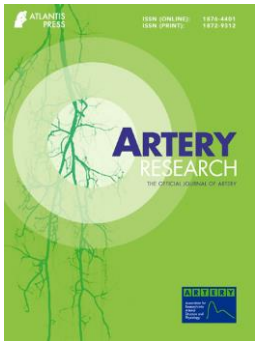

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# P5.1: PROTEOMIC ANALYSIS ON HUMAN ARTERIAL TISSUE: RELATIONS TO ARTERIAL STIFFNESS

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In the population-based Maastricht Study, we evaluated the associations between carotid stiffness (cPWV) and ΔPWV, and glucose metabolism status (GMS). Additionally, we investigated the interdependency of cPWV and ΔPWV in their association with GMS as to find out whether remodeling may act differentially upon cPWV and ΔPWV.
**Methods:** The study consisted of 594 individuals (312 normal glucose metabolism [NGM], 98 impaired glucose metabolism [IGM] and 184 T2D). cPWV and ΔPWV were determined by ultrasonography and tonometry. Regression analyses were used to investigate the associations of cPWV and ΔPWV with GMS (NGM as reference). Models were adjusted for age, sex, mean arterial pressure (MAP), and central pulse pressure, cPWV or ΔPWV as appropriate, and additionally for: anti-hypertensive medication, prior cardiovascular disease, estimated glomerular filtration rate, or body mass index.
**Results:** After adjustment for age, sex and MAP, T2D was associated with greater cPWV (β (95% CI; 0.284 (0.012-0.556)) and ΔPWV (0.299 (-0.005-0.603)). Further adjustments did not change these associations. After additional adjustment for cPWV or ΔPWV the associations with ΔPWV and cPWV attenuated (0.209 (-0.083-0.502) and 0.208 (-0.053-0.470), respectively). IGM was not associated with either cPWV or ΔPWV.
**Conclusions:** In T2D both cPWV and ΔPWV are increased. The associations were only partially interdependent, which suggests that remodeling impacts on both stiffness and its pressure-dependency.

### P4.12
### AGE, WAIST CIRCUMFERENCE AND BLOOD PRESSURE ARE ASSOCIATED WITH SKIN MICROVASCULAR FLOWMOTION: THE MAASTRICHT STUDY

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**Introduction**: Skin microvascular flowmotion (SMF) plays an important role in optimal delivery of nutrients/oxygen to tissue and in maintaining normal peripheral resistance. It is unclear however, which determinants influence SMF. Therefore, we investigated which cardiovascular risk factors are associated with SMF.
**Methods**: We measured SMF in 506 participants without a prior cardiovascular event. SMF was investigated using Fourier transform analysis of skin laser Doppler flowmetry. The associations of the cardiovascular determinants age, sex, waist circumference, 24-h systolic blood pressure (SBP), total-to-HDL cholesterol, fasting plasma glucose (FPG), and cigarette smoking with SMF were analyzed by use of multiple linear regression analysis.
**Results**: The mean age of the study population (n=506) was 58.8 ± 8.5 years, 260 (51.4%) were men, mean waist circumference was 95.7 ± 13.0 cm, mean 24-h SBP was 119 ± 12 mmHg, and 73 (14.4%) were smokers. After adjustment for cardiovascular risk factors and medication, per 1SD higher age SMF was 0.17 SD (95%CI: 0.08; 0.26; $P < 0.001$) higher; per 1SD higher waist circumference SMF was -0.12 SD (-0.23; -0.01; $P = 0.03$) lower; per 1SD higher 24-h SBP SMF was 0.17 SD (0.07; 0.27; $P < 0.001$) higher. No other associations with SMF were found.
**Conclusions**: Age and blood pressure were directly, while waist circumference was inversely associated with SMF. The exact mechanisms underlying these findings remain elusive. The present data support the hypothesis that microvascular dysfunction, specifically, impaired SMF, plays a role in the development of obesity-related insulin resistance and hypertension.

