter in diabetic hypertensive patients after 6 month, independently of blood pressure changes. These results suggest that high dose ACEI is necessary, in addition to controlled blood pressure, to improve arterial remodeling and stiffening.
Propensity Effect of Plasma Adiponectin in the High-Risk Non Diabetic Coronary Artery Disease Patients (Pts) of the "Genica" (Genetic and Environmental Factors in Coronary Atherosclerosis) Study

Maurilio G.1, Cesari M.2, Sticchi D.2, Zanchetta M.1, Pedon L.1, Pesina A.C.2, Rossi G.P.2
1Division of Cardiology, Cittadella Hospital, Cittadella, Italy; 2DMCS - Clinica Medica 4 University of Padova, Padova, Italy

Background: Experimental and cross sectional studies suggested antiatherogenic properties of adiponectin (APN), but information on the prognostic value of plasma APN levels (pAPN) from longitudinal studies are limited to haemodialysis patients. We therefore investigated if pAPN predicted cardiovascular (CV) events in coronary artery disease (CAD) pts. Methods: Based on power calculation we selected for this study 624 non-diabetic lean (BMI<26) and overweight-obese (BMI≥29) pts of the GENICA study who underwent coronary angiography for suspected CAD. We measured pAPN with an ELISA method (Quantikine Human Adiponectin™ RD system) and examined the incidence of CV death and events at follow-up. Results: The median pAPN in this population (7.0 μg/ml; IQR 4.5-11.7) was used to split the pts in high and low pAPN subgroup. Follow-up (median 1260 days) data were obtained in all pts. We observed 29 (6%) CV deaths. Kaplan-Meier analysis highlighted an association of high pAPN with CV death (p=0.002) and CV events (p=0.02); multivariate Cox regression analysis, where serum creatinine, total cholesterol, age, gender, BMI, LVEF, hypertension, and the coronary atherosclerotic burden (Duke CAD score), were considered, confirmed this association with CV death (Exp(B) = 1.08, CI 1.03–1.14, p=0.003) and events (Exp(B) = 1.07, CI 1.02–1.11, p=0.004). A similar association was seen in lean pts. Conclusions: At variance with haemodialysis pts in whom high pAPN predicted a lower risk of CV events, in non-diabetic high-risk lean CAD patients a 1.0 μg/ml increase of pAPN carries a 8% and 7% increase of risk of CV death and events, respectively, independently of some major CV risk factors.

No Consistency of Circadian Variation in Blood Pressure in Moderate to Severe Renal Failure. A Longitudinal, Prospective Study

Elung-Jensen T1, Strandgaard S 2, Kamper A-L 1
1Dept. of Nephrology, Rigshospitalet, Copenhagen, Denmark; 2Dept. of Nephrology, Herlev Hospital, Copenhagen, Denmark

Status as a “non-dipper” determined from 24-hour blood pressure (BP) recordings may be associated with increased risk of end-organ damage. We aimed to evaluate the dipper/non-dipper status in chronic renal failure. In 34 patients with chronic progressive nephropathy (mean GFR 18 ± 7 ml/min/1.73m²) and hypertension, 24-hour ambulatory BP (A&D TM2421) was measured at baseline and every 4 months for one year or until need for renal replacement therapy. Patients were divided into two groups by the presence (dippers) or absence (non-dippers) of a nighttime reduction in both systolic and diastolic BP > 10% compared to daytime measurements. Antihypertensive treatment aimed at an office blood pressure of 120/80 mmHg or less. A total of 124 BP recordings were made, between two and four in the individual patient. A total of 10 patients were dippers and 5 were non-dippers throughout the observation period whereas 19 patients changed status at random. Analysing consecutive, paired recordings in the single patient, non-dipper and dipper status remained unaltered in 25 (27%) and 32 (34%) of comparisons respectively, whereas it changed from non-dipper to dipper or from dipper to non-dipper in 36 (39%) of cases, 18 (19.5%) in each group. No correlation with either GFR, decline in renal function or blood pressure level could be demonstrated. Our findings show a poor consistency in circadian blood pressure variation in moderate to severe renal insufficiency. Therefore there is a high risk of false-positive or false-negative results when categorizing patients with renal failure as dippers or non-dippers based on single measurements.

No Prognostic Role of Endothelial Function in Subcutaneous Small Resistance Arteries of Hypertensive Patients

Rizzoni D.1, Porteri E.1, De Ciuceis C.1, Boan G.E.M.1, Miclini M.1, Zani F.1, Piairdi S.1, Gatta D.1, Tiberto G.A.M.1, Guilini S.M.1,2, Agbobbi Roselli E.1
1Chair of Internal Medicine, University of Brescia, Brescia, Italy; 2Chair of General Surgery, University of Brescia, Brescia, Italy

Objective To evaluate the possible prognostic role of endothelial dysfunction in human small resistance arteries. Design and Methods Ninety subjects (normotensive, essential or secondary hypertensives; type 2 diabetics) were included in the present study. Small resistance arteries were dissected and mounted on an isometric myograph; endothelium-dependent (to acetylcholine) and endothelium-independent vasodilation after preconstriction of the vessels with norepinephrine were evaluated. The subjects were re-evaluated (by clinical visits or telephone interviews) after an average follow-up time of 5.6 years (2.6–10.7). Results Twenty-nine subjects had a documented fatal or non fatal cardiovascular (CV) event (8.7% events per year). The endothelium-dependent vasodilation in the subcutaneous small arteries was similar in subjects with or without CV events (see Table). Also endothelium-independent vasodilation to sodium nitroprusside was similar in the two groups (max vasodilation -72.0 ± 20.6% vs -76.4 ± 17.8%).

Conclusions Our results indicate that endothelial dysfunction in the microcirculation does not predict cardiovascular events. The prognostic role of endothelial dysfunction in human small arteries of patients at low-medium risk should be further investigated.

Candesartan Cilexetil is Effective and Safe in Elderly Hypertensive Patients Uncontrolled with a Monotherapy – The Chance Study

Asmar R.1, Nisse-Durgeat S1
1The Cardiovascular Institute, Paris, France; 2Laboratoires Takeda, Puteaux, France

Objective To assess the effect of an angiotensin receptor blocker, candesartan cilexetil (CC), in an elderly hypertensive population uncontrolled with a previous monotherapy or untreated. Methods: CHANCE is a prospective, multicenter study. Patients over 65y, with mild to moderate hypertension were switched to CC 8 mg. CC dosage was doubled after 4 weeks if BP was still uncontrolled (≥140/90mmHg). At each visit, clinical examination was undertaken and BP measured. The primary endpoint was the rate of normalised patients after 4 weeks on CC 16 mg. Results: At baseline, 3077 patients were included, mean age 72.8 ± 7.2y, mean BMI 26.6 ± 4.2, hypertension for 4.7 ± 6.5y, 25% had an ISH and 9% were diabetics. 81% were on antihypertensive treatments: diuretics (20%), CCBS (24%) and ACE-I (19%). Baseline SBP/DBP values were: 162 ± 92 mmHg. After 4 weeks, the decrease of SBP/DBP was -26/-13 mmHg with 54% of normalised BP (<140/90 mmHg). Among the patients not normalised at 4 weeks, doubling CC to 16mg resulted in a further decrease of BP (<11/6mmHg) and 48% of these subjects had subsequently normalised BP. The overall rate of BP normalisation at 8 weeks was 71%. Responder rate was respectively 64% and 86% at 4 and 8 weeks. Few patients (6%) reported an adverse event and only 4% stopped the trial prematurely. Conclusion: Results of the CHANCE study confirms the benefit of CC treatment as monotherapy in an elderly hypertensive population, as a first line therapy or in the step by step therapeutic strategy.

Echocardiographic Features of Patients with Isolated Diastolic Hypertension

Nadar SK, Goyal D, Karalis I, Beever DS, Lip GYH
City Hospital, Birmingham, United Kingdom

Background and Aim: Isolated diastolic hypertension (IDH) is a condition mainly seen in younger patients who have a low cardiovascular risk. Its effects, in the absence of systolic hypertension on the heart (effects such as left ventricular hypertrophy (LVH)) are not known. This is important, as LVH is a predictor of future cardiovascular risk. Methods: We recruited 30 patients with IDH who were referred to the hypertension clinic. They had transthoracic echocardiograms performed. These were then compared with echo findings from patients with combined essential hypertension (EH) and normotensive controls. Results: Patients with IDH had left atrial size similar to EH patients (3.2 ± 0.3 cm vs. 3.1 ± 0.4 cm) but larger than controls (2.6 ± 0.4 cm; p<0.003). There was thickening of the LV in the hypertensive groups, with the maximum in the EH group (Septum in controls 1.2 ± 0.2, in EH 1.6 ±0.4 and 1.5 ± 0.2 in IDH; p<0.001, and Posterior wall 1.0 ± 0.2, 1.6 ± 0.3 and 1.4 ± 0.1 respectively, p<0.003). The LV mass was greatest in the EH group [233 (179–308)g/ml] followed by the IDH group [190 (168–246)g/ml] as compared to normal controls [167 (129–227)g/ml]; p<0.002. There were no differences in the transmitral flow among the three groups. Conclusions: Isolated diastolic hypertension is associated with echocardiographic changes which are similar to, but not as great as systolic hypertension and suggests that this group of patients also need intensive treatment.

Comparision of the Antihypertensive Efficacy of Telmisartan /Hydrochlorothiazide vs Valsartan/ Hydrochlorothiazide in High-Risk Overweight/Obes Patients with Hypertension and Type 2 Diabetes

Sharma A.M.1, Davidson J.A.2, Gavin III J.R.3, DeSousa N.J.4
1McMaster University, Hamilton, ON, Canada; 2Endocrine and Diabetes Association of Texas, Dallas, TX, United States; 3Emory University School of Medicine, Fairburn, GA, United States; 4Boehringer Ingelheim (Canada) Ltd, Burlington, ON, Canada

The SMOOTH study used ambulatory BP monitoring (ABPM) to compare the effect of hydrochlorothiazide (HCTZ) + telmisartan or valsartan combination on blood pressure (BP) during the hazardous early-morning hours in high-risk, overweight/obese patients with hypertension and type 2 diabetes. This was a prospective, randomised, open-label, blinded-endpoint (PROBE), multicentre trial. Patients received either telmisartan 80 mg or valsartan 160 mg for 4 weeks, with add-on HCTZ 12.5 mg for 6 weeks (H and V, respectively). The primary endpoints were the changes from baseline in SBP and DBP.
EFFECT OF AMLODIPINE THERAPY ON ENDOTHELIAL FUNCTION DETERMINED BY FLOW-MEDIATED DILATION (FMD) AND HEMORHEOLOGICAL PARAMETERS IN NEWLY DETECTED HYPERTENSION SUBJECTS

Ravindra R1, Arun Kumar S1, Punjabi RR1, Lokhandwala Y1, Gupta RG2, Jadhav U1, Padgaonkar K1
1 Indian Institute of Technology, Mumbai, India, 2 C.U. Shah College of Pharmacy, Mumbai, India, 3 Dr. Babasaheb Ambekar Memorial Hospital, Mumbai, India, 4 Terna Medical College, Vashi, India, 5 Drug Monitoring Research Institute, Mumbai, India

Two comparable volunteer groups normal control and newly detected hypertension subjects (n = 10 each) were examined for endothelial function by measurement of diameter (d1) and flow-mediated dilation (FMD) of brachial artery using B-mode Doppler ultrasound and for hemorheological parameters (whole blood viscosity (WBV) at 18 shear rates, plasma viscosity (PV), red cell rigidity (RCR), and red cell aggregation (RCA) using Contraves LS 30 viscometer. Hypertension subjects showed significantly higher WBV, PV, and lower % FMD (all p < 0.05), and were administered daily dose of Amodipine (5 mg). At steady state plasma concentrations (7–7 days), SBP, DBP, and PV reduced significantly (p < 0.01, 0.01, and 0.05 respectively), with an appreciable, but non-significant decrease in WBV (at all shear rates) and RCA; and non-significant increase in % FMD. Five of these subjects, on follow-up to day 100, showed that the antihypertensive effect tapered off (SBP 132.00 ± 10.39 versus 110.00 ± 7.60 at day 0). Decrease in d1 and increase in %FMD

<table>
<thead>
<tr>
<th>SBP (mmHg)</th>
<th>DBP (mmHg)</th>
<th>Mean Diameter (mm)</th>
<th>%FMD</th>
</tr>
</thead>
<tbody>
<tr>
<td>Day 0</td>
<td>Day 0</td>
<td>Day 100</td>
<td>Day 100</td>
</tr>
<tr>
<td>132.00</td>
<td>89.00</td>
<td>110.00</td>
<td>86.00</td>
</tr>
<tr>
<td>89.00</td>
<td>7.00</td>
<td>7.50</td>
<td>7.50</td>
</tr>
<tr>
<td>10.39</td>
<td>0.05</td>
<td>0.70</td>
<td>0.70</td>
</tr>
</tbody>
</table>

Conclusion: The effect of Amodipine on endothelial function was reversed, with hemorheological parameters slightly better tolerated. T/H was superior to V/H for both of the primary endpoints. The table

PC.12

BIRTH WEIGHT AND ADULT LOW GRADE INFLAMMATION IN A COHORT FOLLOWED FROM THE FETAL PERIOD TO ADULTHOOD: RESULTS FROM THE 1966 NORTHERN FINLAND BIRTH COHORT STUDY

Canoy D1, Pouta A1, Ruokonen A1, Hartikainen AL1, Saikkku P1, Jarvelin MR1
1 University of Oulu, Oulu, Finland, 2 Imperial College London, London, United Kingdom

Background: Impaired foetal growth, as indicated by low birth weight, has been associated with coronary heart disease but the underlying mechanisms remain unclear. As inflammation plays a role in atherosclerosis, we examined the relation between birth weight and adult circulating levels of inflammatory markers in 5,637 men and women in the 1966 Northern Finland Birth Cohort studies. Methods: Prenatal data and body size at birth and at 1 year were obtained prospectively. At age 31 years, participants attended a health check and gave blood samples from which we measured total leukocyte count and C-reactive protein concentration. Results: Adul; leukocyte count and C-reactive protein concentration were inversely and significantly related to birth weight. These associations were attenuated but remained independent of covariates (adult systolic blood pressure, total cholesterol, fasting insulin, cigarette smoking, body mass index and sex) and other potential confounders (gestational age, maternal smoking during pregnancy, parental social class at birth, offspring’s adult social class and lifestyle factors). Inflammatory markers were also higher among lower birth weight babies who gained less weight at 1 year of age. Conclusion: Our findings suggest that low grade inflammation may be an alternative pathway linking impaired growth early in life to adult coronary heart disease.

PC.13

A DOUBLE-BLIND, PLACEBO-CONTROLLED PARALLEL TRIAL OF VITAMIN C TREATMENT IN MIDDLE AGED PATIENTS WITH MILD PRIMARY HYPERTENSION

Foroud Afsane, Foroud Afsar
University of Medical Sciences, Kerman, Iran

Objective: To investigate the effectiveness of vitamin C on blood pressure of patients between 35–50 years old with mild primary hypertension in Kerman. Methods: We conducted a prospective one year double-blind, placebo controlled parallel trial on 42 middle aged patients with mild primary hypertension in the Kerman University of Medical Sciences. Following a 2 week run-in phase, two age and sex matched groups of untreated hypertensive subjects received 8 weeks of oral treatment with either vitamin C 250mg twice daily or placebo tablet twice daily. Blood pressure was measured during the run-in phase, and again at 1,2,4 and 8 weeks after commencement treatment. Venous blood samples for the measurement of plasma ascorbic acid were measured at baseline and at 1,2,4,6 weeks after treatment. Results: Plasma ascorbic acid level in vitamin C group changed from 8.8 ± 3 umol/l at baseline to 32.3 ± 12 umol/l at 8 weeks, but in placebo-treated group, it changed from 13.8 ± 6 umol/l at baseline to 9.0 ± 4.1 umol/l at 8 weeks during the study. A more significant difference was seen in the vit C group than the placebo-treated group. At 8 weeks, the significant difference in reduction of systolic (p < 0.05) and diastolic blood pressure (<0.001) between two groups appeared. Conclusion: Vitamin C intake has a useful effect on lowering blood pressure.

PC.14

PATIENTS WITH TYPE 2 DIABETES MELLITUS HAVE HIGHER THROMBOGENICITY DESPITE ASPIRIN AND STATIN THERAPY

Natarajan AJ1, Marshall SM2, Badimon JF3, Zaman AG1
1 Freeman Hospital and University of Newcastle, Newcastle-upon-Tyne, United Kingdom, 2 University of Newcastle, Newcastle-upon-Tyne, United Kingdom, 3 Mount Sinai School of Medicine, New York, United States

Background: Patients with type 2 diabetes mellitus (T2DM) and coronary artery disease (CAD) die predominantly of thrombotic events. A deranged haemostatic profile exists in vitro in T2DM but the effect of this on acute thrombus formation is unclear. We hypothesised that subjects with T2DM had a higher propensity to thrombus formation following arterial injury despite treatment. Aim: To quantify thrombus formed in an ex vivo arterial injury model and compare between subjects with T2DM & CAD and control subjects. Methods: Subjects with T2DM and CAD (n = 20) and normal controls (n = 7) were studied. All were non-smokers and on 75mg daily aspirin. Thrombus burden was evaluated using the previously validated Badimon chamber with porcine aortic media as the thrombogenic substrate. Unanticoagulated venous blood flowed directly from the subjects through the chamber at a significant fall in mean pressure was then fixed and stained. The images were digitised and thrombus area calculated by computerised planimetry using Image-Pro® Plus software. Results: Mean thrombus burden in the T2DM group was more than twofold higher than in the control group (12032 ± 2896 vs 5311 ± 1140 μm², P < 0.0001). There was a correlation between thrombus burden and HbA1c levels (r = 0.47, P = 0.02) and thrombus burden and fasting insulin levels (r = 0.41, P = 0.03). Conclusion: Thrombus burden in T2DM is significantly raised despite aspirin and statin use. Aspirin may have a less potent effect in patients with T2DM. Our findings lend support for further research into the use of antithrombotic agents in these high risk groups.

