Proceedings of the 18th Annual Congress on Atherosclerosis

Organized by The Czech Society for Atherosclerosis
Špindlerův Mlýn, Czech Republic
December 11 - 13, 2014

Abstracts are presented in the alphabetical order of the first author names and are printed without editing in the submitted form. The Editorial Office of Physiological Research disclaim any responsibility for errors that may have been made in abstracts submitted by the authors.
RELATIONSHIP TYPE DENTAL FILLING, ATHEROSCLEROTIC PROCESS DUE TO THE POLYMORPHISM OF MATRIXMETALLOPROTEINASE

V. Adámková1, J. A. Hubáček1, J. Procházková2, Š. Podzimké2, L. Peterková1, J. Bělohlávková, V. Lánská1
1Institution for Clinical and Experimental Medicine, Prague, Czech Republic, 2First Medical Faculty, Charles University, Prague, Czech Republic

Dental health is considered one of the risk factors for developing of the atherosclerosis. Studies are conducted to clarify the relationship of genetic predispositions, type of dental filling and atherosclerosis. We have analysed 169 persons, both gender, aged over 18 years, monitored for response to different type of dental filling, Blood pressure, polymorphisms of matrixmetalloproteinases 13 and 20, and thickening of intima of carotic arteries have been analysed. The lab test were carried out in routine conditions, ultrasound examinations by standard conditions. Statistical method ANOVA was used. The genotype distributions of all MMP polymorphisms were in HWE. The interaction conditions. Statistical method ANOVA was used. The genotype distributions of all MMP polymorphisms were in HWE. The interaction conditions. Statistical method ANOVA was used.

Supported by Internal Grant Agency Ministry of Health, No NT 13087-3/2012.

THE CAROTIC INTIMA THICKNESS, DUE TO THE TYPE OF DENTAL FILLING AND IMMUNOLOGICAL TEST MELISA

J. Bělohlávková1, J. Procházková2, Š. Podzimké2, L. Peterková1, V. Lánská1, V. Adámková1
1Institution for Clinical and Experimental Medicine, Prague, Czech Republic, 2First Medical Faculty, Charles University, Prague, Czech Republic

Many reports are focused on dental health and carotic intima thickness, due to the type of metal in dental fillings. No clear answer for the role of dental health and development of the atherosclerotic process was performed, yet. The patients (n=103) both gender, aged over 18 years, monitored for response to different type of dental filling and MMP variants. In conclusion, the higher value of blood pressure and systolic or diastolic was in the individulas with MMP20 x MMP13 and dental filling with Al2(SO4)3, CH3COOAg but not SnCl2. Dental health seems to have some effect on development of atherosclerosis risk factors.

Supported by Internal Grant Agency Ministry of Health, No NT 13087-3/2012.

LONG-TERM EXPERIENCE WITH SELECTED LDL-CHOLESTEROL ELIMINATION METHODS

M. Bláha1, M. Lánská2, V. Bláha2, L. Sobotka2, P. Žák1
1Fourth Department of Internal Medicine – Hematology, Charles University, Medical Faculty and Teaching Hospital, Hradec Králové, Czech Republic, 2Third Department of Internal Medicine – Geriatrie and Metabolism, Charles University, Medical Faculty and Teaching Hospital, Hradec Králové, Czech Republic

VLDL-apheresis is necessary in more than 1 % of patients (pts) with familial hypercholesterolemia (FH) not responding or not tolerating to conservative treatment or in homozygous FH. We use two methods of LDL-apheresis: immunoadsorption (IA) and rheopheresis (RHF), 14 patients were long-term followed (aged 28-70 years). 10 patients are treated with immunoadsorption (5 homozygous and 5 heterozygous) and 4 patients with RHF. Median follow-up: 8.5 years. Average cholesterol and LDL-cholesterol values before procedure were 5.34 and 3.12 mmol/l in immunoadsorption, 5.07 and 2.86 in rheopheresis; after the procedure 1.73 and 0.72 (72 % and 85 % drop), resp. 1.96 and 0.97 mmol/l (61 % a 66 % drop). Fibrinogen dropped 22 % (3.05 to 2.42 g/l) and 64 % (from 3.48 to 1.2 g/l). Adverse events: 3.1 %, no difference was observed between two methods. In conclusion, treatment was very effective with a a significant decrease in patogenetically important parameters. Both methods are safe with minimum adverse reactions. Immunoadsorption is more effective in CH elimination. Rheopheresis can be used in patients with hyperfibrinogenemia. Care of these patients is costly and requires experienced team and interdisciplininary approach.

Supported by the grants PRVOUK P37/4.12 and IGA MH CZ NT/12287-5, NT/14035-3.

