Cardiovascular Research Oral Presentation
Senior Scientists' Forum

The role of sex specific stress response in heart failure

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Background*:* The stress response is an expensive multi-system effort of the organism to maintain homeostasis. The sympathetic surge of norepinephrine and the adrenergic surge of epinephrine converge on the adrenergic receptors of target organs. Any overwhelming excitation may lead to various changes in the receptor expression: down-regulation, desensitization, internalization, phosphorylation and relocation. The initial changes are adaptive and reversible, but chronic stress leads to maladaptive remodeling and a permanent shift in metabolic regulation.
Objectives*:* Human and animal studies of stress response demonstrate a sex specific difference in central and peripheral reaction. We summarized the findings from the existing studies connecting stress and heart failure.
Results*:* In both sexes stress is linked to the development of obesity, diabetes and heart failure, but the progression of symptoms is delayed in women and connected with reproductive hormones. A direct link between stress and heart dysfunction is established in the case of Takotsubo cardiomyopathy. To date the development of cardiovascular disease has been associated with the following psychosocial risk factors: depression (more frequent in women), anxiety (more frequent in men), character traits (D personality), chronic life stress (from work stress up to PTSD) and social isolation (low social support). The major adverse outcomes of chronically activated hypothalamic-pituitary-adrenal axis are arterial hypertension, atherogenic effect, metabolic changes and increase in blood coagulation diathesis. Individual differences in the development of cardio vascular disease include: genetic background (race, ethnicity), lifestyle habits, sex and social status. The psychosocial stressors studied in primates require a long time of verification, complex design and relatively small groups of animals. It is hard to replicate the typical psychosocial context on the rodent model.
Conclusion*:* The animal studies of pathological changes triggered by stress continue to suffer from a lack of a reliable stress marker, divergent findings in time-frame required for the development of measurable symptoms and a strict distinction between sex specific ageing and sex specific pathology.

The role of connexin 37 gene polymorphism (1019C>T; Pro319Ser) in cardiovascular disease.

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In spite of the strong prognostic value of all traditional cardiovascular risk factors, still striking differences exist in the prevalence of clinical events between patients at apparently similar risk. One of the main reasons is different genetic background. One of recently discussed candidate genes for cardiovascular disease is the gene for the protein Connexin 37 (Cx37). This protein is a part of gap junctions responsible for communications between cells including cells in the vessel wall. Studies focused on the association between Cx37 gene polymorphism (1019C>T; Pro319Ser) and cardiovascular disease demonstrates inconsistent results. Our findings in 1,316 men and women indicated that the Cx37 gene polymorphism (genotype CC) is significantly associated with acute coronary syndrome in non-smoking women. In addition, in urban and rural women from general population (n=1,056) with impaired fasting glycaemia the same genotype is associated with increased intima media thickness of carotid arteries measured by ultrasound. Finally, in 289 women with diabetes type 1 or 2, and in 208 women from general population with central obesity, the CC genotype was associated with lower ankle brachial blood pressure index. These data indicate that Cx37 gene polymorphism could have gender- and smoking- dependent effects on acute coronary events and glucose dependent effect on atherosclerosis in women.

Cardiovascular Research Oral Presentation
Young Scientists' Forum

Comparison of cardiovascular risk factors in women and men patients with ischaemic heart disease – a retrospective study

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Background. Ischemic heart disease is a leading cause of death among women worldwide, responsible approximately for one third of all cause mortality. In the developed countries more women than men die because of IHD. Especially young women (below age of 55 years) are at high risk, since compared to men at similar age they are characterized by a worse outcome.

Objectives. The aim of the present study was to identify possible gender specific cardiovascular risk factors in patients with ischemic heart disease. The study population consisted of patients hospitalized with a diagnosis of ischemic heart disease, undergoing diagnostic coronary angiography at the Department of Cardiology at the University of Debrecen, MHSC in January 2007. The presence of regular cardiovascular risk factors (age, positive family history, hypertension, diabetes mellitus, body weight, hyperlipidemia, smoking) was documented. Relevant laboratory parameters on admission (blood sugar level, glomerular filtration rate (GFR), total cholesterol, LDL-C, HDL-C, triglyceride, hemoglobin (Hob)) were also analyzed and compared between women and men patients.