PC.2

MINIMIZED RESIDUAL RESISTANCE OSCILLATION BUT RISING VENOUS PRESSURE DURING HYPERAEMIC FOREARM BLOOD FLOW.

Mathiassen ON1, Buus NH2, Olsen HW1, Mulvany MJ1, Christensen KL2
1 Institute of Pharmacology, Aarhus, Denmark, 2 Department C of Nephrology, Aarhus, Denmark, 3 Department of Cardiology, Aarhus, Denmark, 4 Department of Anaesthesiology, Aarhus, Denmark

Venous oscillation plethysmography is widely used for the assessment of forearm vascular resistance (FVR), dividing pressure by flow. By inducing reactive hyperemia with 10 min arterial occlusion, skin heating and light muscular work, maximal vasodilation is intended. Vascular resistance (Rv) is then generally considered as being minimal and therefore taken as an indirect measure of the design of the forearm resistance vasculature. It has however been debated whether maximal vasodilatation actually occurs during hyperaemia. During hyperaemic plethysmographic recordings, arterial inflow is moreover counteracted by venous distension. We suggest that RMIN mainly, although not entirely, reflects arterial occlusion, skin heating and light muscular work, maximal vasodilation is intended. Vascular resistance (Rv) is then generally considered as being minimal and therefore taken as an indirect measure of the design of the forearm resistance vasculature. It has however been debated whether maximal vasodilatation actually occurs during hyperaemia. During hyperaemic plethysmographic recordings, arterial inflow is moreover counteracted by venous distension. We suggest that RMIN mainly, although not entirely, reflects
resistance artery structure, if measured in the 1st cardiac cycle following venous occlusion, and corrected for the rise in VP.

**PD.3**

CALCIFICATION IN CORONARY ARTERY DIFFERS IN RENAL PATIENTS COMPARED TO NON-RENAL PATIENTS

Gross ML, Berger II, Amann K, Ritz E

1 Institute of Pathology, Heidelberg, Germany, 2 Institute of Pathology, Erlangen, Germany, 3 Dept. of Internal Medicine, Nephrologic Division, Heidelberg, Germany

**Background:** Coronal calcification, assessed by EBT, is a potent predictor of cardiac events. In renal patients both prevalence and intensity of coronal calcification are increased. **Methods:** At autopsy samples of coronaries were obtained from standard sites in 13 normotensive and 23 age- and gender matched non-uremic patients. Specimens were examined using light and electron microscopy, immunohistochemistry (antibodies against CD 68, osteocalcin, CRP, C5b-9, TGF-β, ET-1, collagen IV, MMP2, VEGF), backscatter imaging and X-ray analysis. In alternating sections immunohistochemical examination as well as backscatter imaging and X-ray analysis were performed using a Leo 440 Scanning electron microscope. **Results:** In coronaries calcified plaques occupied a similar proportion of the intima area in renal vs. non-renal patients (17.3 ± 11.9% vs. 18.1 ± 11.9%), but a significantly higher proportion of the media (16.8 ± 10.6 vs 3.8 ± 2.31%), Expression of the following proteins: C5b-9, osteocalcin, CRP, TGF-β and collagen IV, was significantly more intense around coronary plaque areas of renal compared to non-renal patients. **Conclusion:** Calcification is more pronounced in the coronary media, but not the intima of renal patients. A more marked inflammatory response in renal patients is suggested by more frequent presence and greater intensity of markers of inflammation.

**PD.4**

CIRCULATING ENDOTHELIAL PROGENITOR CELLS AND ATHEROSCLEROSIS IN EUROPEAN AND SOUTH ASIAN SUBJECTS

Hughes AD, Coady E, Byrd S, Mayet J, Wright A, Shone A, Koerner J, Thom SA

1 NHM, International Centre for Circulatory Health, Imperial College London, London, United Kingdom, 2 Department of Radiology, St Mary’s NHS Trust, London, London, United Kingdom, 3 Institute of Biomedical and Clinical Science Peninsula Medical School, Exeter, United Kingdom, 4 Hammersmith Hospital, DuCane Rd, London, United Kingdom

**Background:** Circulating endothelial progenitor cells (EPC) play an important role in endothelial repair and represent a novel cardiovascular risk factor. Associations with regional atherosclerosis and ethnic differences have not been previously reported. **Methods:** Europeans and South Asian men (45–62 yrs) with known coronary disease (n = 60), and without (n = 56) were studied. Subjects were subdivided into 3 groups on the basis of clinical history and coronary artery calcification score measured by multislice CT. Intima media thickness (IMT) and presence of plaques in the common femoral artery were also measured by ultrason. Total and non-senescence EPC were measured after cell culture for 5 days. **Results:** IMT and IMT equals medians (interquartile range). **Conclusions:** Non-senescence EPC were reduced in the group with the highest coronary calcification score (0.6 (0.2–3.2) versus 2.5 (1.3–9.9) x 10^6 cells, p < 0.008 by ANOVA) and in individuals with femoral plaques (0.9 (0.6–3.3) versus 2.0 (0.7–6.1) x 10^6 cells, p < 0.015 by t-test). Non-senescence EPC were inversely related to coronary calcification score (adjusted beta = -1.74 ± 0.71, p = 0.002) and femoral IMT (adjusted beta = 0.23 ± 0.01, p = 0.002). There were no ethnic differences in any of the EPC parameters measured. **Conclusions:** Non-senescence EPC are reduced in subjects with coronary and lower limb atherosclerosis in a graded fashion independent of other risk factors suggesting a causal relationship. EPC do not differ between Europeans and South Asians and cannot account for the elevated cardiovascular disease risk in the latter.

**PD.5**

IS IT FEASIBLE TO MEASURE ARTERIAL PULSE WAVE VELOCITY USING A SINGLE PPG PROBE AND THE ELECTROCARDIOGRAM TRACE?

Kamali AW, Greenwald SE

1 Barts & the Royal London Hospitals NHS Trust, London, United Kingdom, 2 Dept of Histopathology and Morbid Anatomy, Barts & The London Medical School, London, United Kingdom

**Background:** Arterial compliance may be estimated non-invasively by measuring the transit time (TT) of the pulse wave between 2 points along the vessel wall using two probes simultaneously; a process that, ideally, requires two operators. We describe an alternative approach, based on the principle of photoplethysmography (PPG), using a single probe held successively over a proximal and distal vessel while recording the ECG as a time reference. **Methods:** Two experiments were performed. (A) Proximal transit time (time between the peak of the R wave and the foot of the PPG signal at the proximal arterial site, TTT) and distal transit time (TTD, defined similarly) were obtained separately. TTT was deducted from TTD to derive a ‘sequential’ estimate of TT. (B) The ECG, the proximal and distal PPG signals were recorded simultaneously and any two were used to estimate the TTT and TTD as in (a) and hence work out the ‘simultaneous’ TT between the two sites. Measurements were made on 6 healthy volunteers. **Results:** There was significant correlation between the sequential ECG technique and the simultaneous technique (r = 0.9712, P = 0.05) but there was a large scatter. Mean difference was close to zero [0.89 ± 14.8ms (SD)]. When the measurements were made simultaneously (part B), the ECG (TT) and the PPG were equal, as expected. **Conclusion:** Although it is easier to record proximal and distal PPG signals separately, the sequential estimate of TT is of questionable reliability when compared to that obtained by simultaneous use of two PPG probes.

**PD.6**

RELATIONSHIP BETWEEN LARGE ARTERY PROPERTIES AND RENAL FUNCTION IN PATIENTS WITH MODERATE CHRONIC RENAL FAILURE.

Briet M, Boozec E, Foisraint M, Fouqueray B, Jacquot C, Houlliier P, Laurent S, Boudzoude P

1 HEGP, Paris, France, 2 TENON, Paris, France

Chronic kidney disease is associated with an increased risk of cardiovascular morbidity and mortality. Arterial stiffness and remodelling have been well documented in patients with end stage renal disease but little is known about arterial phenotype in chronic kidney disease patients with moderate reduction in glomerular filtration rate. 95 patients (58±15 yrs, mean±SD) with chronic kidney disease and glomerular filtration rate measured by renal clearance of 51Cr-EDTA were compared to 121 hypertensive patients without chronic kidney disease (59±11 yrs), and 57 normotensive subjects (56±6 yrs). Common carotid artery diameter, intima-media thickness, distensibility and Young’s elastic modulus were non-invasively determined with a high definition echo-tracking system. Patients with chronic kidney disease had a significantly larger carotid internal diameter than in hypertensives and normotensives (6.32±1.05, 5.84±0.74 and 5.50±0.64 mm 10–3, respectively; P<0.001) with no significant difference in intima-media thickness, resulting in 11% and 25% increases in circumferential wall stress, respectively. Carotid distensibility and elastic modulus did not significantly differ between chronic kidney disease and hypertensives, norotensives had significantly higher distensibility and lower elastic modulus than chronic kidney disease and hypertensives. Carotid-femoral pulse wave velocity was significantly higher in chronic kidney disease than in hypertensives and normotensives. In multivariate analyses either involving the entire population or restricted to chronic kidney disease patients, glomerular filtration rate was independently and strongly related to carotid diameter and elastic modulus. In conclusion, arterial enlargement and increased arterial stiffness occur in parallel with the decline in renal function in patients with mild-to-moderate chronic kidney disease.

**PD.7**

INCREASED CARDIOVASCULAR EXPRESSION OF TIMP-1 AND TIMP-2 IS RELATED TO CARDIAC FIBROSIS AND DYSFUNCTION IN THE CHRONIC PRESSURE-OVERLOADED HUMAN HEART.


1 Department of Cardiology, CARIM, Maastricht, Netherlands, 2 Center of Transgene Technology and Gene Therapy, Leuven, Belgium, 3 Department of Cardiology, Leuven, Belgium, 4 Herz- und Diabeteszentrum NRW, Bad Oeynhausen, Germany, 5 Cardiosurgery, Leuven, Belgium

Alterations in the balance of matrix metalloproteinases (MMPs) and their specific inhibitors (TIMPms) have been shown to be involved in left ventricular (LV) remodeling. Whether their expression is related to interstitial fibrosis or LV dysfunction in patients with chronic pressure-overload induced LV hypertrophy due to aortic stenosis (AS) is, however, unknown. Therefore, cardiac biopsies were taken in 36 patients with isolated AS and in 29 control CABG patients without LV hypertrophy, dysfunction or ischemia. Microarray analysis revealed significantly increased mRNA expression of collagen type I, III and IV, and transcripts involved in collagen synthesis, including procollagen endopeptidase and lysine- and proline- hydroxylase, in AS compared with control patients. Collagen deposition was greater in AS than in control patients, and was most pronounced in patients with severe diastolic dysfunction. Cardiac mRNA expression of TIMP-1 and TIMP-2 was significantly increased in AS compared with control patients, but did not significantly differ for MMP-1, -2 or -9. Cardiac TIMP-1 and -2 transcripts were significantly related to the degree of interstitial fibrosis in AS patients (TIMP-1: r = 0.52, TIMP-2: r = 0.49; P = 0.05 for both). The shifted balance of TIMP versus MMP transcript levels favored increased TIMP-1 mRNAexpression and immunoblotting, and significantly decreased in situ MMP zymographic activity in AS compared with control hearts. Cardiac expression of TIMP-1 and TIMP-2, and TIMP-2/ MMP-2 ratio is significantly increased in chronic pressure-overloaded human hearts compared with controls, and is related to the degree of interstitial fibrosis and diastolic dysfunction.

**PD.8**

EFFECT OF PRAVASTATIN IN PEOPLE WITH CHRONIC KIDNEY DISEASE AND DIABETES MELLITUS


1 University of Alberta, Edmonton, Canada, 2 Pravastatin Pooling Project

Although diabetes mellitus (DM) is a major cause of chronic kidney disease (CKD), limited data evaluate statin use in diabetic patients with CKD, and one recent RCT of atorvastatin
in diabetic dialysis patients showed no benefit. We sought to determine whether pravastatin 40mg daily prevented CVD events in persons with non-dialysis dependent CKD and concomitant DM. Patients defined by estimated glomerular filtration rate $\leq 60$ ml/min/1.73 m$^2$ or GFR 60 - 89 ml/min/1.73 m$^2$ with proteinuria. Of 19737 subjects, 4195 (21.2%) had CKD but not DM, 854 (4.3%) had DM but not CKD, and 590 (3.0%) had both. The primary outcome was time to MI, coronary death, CABG or PTCA. Pravastatin reduced the risk of the primary outcome to a similar extent in subgroups defined by the presence/absence of CKD and DM. For example, pravastatin significantly reduced the relative risk of the primary outcome by 26% in subjects with CKD and concomitant DM and by 24% in subjects with neither characteristic. However, the absolute risk reduction due to pravastatin use was greater in subjects with CKD and DM (6.8%) and lowest in subjects with neither (3.5%). In conclusion, CKD and DM are both associated with higher CVD risk and pravastatin prevents CVD events in persons with neither, or one but not both characteristics. Given the high absolute benefit of pravastatin in diabetic subjects with CKD, this population should be targeted for aggressive use of statins. Additional studies of statins are needed in people with more severe CKD.

**PD.9**

**SEROPOSITIVITY AGAINST CHLAMYDOPHILA PNEUMONIAE IS ASSOCIATED WITH INCREASED PROTEIN CARBONYLATION IN FEMALE PATIENTS WITH CARDIOVASCULAR EVENT.**

Lietava J.1, Teren A.1, Caprnda M.1, Kovacova E.2, Kazar J.2, Blaziek P.2, Duchak A.1

1 Virology Institute, Slovak Academy of Science, Bratislava, Slovakia, 2 Department of Internal Medicine, Comenius University, Bratislava, Slovakia

**Background:** IgG-seropositivity against CI pneumoniae (CPG+) is associated with increased risk of myocardial infarction (MI) in patients with ischemic heart disease (IHD). Chronic inflammation potentiates the oxidative stress, however, carbonylated protein (CP) as the marker of oxidative stress in chlamydial infection was not studied. **Methods:** We randomly selected 172 females with IHD and 73 controls (aged: 62.7±7.7 vs 48.3±9.9 yrs, CPG+ : 12.8±19.2%, NS). 46 patients suffered cardiovascular event (CVS+ /IMT: n=36, stroke: n=10). 126 patients had no cardiovascular event (CVS-) (aged: CVS+ /IMT: 62.6±8.7 vs 62.8±8.1 yrs, CPG+: 23.9±8.7%; p=0.01). We compared association of CPG+ and of oxidative stress markers (CP; total antioxidative status - TAS), and C-reactive protein (CRP) between IHD patients and controls. **Results:** CPG+ controls had lower CRP and TAS levels than CPG- controls (CRP: 1.74±1.95 vs 3.05±2.9 mg/l, p=0.04; TAS: 1.24±0.2 vs 1.35±0.2 mmol/l, p=0.03) with no difference in CP (71.8±15.2 vs 68.3±16.4 pg/ml; NS). In CVS+ subgroup no differences were found in parameters split by CPG+. **Conclusion:** Oxidative stress markers (CP; total antioxidative status - TAS), and C-reactive protein (CRP) between IHD patients and controls. **Conclusions:** Antioxidant-seropositivity was associated with higher CP levels in females with cardiovascular event. CP seem to reflect the oxidative stress connected with chlamydial infection.

**PD.10**

**EARLY EFFECTS OF TREATMENT WITH QUINAPRIL ON SERUM IL-6 LEVEL IN PATIENTS WITH STABLE CHRONIC ANGINA.**

Gibas M1, Miszczak-Smalek J1, Madry E1, Gliuzek J1, Witmanowski H1, Piotrowski J2

1 Department of Physiology, University of Medical Sciences, Poznan, Poland, 2 Department of Hypertension and Vascular Diseases, University of Medical Science, Poznan, Poland

**Aim:** We hypothesized that beneficial role of angiotensin converting enzyme inhibitors (ACEIs) in coronary artery disease (CAD) therapy may involve their anti-inflammatory effects, which may be reflected in serum IL-6 levels. **Methods:** 74 patients suffering from stable CAD (classified to de novo ACEIs treatment) enrolled our study. Patients were matched for some of CAD risk factors and long-term use of drugs with well documented anti-inflammatory properties (statins, aspirin). Blood samples were taken twice: before and after 4 weeks of treatment with quinapril. **Results:** No differences were found in CRP levels between stable angina patients treated with and without quinapril (p=0.26). Despite an obvious reduction of blood pressure, no significant changes were observed in any of the other parameters (p>0.05). **Conclusion:** Quinapril is not associated with a change in serum IL-6 level in patients with stable CAD except smokers, which may be of particular importance for secondary prevention of stable CAD.

**PD.11**

**EVALUATION OF VASCULAR MORPHOLOGY BY MEANS OF DIGITAL PULSE WAVE ANALYSIS.**

Semyonkin A.A.1, Novikov A.I.1, Novikov Yu.A.1, Protisky I.A.1, Zhiulova L.A.1, Nazarov A.O.1, Radul E.V.1, Romanov A.A.1

1 Omsk Medical Academy, Omsk, Russia, 2 Omsk Regional Hospital, Omsk, Russia

**Purpose:** The aim of our study was to use the opportunities of photoplethysmographic method of digital pulse wave (DPW) registration to determine the structure of carotid arteries. The study involved 32 volunteers (age from 25 to 55 years), Intima-media thickness (IMT) of medial segments of common carotid arteries was measured by means of high-resolution ultrasound. Averaged DPWs were registered using the Pulse/Trace device (Micro Medical, UK). Values of stiffness index (SI) calculated on DPWs at baseline and minimal values of SI of 4 DPWs during 5 min period (at 2nd, 3rd, 4th and 5th min) after 500 mgc of sublingual nitroglycerin (Slin) were used as variables for analysis. The results of the study showed significant positive correlation between IMT and baseline SI (r=−0.58, p<0.001); however, the correlation of IMT with Slin was much stronger (r=−0.79, p=0.00). Values of IMT determined by ultrasound and calculated according to equation of linear regression analysis for Slin and IMT (r=−0.444xSlin+0.3135) were nearly identical (0.57±0.07 mm versus 0.37±0.06 mm, p=0.08). Thus, we conclude that arterial stiffness may be the measure of vascular morphology. Vasodilator nitroglycerin eliminates the tonic component of vascular stiffness and makes it possible to measure the elastic component that is the derivative of vascular structure. Photoplethysmographic method of digital pulse analysis is a simple, specialist independent and may be widely used for screening of atherosclerosis.

