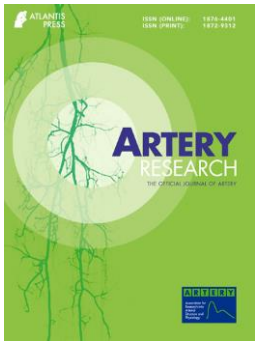

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P2.04: PARADOXICAL INVOLVEMENT OF THE ENDOTHELIAL MINERALOCORTICOID RECEPTOR IN PLATELET ACTIVATION AND VASCULAR THROMBOSIS IN MOUSE

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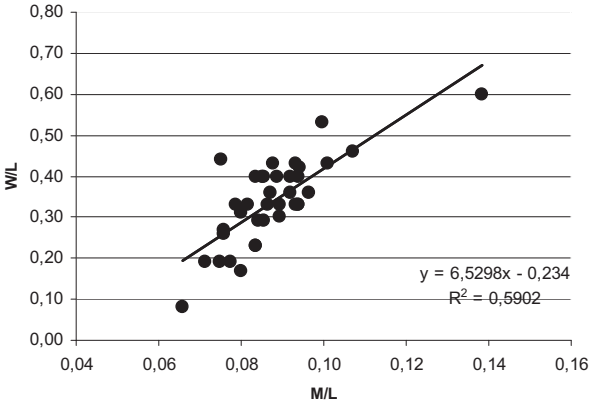
Structural alterations of subcutaneous small resistance arteries, as indicated by an increased media to lumen (M/L) ratio, are frequently present in hypertensive and/or diabetic patients, and may represent the earliest alteration observed. In addition, M/L of small arteries evaluated by micromyography has a strong prognostic significance; however its extensive evaluation is limited by the invasivity of the assessment, since a biopsy of subcutaneous fat is needed. Non-invasive measurement of wall to lumen (W/L) of retinal arterioles using scanning laser doppler flowmetry (SLDF) has been recently introduced (Harazny J et al, Hypertension 2007; 50:623-629). However, this new technique was never compared with micromyographic measurement, considered the gold standard approach.
Methods and Results: We have investigated 37 subjects and patients. Twenty-three of them were hypertensives and 14 normotensives. Blood pressure values were 139/88±17/10 mmHg and 125/71±17/12 mm Hg, respectively ($p<0.05$). All patients underwent a biopsy of subcutaneous fat during an elective surgical intervention. Subcutaneous small resistance arteries were dissected and mounted on a wire myograph, and M/L was measured. In addition, an evaluation of W/L of retinal arterioles by SLDF was performed (Heidelberg Retina Flowmeter, Heidelberg Engineering). A close correlation was observed between M/L of subcutaneous small arteries and WL of retinal arterioles: $r=0.77$, $p<0.001$ (see Figure).
Conclusion: A non invasive and easily repeatable procedure (intraobserver and interobserver variation coefficient around 10%) such as an evaluation of the arterioles in the fundus oculi by SDLF may provide similar information regarding microvascular morphology compared in respect with an invasive, accurate and prognostically relevant micromyographic measurement of M/L.