Results. Average age of women and men patients was comparable (60.6±0.9 vs. 58.7±0.7 years, $p=0.12$), additionally in both patient groups one third of the patients was younger than 55 years. Based on the documented risk factors more women than men suffered from hypertension (82% vs.60%), type 2 diabetes mellitus (32% vs.17%) and were obese (43% vs.26%). However, about twice as many smokers were observed among men than women patients (18% vs.10%). Analysis of laboratory parameters revealed a significantly higher HDL-C (1.59±0.05 vs. 1.32±0.03 moll/l, $p<0.0001$), but a significantly lower GFR (89.4±1.3 vs. 96.4±1.4 ml/min, $p=0.0038$) and Hob (131±1 vs. 144±1 g/l, $p<0.0001$) levels in women. Interestingly, GFR showed a significant decline with age in women ($r=0.66$, $p<0.001$), but not in men patients ($r=0.0016$, $p=0.98$).

Conclusions. Based on the preliminary results of our study differences can be observed between the distribution of cardiovascular risk factors in women and men patients with ischemic heart disease. Our study also emphasizes the crucial importance of the careful patient documentation in the clinical care. This data acquisition was associated with a retrospective study initiated by the Regional Cooperation for Health, Science and Technology (RECOOP HST) Consortium's Women's Health and Cardiovascular Diseases in Central and Eastern Europe Network.

Genetic effects on lung function and their associations with arterial stiffness. Assumption or real link?

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Objective: Central blood pressure (SBP_{ao}), peripherial and aortic pulse pressure (PP, PP_{ao}), the difference between systolic and diastolic blood pressure in the ascending aorta have consistently been shown to be a more powerful predictor of cardiovascular events than traditional cuff blood pressure measurements taken at the arm. Twin studies by comparing identical with non-identical twins produce information on the relative contribution of genes and environment, and how the two interact. **Purpose**: To estimate heritability and environmental effects on SBP_{ao}, PP and PP_{ao} and their correlations with arterial stiffness (augmentation index on brachial artery, Aix_{bra}, augmentation index on aorta, Aix_{ao}, Pulse Wave Velocity on aorta, PWV_{ao}) using a twin sample. **Methods and Materials**: 230 monozygotic (MZ) and 159 dizygotic (DZ) (Italian, Hungarian and American) twin pairs were included in the study as part of International twin study 2009. TensioMed Arteriograph was used to measure all parameters. Aix, SBP_{ao} and PWV_{ao}, measured oscillometrically, showed strong correlation with the invasively obtained values[1]. Statistical analysis was conducted using MPlus Version6. **Results**: Age, sex and country-adjusted heritability of SBP_{ao}, PP and PP_{ao} indicated 45.5% (95% CI, 10.5 to 60.0%), 46.6% (95% CI, 29.8 to 58.0%), and 39.9% (95% CI, 1.4 to 53.9%). Unshared environmental effects accounted for the largest part of variance, respectively (*Table 1*). Model fit was normal. Bivariate saturated model showed high and significant correlations between SBP_{ao}, PP_{ao} and arterial stiffness measures. Non-significant correlations were estimated for PP and Aix (*Table 2*).

Table 1. Parameter Estimates and 95% CIs of the Best-Fitting Univariate Models

Measure	h^2	95% CI	c^2	95% CI	e^2	95% CI	Model fit (p)
SBP_{ao}, mm Hg	0.455	0.105-0.600	0.078	0.000-0.388	0.467	0.382-0.546	0.1464
PP, mm Hg	0.466	0.298-0.580	0.000	0.000-0.027	0.534	0.432-0.625	0.2569
PP_{ao}, mm Hg	0.399	0.014-0.539	0.050	0.000-0.385	0.551	0.447-0.661	0.4399

h^2 indicates heritability; c^2, shared environmental variance component; e^2, unique environmental variance component; and Model fit^2, Chi-square test of Model fit (p value)

Table 2. Correlation (r) parameters and significance (p) values

Correlation values	Aix_{bra}, %	Aix_{ao}, %	PWV_{ao}, m/s
SBP_{ao}, mm Hg	0.588 ($p<0.001$)	0.587 ($p<0.001$)	0.475 ($p<0.001$)
PP, mm Hg	-0.077 ($p=0.057$)	-0.078 ($p=0.055$)	0.083 ($p<0.05$)
PP_{ao}, mm Hg	0.582 ($p<0.001$)	0.581 ($p<0.001$)	0.456 ($p<0.001$)

Conclusions: SBP_{ao}, PP and PP_{ao} are moderately heritable. Unshared environmental effects account for the largest portion of the variance. High significant correlations were estimated between atherosclerosis (arterial stiffness) and SBP_{ao} and PP_{ao} suggesting a genetic background of these correlations. The measurement of central blood pressure and aortic pulse pressure may be the next important advancement in the management of hypertension and increased arterial stiffness.