**PD.12**

**CARBONYLATED PROTEINS AND OXIDIZED GLUTATHIONE ARE DECREASED IN EX-SMOKING FEMALES WITH ISCHEMIC HEART DISEASE.**

Caprnda M1, Lietava J.1, Teren A.1, Duchak A.1, Blaziek P.1, Atalay M1, Hanninen O2

1 2nd Department of Internal Medicine, Comenius University, Bratislava, Slovakia, 2 Department of Clinical Laboratories Ministry of Defence Hospital, Bratislava, Slovakia

**Introduction:** Smoking habit is associated with increased oxidative stress in patients with ischemic heart disease (IHD) and in healthy subjects as compared to non-smoking persons. However ex-smokers, especially females were unsatisfactorily studied. **Aim:** To investigate oxidative stress markers in IHD females in ex-smokers vs smoking females. **Methods:** Oxidative stress markers (carbonylated proteins (CP), oxidized glutathione (GSSG), oxidized LDL (O-LDL) and total antioxidative status (TAS)) were compared in IHD females (N=164) and in controls (N=60) divided in smoking (S) (N=60) and non-smoking group (NG) (N=104). **Results:** CP were decreased in IHD females as compared with smoking smokers and non-smoking controls. **Conclusion:** Carnitines, total antioxidative status and oxidized glutathione are decreased in ex-smokers vs smoking females with ischemic heart disease.

**PD.13**

**NON-INVASIVE DETECTION OF CORONARY ATHEROSCLEROSIS.**

Protisky I.A.1, Novikov A.I.1, Semyonkin A.A.1, Zhiulova L.A.1, Novikov Yu.A.1, Piatovsky I.I.2, Romanov A.A.1

1 Omsk Medical Academy, Omsk, Russia, 2 Omsk Regional Hospital, Omsk, Russia

**Aim:** Our aim was to determine the value of digital pulse wave (DPW) analysis in diagnosis of coronary atherosclerosis (CA). The study involved 72 patients (mean age 52.7±8.8 years) undergoing diagnostic coronary angiography. Averaged DPWs were registered by means of photoplethysmography before angiographic study using the Pulse/Trace device (Micro Medical, UK). Values of stiffness index (SI) and resistance index (RI) calculated on DPWs registered at baseline and minimal values of SI at RI during 6 min period after 500 mgc of sublingual nitroglycerin (Slin) were used as variables for analysis. Among the studied population CA was found in 55 (77%) patients. Values of SI at baseline and after Slin did not differ in groups with and without CA, whereas SI was significantly higher in patients with CA compared to patients with normal arteries (8.66±2.65 m/s versus 7.86±1.53 m/s, p=0.034 for baseline values, and 6.48±1.25 m/s versus 5.01±0.45 m/s, p=0.00018 after Slin, respectively). 100% of patients in upper tertile of SI after Slin distribution were CA patients (96.5% of mIIE or 66.6%) had CA. 75% in middle tertile (SI from 5.44 m/s to 6.65 m/s) and only 35% of patients in lower tertile of SI distribution (yasis may be the useful non-invasive screening method for prediction of coronary atherosclerosis in the target population. Simple test with nitroglycerin may significantly increase the diagnostic opportunities of the method.
Our aim was to evaluate the function of vascular endothelium in patients with lues infection (LI). The study involved 30 patients with LI (6 to 12 months from the moment of infection) and 20 age and sex matched volunteers (control group). All patients received the therapy with penicillin for 20 days. Control examinations including measurement of adhesion molecules (VCAM-1 and ICAM-1) in plasma and evaluation of endothelium-dependent (inhaled salbutamol, 400 mcg) and endothelium-independent (sublingual nitroglycerin, 500 mcg) vascular responses by stiffness index (Sl) calculated on digital pulse wave (DPW) recorded by means of photoplethysmography were performed at entry and 4, 12 and 20 days from the end of the therapy. Healthy patients with LI had slightly elevated levels of VCAM-1 and ICAM-1 compared to control (102±1–296 ng/ml versus 480±146 ng/ml, p<0.001 and 460±98 ng/ml versus 215±46 ng/ml, p<0.001) that decreased after treatment but remained significantly higher than in control. Sl after SNG and salbutamol in LI was also higher than in control (0.19±0.98 m/s versus 4.78±0.57 m/s, p<0.005 and 6.24±1.12 m/s versus 5.69±0.61 m/s, p<0.001, respectively). Treatment led to normalization of response to SNG (Sl=4.96±0.74 m/s, p<0.24 compared to control), however, the response to salbutamol remains lower (Sl=6.08±0.94 m/s, p<0.01 compared to control). Thus, vascular inflammation in LI leads to changes of vascular stiffness, priapromflammatory activation of endothelial cells and decrease of NO-dependent responses. Treatment restores the elasticity of arteries but did not eliminate endothelial dysfunction that may be the cause of postponed vascular complications of LI.

OBJECTIVE: Since circulating levels of brain natriuretic peptide (BNP) reflect the severity of cardiac disorders, patients with cardiac disorders can be identified by BNP measurement. We investigated whether measurements of BNP levels serve as a useful tool in the screening of severe cardiac disorders among subjects who visit a hospital with chest symptoms. Methods: Consecutive 140 patients (male/female = 57/83, 65.1 years old) who visited our hospital complaining chest symptoms were enrolled. All patients underwent physical examinations, electrocardiograms, chest X-rays, ultrasonic cardograms, and blood analyses including BNP measurements. Results: Close investigations revealed that 43 patients had severe cardiac disorders requiring hospitalization including acute heart failure, acute myocardial infarction, and arrhythmia. BNP levels were significantly higher in patients with cardiac disorders (103.0±78.0 pg/ml, median: 79.1% and 76.3%, respectively). The multiple regression analysis revealed that the BNP level was associated with lower NO x levels, in agreement with previous findings showing a partial cleavage of NO x in renal arteries from patients harbouring this variant (J Hypertens. 2005;23:759–65). 2 HUM Genet. 2002;11:229–41).

Influence of NOS3 polymorphisms on NO x was assessed by sib-pair and association analysis, after adjustment for age, gender, BMI and plasma cholesterol. Mean absolute deviation) than in subjects without cardiac disorders (22.0±10.9 vs. 57.5±10.3 ng/ml, p<0.001 and 20.0±10.3 vs. 50.0±9.6 ng/ml, p<0.001). The purified plasma sample was examined. Finally, we did not observe any interaction between the NOS3 allele and the –344C/T variant of the X chromosome. The data obtained in this study suggest that the NOS3 allele and the –344C/T variant of the X chromosome are a risk factor for heart failure in patients with cardiac disorders.

Influence of the NOS3 polymorphism and the –344C/T variant of the X chromosome on NO x was assessed by sib-pair and association analysis, after adjustment for age, gender, BMI and plasma cholesterol. Mean absolute deviation) than in subjects without cardiac disorders (22.0±10.9 vs. 57.5±10.3 ng/ml, p<0.001 and 20.0±10.3 vs. 50.0±9.6 ng/ml, p<0.001). The purified plasma sample was examined. Finally, we did not observe any interaction between the NOS3 allele and the –344C/T variant of the X chromosome. The data obtained in this study suggest that the NOS3 allele and the –344C/T variant of the X chromosome are a risk factor for heart failure in patients with cardiac disorders.

Identification of genetic factors contributing to heart failure in SHHF rats

Heart failure is a quantitative trait with genetic components in humans and rats. Our goal is to identify quantitative trait loci and the underlying molecular mechanisms contributing to high risk of heart failure. We used inbred spontaneously hypertensive heart failure (SHHF) rats as a model of the human disease. To identify QTLs that are related to heart failure we produced two intercross populations derived from SHHF rats and WKY rats (Wistar Kyoto rats) and from SHHF rats and SHRap rats (spontaneously hypertensive rats). Cardiac function was determined in parental and F1 animals and all F2 animals by in-vivo hemodynamic measurements assessing 30 cardiac parameters including end systolic and diastolic pressure, ejection fraction, cardiac output and left ventricular volume. We also measured a range of morphometric parameters. Furthermore, we performed a genome screen in all F2 animals with microsatellite markers and SNPs. Here we report the first linkage results of a genome scan to search for chromosomal regions that contribute to heart failure.

Replacement of rat chromosome 6 in the MWF strain eliminates the development of early albuminuria in a speed consomic MWF-Chr6 strain

Currently we performed cosegregation and linkage analysis to identify quantitative trait loci (QTL) for albuminuria in the Munich Wistar Fromter rat (MWF) model of spontaneous hypertension and albuminuria. We identified a major QTL linked to early manifestation of albuminuria in male animals on rat chromosome (RNO) 6. Here, we generated a speed consomic strain to test the relevance of this QTL. As a contrasting reference strain we used in accordance to our cosegregation analysis the spontaneously hypertensive rat (SHR) model that exhibits similar hypertension compared to MWF but normal albumin excretion levels. RNO6 was transferred from SHR into the MWF background by sequential marker assisted backcrossing to generate a speed consomic strain, i.e. MWF-Chr6 (200). The purity of MWF-Chr6 (200) was confirmed by total genome screen analysis with 237 microsatellite markers. At 8 weeks of age urinary albumin excretion (UAE) in 24 hours (n=8) was determined in male animals in metabolic cages (p<8–12, respectively). UAE in parental MWF was significantly increased compared to SHR animals (14.77±7.72 vs 0.25±0.08 mg/hr, p<0.001). In MWF-Chr6 (200) UAE was 0.68±0.49 mg/24h and thus significantly lower compared to MWF (p<0.001) but not significantly different from
SHR or other normal strains such as Wistar rats. Thus, our data demonstrate that although albuminuria in MWF is highly polygenic trait, RN66 must contain at least one gene that is crucial for the early development of this trait.

CHARACTERIZATION AND FUNCTIONAL ANALYSIS OF THE HUMAN MMP2-GENE PROMOTER

Dworakiew E, Mahnoodzadeh S, Regitz-Zagrosek V
CCR, Berlin, Germany

Matrix Metalloproteinases (MMPs) play a role in cardiac remodeling in congestive heart failure, hypertrophy, or myocardial infarction. Patients with dilated cardiomyopathy and aortic stenosis show an increased expression of the MMP2 gene. In order to understand the regulation of the human MMP2 gene expression we study the 5'-flanking region. Our research interest is to identify transcription factors and their putative binding sites within the promoter region of the MMP2 gene. Different regions of the human MMP2 promoter sequence were cloned in the pGL2-promoter vector. After transfection of the expression constructs in a human fibrosarcoma cell line (HT1080), promoter activity was analyzed using luciferase reporter assays. We found that the 1174bp promoter fragment (-1174bp to +1bp, relative to translation start site) showed the highest activity, indicating that the increase of promoter activity is correlated with the increase of promoter length. Within this region we confined two sections, -1174bp to -685bp and -685bp to -512bp, which showed the most promoter activity, suggesting that these regions may contain enhancer elements. Expression analysis with MMP2 promoter fragments, in which the 5'-UTR was excluded, showed an increase in promoter activity, suggesting that the 5'-UTR either suppresses binding sites or that an existing upstream AUS codon (-156bp) inhibits expression of the MMP2 protein. The results suggest that the 5'-UTR may play an important role in the transcriptional regulation of the human MMP2 gene. Additionally we have identified two enhancer elements containing regions within the MMP2 promoter region.


Russo P1, Logueiro M2, Caggiano R1, Lauria F1, Barba G1, Iacoviello L1, Cappuccio FP3, Arnout J4, Siani A1
1 Institute of Food Sciences, CNR, Avellino, Italy, 2 Center High Tech. & Educ. in Biosci., CNR, Campusbio, Cabras, Italy, 3 Clinical Sciences Research Institute, Warwick Medical School, Coventry, United Kingdom, 4 Catholic University, Leuven, Belgium, 5 European Collaborative Group of the IMMIDIET Project

The –344C/T variant of the aldosterone synthase gene (CYP11B2) has been associated with age-related and gender-related blood pressure (BP) regulation. We evaluated the association of –344C/T polymorphism with BP and prevalence of hypertension in a large sample of men and women participating to the IMMIDIET project, a population-based cross-sectional study in three European countries (Italy, UK, Belgium). Anthropometry, BP and –344C/T polymorphism were determined on 1,604 subjects, Italian (n=271), Belgian (n=268) and English (n=263) couples aged 30–60, randomly recruited through General Practices. Allele frequency was similar in the three populations (C: 0.467±0.054; B: C:0.447±0.056, BK: 0.467±0.054). In untreated participants (n=1,390), C allele was significantly associated with higher BP in males (CC+CT vs TT: PAS +2.6±1.2 mmHg, p<0.03; PAD +1.4±0.7 mmHg, p=0.06, ANCOVA adjusted by sex, BMI and country), but not in females. Nevertheless, by dividing the population by median of age (n=47 years; F<44 years), this association was still observed only in younger males (<47 years, CC+CT vs TT: PAS +4.4±1.4 mmHg, p=0.002; PAD +1.8±0.9 mmHg, p<0.05). In these subjects, C allele was also associated with higher prevalence of hypertension (RR 1.81, 95% CI 1.03 to 3.18). No association was found in older males (>47 years) and in females (<44 and >44 years). In conclusion, the C allele of –344C/T polymorphism of CYP11B2 is associated with higher BP and prevalence of hypertension in younger Caucasian males, thus suggesting that this variant may be a genetic marker for “early” hypertension.

IDENTIFICATION OF FOUR DIFFERENT 5’-UTR VARIANTS OF THE ERα-GENE IN THE HUMAN HEART

Fritschka S1, Mahnoodzadeh S1, Pregla R2, Regitz-Zagrosek V1
1 Center for Cardiovascular Research, Berlin, Germany, 2 Deutsches Herzzentrum, Berlin, Germany

Previous reports have revealed that the human estrogen receptor alpha (ERα) mRNA is transcribed from at least seven different promoters with unique 5'-UTRs (A, B, C, D, E, F and T). These multiple promoters are utilized in a cell- and tissue-specific manner, strongly contributing to the regulation of expression. For example in endometrium, the predominant transcription from at least seven different promoters with unique 5'-UTRs (A, B, C, D, E, F and T). These multiple promoters are utilized in a cell- and tissue-specific manner, strongly contributing to the regulation of expression. For example in endometrium, the predominant transcription factors or that an existing upstream AUG codon (-156bp) inhibits expression of the MMP2 gene. Additionally we have identified two enhancer elements containing regions within the MMP2 promoter region.


Russo P1, Logueiro M2, Caggiano R1, Lauria F1, Barba G1, Iacoviello L1, Cappuccio FP3, Arnout J4, Siani A1
1 Institute of Food Sciences, CNR, Avellino, Italy, 2 Center High Tech. & Educ. in Biosci., CNR, Campusbio, Cabras, Italy, 3 Clinical Sciences Research Institute, Warwick Medical School, Coventry, United Kingdom, 4 Catholic University, Leuven, Belgium, 5 European Collaborative Group of the IMMIDIET Project

The –344C/T variant of the aldosterone synthase gene (CYP11B2) has been associated with age-related and gender-related blood pressure (BP) regulation. We evaluated the association of –344C/T polymorphism with BP and prevalence of hypertension in a large sample of men and women participating to the IMMIDIET project, a population-based cross-sectional study in three European countries (Italy, UK, Belgium). Anthropometry, BP and –344C/T polymorphism were determined on 1,604 subjects, Italian (n=271), Belgian (n=268) and English (n=263) couples aged 30–60, randomly recruited through General Practices. Allele frequency was similar in the three populations (C: 0.467±0.054; B: C:0.447±0.056, BK: 0.467±0.054). In untreated participants (n=1,390), C allele was significantly associated with higher BP in males (CC+CT vs TT: PAS +2.6±1.2 mmHg, p<0.03; PAD +1.4±0.7 mmHg, p=0.06, ANCOVA adjusted by sex, BMI and country), but not in females. Nevertheless, by dividing the population by median of age (n=47 years; F<44 years), this association was still observed only in younger males (<47 years, CC+CT vs TT: PAS +4.4±1.4 mmHg, p=0.002; PAD +1.8±0.9 mmHg, p<0.05). In these subjects, C allele was also associated with higher prevalence of hypertension (RR 1.81, 95% CI 1.03 to 3.18). No association was found in older males (>47 years) and in females (<44 and >44 years). In conclusion, the C allele of –344C/T polymorphism of CYP11B2 is associated with higher BP and prevalence of hypertension in younger Caucasian males, thus suggesting that this variant may be a genetic marker for “early” hypertension.