THE ROLE OF FETUIN-A IN ASSOCIATION TO THE ENDOVASCULAR THERAPY OF SYMPTOMATIC DEGENERATIVE AORTIC VALVE STENOSIS IN DIABETIC PATIENTS

V. Bláha1, J. Bis2, J. Šráske2, J. Fortunato1, C. Andryš1, J. Vojáček1, P. Polanský1, M. Brtko1, L. Sobotka1
1Third Department of Internal Medicine, Metabolism and Gerontology, 2First Department of Internal Medicine, Cardiology, 1Department of Immunology and Allergology, 3Department of Cardio surgery, University Hospital Hradec Králové, Medical Faculty, Charles University, Czech Republic

The aim of the study was to evaluate the role of fetuin-A as an calcification inhibitory glycoprotein in diabetic and non-diabetic patients with an advanced atherosclerosis in association to the endovascular therapy of non-rheumatic aortic stenosis (AS). Patients with an advanced atherosclerosis and non-rheumatic AS, both with diabetes mellitas type 2 (T2DM) (n=21, age 79.2±1.6 years) and non-T2DM (n=23, age 84.4±0.7 years) were studied. Patients were treated by endovascular therapy by TAVI (transcatheter aortic valve implantation, n=36) or balloon angioplasty (BA, n=8). Fetuin-A in serum was analyzed by ELISA (Human Fetuin-A ELISA, BioVendor, CR) before and 72 h after treatment. Fetuin-A in serum both before and 72 h after treatment was significantly increased in T2DM and non-T2DM versus control group (P=0.05). There were not any significant differences among the T2DM and non-T2DM groups in respect to the serum fetuin-A. The serum fetuin-A was decreased 72 h after endovascular procedure; however the results were not statistically significant both in the T2DM, nor in non-T2DM patients. In conclusion, increased serum fetuin-A in geriatric patients with an atherosclerotic non-rheumatic stenosis of aortic valve does not depend on the presence of diabetes and that circulatory fetuin-A might act as an important inhibitor of dystrophic calcification in this group of patients.

Supported by projects IGA MZ CR NT/12287-5 and PRVOUK P37/12.

CIRCADIAN ACTIVITY OF CHOLESTEROL 7A-HYDROXYLASE IS DETERMINED BY -203A/C POLYMORPHISM OF CYP7A1 GENE

T. Blahová1, M. Vlachova1, M. Leniček1, V. Lánská1, L. Viték1, J. Kovář1
1Laboratory for Atherosclerosis Research and 2Department of Statistics, 1Institute for Clinical and Experimental Medicine, Prague, Czech Republic, 2Institute of Medical Biochemistry and Laboratory Diagnostics, Charles University, First Faculty of Medicine, Prague, Czech Republic

The cholesterol 7α-hydroxylase (CYP7A1) activity displays a considerable diurnal variation. To determine whether -203A/C
polymorphism of CYP7A1 gene is involved in circadian regulation of CYP7A1 activity, we analyzed the changes in CYP7A1 activity during the day. The three experiments lasting 15 hours were carried out in 16 healthy male volunteers, 8 homozygous for -203A and 8 homozygous for -203C variant. First of these experiments was carried out after one day treatment with bile acid sequestrant (Questran®), the second after one day treatment with chenodeoxycholic acid (Chenofalk®) and the third one without any treatment (control). The concentration of 7α-hydroxy-4-cholesten-3-one (C4), a serum marker of CYP7A1 activity, was measured from 7AM to 10 PM in 90min intervals. The treatment with bile acid sequestrant resulted in fourfold and eightfold increase of CYP7A1 activity during the day in A and C homozygotes, respectively. The treatment with chenodeoxycholic acid resulted in a pronounced decrease in CYP7A1 activity in carriers of both variants. Importantly, in control experiment, the homozygous carriers of -203A allele manifested a noticeable peak of an enzyme activity around 1PM whereas no such peak could be observed in -203C allele carriers. In conclusion, -203A/C polymorphism of CYP7A1 has a substantial impact on diurnal variation of enzyme activity. The mechanism behind such an effect remains to be determined.

Supported by grant No. NT 13151-4/2012 from IGA MH CR and by MH CZ – DRO („Institute for Clinical and Experimental Medicine – IKEM, IN 00023001“).

PRIORITIES IN PRIMARY AND SECONDARY CARDIOVASCULAR PREVENTION
J. Bruthans1, J. Critchley2
1Center for Cardiovascular Prevention, First Faculty of Medicine, Charles University, Prague, Czech Republic, 2Division of Public Health, University of Liverpool, United Kingdom

The decrease of cardiovascular (CV) mortality, better control of some, and increasing prevalence of other CV risk factors and progress in therapies are changing the importance and priorities of CV prevention. The opinion of specialists and personalities with executive and legislative powers on priorities of CV prevention and treatments is of particular significance. A questionnaire on various aspects of CV prevention was sent to personalities involved in CV prevention and public health in 9 European countries. The addressees were asked to assign priority scales to particular aspects of CV prevention. Here, Czech data are presented. 30 personalities (20 specialists and 10 “politicians”) were approached, 20 of them responded. Top priority was assigned to support of physical activities (90 % of responders), legislation and fiscal policies relating to food (90 %), cooperation with the food industry (80 %), cardiovascular rehabilitation (75 %), smoking cessation initiatives and proper use of drugs in secondary prevention (70 % each), and to legislation and fiscal policies relating to smoking (65 %). The polypill and psychological counselling received less support (25 %). The policy makers were more sceptical about legislation and fiscal policies than experts. In conclusion, experts and policy makers prefer specified, target-oriented preventive measures and increasing prevalence of other CV risk factors and progress in therapies are changing the importance and priorities of CV prevention. The opinion of specialists and personalities with executive and legislative powers on priorities of CV prevention and treatments is of particular significance. A questionnaire on various aspects of CV prevention was sent to personalities involved in CV prevention and public health in 9 European countries. The addressees were asked to assign priority scales to particular aspects of CV prevention. Here, Czech data are presented. 30 personalities (20 specialists and 10 “politicians”) were approached, 20 of them responded. Top priority was assigned to support of physical activities (90 % of responders), legislation and fiscal policies relating to food (90 %), cooperation with the food industry (80 %), cardiovascular rehabilitation (75 %), smoking cessation initiatives and proper use of drugs in secondary prevention (70 % each), and to legislation and fiscal policies relating to smoking (65 %). The polypill and psychological counselling received less support (25 %). The policy makers were more sceptical about legislation and fiscal policies than experts. In conclusion, experts and policy makers prefer specified, target-oriented preventive measures supported by legislation, government, and communities to ill-defined “general” preventive programmes. Primary prevention is considered to be more important than secondary prevention.