### P4.13
### TYPE 2 DIABETES IS ASSOCIATED WITH ALTERED CAROTID ARTERY MECHANICS INDEPENDENTLY OF AGEING AND MEAN ARTERIAL PRESSURE—THE MAASTRICHT STUDY

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**Introduction:** Type 2 diabetes (T2D) is characterised by accelerated vascular ageing, which changes arterial wall structure and hence artery mechanics (e.g., pressure-area (P-A)). Pulse wave velocity (PWV) is affected by both blood pressure and changes in wall mechanics. To better capture underlying changes in wall mechanics in T2D, we aimed to disentangle the vascular ageing phenomena (characterised by PWV) from pressure effects.
**Methods:** We studied young (<55y) and older (>70y) individuals without and with T2D matched at the group level for age, sex and MAP (n=29 each) from the Maastricht Study. Non-linear P-A curves were derived from carotid tonometry and echo-tracking, using Langewouters-model fits. Isobaric PWV (Bramwell-Hill) was determined at MAP.
**Results:** In individuals without T2D, the average P-A curve in older, as compared to younger individuals, was shifted rightward (diastolic area ($A_d$), mean±SD: 48.8±10.3 vs. 42.5±8.3mm$^2$, p=0.003), which led to higher PWV (9.9±2.0 vs. 7.4±1.6m/s, p<0.001). Next, in younger individuals with T2D, as compared to those without, a similar pattern was found ($A_d$: 45.7±9.6 vs. 42.5±8.3mm$^2$, p=0.068 and PWV: 8.2±1.6 vs. 7.4±1.6m/s, p=0.072). Finally, in older individuals with T2D, as compared to those without, the P-A curve was again shifted rightward ($A_d$: 53.1±10.4 vs. 48.8±10.3mm$^2$, p=0.034), but PWV was not significantly different (10.1±1.7 vs. 9.9±2.0m/s, p=0.29).
**Conclusion:** Independently of blood pressure, both ageing and T2D have a dilatory effect on carotid arteries, with ageing also clearly demonstrating stiffening. Although T2D is associated with additional stiffening in individuals younger than 55y, this was not observed in individuals older than 70y.

### P4.14
### THE EFFECT OF GENDER AND BODY SIZE ON ARTERIAL HAEMODYNAMICS AT REST AND DURING EXERCISE

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**Aim**: The positive association between body size and blood pressure (BP) is well recognised. However, not all overweight individuals are hypertensive. This study aimed to examine the influence of body size and gender on the haemodynamic mechanisms driving systolic BP (SBP) at rest and during exercise, in young adults.
**Method**: Detailed anthropometric, biochemical and haemodynamic measurements including BP, cardiac index (CI) and peripheral vascular resistance (PVR) were obtained in 2497 untreated individuals (23±6 years) at rest. Subjects were classified as normal-weight (NW; BMI <25) or overweight (OW; BMI>25). A sub-set of 86 individuals (29±6 years) undertook steady-state, sub-maximal cycling exercise, with detailed haemodynamic measurements re-assessed.
**Results**: At rest, a positive association was found between SBP and cardiac index (CI) in NW but not OW males and females (p<0.001 for both). In contrast, a positive association was found between SBP and PVR in OW but not NW males (P<0.001 for both). Although BMI did not correlate with BP during exercise, body fat (BF) % was inversely associated with exercise-induced changes in PP, even after adjustment for gender. A higher BF% was also associated with a poorer maximum dilatory response to ischaemia.
**Conclusion**: The primary haemodynamic mechanisms driving SBP differ depending on body size in young adults. BF% may be a more useful tool than BMI to further examine the impact of body size on BP in young adults. Structural differences in resistance vessels may underlie the association between body size and BP responses to exercise.

### P5.1
### PROTEOMIC ANALYSIS ON HUMAN ARTERIAL TISSUE: RELATIONS TO ARTERIAL STIFFNESS

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We hypothesized that arterial stiffness is associated with changes in the arterial protein profile, particularly in relation to extracellular matrix (ECM) components, and aimed at determining differentially expressed proteins in human arterial tissue by quantitative proteome analysis in patients with different degrees of arterial stiffness. Arterial stiffness, assessed by carotid-femoral pulse wave velocity (PWV), central blood pressure and augmentation index by pulse wave analysis, as well as carotid intima-media thickness were measured the day prior to surgery in a group of patients undergoing coronary artery bypass grafting. Protein extracts of well-defined, homogenous, non-atherosclerotic individual samples of the left mammary artery from 10 of these patients with high PWV and 9 with low PWV, were compared by quantitative proteome analysis, using iTRAQ-labeling and nano-LC-MSMS. Of 504 quantified proteins, 28 were differentially expressed between groups with high and low PWV (p<0.05). Six out of eight members of the extracellular matrix family of small leucine-rich repeat proteoglycans displayed significant or borderline significant differences between the two groups (p<0.001, Fisher's Exact Test). Only one other of 43 identified ECM proteins were differentially regulated (collagen alpha-1(VIII)). Several proteins related to smooth muscle cell function and structure were found in different amounts between the two groups.