P2.02
INVESTIGATION OF THE EFFECTS INDUCED ON ENDOTHELIAL FUNCTION BY THE HUMAN T2238C ANP GENE MOLECULAR VARIANT

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Objective: The T2238C ANP gene molecular variant has been related to increased risk of cardiovascular events. *In-vitro* investigations of its pathogenetic mechanisms demonstrated that the 2238C allele variant induced detrimental effects on endothelial cells through increased ROS production. Based on these observations we hypothesized that T2238C ANP gene variant could exert a pathogenic impact on endothelial function *in-vivo*.
Design and methods: We enrolled 10 double mutant CC2238/ANP healthy subjects and 10 healthy subjects carrying the 2238TT wild type genotype. NT-proANP levels were measured in a fasting venous sample. Endothelial-dependent vasodilation was assessed through FMD procedure at brachial level and endothelial endothelial-independent vasodilation was evaluated by means of FMD after sublingual nitrate administration. All subjects underwent carotid ultrasonography.
Results: No significant differences were observed between the two groups in terms of prevalence of cardiovascular risk factors. NT-proANP plasma levels were comparable in double mutant and in wild type subjects (2200±190 vs 2353±340, respectively). Endothelial-dependent vasorelaxation was significantly lower in 2238C as compared to 2238T allele carriers (6.97±2.4 vs 9.31±1.6, $p=.02$). No significant differences were observed regarding endothelial-independent vasodilation (13.4±3.4 vs 14.5±5.6, NS). Carotid ultrasonography did not show any significant alteration of arterial wall in either group.
Conclusions: The 2238C ANP molecular variant, responsible for detrimental effects on endothelial cells *in-vitro*, leads to significant early impairment of endothelial function *in-vivo*. The functional derangement induced by T2238C ANP gene variant may contribute to the increased predisposition to cardiovascular events observed in 2238C allele carriers. Further studies are needed to fully explore this issue.

P2.03
RECOMBINANT HUMAN ERYTHROPOIETIN ALTERS SUBCUTANEOUS RESISTANCE ARTERY ENDOTHELIAL FUNCTION THROUGH A MECHANISM INVOLVING OXIDATIVE STRESS AND ENDOTHELIN-1 IN PATIENTS WITH STAGE 4 CHRONIC KIDNEY DISEASE

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Objective: Recent studies have raised concern about the safety of recombinant human erythropoietin (RhuEPO) because of evidence of an increase in the risk of cardiovascular disease in chronic kidney disease (CKD) patients. In the present study we investigated the effect of RhuEPO on the function of resistance arteries isolated from CKD patients.
Design and method: 19 patients (mean age 63 ± 14 years) with stage 4 CKD (mean eGFR 20 ± 5 mL/min/1.73m^2), none treated with RhuEPO, were included. Resistance arteries from gluteal subcutaneous tissue were assessed on a pressurized myograph. Endothelium-dependent and independent relaxations were tested with acetylcholine and sodium nitroprusside respectively, with RhuEPO (0, 1, 10 and 20 UI/mL). Tempol (10^{-3} M), a superoxide dismutase mimetic, was used to inhibit oxidative stress. ABT-627 (10^{-7} M) was used as a selective endothelin subtype A receptor antagonist.
Results: At 20 UI/mL, RhuEPO had no effect on norepinephrine-induced vasoconstriction ($n=10$) nor on sodium nitroprusside-induced relaxation ($n=10$). RhuEPO altered endothelium-dependent relaxation in a dose-dependent manner (maximal relaxation with RhuEPO at 0, 1, 10, 20 UI/mL, 77.7 ± 3.5 %, 68.5 ± 4.7%, 53.2 ± 3.8 %, 45.7 ± 4.6 %, $P<0.001$, $n=7$-14). Tempol and ABT-627 partially reversed the altered endothelial function in presence of RhuEPO 20 UI/mL ($P<0.01$, $P<0.01$, respectively).
Conclusion: RhuEPO alters endothelial function of subcutaneous resistance arteries in predialysis CKD patients via a mechanism in part involving oxidative stress and signaling through endothelin subtype A receptors, which could contribute to the deleterious effect of RhuEPO described in large interventional trials.

P2.04
PARADOXICAL INVOLVEMENT OF THE ENDOTHELIAL MINERALOCORTICOID RECEPTOR IN PLATELET ACTIVATION AND VASCULAR THROMBOSIS IN MOUSE