This study was partly supported by IGA, Ministry of Health, Czech Republic, grant No NT/131366, and an EU grant (EuroHeart II Project).

THE EFFECT OF AMARANTH FLOUR ON PLASMA CHOLESTEROL LEVELS: ANOTHER OPTION FOR CARDIOVASCULAR RISK LOWERING?
Z. Chmelík, M. Vrablík
Third Department of Internal Medicine – Department of Endocrinology and Metabolism, First Faculty of Medicine, Charles University in Prague and General University Hospital in Prague, Czech Republic

The aim of this work was to summarize data related to cholesterol lowering effect of amaranth flour from two pilot studies and to discuss it’s potential to become another option for plasma lipids modification and, thus, cardiovascular disease (CVD) risk reduction in humans. The hypothesis the amaranth flour would have a positive impact on plasma lipid profile in mice with dietary induced hypercholesterolemia was tested. We found that addition of amaranth flour into the feed mixture prevented diet-induced increase in total and LDL-cholesterol levels. In conclusion, our experiments confirmed preventive effect of amaranth flour on the diet-induced increase of total and LDL cholesterol. As for the impact of amaranth flour on HDL cholesterol levels, data remain conflicting. It could be suggested, that amaranth flour contains several substances that can actively participate in its hypocholesterolemic effect. However, it must be accentuated that lipoprotein patterns in mice differ from those in humans, effect of amaranth flour on HDL cholesterol remains unclear. Results from studies performed in men are insufficient to draw conclusions. Nevertheless, amaranth flour continues to be a part of dietary modifications with promising impact on CVD risk that needs to be verified in larger human studies.

GENETICS VARIANTS WITHIN FIVE TELOMERE-ASSOCIATED GENES, LEUKOCYTE TELOMERE LENGTH AND THE RISK OF ACUTE CORONARY SYNDROME IN CZECH WOMEN
D. Dlouhá1, A. Fellnerová1, J. Piňů1, J. Mesanyová1, J. Mrázková1, V. Staněk1, V. Lánská1, J. A. Hubáček1
1Laboratory for Atherosclerosis Research, 2Department of Cardiology, 3Statistical Unit, Institute for Clinical and Experimental Medicine, Prague, Czech Republic

In humans, leukocyte telomere length (TLT) is getting shorter progressively with age and could reflect aging-related diseases. Candidate genes associated with telomere-length maintenance could represent potential risk predictors for cardiovascular disease (CVD). Women’s Genome Health Study identified association of common variants between seven telomere-associated pathway genes and higher risk of CVD. Genetic variants within TERC (rs12696304), TERF2IP (rs37849929, rs8053257), UCP2 (rs6595366, rs622064) were analyzed using TaqMan technology (quantitative PCR-based method) on an AB 7300. The relative telomere length was calculated as the ratio of telomere repeats to single-copy gene (SCG) copies (T/S ratio). 644 healthy women from 3PMFs study and in 524 women with acute coronary syndrome (ACS) were analyzed. TLT in women with ACS was significantly shorter than in 3PMFs women (P<0.0003). The frequencies of the analyzed SNPs didn’t differ between groups. Genotype GG (TERF2IP rs3784929) was more frequent in women with ACS (P<0.02) and these homozygotes had longer TLT. Other SNPs showed in both groups similar trend with TLT. In conclusion, we found significantly shorter TLT in women with ACS in comparison to healthy individuals. Genotype GG (TERF2IP rs3784929) which is associated with greater risk of ischemic stroke were more frequent in ACS women.

Supported by the project (Ministry of Health, Czech Republic) for development of research organization 00023001 (IKEM, Prague, Czech Republic) – Institutional support.