Changes in the arterial amounts of small proteoglycans, known to be involved in collagen fibrillogenesis, are associated with arterial stiffness. In addition, several proteins related to function of the human arterial smooth muscle are changed as well.

### P5.2
### QUANTITATIVE PROTEOMICS REVEAL INCREASED CONTENT OF BASEMENT MEMBRANE PROTEINS IN ARTERIES FROM PATIENTS WITH TYPE 2 DIABETES, BUT REDUCED AMOUNTS AMONG METFORMIN USERS

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We hypothesized that metformin intake influence the molecular composition of arterial tissue from patients with type 2 diabetes.
We analyzed non-atherosclerotic, internal mammary arteries, gathered at coronary by-pass operations from 30 patients with type 2 diabetes (16 treated with metformin, 14 without), as well as from 30 age- and gender-matched non-diabetic individuals. Quantitative proteome analysis was done by iTRAQ-labelling and LC-MS/MS analysis on individual trypsinized extracts of formalin fixed, paraffin embedded tissue. Sections were also analyzed by histology and immunohistochemistry.
We were able to quantitate 129 proteins. The amounts of the basement membrane (BM) component, alpha-1-type IV collagen were increased in patients with diabetes (0.96 +/- 0.05 (non-DM, n=30) vs 1.35 +/- 0.09 (T2DM, n=30), t-test: p= 0.00015, Benjamini-Hochberg correction: p=0.02), as was other BM-components, i.e. laminins and nidogen. The expression of type IV collagen, laminin and other altered proteins were significantly lower among metformin users, compared to patients not treated with metformin (alpha-1-type IV collagen: 1.63 +/- 0.1 (no metformin treatment, n=14)) vs 1.17 +/- 0.10 (metformin treated, n=16) (arb.units)), p=0.013). Patients treated with or without metformin had similar levels of HbA1c, cholesterol and blood pressure.
Accumulation of basement membrane proteins as part of the arteriopathy of type 2 diabetes link the diabetic macro- and micro-angiopathy and provides a molecular substrate for altered functions of the arteries in diabetes, as for example dysfunctional remodeling. Reduced amounts of basement membrane components in metformin treated individuals, despite similar glycemic control, are compatible with the hypothesis that metformin influence the vasculature.

### P5.3
### INFLUENCE OF DIABETES MELLITUS ON ARTERIAL STIFFNESS PARAMETERS, RESPECTIVELY ON CENTRAL SYSTOLIC BLOOD PRESSURE - A MATTER OF SEX?

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Diabetes represents an important cardiovascular risk factor, arterial stiffness being responsible, partially at least, for cardiovascular disease initiation. Objective to evaluate the arterial stiffness changes in diabetic patients, identifying differences between two sexes. Design and method The study included 285 patients, (67% women), with a mean age of 59.27±11.05 years. 69 (42 women) patients (24.2%) were diabetics. All patients underwent biochemical and arterial determinations. Arterial parameters (brachial augmentation index -Aixb-, aortic augmentation index -AixAo-, pulse wave velocity -PWVao-, central systolic blood pressure -SBPAo-, aortic pulse pressure-PPAo-) were determined using the TensioMed™Arteriograph.Results Significant differences were found between diabetic patients vs non diabetic patients regarding PPAo (125.6±44.1 mm Hg vs 97.93±55.3 mm Hg, p=0.017), SBPAo (140.01± 46.05 mm Hg vs 107.37±60.48 mm Hg, p=0.003), but no significant differences were registered regarding Aixb, AixAo, PWVao. In women, significant differences between diabetic vs non-diabetic patients were registered regarding SBPAo (142.1 ± 50.2 mm Hg vs 107.1 ± 61.8 mm Hg, p=0.034) and PWVAo (10.45±1.72 vs 10.07±2.57 m/s , p=0.043). Diabetic men presented significantly greater values for PPAo (121.17±35.67 mm Hg, p= 0.035 ), SBPao (136.27±38.62 mm Hg vs 107.83±57.74 mm Hg, p= 0.034), but not for aortic and brachial augumentation index, nor for PWVao. Conclusion Despite the fact that all parameters quantify aortic and brachial stiffness, the diabetes seems to affect differently men and women, further studies being necessary to elucidate all the involved mechanism. Paper published under the frame of European Social Found, Human Resources Development Operational Programme 2007-2013, project no. POSDRU/159/1.5/S/138776