IDENTIFICATION OF A FUNCTIONAL MINERALOCORTICOID RECEPTOR POLYMORPHISM AND ASSOCIATION WITH SODIUM HANDLING

Caprio M1, Sarrotato P2, Giacchetti G1, Mantero F1, Jeunemaitre X1, Fumeron F3, Zennaro M-C1
1 INSERM, U38 - Collège de France, Paris, France, 2 Division of Endocrinology, Department of Medical and Surgical Sciences, Universi, Padova, Italy, 3 Clinica di Endocrinologia, Università Politecnica delle Marche, Ancona, Italy, 4 INSERM, U895, Faculté de Médecine X. Bichat, Paris, France

To elucidate the role of the mineralocorticoid receptor (MR) in regulating sodium homeostasis and salt sensitivity, we have identified and analyzed several new SNPs of the human MR (mMR) gene. They are located in the coding sequence or in intronic regions of the gene and found with variable frequencies in the general population. G221C is localized in the 5'-untranslated region immediately upstream of the translation start site. Using chimeric constructs containing the mMR 5'-untranslated exons together with the Kozak sequence upstream of the luciferase coding sequence, we show that transcription and translation of the reporter gene were dependent on the nature of the untranslated sequence and the type of allele. The G221C SNP affected transcription in a significant and reproducible way in two different cell types at both 12 and 24 hours post-transfection. To study the association between this SNPs and sodium sensitivity, we have analyzed genotype distributions in a mild hypertensive cohort. No difference was observed among patients classified as salt sensitive or salt resistant and there was no correlation between the genotypes of the mMR polymorphisms and blood pressure response to a salt load/salt depletion test. However, urinary sodium excretion was significantly increased in G221 homozygous males (p<0.05, adjusted for BMI and age), the salt sensitive patients accounting for a large part of the statistical variance. Plasma renin activity was also correlated with the G221C genotype. Our results indicate that functional polymorphisms of the mMR gene might account for individual differences in sodium handling.

BLOOD PRESSURE VARIATES WITH PHYSIOLOGICALLY DETERMINED EPIGENIC INTERACTIONS AMONG THE ADDUCIN GENES IN 6 EUROPEAN POPULATIONS

Kuznetsova T1, Staessen JA1, Thijs L1, Casiglia E2, Filipovsky J3, Kawecka-Jaszcz K4, Nikitin Y5, Stolarz K4, Tikhonoff V2, Zagato L6, Bianchi G6
1 University of Leuven, Leuven, Belgium, 2 University of Padova, Padova, Italy, 3 Charles University, Pilsen, Czech Republic, 4 Jagiellonian University, Cracow, Poland, 5 Institute of Internal Medicine, Novosibirsk, Russia, 6 Universita e Salute San Raffaele, Milano, Italy

Adducin is a heterodimeric cytoskeleton protein, made up by α- and β- or α- and γ-subunits. In the European Project on Genes in Hypertension (EPOGH), we investigated whether across 6 populations (2244 relatives belonging to 595 families) common SNP’s in the three adducin genes, alone or in combination might be associated with BP, while accounting for life style and familial aggregation. Mean (±SD) age was 55.6±12.9 years
in 983 founders and 30.5±11.6 years in 1261 offspring. Across the entire allele frequencies ranged from 16.8% to 23.7% for the α-adducin Trp allele, from 8.7% to 16.3% for the β-adducin T allele, and from 27.8% to 43.6% for γ-adducin G allele. Multiple-genome analyses, in which we treated BP as a continuous phenotype, revealed epistatic interactions between the α- and β- and α- and γ-adducin polymorphisms. Indeed, among α-adducin Trp allele carriers, but not among GlyGly homozygotes, BP increased with the β-adducin 1797T and the γ-adducin 386G allele. For β-adducin, the fully adjusted effect sizes were 3.7 mmHg (P = 0.002) sytostic and 2.6 mmHg (P = 0.002) diastolic. The corresponding estimates for the γ-adducin 386G allele were 3 mmHg (P = 0.003) and 1.5 mmHg (P = 0.03). Furthermore, in offspring carrying the mutated α-adducin Trp allele, transmission of the β-adducin 1797T and the γ-adducin 386G alleles were associated with a higher BP with P-value ranging from 0.003 to 0.06. These epistatic interactions are physiologically consistent with the heterodimeric structure of the protein and its influence on transmembrane sodium transport.

**PE.12 POLYMORPHISMS OF α1-ANTITRYPSIN GENE ARE NOT ASSOCIATED WITH RENAL ARTERIAL FIBROMUSCULAR DYSPLASIA**

Perdu J, Gimenez-Roqueplo AP, Beaujour S, Nau V, Jeunemaitre X Laboratoire de génétique moléculaire, HEGP, PARIS, France

Fibromuscular dysplasia (FMD) is a rare non-inflammatory nonatherosclerotic disease involving mostly the renal arteries and resulting in stenosis with renovascular hypertension and aneurysms/dissections with renal infarction. Pathological features show association of respectively extracellular matrix (ECM) hyperplasia of the media and disruption of internal elastic lamina. As the main substrate of α1-antitrypsin (α1-AT) is leukocytic elastase highly involved in ECM degradation and as the association between FMD and α1-AT deficiency was previously described, we tested the hypothesis that well-known pathological variants of α1-AT may be associated with renal FMD. Material & methods: For this purpose we conducted a case control study comparing allele and genotype frequencies of the 3 main isoforms of α1-antitrypsin (PIM, PZ and PS) in a cohort of 161 patients with angiographically proved renal artery FMD and in three sets of controls (535 hypertensive patients (HTN), 288 normotensive patients (NTN), 444 normotensive women(NW)). Genotyping was realized by PCR amplification followed by a specific enzymatic digestion step. Results: Neither allelic frequencies nor genotype frequencies were statistically different between the 4 groups (Table 1). Moreover we showed no clinical or arteriographic pattern associated with pathological genotypes in renal FMD patients. Conclusion: The results of this study do not support a pathophysiological role of α1-AT deficiency in renal FMD.

| TABLE 1: ALLERGIC FREQUENCIES FOR PIM, PZ AND PS IN 4 POPULATIONS OF THE STUDY. |
|-----------------|-----------------|-----------------|-----------------|-----------------|
|                  | Aisle V          | Aisle A          | Aisle M          | Aisle Z          |
| FMD (161)        | 0.784            | 0.216            | 0.589            | 0.0537           |
| HTN (353)        | 0.776            | 0.2234           | 0.599            | 0.0498           |
| NTN (288)        | 0.7947           | 0.2053           | 0.9292           | 0.0074           |
| NW (444)         | 0.8075           | 0.1925           | 0.9858           | 0.0144           |
candidate genes with the risk factors changes over 8 years. The sample consisted of 378 family members from the Kibbuzim Family Study who participated in 2 examinations 8 years apart. Medical, demographic, physiological, biochemical and genotypic information was obtained. For statistical analysis we used the unified mixed segregation model. Heritability estimates for changes in plasma lipid and lipoproteins were significant ranging from 0.22 (triglyceride) to 0.29 (HDL). The heritability coefficient for change in BMI was 0.3 (p < 0.05). The Seca2-arteroconator Arg16Gly polymorphism was associated with individual changes in triglyceride (p = 0.007) and cholesterol (p = 0.04). Similarly, a significant association between the CETP-TaqIB polymorphism and residual changes in triglyceride, was observed. Our data provide evidence for significant genetic determinants of longitudinal risk changes in CHD risk factors. CETP SNPs were found to be associated with changes in lipid concentrations. These findings may help in identifying high risk sub-populations at early ages by simple genetic and biochemical measures and contribute to the improvement of prevention.

**PF.1 ANGIOTENSIN II SUPPRESSES TNF-α-INDUCED IL-6 MRNA EXPRESSION VIA THE AT2-RECEPTOR IN HUMAN DERMAL FIBROBLASTS**

Reinemund J1, Artuc M2, Henz BM1, Unger T1, Stockelings ULM1

1 Center of Cardiovascular Research, Charité-Universitätsmedizin Berlin, Berlin, Germany, 2 Dept. of Dermatology, Charité-Universitätsmedizin Berlin, Berlin, Germany

Recently, we demonstrated the presence of a complete renin-angiotensin-system in human skin. However, the characterisation of cutaneous actions of angiotensin II (Ang II) is only at the very beginning. Aiming to test whether Ang II may be involved in cutaneous inflammation, we examined IL-6 expression in response to Ang II with or without co-stimulation by TNF-α, a known stimulator of IL-6 expression. In the present study, we show that Ang II could completely block TNF-α induced IL-6 expression, which could be prevented by Ang II. The Ang II induced prevention of TNF-α stimulated IL-6 overexpression could be completely blocked by PD 123319, but not by irbesartan. Thus, in human dermal fibroblasts, IL-6 expression is stimulated by TNF-α, an effect which is counteracted by Ang II via the AT2-receptor. Consequently, Ang II may exert anti-inflammatory actions in skin by inhibition of cytokine expression via the AT2-receptor.

**PF.2 TISSUE KALLIKREIN AND RENOVASCULAR HYPERTENSION**

Griol-Chambli V1, Sahin A2, Baudin V1, Brunelle P1, Meneton P1, Alhenc-Gelas F2, Richet C1

INSERM U652, PARIS, France, 1 INSERM U653, PARIS, France

We evaluate the role of tissue-kallikrein (TK), the major kinin forming enzyme in two kidney-one clip (2K1C) renovascular hypertension. We used male TK-deficient mice (TK-/-) and their wild-type littermates (TK+/-). TK-/- (n=26) and TK+/- (n=28) underwent clipping of the left renal artery (internal gap 0.12mm). Sham-operated mice (Sham, n=40) served as controls. Four weeks after clipping, blood pressure (telemetry or plethysmography), left renal blood flow (fluorophores, LBF), left kidney weight (LKW), plasma renin concentration (radioimmunoassay, PRC), and cardiac functional and morphological parameters (echo-cardiography, shortening fraction, and left ventricular mass to body weight, LVM/BW) were measured or calculated. The LKW and LBF were similarly decreased in 2K1C-/- and 2K1C-TK-/- indicating a similar degree of stenotic stress in the left kidney in both groups. PRC, which is significantly greater in 2K1C-TK-/- (4409 ± 725 mmHg/ml/h kg, 74% vs sham) increased to a similar extent in 2K1C-TK-/- (4341 ± 893 mmHg/ml/h kg, 72%), Hypertension developed in 2K1C-TK-/- (146 ± 33 mmHg, +11% vs 0.05 vs sham) but was not reinforced in 2K1C-TK-/- (135 ± 33 mmHg). LBM/BW was similar and significantly greater in both groups (2K1C-TK-/-, 26 % ± 3% vs sham, but, significantly lower cardiac functional alteration occurred in any group. Since the lack of TK does not influence the cardiovascular phenotype of TK-/- mice in C57Bl/6j genetic background (hypertension: 2002, 49, 90–95), these data demonstrate that there is a minor role, if any, for TK in renovascular hypertension in mice.

**PF.3 DISTRIBUTION OF APJ RECEPTOR mRNA EXPRESSION ALONG THE RAT NEPHRON. EVIDENCE FOR A VASCONSTRICCTOR ROLE OF APELIN IN GLOMERULAR ARTERIOLES.**

Hu-Cihanez A1, Bouty N1, Iltunen X1, Lorences-Cortes C1

INSERM U891, College de France, Paris, France, 1 INSERM U852, Paris, France

Apelin is a vasoactive peptide that has been identified as the endogenous ligand of the orphan G protein-coupled receptor APJ. Apelin and APJ mRNA are expressed in the brain, peripheral tissues and blood vessels, especially in the kidney. However, the distribution of APJ receptor mRNA along the rat nephron is unknown. In this work, we established this distribution using quantitative real-time RT-PCR and evaluated the role of apelin in mediating a vasconstrictor effect in glomerular arterioles by measuring intracellular calcium mobilization ([Ca2+]i) induced by this peptide using Fura 2 fluorescence. The highest expression of APJ mRNA was found in glomerulus -> proximal tubule -> cortical and medullary thick ascending limb -> cortical and medullary collecting duct. Previously, it was shown that vasoconstriction is mediated by an increase in [Ca2+]i, whereas vascular smooth muscle relaxation results from a [Ca2+]i decrease. In this context, bradykinin attenuates the response to angiotensin II in arterioles (Marchetti et al, Br. J. Pharmacol, 2001). Here, we showed in arterioles that 5x10^-7M apelin-17 induced an increase of [Ca2+]i, followed by a plateau phase (102 ± 9 nM) and application of 5x10^-6 M bradykinin during this plateau phase caused a rapid drop in [Ca2+]i by 67 ± 11 nM. Taken together, these data indicate that the predominant expression of APJ mRNA in glomeruli and to a lesser extent in nephron segments suggest a putative role for apelin in glomerular filtration and fluid reabsorption. In addition, vasoconstriction observed in arterioles suggests that apelin could regulate vascular tone in the kidney.
CONTRIBUTION OF MOLECULAR MODELING AND SITE-DIRECTED MUTAGENESIS TO THE IDENTIFICATION OF RESIDUES INVOLVED IN APelin BINDING AND APelin RECEPTOR ACTIVATION

Iiturroz X1, Maigret B2, Llorens-Cortés C1
1 INSERM U691, Collège de France, Paris, France, 2 CNRS UMR UHP7565, Vandœuvres-les-Nancy, France

The APJ receptor was originally isolated as an orphan seven transmembrane-domain G protein-coupled receptor in humans. Interest in this receptor in mammals increased with the identification, in 1998, of its endogenous ligand, apelin. Apelin is a peptide generated from a 77-amino acid precursor, proapelin. Apelin and its receptor are strongly expressed in the brain particularly in vasopressinergic neurons in the hypothalamus and in peripheral organs such as the heart, the lung and the kidney. Apelin and its receptor, appear to play an important role in body-fluid homeostasis via a direct inhibitory action of central apelin on vasopression secretion, leading to water diuresis. Moreover, intravenous administration of apelin decreases mean arterial blood pressure and apelin also improves cardiac contractility and reduce cardiac loading in vivo. Therefore, the development of non-peptide agonists of the apelin receptor could lead to new therapeutic tools for the treatment of the SIADH and heart and kidney failure. The development of such molecules requires to explore the mode of interaction of apelin with its receptor as well as the mechanisms of activation of apelin receptor. For this purpose, we have built a three-dimensional model of apelin receptor by homology with the experimentally validated three-dimensional model of the Cholecystokinin receptor type 1. Apelin peptide (pE13F) has been docked into this receptor model. Based on this model, the role of several residues of the receptor has been investigated by site-directed mutagenesis studies. This allowed us to define the residues involved in apelin binding, receptor activation and internalization.

Evidence for the role of apelin receptor in the vascular system is provided by the results of site-directed mutagenesis of residues critical for the binding of the apelin peptide to the receptor. The introduction of charged residues into the receptor in the vicinity of the apelin binding site significantly increased the potency of the peptide, whereas the introduction of hydrophobic residues decreased the affinity of the peptide for the receptor.

In conclusion, the results obtained in this study suggest that the apelin receptor is a potential target for the development of new therapeutic agents for the treatment of cardiovascular diseases.

Evidence for the Role of Apelin in Cardiovascular Diseases

Apelin has been implicated in various cardiovascular processes, including blood pressure regulation, sodium homeostasis, and renal function. The role of apelin in cardiovascular diseases is supported by several lines of evidence:

1. Apelin expression is upregulated in the heart and kidney in response to hypertensive stimuli.
2. Apelin receptor activation inhibits cardiac hypertrophy and reduces cardiac remodeling.
3. Apelin has been shown to have anti-fibrotic effects in the heart and lung.
4. Apelin and its receptor are expressed in the vasculature, and their levels are increased in various vascular diseases.
5. Apelin receptor activation has been shown to reduce fibroblast proliferation and collagen deposition in the vasculature.

These findings suggest that apelin and its receptor play a role in the regulation of cardiovascular homeostasis and provide a potential therapeutic target for the treatment of cardiovascular diseases.

In summary, the apelin receptor is a promising target for the development of new therapeutic agents for the treatment of cardiovascular diseases. Further studies are needed to fully understand the role of apelin in cardiovascular physiology and pathophysiology.
expression of p53, Bax and caspase-3 but enhanced the expression of IL-10, whereas blocking AT2 receptors by PD 123319 failed to influence these proapoptotic proteins, but significantly attenuated IL-10 expression. These results suggest the divergent roles of AT1 and AT2 receptors in cardiac apoptosis and inflammation. AT1 receptors may initiate cardiomyocyte apoptosis by inducing p53-triggered apoptotic cascade whereas AT2 receptors may exert an anti-inflammatory effect by mediating cardiac IL-10 expression in myocardial infiltrating macrophages.

**PF.12**

**AT2R BINDING PROTEIN (ATBP) – A NOVEL PROTEIN INVOLVED IN AT2R FUNCTION.**

Funke-Kaiser H.1, Wruck C.J.2, Pufe T.2, Kusserow H.1, Menk M.1, Scheife J.H.1, Kruse M.L.1, Stoll M.1, Unger T.2

1 Center for Cardiovascular Research (CCR), Charité, Berlin, Germany, 2 Institute of Pharmacology, University Hospital Schleswig-Holstein, Kiel, Germany, 3 Institute of Anatomy, University of Kiel, Kiel, Germany, 4 First Department of Medicine, University Hospital Schleswig-Holstein, Kiel, Germany, 5 Institute for Arteriosclerosis Research at the University of Muenster, Münster, Germany

Synthesis and maturation of G protein-coupled receptors (GPCR) are complex events that require an intricate combination of processes including protein folding, post-translational modifications, and transport through distinct cellular compartments. Little is known concerning the regulation of GPCR transport from the endoplasmic reticulum (ER) to the cell surface. Here we show that the cytoplasmic carboxy-terminal of the angiotensin AT2 receptor (AT2R) acts independently as an ER-export signal. Using a yeast two-hybrid system we identified a murine Goji-membrane-associated protein termed ATBSP50 (for AT2R binding protein of 50 kDa) that binds to this motif. We also cloned ATBSP50 and ATBPI35 encoded by the same gene as ATBSP50 that mapped to mouse chromosomes 8p21.3. Downregulation of ATBSP50 using siRNA leads to retention of AT2R in inner compartments, reduced cell surface expression, and decreased antiproliferative effects of the receptor. Concerning the human ATBSP50 analogue ATIP1, we analyzed mRNA expression in different cell lines, determined the transcriptional start sites and measured its promoter activity, indicating housekeeping features of this human isoform. Our results indicate that ATBSP50 regulates the transport of the AT2R to cell membrane by binding to a specific signal within its cytoplasmic carboxy-terminal and thereby enabling the antiproliferative effects of the receptor.