VARIANT WITHIN THE APOLIPOPROTEIN B AND ITS ROLE IN PSEUDO-FH DEVELOPMENT
A. Fellnerová1, D. Dlouhá1, J. A. Hubáček1, L. Tichý2, L. Fajkusová1, T. Freiberger1
1Center for Experimental Medicine, IKEM, Prague, Czech Republic, 2Centre of Molecular Biology and Gene Therapy, University Hospital Brno, Czech Republic, 3Molecular Genetics Laboratory, Centre for Cardiovascular Surgery and Transplantation, Brno, Czech Republic

Familiar hypercholesterolemia (FH) is a serious disease, leading (if not treated) to premature myocardial infarction. FH is caused predominantly by mutations within the LDL receptor and APOB genes. Recently, gene score from 12 SNPs within different genes have been defined, suggesting the existence of “pseudo-FH”, clinically indistinguishable from classical FH, but without one causal mutation. We have analyzed one from these SNPs in Czech patients with FH. APOB rs1367717 (G>A) variant was genotyped using Taqman technology on an AB 7300 RT PCR cycler in 298 FH patients without the causal mutation and in
HMG-COA ENZYME A REDUCTASE INHIBITION AND RISK OF TYPE 2 DIABETES DEVELOPMENT: EVIDENCE FROM RANDOMISED TRIALS

J. A. Hubacek for Statins/Diabetes consorcium
CIM, Institute for Clinical and Experimental Medicine, Prague, Czech Republic

Statins are suspected to enhance the risk of new-onset type 2 diabetes mellitus (T2DM). We aimed to assess the effect size and whether this increase in risk is a causal and direct consequence of inhibition of 3-hydroxy-3-methylglutaryl-CoA reductase (HMGCoAR) by statins. Data were available from more than 220,000 individuals from 43 studies. Polymorphisms within the HMGCoAR were used as proxies for HMGCoAR inhibition by statins. Meta-analysis was performed to estimate the risk of new-onset T2DM in individuals on statins. HMGCoAR alleles associated with lower enzyme activity and lower plasma cholesterols levels increase significantly the risk of new-onset T2DM for (OR) 1.02 and 1.06 per allele, confirming the causality between the HMGCoAR inhibition and T2DM development. In randomized trials, statins increased the odds of new-onset T2DM (OR 1.12, 95 % CI 1.01-1.24) in placebo or standard care controlled trials and 1.12, 95 % CI 1.04-1.22 in intensive-dose versus moderate dose trials (P<0.006). Minor allele homozygotes occurs more often among the placebo or standard care controlled trials. In conclusion, the increased risk of new-onset T2DM noted with statin use exists and is at least primarily explained by HMGCoAR inhibition. The cardiovascular benefits of statin treatment outweigh the risk of new-onset T2DM.

1Lancet 2014; doi 10.1016/S0140-6736(14)61183-1.

STRONG ASSOCIATION BETWEEN SNPS WITHIN THE CHOLINERGIC RECEPTORS GENES AND TOBACCO DEPENDENCE

J. A. Hubacek1, A. Kmetova2, L. Stepankova, K. Zvolska1, V. Adamkova1, E. Kralkova1
1Institute of Clinical and Experimental Medicine, Prague, Czech Republic
2Centre for Tobacco-Dependent, Third Medical Department and Institute of Hygiene and Epidemiology, First Faculty of Medicine and the General University Hospital in Prague, Czech Republic

Cigarette smoking is the most common form of tobacco use and is a major preventable cause of cancer and cardiovascular disease. Tobacco dependence has a significant heritable component of about 30-60 %. Recent genome wide association studies have associated single nucleotide polymorphisms (SNPs) within the cholinergic receptors, nicotinic, a5 (rs578776), a6 (rs16969968) and b3 (rs6474412) with nicotine dependence in Western European populations. We have analysed, if these cholinergic receptors SNPs influence tobacco dependence in Czech middle European population. Variants within the cholinergic receptors were analysed by PCR-RFLP in 568 adult tobacco dependent smokers – patients of the Centre for Tobacco Dependent, and 1,323 never smokers (population sample of post-MONICA study). All three analysed variants significantly (all P<0.02) influence tobacco dependence, enhancing the risk for about 30 %. TT homozygotes for rs6474412 have OR (96 %CI) 1.33 (1.08-1.64); T allele carriers for rs16969968 have 1.32 (1.08-1.62) and finally CC homozygotes for rs578776 have the values of 1.27 (1.04-1.56). In conclusion, all three variants within the nicotine-
acetylcholine receptors (-α3, -α5 and -β3) are strong predictors of tobacco dependence development in Czech population.

Supported by the projects No. NT/112170-5 (IGA, MH, Czech Republic) and PRVOUK P25/LF1/2.

**TOTAL MORTALITY AND PLASMA CHOLESTEROL LEVELS. CZECH HAPIEE STUDY**

J. A. Habáček1, R. Kubinová2, A. Peasey3, M. Bobáč1, H. Pikhart2

1Center for Experimental Medicine, IEME, Prague, Czech Republic, 2National Health Institute, Prague, Czech Republic, 3Department of Epidemiology and Public Health, UCL, London, United Kingdom

Based on the results of studies performed over forty years ago, the high levels of plasma cholesterol are considered to be a risk factor for cardiovascular and total mortality. However, data’s from large studies of the third millennium are sparse and controversial. We followed a link between plasma levels of total and LDL cholesterol and an eight-year mortality (total, cardiovascular and cancer) in a sample of 6,653 individuals (age range 49-65 years; 627 deaths, including 233 from cardiovascular disease and 283 from cancer) of Czech studies HAPIEE. The lowest total and cardiovascular mortality (P<0.01) were associated with total cholesterol levels between 5.15 and 6.18 mmol/L. Cancer mortality was highest (P=0.01) in the group with total cholesterol 5.15 mmol/l or less. Analysis of LDL-cholesterol showed similar results. All relationships exhibit the typical "U" shape curve. In conclusion, the results from the HAPIEE study do not support the assertion, that elevated levels of total and LDL cholesterol levels are linearly associated with a higher total and cardiovascular mortality.