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The analysis of hepatic transcriptome in Prague Hereditary Hypercholesterolemic (PHHC) rat – an experimental model of hypercholesterolemia

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Most of the hypercholesterolemic patients have hypercholesterolemia of polygenic origin and genes involved have not been well characterized yet. Unfortunately, there are almost no animal models that could be used for study of polygenic hypercholesterolemia. Prague Hereditary Hypercholesterolemic (PHHC) rat model may be an exception – PHHC rats develop polygenic hypercholesterolemia after dietary cholesterol.

Objectives*:* To characterize pathogenesis of hypercholesterolemia and identify genes involved in development of hypercholesterolemia in PHHC rat.

Methods*:* Firstly, nascent VLDL of PHHC and Wistar rats were isolated from serum obtained 2 hours after intravenous application of tyloxapol and characterized. In another experiment, serum VLDL was isolated from both PHHC and Wistar rats, labeled with ^{125}I and the rate of VLDL disappearance was determined after their application into Wistar rats. Secondly, male PHHC and parental Wistar rats were fed chow (C) or 1% cholesterol (CHOL) diet for three weeks. Hepatic transcriptome analysis was evaluated using Affymetrix GeneChip arrays. The gene expression of selected genes was validated by qPCR.

Results*:* On CHOL diet, cholesterol and triglycerides accumulated in the liver of both PHHC and Wistar rats; cholesterolemia rose significantly only in PHHC rats. Nascent VLDL of PHHC rats carry twice as much cholesterol than VLDL of Wistar rats on CHOL diet and VLDL of PHHC rats are catabolized more slowly than those of Wistar rats when injected in vivo. Nevertheless, the gene expression of both strains responded to CHOL diet exactly in the same way - genes of cholesterol synthetic pathway were down regulated. On the other hand, several genes were found to be differently expressed between both strains independently of the diet (*Aldh1a7*, *Yc2*, *Apof*, *Ugt2b*, *Cdh17*, *Ltc4s*, etc.). *Apof* as the only gene directly involved in lipoprotein metabolism was then sequenced and the homozygous 17bp insertion was identified in the coding region in all PHHC rats. This insertion was found also in Wistar rats with allelic frequency only 12 %.

Conclusions: PHHC rats secrete cholesterol-enriched VLDL from the liver and these VLDL are catabolized more slowly. This can explain the accumulation of cholesterol in serum of PHHC rats. The analysis of transcriptome revealed several new candidate genes for hypercholesterolemia in our experimental model. Their exact role in pathogenesis remains to be determined.

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Cardiovascular Research Network Meeting Oral Presentation

Genetic Determinants of Cardiovascular Disease and its Risk Factors

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Background: Cardiovascular diseases (CVD) are the leading cause of death among non-communicable diseases. A cross-sectional study of 2403 randomly selected subjects was conducted to assess the relationship between several allelic variants of the genes previously associated with the disease risk and CVD related quantitative traits.
Objectives: Variants of the Apo lipoprotein E (ApoE; rs429358 and rs7412), methylenetetrahydrofolate reductase (NAD(P)H) (MTHFR; rs1801133), methylenetetrahydrofolate dehydrogenase (NADP+ dependent) 1-like (MTHFD1L; rs6922269), chromosome 9p21.3 polymorphism (rs1333049), ATG16 autophagy related 16-like 1 (ATG16L1; rs2241880) and ATP-binding cassette, sub-family G (WHITE), member 5 (ABCG8; rs11887534) were analyzed in relation to CVD related quantitative risk factors. Studied variables were: body mass index, waist circumference, blood pressure, serum total cholesterol (TC), low density lipoprotein-cholesterol (LDL-C), triglycerides (Tg), high density lipoprotein-cholesterol (HDL-C), Non-HDL cholesterol (non HDL-C), Total to HDL cholesterol ratio (TC/HDL) and Glucose levels. Together with positive personal and family anamnesis (case history) they were used for determination of the classic CVD risk factors: obesity, dyslipidemia, hypertension, diabetes, metabolic syndrome, smoking habit, and positive personal and family history.
Results: Variant rs429358 of the ApoE gene was strongly associated with elevated serum lipid levels (TC P=0.000; LDL cholesterol P=0.000; triglycerides P=0.007), lowered HDL-C levels (P=0.02) and higher nonHDL-C (P=0.000) and TC/ HDL-C ratio (P=0.000). Rs7412 variant of the ApoE gene showed opposite effect on the serum lipids (TC and LDL-C levels (P=0.000), HDL-C (P=0-048) also on nonHDL-C (P=0.000) and TC/ HDL-C ratio (P=0.000). Higher frequency of variant allele rs429358 (P=0.001) and lower frequency of the rs7412 (P=0.000) of the ApoE gene was found in the group of dyslipidemic subjects. Variant rs2241880 of the ATG16L1 gene was associated with higher BMI values (P=0.035) and waist circumference (0.02) in subjects with wild type allele, who had lowest HDL-C and higher TC/HDL ratio (P<0.001). Similar results were found for carriers of the rs11887534 variant of the ABCG8 gene. Wild type homozygotes had elevated LDL-C (P=0.038) as well as both non HDL-C (P=0.014) and TC/HDL ratio (P<0.009). None of the other variants was associated at p< 0.05 with the quantitative traits tested here.
Conclusion: To summarize, we have identified four variants: rs429358 and rs7412 of the ApoE, rs2241880 of the ATG16L1 and rs11887534 variant of the ABCG8 gene that may affect risk of CVD and/or associate with quantitative CVD-risk factors.