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Our aim was to investigate whether mineralocorticoid receptor (MR) overexpression in the vascular endothelium modifies the *in vitro* thrombin generation using thrombography and enhances the thrombotic risk *in vivo*. We used our mouse model with conditional overexpression of the MR in endothelial cells (MR-EC) (Nguyen Dinh Cat et al, FASEB J. 2010;24:2454-63).
The plasma level of von Willebrand factor was significantly increased in 3 month-old MR-EC mice compared with that in control mice (CT). In the presence of the activated protein C (APC) anticoagulant system, thrombin generation was lower in the plasma of MR-EC mice than in CT. Maximal platelet aggregation in

response to collagen was lower in MR-EC than in CT. To address the role of endothelial cells as cellular surfaces involved in the coagulation process, *in vitro* thrombin generation was assessed at the surface of cultured human aortic endothelial cells. Treatment of these cells with 10^{-8} M aldosterone resulted in a significant reduction of thrombin generation prevented by the MR antagonist RU28318. *In vivo*, vessel occlusion times after exposure of the carotid artery surface to ferric chloride was delayed in MR-EC compared with CT mice.
These results demonstrated that enhanced endothelial MR activation induced endothelial dysfunction. Paradoxically, MR-EC mice exhibited a decreased risk of thrombosis. Our results suggested that MR activation in the endothelium affected coagulation by enhancing the APC anticoagulant system and decreasing platelet aggregation. This finding raised interesting prospects on the potential mechanisms of action of new anti-thrombotic drugs and their interference with the mineralocorticoids.

P2.05
ARE RETINAL MICROVASCULAR PHENOTYPES ASSOCIATED WITH 1675G/A POLYMORPHISM IN ANGIOTENSIN II RECEPTOR-2 GENE?

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Background: The X-linked *AT2R G1675A* polymorphism is located in the short intron 1 of the *AT2R* gene within a sequence motif conforming to a splice branch site. AT2R is expressed in the human retina, but no previous study examined the association between retinal microvascular phenotypes and the *AT2R G1675A* polymorphism.
Methods: In 340 subjects randomly selected from a Flemish population (mean age, 51.9 years; 51.5% women), we post-processed retinal images (Canon Cr-DGi) using IVAN software to generate the retinal arteriole and venule equivalents (CRAE and CRVE) and the arteriole-to-venule-ratio (AVR). DNA fragments including the *AT2R G1675A*, *AT1R A1166C*, *ACE I/D* and *CYP11B2 C*−344T polymorphisms, were amplified by PCR. We applied a mixed model to assess phenotype-genotype associations while accounting for relatedness and covariables.
Results: CRAE, CRVE and AVR averaged 151.9 μm, 215.2 μm and 0.710, respectively. CRAE was 5.5 μm greater in women than men and decreased with age ($P<0.05$). In multivariable-adjusted analyses, CRAE was higher in hemizygous and homozygous carriers of the *AT2R A* allele than in their *G* allele counterparts in both sexes combined (+4.49 μm; $P=0.014$) and in men (+4.91 μm; $P=0.032$) with a similar trend in women (+3.41 μm; $P=0.14$). AVR was increased in the presence of the *AT2R A* allele compared with *AT2R G* hemizygotes and homozygotes (+0.024; $P=0.0082$). The associations of CRAE and CRVE with other polymorphisms was not significant.
Conclusions: Pending confirmation in experimental and epidemiological studies, our findings suggest that diameter of the retinal arterioles might be associated with the *AT2R 1675G/A* polymorphism.

P2.06
AN IMPAIRED ROLE OF EPOXYEICOSATRIENOIC ACIDS CONTRIBUTES WITH ALTERED NO AND ENDOTHELIN-1 PATHWAYS TO CONDUIT ARTERY ENDOTHELIAL DYSFUNCTION IN ESSENTIAL HYPERTENSION