Supported by project MH CR - IN 00230001, IKEM.

**A POSSIBLE PROTECTION AGAINST HIGH-FAT DIET INDUCED ENDOTHELIAL DYSFUNCTION BY SOLUBLE ENDOGLIN**

K. Jeżkova1, A. Serwańczak2, I. Nemeckova1, J. Rathouska1, S. Chlopicki2, P. Nachitagil1

1Department of Biological and Medical Sciences, Charles University in Prague, Faculty of Pharmacy in Hradec Králové, Hradec Králové, Czech Republic, 2Jagiellonian Centre for Experimental Therapeutics, Krakow, Poland

Soluble endoglin (sEng) is plasma biomarker generated by the cleavage of the extracellular domain by membrane-type metalloproteinase 1 from membrane endoglin (CD105) expressed by vascular endothelium. Increased levels of sEng were found in patients with preeclampsia, type II diabetes, hypertension and were also related to hypercholesterolemia. Soluble endoglin (sEng) background were fed high fat/cholesterol rich diet for 3 month. The level of human sEng was determined by ELISA.

Supported by grant Agency of CU (Prague) n. 1284214/3 and grant SV/2014/260064, by EU from the resources of the ER Development Fund under the IEP (coordinated by JETC-UJ. No POIG.01.02.00-069/09). Transgenic mice were kindly provided by Prof. Lopes-Novoa (University of Salamanca/Spain) and Dr. Bernabeu (CSIC Madrid).

**IDENTIFICATION OF HIGH-RISK PATIENTS WITH MULTIVESSEL CORONARY ARTERY DISEASE BY COMBINED CARDIAC GATED SPECT IMAGING AND CORONARY ARTERY CALCIUM SCORE**

M. Kaminek1, I. Metelková1, M. Budíková1, P. Koranda1, L. Henzlová1, E. Sova2

1Department of Nuclear Medicine and 2Department of Internal Medicine – Cardiology, University Hospital, Olomouc, Czech Republic

To investigate the added value of the coronary artery calcium (CAC) score as an adjunct to cardiac SPECT in the detection of multivessel coronary artery disease (CAD). The study group consisted of 164 prospectively recruited patients who underwent SPECT, CAC, and coronary angiography. Patients with known CAD, after myocardial infarction, and coronary revascularization were excluded. We calculated the extent of ischemic myocardium, stress and rest rest left ventricular ejection fraction (EF), and transient ischemic dilatation (TID) ratio. CAD was defined as ≥50 % stenosis of epicardial coronary arteries or their major branch. We observed significantly lower sensitivity of perfusion SPECT alone in patients with 1-vessel disease (VD) in comparison with 2VD, and 3VD (76 % vs. 94 % vs. 95 %, P<0.05). However, only 38 of 74 patients with multivessel CAD had reversible defects in multiple territories. Patients with multivessel CAD had more frequently severe ischemia ≥10 % of the left ventricle, stress worsening of the EF ≥5 %, TID ratio ≥1.17, and CAC score >1000. In the detection of multivessel CAD, the sensitivity of combined assessment of perfusion, function, and CAD (i.e., multiple and/or ≥10 % ischemia, and/or worsening of the LVEF ≥5 %, and/or TID ratio ≥1.17, and/or CAC score >1000) was significantly higher than sensitivity of perfusion alone or perfusion and function alone (81 % vs. 55 % vs. 65 %, P<0.05). In conclusion, combined perfusion, function and CAC score can help for identifying of high-risk patients with multivessel CAD.

**FIBROBLAST GROWTH FACTOR 21, ADIPOCYTE FATTY ACID BINDING PROTEIN AND THEIR RELATIONSHIP TO THE LEVELS OF ENDOTHELIAL/HEMOSTATIC MARKERS IN SUBJECTS WITH UNFAVORABLE DYSLIPIDEMIC PROFILE**

D. Karasek1, D. Novotny2, H. Vaverková1, J. Orsag1, V. Kubíčková1

1Third Department of Internal Medicine – Nephrology, Rheumatology and Endocrinology, Faculty of Medicine and Dentistry, Palacký University, Olomouc, Czech Republic, 2Department of Clinical Biochemistry, University Hospital Olomouc, Czech Republic