Age related changes in rat cardiac spinal afferent neurons

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Background: Dorsal root ganglion (DRG) is a structure that contains ~90% of somatic afferent neurons and only 5-10% of visceral afferent neurons. Within visceral afferent neurons is a subpopulation responsible for the heart innervations, known as cardiac spinal afferent neurons. Like many other cell types, the neurons constituting DRG are susceptible to changes related to ageing. Previous studies in rat have shown that the process of ageing does not affect the whole population of DRG neurons equally. In addition, there was no change in proportion of isolectin B_4 (IB_4)-positive (likely unmyelinated) and neurofilament 200 (N52)-positive (likely myelinated) neurons, while the total number of neurons is slightly decreased or remains unchanged. However, to the best of our knowledge, there are no studies about age-related changes of cardiac spinal afferent neurons.
Objectives: The aim of this study was to investigate the effect of ageing on cardiac spinal afferent neurons in the rat.
Materials and Methods: A patch loaded with retrograde tracer Fast Blue (FB) was applied to all chambers of the rat heart. Morphological and neurochemical characteristics of labeled cardiac spinal afferent neurons were assessed in young (2 months) and old (2 years) rats using IB_4 and N52.
Results: Our study shows that the number of cardiac spinal afferent neurons decreased with age and it was reduced to 15% of the total number of neurons found in young rats (1604 vs. 248). In addition, we have observed a difference in myelinated and unmyelinated neurons rate. The number of IB_4-positive neurons increased significantly, whereas the proportion of N52-positive ones decreased significantly during the ageing process. The size of neuronal soma of IB_4-positive neurons increased, while the size of N52-positive neurons remained unchanged.
Conclusion: Unlike somatic spinal afferents, cardiac spinal afferent neurons underwent morphological and neurochemical changes during ageing process. Although there is a major decrease in total number of cardiac spinal afferent neurons, the innervation density and nociceptive capacity are preserved due to both, an increased proportion and compensatory growth of IB_4-positive neurons.

Gender specifics of off-pump coronary bypass grafting

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The **aim** of this work was to examine and compare the effect of gender on beating heart CABG operations results, using compression type stabilizer.
Materials and Methods. In this study we used the database of the National M.M.Amosov Institute of Cardiovascular Surgery. The study population consisted of 5113 patients undergoing isolated CABG between 2000 and 2009; 631 were female and 4482 were male. The patients were compared by gender, age, height, weight, ejection fraction, amount of vessel disease and the usage of IMA. Amongst the 5113 surgeries conducted, 4899 (95.8%) are done by the off-pump technique using compression type stabilizer and only 214 (4,2%) are done by the on-pump technology mainly due to the severity of the patients state. Amongst the on-pump CABG surgery patients, 87.1% were men and 12.9% were women.
Results. Women tend to have more problems due to age, stature and different vessel effect. Stature and age tend to be the worse predictors for CABG. The comparison of male and female patients undergoing CABG shows that there were certain differences in male and female patients. Women were older, of shorter stature, had higher ejection fraction and had less one-vessel diseases. The most frequently grafted artery for both groups (nearly 31 percent for both sex at all age) was the anterior interventricular artery. In women on early stages (40-60 years old) the distal anastomosis is more frequently conducted on artery marginalis (25.5% of all distal anastomosis), but in late stage (more then 70 years old) in women the distal anastomosis is more frequently conducted on the posterior interventricular artery (19.6% of all distal anastomosis). While the distal anastomosis is more frequently conducted on artery marginalis (24.5% of all distal anastomosis) for men in late stages (more then 70 years old), mortality between 2000-2009 was 0.6% for men and 0.63% for women. Regarding off-pump technique, there was no difference in mortality amongst patients off different sex. The main causes of death are preoperative myocardial infarction and stroke.
Conclusion. The usage of beating heart technology using compression type stabilizer allowed us to eliminate the influence of gender on coronary artery bypass grafting results.