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Background: The mechanisms involved in the endothelial dysfunction of conduit arteries, which is an independent contributor to the high incidence of cardiovascular events during essential hypertension, remains to be fully elucidated.
Methods and results. Radial artery diameter, blood flow and mean wall shear stress were determined in 28 non-treated essential hypertensive patients and 30 normotensive control subjects, during endothelium-dependent flow-mediated dilatation (FMD) induced by hand skin heating. The role of epoxyeicosatrienoic acids (EETs) and NO was assessed during heating using the brachial infusion of inhibitors of cytochrome P450 epoxygenases (fluconazole) and NO-synthase (L-NMMA). First, as compared with controls, hypertensive patients exhibited a decreased FMD in response to post-ischemic hyperemia as well as to heating, as shown by the lesser slope of their diameter-shear stress relationship, with no modification in endothelium-independent dilatation. In controls, heating-induced FMD was reduced by fluconazole, L-NMMA and, to a larger extent, by L-NMMA+fluconazole. In patients, FMD was not affected by fluconazole and was reduced by L-NMMA and L-NMMA+fluconazole to a lesser extent than in controls. Local plasma EETs level increased during heating in controls (an effect diminished by fluconazole), but not in patients. Plasma nitrite level, an indicator of NO availability, increased during heating in controls (an effect abolished by L-NMMA), and to a lesser extent in patients. Plasma endothelin-1 level decreased during heating in controls but not in patients.
Conclusions. These results show that an impaired role of EETs contributes with alteration in NO and endothelin-1 pathways to conduit artery endothelial dysfunction in essential hypertension.

P2.07
ENDOTHELIUM DEPENDENT AND INDEPENDENT DILATATION IN DIFFERENT VASCULAR BEDS

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Objective Several methods have been proposed for the evaluation of endothelial dysfunction in patients with cardiovascular risk factors. Whether the flow-mediated dilation (FMD) in medium size arteries is related to the vasodilating response to different agonists in small resistance arteries has not been adequately evaluated. Aim of the present study was to assess the endothelial dysfunction in subcutaneous small resistance arteries (response to acetylcholine, Ach or bradikinin, BK) and in the brachial artery (FMD) in normotensive subjects (NT), essential hypertensives (EHT), patients with primary aldosteronism (PA) and patients with type2 diabetes (DM). Methods 46 DM (20 F, age 39-77 yrs, 14 NT and 32 HT), 6 EHT (3 F, age 40-66 yrs), 6 PA (4 F, age 40- 57yrs), and 4 NT (2 F, age 16-64 yrs) underwent a biopsy of the subcutaneous fat. Small resistance arteries were mounted on a micromyograph and a concentration-response curve to Ach (from 10^{-9} to 10^{-5}) and to BK (from 10^{-10} to 10^{-6}) was performed. In all patients we measured, by a high resolution ultrasound, the brachial artery (BA) diameter at rest, during reactive hyperemia (5 mis of BA occlusion); BA flow velocity was measured by pulsed doppler
Results:

	NIDDM (NT)	NIDDM (EH)	EH	PA
Ach 10^{-5} mol/L (%)	-37 ± 20	-56 ± 22	- 63 ± 35	- 63 ± 13
BK 10^{-6} mol/L (%)	-35.2 ± 25	-44 ± 27	- 54 ± 39	- 58 ± 15
FMD %	8.56± 4.6	5.19 ± 3.0	6.12 ± 6.8	4.76 ± 2.82

There was a small, although statistically significant, correlation between FMD and the maximal response to BK ($r = 0.34$, $p < 0.05$) while no significant correlation was observed between FMD and the maximal response to Ach ($r= 0.19$, ns) in all patients. A significant correlation was observed between BA dilatation after NTG 40 mcg s.l and the maximal response to NTP ($r= 0.30$, $p = 0.05$). Conclusions These results indicate that, although endothelial dysfunction may be observed in both small resistance arteries and medium size arteries, the degree of impairment may differ according to the vascular bed observed, and to the pathophysiology of the disease.

P2.08
ENDOTHELIAL FUNCTION AND RENAL VASODILATION, BUT NOT ARTERIAL STIFFNESS, ARE IMPAIRED IN LEAN, NORMOTENSIVE PATIENTS WITH OBSTRUCTIVE SLEEP-APNEA

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Background: Patients with obstructive sleep apnea (OSA), a condition with a strong comorbidity with hypertension and obesity, exhibit an accelerated vascular aging and renal damage. The aim of the study was to evaluate