**PF.13**

**PLASMA AND KIDNEY ANGiotensIN II (ANG II) LEVELS IN ANESTHETIZED AND CONSCIOUS REN-2 TRANSGENIC RATS (TGR) **

Huskova Z.1, Kramer H.J.2, Vanourkova Z.1, Cervenka L.1

1 Institute for Clinical and Experimental Medicine, Prague, Czech Republic, 2 Medical Policlinic, Bonn, Germany

In the present study we assessed ANG II in plasma and kidney of pentobarbital-anesthetized heterozygous male TGR during the prehypertensive (at 32 days of age), developing (38 days), and early (52 days) and late (90 days) phases of hypertension as compared with nonanesthetized TGR. Plasma and kidney ANG II levels were lower in 32 days old anesthetized TGR than in HanSD rats, but at 38, 52 and 90 days significantly higher than in HanSD rats. ANG II levels were higher in 32, 38, 52 and 90 days old untreated TGR than in HanSD rats, but were significantly lower in 32 and 52 days old TGR after AT1R blockade (losartan). In contrast, plasma ANG II immediately after decapitation was higher in 32, 38, 52 and 90 days old untreated TGR than in HanSD rats. Also ANG II in kidney obtained after decapitation in 32, 38, 52, 52 and 90 days old TGR were higher than in HanSD rats. The present results indicate, first, that pentobarbital anesthesia increases ANG II in HanSD rats to greater extend than in TGR. Second, based on our data obtained in unanesthetized animals, we conclude that enhanced plasma and kidney ANG II levels during all phases of hypertension in TGR probably represent the most important mechanism leading to high blood pressure in this model of renin-dependent hypertension.

**PF.14**

**EFFECTS OF EARLY-ONSET AND LATE-ONSET ENDOThELIN-A RECEPTOR BLOCKADE ON BLOOD PRESSURE, END-ORGAN DAMAGE AND SURVIVAL RATE IN HOMOZYGOUS REN-2 RATS**

Vanecova L.1, Kramer H.J.1, Backer A.1, Vernerova Z.2, Eis V.1, Cervenka L.1

1 Institute for Clinical and Experimental Medicine, Prague, Czech Republic, 2 Medical Policlinic, University of Bonn, Bonn, Germany, 3 3rd Medical Faculty, Charles University, Prague, Czech Republic

We have recently found that nonselective endothelin ETA/ETB receptor blockade markedly improves survival rate and ameliorates end-organ damage in male homozygous rats transgenic for the mouse Ren-2 renin gene without lowering blood pressure. Since for the detrimental effects of ET in the development of hypertension the activation of ETA receptors may be responsible and usually antihypertensive therapy is more effective when started early in the life, the aims of the study was to compare the efficacy of nonselective ETA/ETB versus selective ETA receptor blockade, and the early-onset (prevention protocol-

**PF.15**

**SPECIFIC ANG II TYPE 2 RECEPTOR BLOCKADE, SIMILARLY TO ANG II TYPE 1 RECEPTOR BLOCKADE IMPROVES POSTISCHEMIC KIDNEY BLOOD FLOW**

Kunicka A1, Zvolinska-Bernat A1, Lodzinska J1, Bojakowski K2, Tyszkwicz J1, Sirski M1, Abramczyk P2

1 Department of Experimental and Clinical Physiology, Medical University of Warsaw, Warsaw, Poland, 2 Dept of Internal Med, Hypertension and Vascular Dis, Medical University of Warsaw, Warsaw, Warsaw, Poland

Objective: Previous studies show that blockade of Ang II type 1 receptor (AT1R) increases kidney blood flow. It is also assumed that stimulation of Ang II type 2 receptor (AT2R) has opposite effect to AT1R activation. The aim of the present study was to investigate the influence of angiotensin receptors blockade on renal blood flow after kidney ischemia in the rat. Methods: Experiments were performed on 24 male Sprague-Dawley rats divided into three groups receiving either saline solution, AT1R blocker – losartan or AT2R blocker – P – PD 123319. The systemic blood pressure was measured invasively and renal blood flow (RBF) was measured on left renal artery with ultrasound Doppler flowmeter. Kidney ischemia was produced by 60 minutes clamping of renal artery. Renal blood flow was measured before ischemia and during 60 min of reperfusion. Results: During reperfusion in all groups RBF was significantly lower as compared to the initial values. In the experimental groups (losartan and PD 123319), RBF was significantly higher during reperfusion period than in the control group. There were no differences in RBF and MAP between losartan and PD 123319 groups. Conclusions: We conclude that AT2R blockade, similarly to AT1R blockade, improves blood flow through ischemic kidney.
to characterise the action of Ap nA and Ap 5A on endocardial tissue and to investigate whether Ap nA induced action potential prolongation is variable in different regions of the heart (e.g. endocardium vs. epicardium), which could exaggerate arrhythmias. Left papillary muscles were isolated from hearts of male guinea pigs and mounted in horizontal organ baths. Isometric force of contraction was measured after stretching each muscle to optimal length. The overall increases in endocardial APD by Ap nA and Ap 5A at different stimulation frequencies indicate that the effects of Ap nA are not frequency dependent and that Ap 5A induced prolongation is evident in different regions of the heart. At a stimulation frequency of 1 Hz, Ap nA increased APD95 by (from 198.8 ± 7.85 ms to 201.4 ± 10.67 ms with 10-6M, and to 290.1 ± 14.35 ms, P < 0.01 with 10-6 M. Pacing at 3.3 Hz increased APD95 (from 177.9 ± 21.1 ms to 186.8 ± 1.9 ms, P < 0.01 with 10-6 M Ap nA. At a frequency of 1 Hz, 10-6 M Ap 5A increased APD (from 204.3 ± 13.70 ms to 206.7 ± 15.31 ms with 10-6 M, and to 294.8 ± 27.32 ms with 10-6 M. P < 0.01. Ap 5A also increased APD95 (from 172.9 ± 2.6 to 179.8 ± 3.6 ms with 10-6M, to 188.4 ± 3.6 ms, P < 0.01, with 10-6 M. At 3 M Hz, Ap 5A and Ap nA increased action potential duration in superfused papillary muscle and induced a positive inotropic effect. The latter effect could be attributed in part to an increase in Ca2+ current or Na+Ca2+ exchange activity.

**PG.3**

**EXPRESSION OF ENDOTHELIAL-DIFFERENTIATION-GENE-RECEPTORS EDG-1 AND EDG-2 IS INCREASED CELL-SPECIFIC IN PATIENTS WITH DILATATIVE CARDIOMYOPATHY**

Bechar E1, Weissbach J2, Steenge A1, Stuke T1, Delco J1, Regitz-Zagrosek V1,2
1 Charte University Medicine, Berlin, Germany, 2 Aventis Pharma Deutschland, Frankfurt, Germany, 3 German Heart Institute, Berlin, Germany

Endothelial-Differentiation-Gene Receptors (EDG) mediate proinflammatory and antiapoptotic effects in the cardiovascular system. EDG are important in ischemic and inflammatory processes in the human myocardium. We investigated the total expression and the cell-specific expression of EDG-1 and EDG-2 in human myocardial samples from patients with DCM and from donor hearts. Real-Time RT-PCR (Taqman) and Western blot analysis was performed with 16 control hearts and 24 hearts with DCM with EDG-1 and EDG-2 specific primer pairs, respectively antibodies. Paraffin sections of 10 control hearts and 10 DCM-patients were stained with antibodies against EDG-1, EDG-2 and costained with cell specific antibodies for fibroblasts, VSMCs, fibroblasts and macrophages. Fluorescence intensity was evaluated semiquantitatively in four categories. DCM-hearts showed a significant stronger (twofold, p<0.005) expression of EDG-1 and EDG-2 compared to healthy donor hearts on mRNA and protein level. Immunofluorescence analysis demonstrated the upregulation of EDG-1 and EDG-2 in endocardial cells and in myocytes. Furthermore, EDG-1 and EDG-2 were expressed in the myocardium of endotoxemia, whereas EDG-2 was localized in the nucleus and cytoplasm. The expression of EDG-1 and EDG-2 increased with 10-6 M Ap nA, to 294.8 ± 27.3 ms

**PG.4**

**ERBB2 SIGNALLING IN THE HEART: ROLE OF THE ADAPTOR PROTEIN NCK2**

Kettel K, Effertz K, Ruiz P

Center for Cardiovascular Research, Charité University Medicine, Berlin, Germany

The receptor tyrosine kinase Erbb2, as a member of the EGF-family, is overexpressed in many epithelial tumors. It is also essential for normal heart development and function: conditional mutant mice develop a severe dilated cardiomyopathy [1]. Clinical testing of the Erbb2--/--antibody Hecoplin on cancer patients revealed a cardiac dysfunction in 30% of the patients when combined with anthracyclines. In order to further untangle the Erbb2 signalling network in the heart we searched for intracellular interaction partners. By a yeast-s-two hybrid screen with a heart cDNA library we identified the adaptor protein Nck2 to interact with Erbb2. We confirmed this interaction in mammalian cells and found it to be dependent on the phosphorylation (activation) of Erbb2. We also showed that Erbb2 is localized at the membrane of adult rat cardiomyocytes. Nck2 is involved in integrin-mediated signalling and has been described as a potential convergence point between growth factor and integrin signalling pathways. Indeed, integrin signalling is activated during cardiac remodelling at the onset of cardiac hypertrophy [2].


**PG.5**

**IN VITRO AND IN VIVO EVIDENCE FOR A ROLE OF THE CD40/CD40L SYSTEM IN CHRONIC HEART FAILURE**

Varo N1, Natal C1, López N1, López B1, González A1, Beloqui O2, Larmán M1, Querejeta R2, Díez J1, Fortuño MA1,
1 Centre for Applied Medical Research (CIMA), Pamplona, Spain, 2 University Clinic of Navarra, Pamplona, Spain, 3 Policlinica Guipuzcoa, San Sebastian, Spain

The mechanisms by which the proinflammatory cytokine CD40L and its receptor, CD40, may play a role in CHF have not been investigated. This work aimed 1) to analyze the expression and function of CD40/CD40L on isolated adult Wistar rat cardiomyocytes and fibroblasts and 2) to quantify the soluble form (sCD40L) in patients with CHF (n=39) and healthy individuals (n=3). CD40/CD40L expression was shown in whole cardiac tissue and isolated cardiac cells by western blotting, real time PCR and immunocytochemistry. Recombinant and platelet derived CD40L induced (p<0.01) cardiomyocyte death (2.7±0.1 and 2.0±0.4-fold). CD40 was functional in cardiac fibroblasts as stimulation with CD40L induced proliferation (1.4±0.5-fold, p<0.05) and gelatinase activity. Serum sCD40L was higher (p<0.05) in patients with CHF than in controls (8.0±5.4 vs 4.8±3.5 ng/ml), and negatively correlated with the ejection fraction (r=-0.36, p<0.05). Thus, patients with systolic dysfunction had higher sCD40L levels than those with normal function (9.7±3.6 vs 6.5±2.7 ng/ml, p<0.05). Patients with diastolic dysfunction had higher sCD40L levels than those with normal function (6.6±2.6 vs 5.1±3.5 ng/ml).

This study shows increased sCD40L in human CHF associated with poor cardiac function and that CD40 ligation on cardiac cells in vitro promotes cellular responses that lead to cardiac damage. These data suggest that CD40/CD40L may play a pathogenic role in CHF and raise the possibility of the use of sCD40L as a marker in this disease.

**PG.6**

**PLAQUE-PRONE HEMODYNAMICS INDUCE PROPRENDOTHELIN-1 GENE EXPRESSION IS MEDIATED BY AN ACTIVATION OF AP-1 AND A STABILIZATION OF MRNA.**

Da Silva R, Chambaz C, Stengelouis N, Silacci P

Swiss Federal Institute of Technology, Lausanne, Switzerland

Hemodynamic forces play an active role in vascular pathologies, particularly in the focal localization of atherosclerotic lesions. Plaque-prone regions are exposed to a hemodynamic environment characterized by a low mean shear stress value and a cyclic reversal of flow direction (oscillatory shear stress). Plaque-prone conditions were shown to affect endothelial cell function by decreasing endothelial nitric oxide synthase gene expression, nitric oxide-mediated vasorelaxation and by increasing the expression of prorenodothelin1 gene. By using a specialized in vitro flow system we investigated the effects oscillatory shear stress on the activity of prorenodothelin1 promoter and messenger stability. A 156-bp proximal promoter was shown to contain all the necessary cis-acting elements conferring oscillatory shear stress sensitivity. Mutational analysis of this proximal promoter allowed the identification of a crucial role of an AP-1 binding site present in this fragment. Interestingly, mutation of this AP-1 site abrogated both the inducible as well as the basal expression of prorenodothelin1 promoter activity. In addition, oscillatory shear stress induced the appearance of an AP-1-binding complex as revealed by electrophoretic mobility shift assay. Another important regulatory pathway determining the level of prorenodothelin1 gene expression is post-transcriptional and involves the stabilization of prorenodothelin1 mRNA. Preliminary results suggested that oscillatory shear stress also act through such mechanism to induce an up-regulation of prorenodothelin1 expression in endothelial cells. In conclusion, these results further elucidate the regulatory mechanisms involved in ET-1 gene expression by plaque-prone hemodynamics, showing that both transcriptional as well as post-transcriptional mechanisms are involved.

**PG.7**

**EVALUATING THE EFFECTS OF PKC, JNK, AND ERK INHIBITORS ON THE UPRGULATION OF ETA RECEPTOR IN RAT BASILAR AND MENSETERARY ARTERIES**

Jamali Roya, Edvinsson Johan, Edvinsson Lars

Lund University, Lund, Sweden

The present study was designed to evaluate the importance of protein kinase C (PKC), extracellular-calculated kinase (ERK 1/2) and c-jun N-terminal kinase (JNK) and the time dependency of the inhibitory effect of PKC, JNK, and ERK inhibitors on upregulation of endothelin B (ET B) receptors in an organ culture model. Methods: Rat basilar arteries were incubated for 24 hours and the PKC inhibitor R0-31–7549, the ERK 1/2 inhibitor SB202190, and the JNK inhibitor SP600125 were added after 3, 6, or 12 h of incubation. Vessel segments were mounted in isometric tissue baths and challenged with endothelin B (ET B) receptor agonists and sarafotoxin 6C (S C; ET B receptor agonist) were studied. The ET B and ET A receptor mRNA levels were determined with a real-time polymerase chain reaction (PCR). Results: The PKC and ERK inhibitors attenuated the contractile induction by ET B but not ET A in the basilar artery. This effect was less pronounced in mesenteric arteries. The efficiency of the inhibitors was proportional to the incubation time. The real-time PCR showed a decrease of the ET B receptor mRNA levels in arteries treated with PKC or ERK inhibitors, while the ET A mRNA levels were unchanged. The JNK inhibitor had a significant inhibitory effect on ET B receptor upregulation and mRNA levels in the basilar artery but not in the mesenteric artery. Conclusion: Our results show that the PKC, ERK, and JNK are more important in the upregulation of contractile ET B receptors in cerebral arteries compared to mesenteric arteries.
Background: Tyrosine kinases are believed to play a role in the pathogenesis of sepsis. There is, however, uncertainty about the identity of the tyrosine kinase(s) and in what way they are involved in the sepsis cascade. We investigated involvement of src-family tyrosine kinases in the effect of lipopolysaccharide (LPS) on contractility of rat tail artery using selective src-family inhibitors. Methods: Isometric tension was measured in rat tail artery segments mounted in a wire myograph. The effect of incubation with LPS (20 mg/ml), with and without the src-family inhibitors, SU6656 (10 μM) and PPI (16 μM), was examined on phenylephrine (PE) and high potassium (KPS)-induced contraction. Results: LPS greatly diminished maximum response to PE and KPS compared to controls (PE; 1.96 ± 0.23 Nm vs. 1.48 ± 0.18 Nm, P < 0.0001; KPS; 2.14 ± 0.17 Nm vs. 10.18 ± 0.54 Nm, P < 0.0001). Incubation with LPS and SU6656 partially restored PE and KPS responsiveness compared to incubation with PE (PE; 4.77 ± 0.63 Nm vs. 1.96 ± 0.23 Nm, P < 0.001; KPS; 5.16 ± 0.31 Nm vs. 2.14 ± 0.17 Nm, P < 0.0001). SU6656 did not affect contractility in vessels untreated with LPS. In contrast, PPI did not prevent the effect of LPS; moreover, it also diminished contractility in vessels not exposed to LPS. Conclusion: The effects of LPS may be mediated, in part, by a member of the src-family of tyrosine kinases. The mechanism of the inhibitory effect of PPI on contraction remains to be established.

INTERACTION BETWEEN THE NA+/K+ -ATPASE AND THE NA+/Ca2+ EXCHANGER MODULATES INTERCELLULAR COMMUNICATION VIA INTRACELLULAR CALCIUM

MATCHIKOV V.U., AASKJAAE C., NILSSON H. 
Institute of Physiology and Biophysics, University of Aarhus, Aarhus, Denmark

Oxubain, a specific inhibitor of Na+/K+ ATPase, has previously been suggested to interrupt intercellular communication in the vascular wall at concentrations lower than are necessary for significant changes of global intracellular sodium. We have here tested the hypothesis that regulation of intercellular communication occurs via a functional interaction between the Na+/K+ ATPase and the Na+/Ca2+ exchanger in restricted spaces near the plasma membrane. Simultaneous confocal imaging of [Ca2+]i in individual smooth muscle cells in the wall of rat mesenteric small artery and isometric force measurements have been performed. Membrane capacitance measured in voltage-clamped cultured rat aortic At75 cells has been used to evaluate electrical coupling of paired cells. The inhibition of the Na+/K+ ATPase with a low concentration of ouabain, ATP depletion and [K+]o-free solution as well as the inhibition of the Na+/Ca2+ exchanger with a specific inhibitor -2,3-ECO and reduction of Na+/Ca2+ uncoupled the paired cells. Depletion of [Na+]i and clamping of [Ca2+]i at low concentrations prevented the uncoupling. We therefore suggest that modulation of the Na+/K+ ATPase activity affects gap junctional conductance through an effect on the Na+/Ca2+ exchanger which regulates the local [Ca2+]i.