Cardiovascular Research Young Scientists' Poster Presentation

SCLO1B1 transporter and statin treatment efficacy

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Introduction: Statins belong to drugs of first choice in patients with increased cardiovascular risk. There exists a significant inter-individual variability in statin treatment efficacy that is likely to have a strong genetic background. Gene for SCLO1B1 belongs to the candidates with potential to influence the statin treatment efficacy. SCLO1B1 codes for the solute carrier organic anion transporter **(**which has been shown to regulate the hepatic uptake of statins and some other drugs).
Materials and methods: SCLO1B1 rs4149056 (T>C) polymorphism was analyzed on heterogeneous group of 310 (141 males) patients with dyslipidemia treated by statins (~90% on simvastin or atorvastatin, 10 or 20 mg per day). Polymorphism was analyzed using PCR and restriction analysis. Lipid values (total-, LDL- and HDL- cholesterol, triglycerides) were analyzed before the treatment and after 8 - 12 weeks of treatment.
Results: After treatment, there was a significant decrease both in total (7.4±1.3 → 5.4±1.0 mmol/L, $P < 0.0001$) and LDL-cholesterol (4.7±1.2 → 3.2±0.9 mmol/L, $P < 0.0005$). Distribution of the individual genotypes in Czech patients (TT = 62%, CT = 32%, CC = 6%) was similar to the distributions found previously in west European populations. Decrease of both total cholesterol (29% vs. 24%) and LDL cholesterol (34% vs. 30%) was non - significantly higher in the carriers of the CC genotype in comparison with the commonest TT homozygotes. Changes in values of the HDL - cholesterol and triglycerides were not associated with analyzed polymorphism.
Conclusions: Results of the pilot study suggests that the rs4149056 variant within the gene for SCLO1B1 transporter could be one of the genetic determinants of statin treatment efficacy in Czech patients with dyslipidemia. Because of the low frequency of the potentially advantageous genotype, it is necessary to analyze larger group of patients
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Adrenergic receptors upregulation in ovariectomized rats

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Stress is gender specific response aimed to preserve physiological homeostasis. Although it is whole organism reaction, the stimulation originates from two sides: hypothalamic-pituitary-adrenal axis (HPA) and sympathetic-adrenal medullary system. Both sides, either through norepinephrine or epinephrine, converge on Beta-1 adrenergic receptor, which is G-protein coupled membrane receptor. Activation of the heart Beta-1 receptors cause increased cardiac output by rising heart rate, increasing impulse conduction and ejection fraction – all required in stress response, but inadequate in the state without real jeopardy.

Three-month-old rats were ovariectomized and kept for a month in the unstressful environment. Animals were sacrificed and compared with age-matched male and female controls. Sampled heart, hypothalamic and fat tissue were immunoprobed for Beta-1 and Beta-3 receptors. The heart tissue of ovariectomized animals had highest concentration of Beta-1 receptors.

The Beta-1 receptor upregulation in a condition of ovarian hormone deprivation could have serious consequence on cardiac function, particularly in stress response. We assume that heart becomes more sensitive to levels of circulating norepinephrine and epinephrine, overreacting even on mild provocation. Similar changes might be pathophysiological mechanism in the cardiovascular disease in human premature ovarian failure and polycystic ovary syndrome.