### P5.4
### IDENTIFICATION OF FACTORS THAT INFLUENCE AORTIC AND BRACHIAL STIFFNESS

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Knowing the predictive value of arterial stiffness for cardiovascular events, it is mandatory to identify the factors responsible for the increase in arterial stiffness.
**Objective:** Identification of factors that influence arterial stiffness, i.e. brachial and aortic augmentation index (AixAo), pulse wave velocity (PWVao), respectively central systolic blood pressure (SBPAo).
**Design and method:** The study included 285 patients with a mean age of 59.27±11.05 years. All patients underwent anthropometric measurements, biochemical and arterial determinations. Arterial parameters were determined using the TensioMed™Arteriograph.
**Results:** 74% of patients were hypertensive, 24.2% diabetics, 40% obese, 16.8% smokers, 68.1% with dyslipidaemia, 70.9% with metabolic syndrome, 37.8% with cardiovascular diseases. A significant correlation ($p<0.05$) was found between brachial augmentation index and age (r=0.375), weight (r= - 0.427), abdominal circumference (r= - 0.286), systolic blood pressure (r=0.359), HDL-cholesterol (r=0.352). In addition aortic augmentation index correlated with diastolic blood pressure (r=0.173). Pulse wave velocity significantly correlated with age (r=0.266), systolic blood pressure (r=0.376), diastolic blood pressure (r=0.168), triglycerides (r=0.192). A relationship was found between SBPAo and age (r=0.155), systolic blood pressure (r=0.423), diastolic blood pressure (r=0.390), glycemia (r=0.155). All arterial stiffness parameters correlated with SBPAo (for AixAo r=0.405, for Aixb r=0.291, for PWVao r=0.214).
**Conclusion:** Despite the fact that all parameters quantify aortic and brachial stiffness, they seems to be different influence by anthropometric and biochemical parameters.
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### P5.5
### IN VIVO ASSESSMENT OF REGULATORY MECHANISM OF THE SYSTEMIC ARTERIAL AND VENOUS SYSTEM FOR THE PREVENTION OF ORTHOSTATIC INTOLERANCE

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To reduce the risk of post-flight orthostatic intolerance for astronauts, a better understanding of the response of the cardiovascular system to changes in hydrostatic pressure is essential. The objective of this study is to assess the regulatory mechanisms of the arterial and venous system in response to acute changes in hydrostatic pressure.
The experimental protocol applied to twelve volunteers consisted of: 3x3min 70-degree head-up tilt (HUT) and 3x3min 45-mmHg Lower Body Negative Pressure (LBNP). Blood Pressure, 4-segment electrical impedance (thorax, splanchnic, upper leg, and lower leg-$Z_{leg}$) and Femoral artery blood flow (FABF) was assessed.
FABF responses were characterized by a half-time decay constant, $\tau$, of 6.10±0.34s and a volume increase of 27.2±3.4mL. $Z_{leg}$ after tilting and LBNP onset decreases faster with HUT ($\tau$=6.9±0.7s LBNP and 2.7±0.7s HUT, $p<.0001$), whereas upon tilting-back and removal of LBNP no differences were obtained ($\tau$=3.3±0.7s LBNP and 2.1±0.7s HUT).
We can assume that the extra FABF volume and dynamics is mainly related to a vasoconstriction sympathetic reaction independent of the stress conditions. The faster decrease in $Z_{leg}$ for HUT indicates a faster fluid shift in the lower leg than with LBNP, whereas the similar time course upon tilt-back and removal of LBNP may mainly be related to the compliance of the tissues in the lower limb, and thus independent of the stress conditions. With this study, we have shown that LBNP onset induces a delayed blood fluid shift compared to HUT whereas fluid emptying on removal coincided with lower limb tissue properties.