FEED-BACK INHIBITION OF ERK1/2 BY ANGIOTENSIN II VIA INDUCTION OF MAP KINASE PHOSPHATASE-1 (MKP-1) EXPRESSION IN BOVINE ADRENAL GLOMERULOSA CELLS

Casal A, Capponi A M 
University Hospital, Geneva, Switzerland

Angiotensin II (AngII) stimulates aldosterone biosynthesis in the adrenal zona glomerulosa through the expression of the Steroidogenic Acute Regulatory (STAR) protein which promotes intramitochondrial cholesterol transfer. The intracellular signaling pathways leading to STAR gene activation and aldosterone production are poorly understood. The activation of the AT1 receptor subtype by AngII transiently activates extracellular signal-regulated kinase (ERK1/2). We have examined the role of the dual specificity (threonine/tyrosine) MAP kinase phosphatase-1 (MKP-1) in this transient activation. MKP-1 is present in bovine adrenal glomerulosa cells in primary culture and AngII treatment markedly stimulates its expression in a time- and concentration-dependent manner (IC50 ~ 1 mM), a maximum of 548 ± 10% of control being reached with 10 nM AngII after 3 h (n = 3, p < 0.01); this effect is completely abolished by blockade of the AT1 receptor subtype. Moreover, this AngII-induced MKP-1 expression is reduced to 25% of control (n = 3, p < 0.01) in the presence of an inhibitor of ERK1/2 phosphorylation, suggesting an interaction between the ERK1/2 pathway and the MKP-1 phosphatase. Indeed, under AngII challenge, the kinetics of MKP-1 expression was a mirror image of ERK1/2 phosphorylation. The increase in MKP-1 protein levels resulted from both a transcriptional activation of the MKP-1 promoter and a reduction of MKP-1 mRNA degradation rate by AngII, suggesting that the hormone stabilizes the mRNA for MKP-1. These data strongly indicate that MKP-1 could be the particular phosphatase involved in the negative feed-back mechanism on AngII-stimulated aldosterone production via the ERK1/2 pathway.

INVOLVEMENT OF CYCLIC NUCLEOTIDES AND POTASSIUM CHANNELS IN HYPOXIC VASODILATION IN PIG CORONARY ARTERIES

Nielsen BD1, Frobert O1, Clunn G2, Hughes AD2, Simmons U1
1Department of Pharmacology, University of Aarhus, Aarhus, Denmark, 2Department of Clinical Pharmacology, National Heart and Lung Institute, London, United Kingdom

In coronary arteries hypoxia leads to vasodilation but there is disagreement concerning the mechanisms involved. Objective: We hypothesized that the cyclic nucleotide-dependent pathways are pivotal for hypoxic vasodilation. Methods: Segments of left anterior descending coronary arteries from pigs were mounted in myographs for isometric tension recording. Vessels were contracted with either 50 mM K+ or phenylephrine (10−5M). A relaxation was induced by reducing oxygen from 95% to 0%. Studies were performed in the absence and presence of inhibitors of protein kinases and K+ channels and intracellular calcium was measured by loading arteries with Fura-2-AM. Results: Followings PDE5 inhibition, arteries relaxed in proportion to level of hypoxia. An inhibitor of protein kinase A, Rp-CPT-CAMPS (100μM), reduced hypoxic relaxation. Hypoxic relaxation was also diminished by blockers of voltage-dependent K channels, 4-aminopyridine (0.5mM) and a blocker of large-conductance calcium-activated K channels,iberiotoxin (100nM) and in arteries contracted with 30mM K+ or phenylephrine (10−5M), a blocker of ATP-sensitive K channels, did not change relaxation to hypoxia, although it inhibited cromakalim relaxation. Hypoxic induced relaxation was associated with reduced intracellular calcium, but calcium concentration remained high in 30mM K+ contracted as compared to PGF2α contracted arteries. Conclusion: Hypoxia relaxes porcine coronary
arteries by activation of protein kinase A resulting in opening of potassium channels and lowering of intracellular calcium. Desensitization of the contractile apparatus may also contribute to hypoxic vasodilatation.

**LACK OF UPREGULATION OF NA⁺-CA²⁺ EXCHANGE IN PRESSURE-OVERLOADED HEARTS OF SERCA2 OVEREXPRESSION TRANSIENT RATS**

Vetter R, Reisfelder C, Weiss W, Rehfeldt U, Paul M

Institute of Clinical Pharmacology and Toxicology, Charite University Medicine, Berlin, Germany

Ventricular arrhythmias and contractile dysfunction are the main causes of death in human heart failure (HF). In HF, downregulation of the sarcoplasmic reticulum (SR) Ca²⁺ ATPase (SERCA2) is linked to a functional upregulation of the electrogenic Na⁺-Ca²⁺ exchange (NaCaX) which is thought to be critical for arrhythmogenesis and contractile dysfunction. To examine whether constitutive cardiac SERCA2 overexpression can protect against upregulation of NaCaX in experimental HF due to severe pressure overload (PO) the rates of Na⁺-Ca²⁺ exchange were measured in isolated myocytes of homozygous or membranes from left ventricular (LV) myocardium of transient rats (TGR) overexpressing SERCA2 and wild-type animals (WT). PO was induced in anesthetized, thoracotomized 5 weeks old male TGR and WT by a 20% coarctation of the ascending aorta using a hemoclip (n=10 each). Sham-operated rats (Sh) were used as controls. Measurements were performed 12 weeks after surgery. PO caused an increase in LV mass by 45 and 65% in TGR and WT, respectively (p<0.05 vs. Sh). Decrease of SR Ca²⁺-uptake in PO was attenuated in TGR (34% vs. WT with PO, p<0.05). By contrast, the NaCaX activity was markedly increased in WT with PO (+78% vs. WT-Sh, p<0.05). This did not occur in SERCA2 overexpressors with PO. This indicates that cardiac overexpression of SERCA2 in the TGR model prevents functional upregulation of NaCaX in experimental HF due to PO. It suggests that SERCA2 overexpression could protect against arrhythmias and contractile dysfunction that are related to an upregulation of NaCaX in HF.

**INCREASED EXPRESSION OF SYNDECAN-1 PROTECTS AGAINST CARDIAC DILATATION AND DYSFUNCTION AFTER MYOCARDIAL INFARCTION**

Schellings M¹, Vanhoutte D¹, Herias V¹, Carmeliet P², Stepp MA³, Heymans S¹

¹ Department of Cardiology/CARIM, Maastricht, Netherlands, ² Center of Transgene Technology and Gene Therapy, Leuven, Belgium, ³ George Washington University Medical Center, Washington, United States

The cell-associated proteoglycan syndecan-1 (Synd1) strongly regulates cell-matrix interactions. Whether Synd1 also regulates extracellular matrix homogenates or membranes from left ventricular (LV) myocardium of transient rats (TGR) overexpressing Synd1 and wild-type animals (WT). PO was induced in anesthetized, thoracotomized 5 weeks old male TGR and WT by a 20% coarctation of the ascending aorta using a hemoclip (n=10 each). Sham-operated rats (Sh) were used as controls. Measurements were performed 12 weeks after surgery. PO caused an increase in LV mass by 45 and 65% in TGR and WT, respectively (p<0.05 vs. Sh). Decrease of SR Ca²⁺-uptake in PO was attenuated in TGR (34% vs. WT with PO, p<0.05). By contrast, the NaCaX activity was markedly increased in WT with PO (+78% vs. WT-Sh, p<0.05). This did not occur in Synd1 overexpressors with PO. This indicates that cardiac overexpression of Synd1 in the TGR model prevents functional upregulation of NaCaX in experimental HF due to PO. It suggests that Synd1 overexpression could protect against arrhythmias and contractile dysfunction that are related to an upregulation of NaCaX in HF.

**CHANGE IN PULMONARY AQUaporIN EXPRESSION IN An AORTIC-BANDED Model OF CARDiac HYPERTROPHY AND FAILURE.**

Christofi F, Turner M, Sheridan D, Kingsbury M

Imperial College, London, United Kingdom

We have previously reported a reduction in pulmonary capillary filtration and changes in lung structure, suggesting an increased barrier to paracapillary water transport in lungs adapted to chronic heart failure. Transgenic studies have shown pulmonary aquaporin (AQP) water channels are important in lung water transudation, particularly AQP1, which is expressed in basolateral membranes of pulmonary vascular endothelium, and AQP5, which is localized on the membrane of type I pneumocytes. In light of this we investigated pulmonary AQP1 and AQP5 expression during developing heart failure. Heart failure was induced in guinea-pigs (n=12) by aortic banding as previously described; pulmonary AQP mRNA quantified with “Real Time PCR” using a fluorogenic probe system and Western blot analysis used to estimate protein content. There was an initial decrease in AQP1 mRNA (p<0.001) and protein (p<0.05) levels in the lung acutely (7±1 days) post banding, although there was no apparent decrease in AQP5 mRNA or protein levels during chronic heart failure. There was no difference in either AQP1 or AQP5 mRNA and protein levels between lungs from banded and control animals. Similarly there was no change in AQP mRNA or protein levels in chronic heart failure lungs 152±1 days post banding. In conclusion, we have shown a reduction of AQP1 protein and mRNA acutely after banding. We speculate that this may be in response to the oedema resulting from the initial haemodynamic effects of banding.

**LOSS OF UKRINOASE-TYPE PLASMINOGEN ACTIVATOR REDUCES MMP-ACTIVITY, CYTOKINE EXPRESSION AND INFLAMMATION, THEREFORE PROTECTING AGAINST CARDIAC DYSFUNCTION DURING VIRAL MYOCARDITIS.**

Heymans S¹, Rutschow S², Kallweiss A³, Torpai R³, Schultheiss HP³, Bauchinger M³, Pinto YM²

¹ Department of Cardiology/CARIM, Maastricht, Netherlands, ² Charité University Hospital, Berlin, Germany

Acute viral myocarditis is an important cause of dilated cardiomyopathy. The present study aimed to investigate whether urkinase (uPA)-gene inactivation may decrease matrix...
Mechanisms of Transition from Hypertension-Induced Hypertrophy to Heart Failure in the Spontaneously Hypertensive Rat Phone to Heart Failure.

Cohuet GMS, Hermans JIR, Smits JFM, Struijer-Boudier HAF

1 Pharmacology and toxicology, university of Maastricht, Maastricht, Netherlands, 2 Cardiovascular Research Institute of Maastricht, Maastricht, Netherlands

The contribution of hypertension to the pathogenesis of heart failure is relatively high. The heart develops structural adaptations like left ventricular hypertrophy (LVH) and increased collagen (col) deposition. Methods: In this study, we used spontaneously hypertensive rats prone to heart failure (SHHF) of 40 weeks (N=7) and of 52 weeks (N=8) compared to age-matched normotensive control Sprague Dawley rats (SD40-52). Rats underwent echocardiography to assess LV wall thickness (WT) and functional parameters. Maximal coronary flow was measured ex vivo and capillary densities, myocyte size and collagen deposition were determined by histology. Results: The heart/body weight ratios (mg/g) were significantly higher in the SHHF vs SD at p<0.001; 40 wks: 3.98±0.14 vs 2.80±0.15 and 52 wks: 4.21±0.15 vs 2.63±0.17. Fractional shortening was significantly decreased in the SHHF between 40 and 52 wks (p<0.001; 33±2 % vs 22±3 %) and maximal coronary flow lower in the SHHF vs SD (p<0.01; 40 wks: 0.19±0.01 vs 0.39±0.04 and 52 wks: 0.15±0.01 vs 0.27±0.02 ml/min/g respectively). Col deposition increased in the SHHF vs SD (p<0.001; 10% vs 2%). Capillary densities (per mm²) were lower in the SHHF rats (p<0.001; 2088±35 at 40wks and 1604±124 at 52 wks) vs SD (2658±116 and 2618±133 respectively). Cardiomyocytes were hypertrophic (p<0.01; μm²: 700±26 at 40wks and 982±85 at 52 wks in the SHHF vs 540±20 in SD rats). Conclusion: SHHF rats showed signs of progressive cardiac remodeling in terms of fibrosis and microvascular changes which can explain of transition from LVH to heart failure.
normalization with ACEI treatment in VHU is not due to reduction in cardiac interstitial collagen.

**THE AT, ANTAGONIST VALSARTAN ATTENUATES PATHOLOGICAL VASCULAR HYPERPLASIA IN RATS WITH HYPERHOMOCYSTEINEMIA**

Kassab SE, Garadah TS, Abu-Hijleh MF, Das NS, Gumaa K

College of Medicine and Medical Sciences, Arabian Gulf University, Manama, Bahrain, 2 Saimaney Medical Complex, Manama, Bahrain

Recent studies indicated that experimental hyperhomocysteinemia (Hhe) causes ventricular hypertrophy and remodelling in the absence of other hypertrophic stimuli. We examined the role of angiotensin-II type 1 (AT1) receptors in mediating ventricular hypertrophy and cardiac remodeling induced by Hhe in rats. The study was conducted on rats fed with methionine (Met) (1.5 mg/kg/day) in a Met-based diet and with methionine plus an oral AT antagonist (valsartan, 60 mg/kg/day) (Met-Val) (n = 8) for 7 weeks. After baseline echocardiography and systolic pressures, rats were subjected to further measurements 3 and 7 weeks after induction of Hhe. After seven weeks, blood samples were obtained under anaesthesia and rats were subsequently sacrificed for histopathological and biochemical assessment of cardiac structure. High methionine intake induced comparable degree of Hhe in both groups. In the Met group, Hhe caused a significant increase in left ventricular wall thickness (LWTH) associated with increased perivascular and interstitial collagen, in the absence of significant blood pressure changes. However, LWTH in the Met-Val group (4.36 ± 0.11 mm) was significantly lower compared to the Met group (5.00 ± 0.23 mm, P = 0.030). Furthermore, cardiac collagen to total protein was significantly lower in the Met-Val group compared with the Met group (2.29 ± 0.11 % vs. 2.64 ± 0.08 %, respectively, P = 0.026). Fractional shortening (FS) was not significantly different between both groups. We conclude that angiotensin-II, through type I receptors, may play an important role in mediating ventricular hypertrophy and remodelling in hyperhomocysteinemic rats.

**SPONTANEOUSLY HYPERTENSIVE RATS EXHIBIT A REDUCTION IN THE AMOUNT AND VASODILATORY EFFECT OF PERIVASCULAR MESENTERIC ADIPOSE TISSUE WHICH IS PREVIOUS TO HYPERTEENSION DEVELOPMENT**

Galvez-Prieto B, Arribas SM, Gonzalez MC, Fernandez-Alfonso MS

1 Unidad de Carotogafia Cerebral, Instituto pluridisciplinar UCM, Madrid, Spain, 2 Departamento de Fisiologia, Fuctudad de Medicina UAM, Madrid, Spain

We have previously described that 3-month-old spontaneously hypertensive rats (SHR) have a lower amount of mesenteric adipose tissue that correlates with a reduced anticontractile effect of perivascular fat (Galvez-Prieto et al, 2004). The aim of this study is to determine if alterations in perivascular adipose tissue and its anticontractile effect are prior to development of hypertension. We have used 4-week-old male Wistar Kyoto (WKY) and prehypertensive SHR (systolic blood pressure: WKY 0.3 ng/ml; WKY 4.4 mmHg) hypertensive rats. After 8 weeks, rats were sacrificed and an in vitro perfusion study with vasopressin (VAP) (10-5 M) was carried out. SHR showed a carotid diameter and a mechanical resistance significantly diminished, an arterial stiffness increased and carotids appeared more contracted. Structural alterations were widely observed in elastic lamellae with a reduction of mature cross-linked elastin in thoracic aorta. This suggested a more advanced maturation of elastic and collagen fibers and/or in VSMC activation. Because SCZ induces a non specific inhibition of LO, we then examined mechanical and biochemical properties of large arteries in SSAO knockout mice. Results have shown a decreased arterial distensibility and an increased carotid diameter in knockout mice, independently of any changes in cross-linked elastin and total collagen. Thus, our results suggest that the absence or the inhibition of SSAO could be implicated in the arterial stiffness in modifying the phenotype and the vasomotor tone of VSMC without affecting collagen and elastin cross-links.

**ROLE OF SEMICARBAZIDE-SENSITIVE AMINE OXIDASE (SSAO) IN ARTERIAL WALL : USE OF SEMICARBAZIDE IN RAT AND A MURIN SSAO KNOCK-OUT MODEL**


1 Medicity- University of Turku, Turku, Finland, 2 Inserm U684, Nancy, France, 3 CNRS-UMR 7079, Paris, France, 4 Inserm U460, Paris, France

Mechanical properties of elastic arteries depend on cross-linking established in elastin and collagen by lysyl oxidase (LO). Differetniated vascular smooth muscle cells (VSMC) express the membrane-associated semicarbazide-sensitive amine oxidase (SSAO) that is involved in the oxidation of a variety of substrates, including L-arginine and L-lysine. These substrates generate nitric oxide (NO) and the antiinflammatory and antihypertensive agent L-arginine. We then examined mechanical and biochemical properties of large arteries in SSAO knockout mice. SSAO activity was reduced by 90 % whereas LO activity was partially decreased (40-60%) in carotid and aorta. SCZ-treated rats had a carotid diameter and a mechanical resistance significantly diminished, an arterial stiffnes increased and carotids appeared more contracted. Structural alterations were widely observed in elastic lamellae with a reduction of mature cross-linked elastin in thoracic aorta. This suggested a more advanced maturation of elastic and collagen fibers and/or in VSMC activation. Because SCZ induces a non specific inhibition of LO, we then examined mechanical and biochemical properties of large arteries in SSAO knockout mice. Results have shown a decreased arterial distensibility and an increased carotid diameter in knockout mice, independently of any changes in cross-linked elastin and total collagen. Thus, our results suggest that the absence or the inhibition of SSAO could be implicated in the arterial stiffness in modifying the phenotype and the vasomotor tone of VSMC without affecting collagen and elastin cross-links.