Fibroblast growth factor 21 (FGF 21) has been suggested as an independent factor for protection of cardiovascular system cells. Adipocyte fatty acid binding protein (A-FABP) is considered a key proinflammatory mediator. We analysed an association of FGF 21 and A-FABP levels with endothelial/hemostatic markers and parameters of insulin resistance in dyslipidemic subjects. The study was realized with asymptomatic dyslipidemic patients (n=214), divided into two groups (DLP1, n=66, 38 males; DLP2, n=148, 70 males), according to sex, age and BMI, we observed no differences in parameters of insulin resistance between groups. The multiple regression analysis revealed that FGF 21 was significantly associated with von Willebrand factor (vWF, p<0.01), tissue plasminogen activator (tPA, p<0.01), and glucose (p<0.05) only in DLP2 individuals. In the same group, A-FABP was positively associated with vWF (p<0.001). In conclusion, there is significant elevation of the FGF 21 levels in asymptomatic dyslipidemic patients with elevated TG and/or ApoB ≥1.2 g/l, plasma fibrinogen and FGF 21 levels were significantly higher (p<0.01 and p<0.001, respectively) compared to DLP1 group. After adjustment for sex, age and BMI we observed no differences in parameters of insulin resistance between groups. The multiple regression analysis revealed that FGF 21 was positively associated with von Willebrand factor (vWF, p<0.01), tissue plasminogen activator (tPA, p<0.01), and glucose (p<0.05) only in DLP2 individuals. In the same group, A-FABP was positively associated with vWF (p<0.001).

Supported by grant Nr. LF-2014-011.
THE INFLUENCE OF ADIPOSE TISSUE INFLAMMATION IN THE PATHOGENESIS OF ATHEROSCLEROSIS

A. Králová, I. Králová Lesná, A. Sekerková, J. Ždíčková, J. Froněk, L. Janoušek, R. Poludne
Laboratory for Atherosclerosis Research, Centre for Experimental Medicine, Department of Transplantation Surgery, Institute for Clinical and Experimental Medicine (IKEM), Prague, Czech Republic

In the last decade the significant participation of immune system in the pathogenesis of atherosclerosis has been proved with substantial role of adipose tissue. Using the large transplantation program of living kidney donors at IKEM subcutaneous, perirenal and perivascular adipose tissue of healthy donors were analysed. The stroma vascular fraction of adipose tissue samples were labeled with monoclonal antibodies conjugated with fluorochromes (CD14, CD16, CD36, CD163, CD68 and calprotectin) and subsequently analyzed by flow cytometry. The same surface markers were determined in blood samples of the subjects. All clinical data of enrolled subjects were collected. Significantly higher proportion of proinflammatory macrophages (CD16+ and calprotectin+) was found in all types of adipose tissues compared to blood. The number of CD14+macrophages in the subcutaneous adipose tissue positively correlated (p<0.05) with BMI. In conclusion, the aim of the project is to compare phenotypes of macrophages isolated from adipose tissue of healthy donors to different risk factors of atherosclerosis and finally to patients with proved atherosclerosis.

This project is supported by grant IGA MZ CR NT 14009/3.

MORPHOLOGIC CHARACTERISTICS OF THE CORONARY ARTERY WALL AND PERIVASCULAR ADIPOSE TISSUE AND THEIR RELATIONSHIP TO CORONARY HEART DISEASE

Laboratory for Atherosclerosis Research, 1Clinical and Transplant Pathology Department, 2Cardiovascular Surgery Department, 3Cardiology Department, 4Statistical Department, Institute for Clinical and Experimental Medicine (IKEM), Prague, Czech Republic and 5Charles University, Faculty of Medicine, Department of Histology and Embryology, Pilsen, Czech Republic

This project was focused on the relationship between coronary heart disease (CHD), the amount of perivascular fat, coronary artery (CA) diameter and macrophage content (MF) both in the artery wall and in the adjacent adipose tissue. 49 subjects with CHD and 47 subjects with cardiomyopathy (CMP) were enrolled in the study. Patient clinical data were obtained from clinical documentation. Two samples of CA were taken and CA wall and perivascular adipose tissue size (PVT) and the area of MF in the artery wall was also significantly higher in both parts of CHD subjects’ arteries. There were no differences in the amount of PVT in CHD in comparison with CMP subjects. The surface area of MF in the artery wall was also significantly higher in both parts of CHD subjects’ arteries. There were no differences in the amount of PVT in CHD in comparison with CMP subjects. The surface area of perivascular adipose tissue MF also did not differ between groups. A highly significant relation between the MF surface area in PVT and in the CA wall was found, whereas there was no such relationship in the CMP group. In conclusion, we proved our early results that PVT size does not relate to atherosclerosis. However, a significant correlation of MF in the coronary artery and PVT in CHD (contrary to CMP) suggests interplay of these cells in atherogenesis.

Supported by the grant IGA MZ CR NT 14009/3.

IDENTIFICATION OF A 14-GENE REGION AFFECTING SUCROSE-DIET-INDUCED GLUCOSE INTOLERANCE AND DYSLIPIDEMIA

M. Krupková, M. Janků, M. Hodílková, L. Šedoň, F. Liška, L. Kozdová, D. Křenová, V. Křen, O. Šedá

1Institute of Biology and Medical Genetics, First Faculty of Medicine, Charles University and General Teaching Hospital, Prague, Czech Republic, 2Department of Metabolism and Diabetes, Institute for Clinical and Experimental Medicine, Prague, Czech Republic