High salt diet is associated with impaired microvascular reactivity in young healthy female human subjects

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Studies on experimental animals have shown that changes in salt intake significantly alter vascular reactivity to different physiological stimuli, in conduit vessels, resistance arteries, and in the microcirculation. However, the effects of high salt (HS) intake on microvascular endothelial response in young healthy human subjects without pre-existing conditions such as diabetes and hypertension are still unknown.
The aim of this study was to assess effects of acute salt loading on microvascular reactivity during reactive hyperemia in young healthy women, using non-invasive Laser Doppler Flowmetry (LDF). Eleven normotensive women (21±3 years) were instructed to maintain a low-salt (LS) diet (less than 40 mmol Na/daily) during 7 days. Simultaneously they were divided into HS group (N=6) (intake of 200 mmol Na/daily) and placebo group (N=5). LDF was performed before and after salt diet protocol as measurement of relative changes in blood flow between baseline flow and reperfusion that follows 1- and 2-minute occlusion. Blood sampling for plasma electrolytes, aldosterone and plasma renin activity, just as 24-hour urine sodium and potassium excretion took place before and after diet protocol.
In the HS group there was a statistically significant decrease in micro vascular reactivity after 1-minute occlusion (endothelium-dependant mechanisms) with no difference after a 2-minute occlusion (maximum dilation ability), before and after HS diet. The increased urinary volume, decreased urinary sodium, and increased urinary potassium concentration in subjects on LS diet, and increased urinary sodium concentration in subjects on HS diet confirmed consistency of experimental protocol and subjects' adherence to diet. Although plasma renin activity and serum aldosterone concentration decreased in HS diet and increased in women on LS diet, they didn't reach statistical significance.
This study shows that even one week of HS intake may have negative effect on micro vascular reactivity, decreasing blood flow during reactive hyperemia by affecting endothelial function. Increased number of subjects in further study is needed.

Antioxidant status in the context of obesity in humans.

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Background: Obesity is multifactorial chronic disorder defined as abnormal, excessive body fat accumulation, leading to increased health problems, reduced life expectancy and discrimination. Genetic predisposition, metabolic, hormonal and mostly behavioral aspects are implied in the pathogenesis of this phenomenon, which is regarded to be global epidemic of recent time. Overweight and obesity represent major risk factors for chronic diseases such as type 2 diabetes, hypertension, cardiovascular diseases, osteoarthritis and certain cancers. Increased systemic oxidative stress in accumulated adipose tissue is one of pathogenic mechanisms of obesity-associated metabolic syndrome and cardiovascular diseases.

Objectives*:* For our purposes, 97 normal weight, 95 overweight and 94 subjects with BMI>30 were selected from cross-section study of 40 years old probands from the Slovak population. Spectrum of biochemical parameters, lipid profile, antioxidant status and other markers were analyzed in blood of all volunteers. Polymorphisms in 27 candidate genes involved in etiology of common chronic diseases and in genes for defense mechanisms were determined to assess individual genetic susceptibility.

The aim of this study was to investigate markers of antioxidant defense and oxidative stress, and seek for associations between genetic background and clinical, biochemical parameters in obese population.

Results*:* In 73% of overweight and 90% of obese subjects, systolic and diastolic blood pressure values were above recommended reference 120/80 mmHg. Individuals with BMI>30 showed increased activity of ceruloplasmin oxidase ($p<0.05$) and decreased level of cysteine ($p<0.05$). Reduced glutathione was decreased in overweight subjects too ($p<0.05$). Concentrations of retinol ($p<0.05$) and tocopherols ($p<0.05$) in plasma were elevated when BMI>25. Reversely, levels of xanthophylls ($p<0.01$) and β-carotene ($p<0.001$) were decreased in subjects with BMI>30. In all overweight and obese subjects was observed markedly decreased protection of fatty acids against peroxidation, calculated from vitamin E/triacylglycerol´s ratio ($p<0.001$).

Subjects with deletion of detoxification and antioxidant enzyme GSTT1 displayed lower activity of glutathione peroxidase in erythrocytes, compared with subject owning efficient enzyme ($p<0.05$).

Conclusions*:* Several parameters of antioxidant state were found changed in overweight and obese subjects, which indicate elevation of oxidative stress in these groups. Redox state is an early marker and could serve as potential therapeutic target for obesity-related cardiovascular disorders.

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Adenosine triphosphate concentration of exhaled breath condensate in asthma

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The pathogenesis of bronchial asthma, a common obstructive inflammatory airway disease affecting 300 million people worldwide, is not clearly understood. Adenosine triphosphate (ATP), a mediator of purinergic signaling, might play a role in disease development by triggering airway inflammation and inducing bronchoconstriction. However, supporting human evidence is scarce. ATP can be measured in exhaled breath condensate (EBC), a non-invasive airway sample generally suitable for disease monitoring. For the first time, we aimed to determine EBC ATP concentration in asthma, and to study its relation to disease parameters and calculate airway ATP level.