**MECHANISM OF INDAPAMIDE-INDUCED PREVENTION OF SPONTANEOUS HYPERTENSION.**

Pechanova O, Kojsova S, Jendekova L, Zicha J

1 Institute of Normal and Pathological Physiology, SAS, Bratislava, Slovakia, 2 CRC, Institute of Physiology AS CR, Prague, Czech Republic

Diuretics are commonly used drugs with antihypertensive efficacy through combined diuretic and vasodilator effect. This study was aimed to compare the preventive effect of thiazide-like diuretics hydrochlorothiazide (HTC) and indapamide on blood pressure development in SHR. Young 5-week-old male SHR were treated with HTC (10 mg/kg/day) or indapamide (1 mg/kg/day) for 6 weeks. Nitric oxide synthase (NOS) activity, eNOS and nuclear factor NF-κB protein expression and conjugated diene (CD) concentration were determined in the aorta, heart and kidney. Both drugs partially attenuated systolic blood pressure rise in young SHR (control: 181 ± 2, HTC: 154 ± 4, indapamide: 157 ± 3 mmHg). Indapamide, in contrast to HTC, significantly increased NOS activity in the aorta without modulating eNOS protein expression in this tissue. Both indapamide and HTC failed to modify NOS activity and/or eNOS protein expression in the heart and kidney. Indapamide attenuated concentration of reactive oxygen species (ROS) measured as decreased concentration of CD and proportional increase of NF-κB and CD concentration in the heart, whereas HTC treatment did not show antioxidant effect in any tissue investigated. Our study demonstrated that HTC and indapamide (even if used in ten times lower dose than HTC) decreased blood pressure similarly. However, only indapamide increased NOS activity in the aorta and decreased ROS production in the aorta and kidney. In conclusion, the antihypertensive effect of indapamide involves not only its diuretic effect, but also the attenuation of the imbalance between NO and ROS production. Supported by VEGA: 2/3185/24, 1/1171/24, 1/0540/24 APVT: 51–017902.

**TRANSIENT CAPTOPRIL TREATMENT OF SHR IN JUVENILE DEVELOPMENTAL WINDOW: MECHANISM OF PERSISTENT BP REDUCTION**

Zicha J, Dobesova Z, Hojna S, Kunes J

CRC, Institute of Physiology AS CR, Prague, Czech Republic

Transient treatment of young SHR with ACE inhibitors prevents hypertension development and attenuates BP recovery after drug withdrawal. Our study was aimed to find mechanism(s) responsible for captopril-induced prevention of spontaneous hypertension and the cause of long-term BP reduction after early captopril treatment. Young and adult (4- and 24-week-old) SHR were treated with captopril (10 mg/kg/day) or placebo for 6 weeks. Basal BP, BP response to i.v. nifedipine or consecutive blockade of RAS (insarant, SNS (pentolim) and NOS (L-NMMA) as well as residual BP after nitroprusside were measured at the end of active treatment and after 4 or 20 weeks of drug withdrawal. Compared to WKY, SHR had increased residual BP, enhanced sympathetic vasoconstriction, augmented nifedipine-induced BP fall and greater vasodilator deficit. Captopril treatment of young SHR prevented all above alterations except of vasodilator deficit. Low residual BP and normalized BP response to nifedipine persisted after captopril withdrawal and could be detected during long-term attenuation of hypertension development (50 weeks after drug withdrawal). Compared to young rats, captopril treatment of adult SHR caused similar but less pronounced changes which were completely reversible after drug withdrawal. Faster BP recovery after hydralazine withdrawal can be explained by the absence of its effect on structural changes of resistance vessels. Thus the blockade of central angiotensin effects in juvenile SHR decreases enhanced sympathetic tone and resulting in reduced Ca2+ influx during tonic vascular contraction and attenuated hypertrophic vascular remodeling. (NR/786-3/2004).

**ANTIHYPERTENSIVE MECHANISMS OF CHRONIC CAPTOPRIL (CP) OR N-ACETYLCYSTEINE (NAC) TREATMENT IN L-NAMEN HYPERTENSIVE RATS**

Dobesova Z, Zicha J, Pechanova O, Kunes J

CRC, Institute of Physiology AS CR, Prague, Czech Republic

Chronic L-NMMA hypertension is characterized by both impaired N0-dependent vasodilation and enhanced sympathetic vasoconstriction. Our study was aimed to evaluate the mechanisms of antihypertensive action exerted by chronic captopril or NAC treatment in this model. Three-month-old Wistar males treated with L-NMMA (60 mg/kg/day) for 5 weeks were compared to rats in which L-NMMA (LN) was combined with simultaneous administration of captopril (LN-CP, 100 mg/kg/day) or NAC (LN-NAC, 10 mg/kg/day). Basal
BP and its acute responses to consecutive i.v. injection of losartan (10 mg/kg), pentololium (5 mg/kg), L-NMMA (50 mg/kg) and nitroprusside (NP, 20 μg/kg) were determined in conscious, cannulated rats at the end of the study. The development of L-NMMA hypertension (MAP 164±5 vs 122±3 mmHg in controls) was prevented by CPT treatment (development 126±3 mmHg), whereas NAC treatment caused only moderate BP reduction (148±3 mmHg). Major effects of chronic captopril treatment was caused by significant reduction of both sympathetic BP component (by 23%) and residual BP measured at full NP-induced vasodilation (by 14%). In contrast, chronic NAC treatment did not modify sympathetic BP component or residual BP, but significantly attenuated vasodilator deficit (by 35%) due to enhanced NO-dependent vasodilation. In conclusions, chronic captopril treatment prevents L-NMMA hypertension by lowering of central sympathetic tone and by reduction of structural component of vascular resistance, whereas chronic NAC treatment attenuates L-NMMA hypertension by enhancement of NO-dependent vasodilation. (NRJ778–6/2004).

P.J.6
EXAGGERATED RESPONSE TO ENDOTOXIC SHOCK IN TRANSGENIC RATS WITH ENDOTHELIO-SPECIFIC KININ B1 RECEPTOR OVEREXPRESS

Merino V.F.1, Todiras M.1, Campos L.A.1, Baltatu O.C.1, Popova E.1, Pesquero J.B.2, Bader M.3
1 Max-Debruck-Center for Molecular Medicine (MDC), Berlin, Germany, 2 Universidade Federal de S˜ao Paulo, S˜ao Paulo, Brazil

The kallikrein-kinin system has been implicated in the pathogenesis of sepsis by the activation of both kinin receptors, B1 and B2. The B1 receptor, but not the constitutive B2 receptor, is highly induced in a sepsis model, the endotoxic shock caused by bacterial lipopolysaccharide (LPS). To clarify the pathophysiological role of this receptor in sepsis, transgenic rats expressing the B1 receptor in the vascular endothelium were generated. To this purpose a construct harboring the B1 receptor cDNA under the control of the Tie2 promoter/enhancer sequence was used for microinjection in rat zygotes. The correct orientation of the transgene was confirmed by the vasomotor response to the B1 agonist des-Arg9-bradykinin in vitro and in vivo only in TGR(Tie2B1) rats but not in controls. Endothelial-cell specific transgene expression was confirmed by denudation of the aorta ablation this vasorelaxation, which is mediated by NO and endothelial-derived hyperpolarizing factors and not by NO synthase but normal amounts of the endothelial isoform. TGR(Tie2B1) rats are normoten-
sive, but present a more pronounced hypotensive response to LPS treatment when compared to controls (first phase, 10 min after LPS, WT: -39 mmHg, TGR(Tie2B1): -57 mmHg; second phase, 60 min, WT: -45 mmHg, TGR(Tie2B1): -71 mmHg). Further-
more, TGR(Tie2B1) rats are more susceptible to LPS exhibiting a higher mortality than wild-type animals. Thus, the endothelin-kinin B1 receptor plays an important role in endotoxic shock and the kallikrein-kinin system may be a valid target in sepsis.

P.J.7
DEFECTIVE PRESSURE- AND FLOW-DEPENDENT VASCULAR REMODELING IN MICE DEFICIENT FOR TISSUE-TYPE TRANSGLUTAMINASE

Bakker ENTP, Pitsa A, Spaan JAE, VanBavel E
Academic Medical Center, Amsterdam, The Netherlands

Tissue-type transglutaminase (tTG) is a cross-linking enzyme and G-protein. We tested the hypothesis that tTG plays a role in the inward remodeling of small arteries in hypertension and reduced blood flow. Mice were given L-NMMA to induce hypertension, Blood pressure increased similarly in wild type (WT) and tTG null mice. After one week, mesenteric arteries from tTG null mice did not (0%): however, after 7 days inward remodeling in WT and tTG null mice was similar: -17% vs. –21%. The tTG null mice lacked tTG expression and protein but showed the cross-link specific for transglutaminases. In conclusion tissue-type transglutaminase (tTG) is important for the inward remodeling of small arteries in vivo independent of hypertension (2 months after birth) and the animals were fed for 8 weeks.

P.J.8
DIETARY ZN AFFECTS THE ACTIVITY OF Cu/ZNSOD AND ATHEROSCLEROTIC PLAQUE FORMATION IN SPONTANEOUSLY HYPERTENSIVE RATS

Dimitrova A.A.1, Strashimirov D.2, Nachev C.K.1, Russeva A.1, Apostolova M.O.1
1 Medical University Department of Pathophysiology, Ploven, Bulgaria, 2 St. Anna Hospital Department of Internal Medicine, Sofia, Bulgaria, 3 Medical University Department of Clinical Laboratory, Ploven, Bulgaria, 4 Institute of Molecular Biology, Lab for Medical and Biological Research, Sofia, Bulgaria

The present study was undertaken to determine the interactions between dietary supplements of Zn containing diet (52 or 155 or 236 mg Zn/kg lab chow) on the activity of Cu/ZNSOD (SOD) and atherosclerotic plaques formation in the aorta of spontaneously hypertensive rats (SHR). Male SHR (n = 52) and their normotensive ancestors, Wistar-Kyoto rats (WKY, n = 53), were studied. The diets started at the beginning of the development of hypertension (2 months after birth) and the animals were fed for 8 weeks.

The formation of atherosclerotic plaques was evaluated by using different immunohistochem-ical and electron microscopy techniques. Atomic-absorption spectrometry was applied for determination of metal content (Zn and/or Cu) in the serum. SOD activity was measured by RANSOD (Randox), in comparison with the data from WKY a significant increase of atherosclerotic plaques was found in the SHR (P < 0.01) during the observation period. The level of endothelial dysfunction in SHR was greater compared to the WKY. There was a protective effect of the increasing Zn concentrations in the diet on the development of the atherosclerotic plaques in the SHR. Many lipid droplets were observed in the aorta of SHR and its number was diminished following Zn treatment. The activity of SOD was significantly increased in SHR fed a Zn diet (236mg/kg) in comparison with SHR fed a Zn diet (52 mg/kg). Increased Zn content in the diet decreased the number of formed atheromas in SHR and at the same time increased the activity of SOD.

P.J.9
HYDROXYL RADICAL CAUSES RAPID VASODILATION IN THE MOUSE AORTA: MODULATION BY ENDOGENOUS LEPTIN

Mundy A.L., Widmer C.C., Kretz M., Barton M.
Medical Polyclinic, Department of Internal Medicine, University Hospital Zurich, Zurich, Switzerland

Reactive oxygen species are increasingly implicated both in the maintenance of vascular tone and cell signalling. The aim of this study was to characterize vascular responses to endogenous and exogenous hydrogen peroxide (H2O2) in the mouse aorta. Aortic rings of C57/B6J mice and leptin-deficient ob/ob mice were mounted in organ baths. Endogenous OH was assessed by recording responses in quiescent rings in the absence or presence of EPC-XI (10-7 mg ml-1), a specific OH scavenger. The vascular response to exogenous OH (generated by vitamin C and Fe2+) was recorded in rings preconstricted with phenylephrine (50% of 100 mM KCl). Scavenging of hydroxyl radical resulted in an increase in basal tone in both control and ob/ob aortas (p > 0.05 vs. untreated for both). Addition of exogenous OH to precontracted rings caused a rapid, transient dilation that was markedly greater in leptin-deficient mice (> 5 fold, p < 0.05). These data indicate a vasodilator role for OH in the mouse vasculature under basal and stimulated conditions. The OH-induced vasodilation is enhanced in the absence of the leptin gene. This can provide a novel mechanism to antagonize vasocostriction in states of leptin resistance and/or deficiency present in obesity. Tosaka, M. et al. Acta Neurochir (Wien) 144: 1305–10.

P.J.10
TISSUE TRANSGLUTAMINASE REGulates INWARD REMODELING INDUCED BY VASOCONSTRICTOR.

Effekhtah A., Buus CB.1, Bakker ENTP1, van Bavel E.2, Mulvany MJ.3
1Dept. Pharmacology, University of Aarhus, Aarhus, Denmark, 2 Dept. Medical Physics, University of Amsterdam, Amsterdam, Netherlands

Essential hypertension is associated with inward remodeling of the small arteries. Tissue transglutaminase (tTG) has been shown to play an important role in small artery inward remodel-ling (Bakker et al. Circ Res 2005; 96: 119–126). Here we have investigated the role of tTG in vivo role of tTG in inward remodelling. Male Wistar rats (9 weeks 260–270g) had two crometic pumps (Alzet 2002 and 2ML2, Scareatur 8K, Denmark) implanted subcutaneously for infusion of phenylephrine (PE, 1.0 μg/kg/min, n=8), or PE plus the tTG inhibitor cystamine (PE + CY5 28 μg/kg/min, n=6) and control (n=7). Statistical analysis for all parameters was performed by one-way ANOVA. Systolic and diastolic blood pressure at Day 6 were similar in all groups (P=0.17). On Day 7 the rats were sacrificed and the mesenteric bed harvested. Three mid-segment 2nd branch arteries from pre-assigned locations were taken from each rat for relaxed pressure-volume measurement in the pressure myograph (111P, Danish Myotechnology, Denmark) with the blind-sack method. The lumen diameter (measured with intravascular pressure 60 mmHg under relaxed conditions) was decreased in PE group (270±6 μm) and unaltered in the PE + CY5 group (232±8 μm) compared to the control group (314±12 μm, P<0.0005). Moreover, the wall–lumen ratio and wall cross-sectional area were increased 73% and 46%, respectively, in the PE group and unaltered in the PE + CY5 group (P<0.0001 and P<0.001, respectively). Left ventricular weight was similar in all groups (P<0.59). The results supported a role for tTG in regulating small artery inward remodelling in vivo independent of blood pressure.

P.J.11
A PROTECTIVE ROLE FOR VAGAL ACTIVITY IN ARTERIAL RIGIDITY?

Cossin E., Valensi P., Laude D., Hérissé M., Attali J.R., Gabrière H.
INSERM U0107, Paris, France, 3 Paris Nord University, Bondy, France

An increase in sympathetic nervous system activity is common in hypertension and type 2 diabetes. Vagal activity has been suggested to be protective against high blood pressure (BP) and arterial rigidity. The aim of the study was to determine the correlation of vagal and sympathetic activities with BP and arterial rigidity in 24-week old SHR rats.
SHR/NICrBR, a model of hypertension, and Zucker Diabetic Fatty rat (ZDF-fmi-fa/fl), a model of type 2 diabetes, and their controls (WKY/NCr and lean (7/6) respectively). Two catheters were placed into the femoral artery and one into the femoral vein. Two days later, BP waves and heart rate were simultaneously displayed from conscious rats. Then, the distance between the tips of the 2 catheters was measured to calculate pulse wave velocity (PWV), an index of arterial rigidity. Spectral analysis of the variations in pulse interval (high frequency: HF-P, representing vagal activity) and systolic BP (low frequency: LF-SSP, an index of sympathetic activity) was performed. Mean BP (p < 0.0001), weight (p < 0.01), heart rate (p < 0.05), and PWV with weight (r = 0.489, p < 0.01) and heart rate (r = 0.498, p < 0.01) were significantly correlated. The results suggest that sympathetic activity plays a central role in regulating BP, and that vagal activity may be protective against arterial rigidity in rat models with hypertension or diabetes.

Two days later, BP waves and heart rate were simultaneously displayed from conscious subjects of this study. Hypertension was induced by DOCA-salt injection (20 mg/kg, twice weekly, for 5 weeks, s.c.) and drinking water was replaced by NaCl (1%). Five weeks later, animals were anesthetized with sodium thiopental (30 mg/kg, i.p.), then systemic arterial blood pressure (systolic, diastolic and mean) was directly measured. Mean arterial blood pressure in hypertensive rats was 192 ± 6. Administration of aqueous extracts of B. vulgaris reduced the arterial blood pressure in a dose-dependent manner. In other sets of experiments, mesenteric beds were removed and precontracted with KCl (40 mM), then different concentrations of the aqueous extract were added. In this preparation, addition of dexamethasone (10−6 M, for 20 min) and L-NAME (10−6 M, for 20 min) did not change the response to B. vulgaris. The findings suggest that the antihypertensive effects of B. vulgaris extract are implemented through the reduction in total peripheral resistance and this effect is not mediated by activation of nitric oxide pathway and prostaglandins.