The spontaneously hypertensive rat (SHR) and the polydactylous rat (PD-Cub) are two established rodent models of human metabolic syndrome. We have produced the SHR-Lx PD5 congenic strain carrying approximately 1.4 Mb PD-derived region encompassing only 14 genes. We have compared the sensitivity of SHR and SHR-Lx strains to high-sucrose diet (HSD) challenge. Male rats (n=12/strain) of SHR and SHR-Lx strains were fed standard diet for 15 weeks, followed by HSD for 2 weeks. We assessed their metabolic profiles. We observed several significant interactions between the strain and diet factors, e.g. for fasting glucose and area under the glycaemic curve with SHR showing more pronounced sucrose-induced deterioration of glucose tolerance compared to SHR-Lx. VLDL triglyceride concentrations were higher in SHR on standard diet (SHR 24±3 vs SHR-Lx 13±1 mg/dl) but HSD induced their more robust increase in SHR-Lx rendering the levels to be comparable between the two strains (SHR 39±4 vs SHR-Lx 41±3 mg/dl). SHR-Lx animals also showed steeper fall of cholesterol in total (SHR 2 % vs SHR-Lx 14 %), LDL (SHR 14 % vs SHR-Lx 50 %) and HDL fractions compared to SHR. In conclusion, we have identified a 14-gene region involved in an nutrientigenic interaction with high-sucrose diet affecting glucose tolerance, triglycerides and cholesterol distribution into lipoprotein fractions.

Supported by GAUK 434313.

WHICH IS ACTUALLY IMPORTANT OF THE MTHFR MUTATIONS?

M. Kuklík, V. Helešic, M. Tothová
1Genetic Department, Institute of Endocrinology, Prague, Czech Republic, 2Department of Molecular Endocrinology, Institute of Endocrinology, Prague, Czech Republic, 3Genvia, Molecular Genetic Laboratory, Prague, Czech Republic

Mutations at 677 and 1297 of methylenetetrahydrofolate reductase represents important human polymorphisms. Both mutations are in the connection to the homocystein level and negative influence the vessels endothelium. MTHFR mutations are each only one from many others factors in the atherosclerosis pathogenesis. The C677T genotype of MTHFR, has been linked to an increased risk of trobomtic episodes or unexplained pregnancy loss. Hyperhomocysteinemia is an established, independent risk factor in cardiovascular disease. We examined cohort of 700 persons (300 males), which were designed as risky individuals according to clinical, familiar and personal anamnestic dates. DNAs were analysed according to real-time PCR methods with help Gene Proof R kits for II protrombin, FV Leiden, MTHFR C677T and A1298C. We found MTHFR677 52.3 % heterozygotes and 47.7 % homozygotes in investigated persons and at MTHFR 1298 heterozygotes 49.5 % were found. Homozygotes: MTHFR677 7.3 %, MTHFR 1298 8.3 % from all examined persons. Double heterozygous status included 27.5 % individuals, according to the following familiar study probably in the trans position. Without MTHFR mutations are only 10.1 % individuals. In conclusion, our dates indicates abbreviation to the Hardy-Weinberg-Castle equilibrium. The MTHFR deficiency is not true coagulation trombophilic factors, as FV and FII mutations but slow acting risky factor. We discuss the connection MTHFR mutations situation to the other pathology (midline defects) and more complicated trombophilic situations.

Correlation of blood lipids and lipoproteins with the extent of coronary artery disease in patients with established coronary atherosclerosis – gender differences

1Cardiovascular Center, Na Homolce Hospital, Prague, Czech Republic, 2Czech National Institute of Health, Prague, Czech Republic,
Male Wistar rats were fed for two months a high sucrose diet (HSD) which has a central role in lipid biosynthesis. Adult composition of phospholipids and expression of stearoyl-CoA desaturase (SCD), which has a modified angiographic Gensini Score (GS) was used to reflect the extent of coronary atherosclerosis. Significant differences between men and women were identified for HDL cholesterol level (1.05 vs. 1.18 mmol/l), apolipoprotein B (1.28 vs. 0.85 g/l), apolipoprotein A (1.41 vs. 1.57 g/l), lipidoprotein(a) (307.1 vs. 282.7 mg/l) and fasting glucose (6.82 vs. 6.65 mmol/l) (p<0.05 for all). Mean HR was 69.9/min in men and 69.2 in women, mean LVEF was 55.9 % in men and 60.5 % in women. Mean angiographic score was 19.6 in men and 15.0 in women. Lp(a) level correlated with GS in men (r=0.168, p<0.05), HDL cholesterol and apo A level correlated with GS in women (r=-0.23, p<0.05, resp. r=-0.26, p<0.05). In conclusion, in the patients with established coronary atherosclerosis gender differences were identified in the relation of Lp(a) and other lipoproteins with the extent of coronary artery disease.