EBC was collected from 45 patients with persistent asthma (age 34.7 (yr) ± 13.2; FEV_1 % predicted 87.0 ± 15.5; mean ± SD) and 32 healthy controls (age 36.9 ± 12.6; FEV1 % predicted 98.9 ± 9.9). Exhaled nitric oxide and spirometry were measured; disease control was assessed with the Asthma Control Test. ATP was measured in luciferin-luciferase assay. Airway ATP concentration was calculated using dilution estimated from conductivity of vacuum-treated EBC samples. Parametric tests were applied; ATP concentrations and nitric oxide levels were logarithmically transformed (geometric mean [95% confidence interval]).

EBC ATP concentrations were similar in asthmatic patients and healthy controls (4.12 pM [3.13-5.41]
vs. 3.18 pM [2.32-4.36], p=0.21) and it was not related to lung function variables, exhaled nitric oxide level or disease control. EBC ATP concentration was correlated to airway droplet dilution in the sample ($r=-0.32$, $p<0.05$). The calculated airway ATP level was not elevated in patients compared to controls (24.79 nM [17.27-35.58] vs. 20.70 nM [13.04-32.86], p=0.52), but it was negatively related to FEV1 in study subjects ($r=-0.35$, $p<0.05$). The estimated airway ATP concentration was similar to that previously measured in airway epithelial cultures.

EBC ATP concentration might not serve as a biomarker of bronchial asthma. Nevertheless our data provide the first evidence that the assessment of respiratory droplet dilution in EBC can be reliably applied to calculate real mediator level in the airways thereby increasing the applicability of EBC technique for monitoring of the airways. Our results also demonstrate that extracellular ATP might be involved in a general mechanism regulating airway caliber.

Trend of change in gender distribution among patients with acute coronary syndrome in Slavonia and Baranja region (Croatia)

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First catheterization laboratory in Osijek region was opened in 2005 and was followed by institution of cardiosurgery during 2007. The laboratory had managed 88 myocardial infarctions with ST elevation (STEMI) by primary percutaneous coronary intervention (PCI) before the cardiosurgical treatment was available. With the surgeon in site the number of the patients has rapidly raised up to 106 primary PCIs in just two months. Most of the patients with STEMI managed with primary PCI (PPCI) before 2007 (70.7%) and during 2008 were males (80.6 %). Mean age of the male patients was 55.2 before 2007 and 58.1 years in 2008, while the mean age of the female patients was 65.8 and 59.9 years, respectively.

Looking closely the patients undergoing coronarography (48 in total) during January 2007 we found that 54% of them were males (mean age 57.5 years). Females' mean age was 59.1 years. Long lesions (≥20 mm) were found in 2 males and in 1 female patient. Interestingly, the lesions in small vessels were more frequently found in females (n=13) than in males (n=7). There were 38 coronary stents placed during the PCI (36 bare metal stents and 2 drug-eluting stents). There was only one female patient without significant coronary artery disease. Three female patients experienced artificially caused coronary artery dissection. The outcome of the coronarography was coronary artery bypass graft (CABG) surgery in 9 males and in 4 female patients. One male and one female patient underwent urgent CABG surgery. Equal number of male and female patients was treated with optimal medical therapy (4).

The total number of the patients managed by coronarography in 2007 was 492. Thereof were 182 PCI cases (102 for ongoing infarction, 69 during the diagnostic study and 3 with multivessel PCI during the one session) and 237 stents placements.

In 2009 and 2010 the catheterization laboratory managed 906 and 1056 patients, respectively, out of which 60 % were males. In 30 % of patients the diagnostic procedure was followed by PCI and percutaneous transluminal coronary angioplasty. Nevertheless, the number of urgent PCIs rose up to 96 in 2009. We observed a trend of rising number of female patients, accompanied with younger age and pathology of small vessels. This phenomenon is contrary to the predominant clinical expectations and suggests some drastic changes in gender specific epidemiology of coronary disease.