In this study, we investigated the effects of Berberis vulgaris (B. vulgaris) on blood pressure in anesthetized hypertensive rats and also on responses of the rats isolated perfused mesenteric bed. 17 male rats of Sprague-Dawley race (200–250 g) were subgroups of this study. Hypertension was induced by DOCA-salt injection (20 mg/kg, twice weekly, for 5 weeks, s.c.) and drinking water was replaced by NaCl (1%). Five weeks later, animals were anesthetized with sodium thiopental (30 mg/kg, i.p.), then systemic arterial blood pressure (systolic, diastolic and mean) was directly measured. Mean arterial blood pressure in hypertensive rats was 192 ± 6. Administration of aqueous extracts of B. vulgaris reduced the arterial blood pressure in a dose-dependent manner. In other sets of experiments, mesenteric beds were removed and precontracted with KCl (40 mM), then different concentrations of the aqueous extract were added. In this preparation, addition of dexamethasone (10−6 M, for 20 min) and L-NAME (10−6 M, for 20 min) did not change the response to B. vulgaris. The findings suggest that the antihypertensive effects of B. vulgaris extract are implemented through the reduction in total peripheral resistance and this effect is not mediated by activation of nitric oxide pathway and prostaglandins.
STRUCTURAL AND FUNCTIONAL COMPARISON OF NATIVE AND MONOMERIC C-REACTIVE PROTEIN.

Taylor KE, van den Berg CW
Cardiff University, Cardiff, United Kingdom

Introduction. C-reactive protein (CRP) is considered a risk factor for cardiovascular events. CRP is a pentameric molecule and it has been suggested that the monomeric form of CRP (mCRP) is responsible for certain endothelial cell (EC) activation events. Some studies have aimed to inactivate CRP’s putative ability to activate EC by trypsinisation. The aims of our study are to develop methods to distinguish between native CRP and mCRP, to assess the stability of CRP, to compare the binding characteristics of CRP and mCRP to natural ligands and EC cells and the ability of both forms of CRP to activate EC. Methods and Results. Modifications of the Laemmli SDS-PAGE method were employed to distinguish between native CRP and mCRP. mCRP but not native CRP was sensitive to degradation by trypsin, suggesting that this is not an appropriate control in studies investigating the ability of CRP to activate cells. mCRP had lost the ability to bind to its natural ligand phosphorylcholine but attained the ability to bind to EC. Neither form of CRP showed the ability to alter the expression of adhesion molecules, IL-6, MCP-1 by EC. Conclusions. The modified SDS-PAGE provides a simple method to distinguish between different forms of CRP. Neither mCRP nor native CRP were found to have any effect on EC activation, suggesting that previous reports attributing EC activation events to CRP or mCRP are likely artefacts caused by the presence of contaminants in the recombinant CRP preparations used in these studies.

SODIUM AZIDE IN COMMERCIAL C-REACTIVE PROTEIN (CRP) PREPARATIONS IS RESPONSIBLE FOR REPORTED In VITRO EFFECTS ATTRIBUTED TO CRP

Taylor KE, Lang D, Giddings JC, van den Berg CW
Cardiff University, Cardiff, United Kingdom

Objective: C-reactive protein (CRP) has been proposed to be an independent risk factor for cardiovascular disease. In vitro studies have sought to address the mechanism behind this by studying the effect of purified CRP on endothelial cell activation. Most studies have used commercially sourced CRP for which there has been no characterisation of purity or integrity. Few studies include robust controls, which are essential if observations are to be attributed to CRP. We have aimed to investigate if its reported effects are due to contaminants in commercial CRP preparations. Methods: Endothelial cells were incubated with commercial CRP, in house generated azide-free recombinant CRP and ascites purified CRP or azide or LPS equivalent to the concentration present in commercial CRP preparations. Cell supernatants were assessed for MCP-1, IL-8, WfSF secretion and pH change. Results: In house recombinant CRP and ascites purified CRP did not mirror the effects of commercial CRP. Commercial CRP was able to induce all activation events analysed, however this ability was lost upon extensive dialysis, suggesting that low molecular weight contaminants were responsible for these events. Indeed, azide or LPS mirrored the effects of CRP.

CONCLUSIONS: CRP does not activate endothelial cells. All studies published using commercial CRP preparations should be interpreted with great care, as these effects are most likely due the contaminating azide and/or LPS.

ACTIVATION OF DIFFERENT PROTEOLYTIC SYSTEMS IN HUMAN ABDOMINAL AORTIC ANEURYSMS

Kaschina E1, Scholz H2, Sommerfeld M3, Vosgerau U4, Doerfel N4, Kintscher U5, Schmidt ST, Unger T1
1 Center for Cardiovascular Research, Charité University Medicine Berlin, Berlin, Germany
2 Vascular Center Berlin, Berlin, Germany
3 Institute of Forensic Medicine, Charité University Berlin, Berlin, Germany

Abdominal aortic aneurysm is a complex vascular disorder which carries a significant mortality. Our study was focused on the role of protease-antiprotease cascades (cathepsins, kinasins, MMPs, TIMPs) in the elastolysis and apoptosis of the aorta. We examined aneurysmotic abdominal aortic tissue from patients undergoing surgery and healthy aortic tissue (n=12 in each group) using Western blot protein analysis, immuno histochemistry and real-time PCR. Protein analysis demonstrated an up-regulation of the active forms of cathepsin B, L, H and metalloproteinases (MMPs) pro-MMP3, MMP9, pro-MMP12 in the aneurysmal- as compared to healthy aortic tissue. Consistently with the MMP up-regulation, the active form of TIMP-4 was downregulated.

Cathepsins B, K and neutrophil elastase were not significantly changed. Immunohistochemistry revealed a co-localization of cathepsins D and chymases, suggesting that these effects are most likely due the contaminating azide and/or LPS.

SNKE VENOM DISINTEGRIN, FITC- DENDROASPIN AS A FUNCTIONAL PROBE FOR PLATELET-DERIVED MICROPARTICLE ANALYSIS

Dotsenko O, Scully MF, Clutterbuck A, Lu X, Kakkar VV
Thrombosis Research Institute, London, United Kingdom

Platelet-derived microparticles (MPs), bearing the most prominent platelet adhesion receptor integrin αIIbβ3, are thought to play a significant role in the processes of thrombosis and inflammation. Snake venom protein dendroaspin, containing Agr-Gly-Asp (RGD) motif within its molecule has been shown to block platelet aggregation and adhesion, however its effects on MPs are not known. Aim: To develop an approach of measurement of MPs using disintegrin, dendroaspin as a functional probe. Methods: A normal pool (5 replicates) and 25 individual samples of platelet free plasma were subjected to two consecutive ultracentrifugations. Aspirated pellets were used for flow cytometric analysis, which included measurement of the percentage (%) and total numbers of MPs expressing αIIbβ3 either as the IIIa subunit (detectable with monoclonal antibody (MoAb) CD61) or as the activated form (detectable with MoAb PAC-1). This was compared to the binding of FITC-dendroaspin. Results: Back titration with unlabelled dendroaspin revealed that dendroaspin acts as a potent inhibitor of PAC-1 binding to MPs. Although pre-treatment of the platelets with RGDS peptides completely blocked FITC-PAC-1 binding, it only partially decreased dendroaspin binding coinciding with our previous conclusions about the broad structural nature of the interaction between RGD snake venoms and the integrin (Lu et al, JBC, 1996). An assay was devised in which a saturating concentration of unlabelled dendroaspin was used as a control for the measurement of FITC-dendroaspin binding. The replicate analysis (n=5) found good reproducibility of detecting dendroaspin-MPs (% of dendroaspin-MPs mean ± SD = 75.7 ± 4.4). The absolute number of CD61-positive MPs ("standard" MPs marker) and dendroaspin-positive MPs were significantly correlated (R2 = 0.81) Conclusions: Platelet integrin αIIbβ3 ligand dendroaspin can be used as a probe in the analysis of the functional status of RGD integrins on the surface of MPs analysis.

GENE EXPRESSION PROFILING OF THORACIC AORTA IN PATIENTS WITH MODERATE CHRONIC RENAL FAILURE: RELATIONSHIP WITH ARTERIAL FUNCTION AND REMODELLING.

Farest C1, Briet M1, Boutouyrie P1, Perret C2, Rostagno P1, Barbry P1, Laurent S1
1 Inserm U562, Paris, France. 2 Hospital Georges Pompidou, Paris, France, 3 CHNS UMR 6097, Sophia-Antipolis, France

Arterial stiffness, an independent cardiovascular risk factor, is influenced by genetic factors. We already showed that patients with an abnormally high aortic stiffness show a specific gene expression profile in aortic tissues. Chronic renal failure (CRF) is considered a model of accelerated arterial stiffness, through arterial calcifications. We hypothesized that, in patients with CRF, the gene expression profile includes genes involved in arterial calcifications. We analysed human aortic tissues with the "GeneChip Microarray" technology, in 20 patients, with or without CRF, scheduled for a coronary artery bypass graft. The total RNA of each punch biopsy was extracted and hybridized on oligonucleotides chips (25.000 human genes). Cardiolfemoral pulse wave velocity, a robust index of arterial stiffness, was measured before surgery in all patients. In patients with high arterial
stiffness and no CRF, we observed a significant overexpression of lymna and nc2a (proteoglycans) and underexpression of fiblin-1 or pdradin, compared to patients with low arterial stiffness. Genes implicated in vascular calcifications were not differentially expressed between patients with and without CRF. However, CRF was associated with significant changes in genes involved in the regulation of actin polymerisation (CAPZA1, CA1, ARPC3). The presence in the arterial wall of proteins encoded by most of differentially expressed genes was confirmed by immunohistochemistry. In conclusion, CRF patients have a specific gene expression profile of aortic tissue, which can be related to the observed outward arterial remodelling and increased arterial stiffness.

Hypertension represents one of the main risk factor of vascular diseases, and genetic susceptibility can influence the rate of vascular complications in hypertension. The aim of this study is to compare the patterns of proteins secreted by the aorta of control versus L-NAMEn hyper tension rats in two strains differently sensitive to vascular complications of hypertension. Fisher and Brown-Norway rats were divided into two groups: a control group and a group intoxicated by L-NAME (50 mg/kg/d). Survival rate was established on a separated series of Fisher or Brown-Norway L-NAME rats. Systolic blood pressure was monitored and the animals were sacrificed after 1 months of treatment. Secreted proteins from aortas were analyzed using the SELDI-TOF Mass Spectrometry technology. In spite of a significant elevation of blood pressure observed in both strains in response to L-NAMEn, BN survival was 80% after 90 days whereas all Fisher rats had died. L-NAME promoted the secretion of proteins by the arterial wall only in Fisher rats. SELDI-TOF allowed us to detect 4 proteins (4.9 kDa, 8.5 kDa, 10.7 kDa and 22.4 kDa) differentially secreted in L-NAME versus control rats. These proteins were identified as members of the proteasome pathway and the others are associated with the cytoskeleton. The secretion of these proteins could reflect the enhance in proteins turn-over observed during the remodelling of arterial wall linked to cardiovascular morbidity in experimental hypertension.

A COMPARISON OF THE CORONARY FLOW AND REACTIVITY OF LARGE EPICARDIAL CORONARY ARTERY RINGS DURING HYPOXIA AND REOXYGENATION
Japeli J.
Department of Pharmacology and Toxicology, Ljubljana, Slovenia

Introduction: Hypoxia is by definition a reduced partial pressure of O2 in the tissue. Aim: In the present study I compared reactions of left anter descending (LAD) porcine coronary artery rings with the coronary flow in perfused rat hearts according to Langendorff during hypoxia and reoxygenation. Material and methods: Rat (m=250–400 g, n=5–6) hearts were isolated and perfused according to Langendorff at a constant pressure (60 mmHg) with the normoxic (95% O2 + 5% CO2) Krebs-Henseleit (K-H) solution (37°C). After the equilibration period (40 min) the hearts were perfused for 30 min with the hypoxic (95% N2 + 5% CO2) K-H solution and after that reoxygenated for 80 min. Heart rate, left ventricular pressure and coronary flow were measured. Rings (n=5–5 mm wide) of the LAD porcine coronary artery rings were equilibrated in the normoxic (95% O2 + 5% CO2) K-H solution (37°C). For 60 min and twice contracted with 30 mM KCl. On the plateau of the second contraction with 30 mM KCl the aeration was switched to the hypoxic (95% N2 + 5% CO2) K-H solution and after 30 min again to normoxic aeration. Results and conclusion: Except at the beginning of reoxygenation, when the coronary vessels relax as the coronary flow increases, in all other phases during hypoxia and reoxygenation these two parameters are not in accordance. It is obvious that the reactions of the large epicardial coronary artery do not predict well the coronary flow during hypoxia and reoxygenation, and that the coronary flow depends more on extravascular factors which have an influence on small resistance vessels in the myocardium.

ROLE OF HEME OXYGENASE IN MODULATING ENDOThELIAL FUNCTION IN MESENTERIC SMALL RESISTANCE ARTERIES OF SPONTANEOUSLY HYPERTENSIVE RATS
Rizzoni D.1, Porteri E, Rodella L1, Rezzani R2, Piaardi S1, De Cucchi C1, Zani F1, Micliti M1, Boari G.E.M.1, Bianchi R2, Abrahams N.G.3, Agabiti Rosei E1
1 Chair of Internal Medicine, University of Brescia, Brescia, Italy, 2 Chair of Human Anatomy, University of Brescia, Brescia, Italy, 3 New York Medical College, Valhalla, New York, United States

The enzyme heme-oxygenase (HeOx) seems to exert a protective effect toward oxidative stress in the vasculature. Objective To evaluate the effects of inhibition or activation of Heme oxygenase (HeOx) on endothelial function in mesenteric small resistance arteries.

Material and Methods SHR and Wistar Kyoto rats (WKY) (6 rats in every group) were treated with cobalt protoporphyrin (CoPP, activator of HeOx), with stannous stannousporphyrin (SnMP, inhibitor of HeOx), or saline (Unt). Drugs were injected in the peritoneum once a week for 2 weeks. SBP was measured (tail cuff method) before and after treatment. Mesenteric small resistance arteries were mounted on a myograph. Endothelial function was evaluated as cumulative concentration-response curve to acetylcholine (ACH) (10–7–10–5 mol/L) before and after preincubation with L-NMMA (inhibitor of NO synthase).

Results (see figure): In SHR treatment with CoPP improved ACH induced vasodilatation (ANOVA P<0.001) and this improvement was abolished by L-NMMA (ANOVA P<0.001). SnMP was devoid of effects on endothelial function. In WKY rats, endothelium-mediated vasodilatation was not substantially affected by both treatments. Conclusions The stimulation of HeOx seems to improve endothelial function in SHR, possibly by reducing oxidative stress and increasing NO availability.

OLEANOLIC ACID, A COMPONENT IN RESIDUES OF OLIVE OIL, DILATES ISOLATED ARTERIES BY RELEASE OF ENDOTHELIUM-DERIVED RELAXING FACTORS
Rodriguez-Rodriguez R1, Herrera MD1, Petersen LO2, Ruiz-Gutierrez V2, Simonsson U2
1 Instituto de la Grasa (CSIC), Seville, Spain, 2 Dept. Pharmacology, University Aarhus, Aarhus, Denmark

Emerging interest has been directed to the role of diet components and olive oil in preventing cardiovascular diseases. The present study investigated whether oleanolic acid, a component of olive oil residues, dilates different size-arteries from the mesenteric of the rat. Method: superior (0.9–1.2 mm) and mesenteric small arteries (~200 μm diameter) were mounted in microvascular myographs, and relaxation curves were constructed for oleanolic acid and acetylsalicylic acid. Results: In noradrenaline-contraction arteries, oleanolic acid induced concentration-dependent relaxations with pD2-values of 5.8±0.2 and 5.2±0.2, and maximal relaxations of 74±5% and 81±7%, in superior and mesenteric small arteries, respectively. In preparations without endothelium this relaxation was markedly reduced. In contrast to the cyclooxygenase inhibitor, indomethacin, a nitric oxide (NO) synthase inhibitor, Nω-asymmetric dimethyl-L-arginine (300 μM) blunted oleanolic acid relaxation to the same degree as endothelium denudation. Measurements with an NO sensitive electrode revealed oleanolic acid increased NO concentration to same degree as acetylsalicylic acid in superior mesenteric artery. In contrast to superior, in small arteries, oleanolic acid induced less relaxation in the presence of 60 μM K+ or the Ca2+–activated K+ channels blockers, charybdotoxin (0.1 M) and apamin (0.5 μM). Conclusion: This study suggests that oleanolic acid increases NO in large arteries, while endothelium-derived hyperpolarization type release also contributes in small arteries. The increased bioavailability of NO in large arteries exposed to oleanolic acid may counteract the endothelial dysfunction in arteriosclerosis.

NON-GENOMIC VASCULAR EFFECTS OF 17β-ESTRADIOL, PROGESTERONE AND TESTOSTERONE ON HUMAN UMBILICAL ARTERY
Cairrão E, Carval J, Álvarez E, Santos-Silva AJ, Verde I
Universidade da Beira Interior. Centro de Investigação em Ciências da Saúde, Covilhã, Portugal

Many epidemiological studies suggest different gender incidence of cardiovascular diseases, probably caused by cardioprotective effects of sex hormones. The aim of this study was to investigate the rapid (non-genomic) effects of progesterone (PRG), 17β-estradiol (EST) and testosterone (TES) on human umbilical artery (HUA) contractility. Isometric tension of HUA rings without endothelium from normal term pregnancies was measured in an organ bath system. The HUA were contracted with serotonin (1 μM), histamine (10μM), or KCl depolarising concentrations (60 mM). PROG, EST and TES (1–100μM) produced a partial, and dose-dependent relaxation of serotonin and histamine contracted rings. However, KCl-induced contractions were resistant to PROG and EST vaso relaxing effects, while TES almost totally relaxed contractions elicited by KCl. The steroids hormones studied relaxed in a same extent the agonists-induced contractions. However, voltage-operated calcium channels-dependent contractions were unaffected by PROG and EST, while TES practically totally relaxed them. This data suggest that the vasodilator mechanisms of EST and TES are different of that produced by PROG, which affects voltage-dependent calcium channel inhibition. No gender differences were observed neither on the contractile response to vasoconstrictor agents, nor on the vasorelaxing effects of sex hormones.