Metabolic background of heart rate variability: Evidence achieved by supplementation with amaranth oil in type 2 diabetes mellitus patients

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Heart rate variability (HRV) is a modern noninvasive tool frequently used in clinical practice to establish functional capacity and prognosis in patients with various illnesses, particularly diabetes mellitus (DM). This disorder is associated with significant reduction of HRV indexes and oxidative stress (OS) related to excessive accumulation of oxidative destruction products with depression of redox reactions in cells and tissues. There is increasing evidence about close relationships between HRV and certain metabolic parameters reflecting the depth of OS, but biochemical mechanisms of these relationships remain to be established. The aim of present research was to study the changes of HRV and some parameters of aerobic metabolism in type 2 DM patients achieved with supplementation with oil derived from the seeds of *Amaranthus cruentus L.* (AmO).

The impact of concentrated AmO (1.0 ml per 60 kg of body weight daily for one month) on the time and frequency domain HRV indexes and some parameters reflecting OS in blood (levels of TBARS, oxidative modification proteins (OMP), hydro peroxides (HP), LDL-cholesterol, middle mass molecules (MMM), activities of catalase and superoxide dismutase (SOD)) were evaluated in 36 patients with moderate and severe type 2 DM aged 51-67 years with disease duration up to 10 year. Control group consisted of 35 apparently healthy volunteers of the same age. Severe manifestations of OS were found in all DM patients despite increased activities of catalase and SOD. These biochemical changes were associated with dramatically decreased HRV. Because of wide range of total power (TP) values obtained, the patients were divided into 2 groups, with group 1 including the subjects with low (600-1000ms^2) and group 2 very low (100-600ms^2) TP indexes. Administration of AmO caused activation of aerobic metabolism with significant decrease in TBARS, HP, LDL, and OMP levels in group 1 patients. Simultaneously, significant increase in most HRV parameters, predominantly due to autonomic up regulation was observed in this group. The patients with initial low TP values (group 2) demonstrated normalization of antioxidant enzymes activities, however, no changes were observed in patients with initially high levels of TBARS, MMM, and OMP. Notably, marked decrease in HP, LDL was shown in group 2 due to better utilization of these OS markers in redox reactions, which was accompanied by improvement of HRV parameters.

In conclusion, activation of aerobic metabolism with AmO supplementation in type 2 DM patients caused more prominent increase in HRV indexes in group 1 (with initially higher TP) patients. That was associated with the improved intensity of aerobic metabolism and reduction of OS manifestations. Thus, combination of biochemical studies and HRV provides more precise assessment of functional and metabolic conditions, more adequate follow up, and control of treatment.

Genetic effects on lung function and their associations with arterial stiffness. Assumption or real link?

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Background: An association between reduced lung function and an increased risk of vascular events has been reported but the underlying mechanisms are unknown. So far no twin studies have examined the association between atherosclerosis and lung function. **Objectives**: To estimate association of arterial stiffness and lung function and to assess heritability and environmental effects on these parameters. **Subjects and Methods**: 380 (232 monozygotic /MZ/ and 148 dizygotic /DZ/) twin pairs (age yrs 50.5±15.4; mean±SD) were included in this classical twin study as part of International Twin Study 2009. Augmentation index on brachial artery (Aix_{bra}) and Pulse Wave Velocity on aorta (PWV_{ao}) were measured to reflect arterial stiffness (TensioMed Arteriograph) and forced vital capacity (FVC) with forced expiratory volume in one second (FEV1) (MIR Minispir) for lung function. Heritability and Pearson correlation coefficient (β) between variables were determined. **Results**: Age and gender-corrected heritability of FVC and FEV1 were 0.74 and 0.75 ($p<0.01$). Shared and unshared environmental effects were found to be 0, 0.26 ($p<0.01$) and 0, 0.25 ($p<0.01$) for FVC and FEV1, respectively (*Table 1*). β between FVC and PWV_{ao} were -0.42 ($p<0.001$) and -0.29 ($p<0.001$), respectively. β between FEV1 and Aix_{bra} and PWV_{ao} were found to be -0.47 ($p<0.001$) and -0.38 ($p<0.001$), respectively. Table 1. Heritability (A), common (C) and unique (E) environmental effects of FVC and FEV1 **Conclusions**: Lung function is strongly heritable and is associated with arterial stiffness. The observed relationship can aid to understand the background of vascular changes in different airway diseases.

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	A	C	E
FVC	0.74 ($p<0.01$)	0	0.26 ($p<0.01$)
FVC %predicted	0.422	0.107	0.471 ($p<0.01$)
FEV1	0.747 ($p<0.01$)	0	0.253 ($p<0.01$)
FEV1 %predicted	0.002	0.003	0.995 ($p<0.01$)