ADVERSE EFFECT OF OVARIECTOMY ON BROWN ADIPOSE TISSUE ACTIVITY

H. Malinská, J. Tmovská, V. Škop, L. Kazdová
Center for Experimental Medicine, Institute for Clinical and Experimental Medicine, Prague, Czech Republic

Ovariectomized rats are used as a model of postmenopausal metabolic syndrome, which is associated with increased visceral adiposity and insulin resistance. Brown adipose tissue (BAT) plays a key role in energy expenditure and its activity could be regulated by sexual hormones. The aim of this study was to evaluate the effect of ovariectomy on BAT activity. In this study, female Wistar rats were ovariectomized (OVX) at 8 weeks of age and were fed by standard diet for 4 months. Compared with sham operated control Wistar rats, OVX rats exhibited markedly increased body weight (+10 %, p<0.05), visceral adipose tissue weight (p<0.05), hepatic triacylglycerols accumulation (+220, p<0.001) and insulin resistance in visceral adipose and muscle tissue measured according to glucose incorporation into lipids and glycogen, respectively. BAT ovariectomized rats after 4 months after ovariectomy exhibited reduced fatty acid utilization for oxidation (~72 %, p<0.05) and lipogenesis (~26 %, p<0.05) and decreased lipolysis (~25 %, p<0.05), despite BAT weight was significantly increased (p<0.05) in OVX rats. In conclusion, our results indicate that ovariectomy reduces fuel utilization and lipolysis in BAT, which supports hypothesis that low metabolic activity of BAT may contribute to development of obesity and postmenopausal metabolic syndrome.

Supported by MH CZ - DRO („Institute for Clinical and Experimental Medicine – IKEM, IN 00023001“).

SOLUBLE ENDOGLIN AS BIOMARKER OR INDUCER OF ENDOTHELIAL DYSFUNCTION

P. Nachtigal1, I. Němečková1, K. Ježková1, J. Rathouská2, M. Vařejková2, P. Fikrová2, A. Serwadzak2, B. Oujo2, S. Chlopicki2
1 Department of Biological and Medical Sciences, Faculty of Pharmacy in Hradec Kralove, Charles University in Prague, Hradec Kralove, Czech Republic, 2 Jagiellonian Centre for Experimental Therapeutics (JCET), Krakow, Poland

A soluble endoglin (Sol-Eng) circulating in plasma is cleaved from membrane endoglin in various pathological conditions including atherosclerosis and endothelial dysfunction. High concentration of sEng was considered as biomarker related to hypercholesterolemia, type II diabetes and arterioles hypertension. Sol-Eng might contribute to the development of endothelial dysfunction, but there is no direct evidence. We hypothesized that high sEng levels both in vitro and in vivo induce endothelial dysfunction or upregulate expression of pro-inflammatory proteins in both aorta and/or endothelial cells (HUVEC). Functional analysis either in vivo or ex vivo in isolated aorta, and Western blot analysis in aorta and HUVECs were used for evaluation of possible changes in relation to high levels of Sol-Eng. Endothelium-dependent vascular function was similar in Sol-Eng and control mice including in vivo analyses in aorta and HUVECs were used for evaluation of possible changes in relation to high levels of Sol-Eng. Endothelium-dependent functional analysis either in vivo or ex vivo in isolated aorta, and Western blot analysis in aorta and HUVECs were used for evaluation of possible changes in relation to high levels of Sol-Eng. Endothelium-dependent vascular function was similar in Sol-Eng and control mice including in vivo analyses in aorta and HUVECs were used for evaluation of possible changes in relation to high levels of Sol-Eng. Endothelium-dependent vascular function was similar in Sol-Eng and control mice including in vivo analyses in aorta. In conclusion, our results demonstrate that high concentration of sEng was also proposed to contribute to the development of endothelial dysfunction, however there is now evidence.

Supported by grants from the Grant Agency of Charles University in Prague (306811/C and 1158413/C), Charles University in Prague (SVU/2014/260064), European Regional Development Fund under the Innovative Economy Program of the European Union (grant coordinated by JCET-UK, No POIG.01.01.02-00-069/09).

HIGH SOLUBLE ENDOGLIN LEVELS DO NOT INDUCE ENDOTHELIAL DYSFUNCTION IN MOUSE AORTA

I. Němečková1, A. Serwadzak2, B. Oujo2, K. Ježková1, J. Rathouská2, P. Fikrová2, M. Vařejková1, C. Bernabev1, J. M. Lopez-Novoa1, S. Chlopicki2, P. Nachtigal1
1 Department of Biological and Medical Sciences, Faculty of Pharmacy in Hradec Kralove, Charles University in Prague, Hradec Kralove, Czech Republic, 2 JCE, Krakow, Poland

A soluble form of endoglin (sEng) and its increased levels has been detected in various pathological conditions related to cardiovascular system. High concentration of sEng was also proposed to contribute to the development of endothelial dysfunction, however there is now evidence.
Evidence that this happens in atherosclerotic prone vessels. Therefore, in the present work we analyzed whether high sEng levels induce endothelial dysfunction in mouse aorta. Four to six month old transgenic mice with high expression of human sEng on CBAxC57Bl/6J background (Sol-Eng') and age-matched transgenic littermates that do not develop high levels of human sEng (control animals) on chow diet were used. Sol-Eng' transgenic mice showed higher levels of human sEng as well as increased blood arterial pressure, as compared to controls. Functional analysis either in vivo or ex vivo in isolated aorta demonstrated that the endothelium-dependent vascular function was similar in Sol-Eng' and control mice. Western blot analysis showed no differences between Sol-Eng' and control mice in the protein expression of endoglin, eNOS ICAM-1 and VCAM-1. In conclusion, high levels of sEng alone do not induce endothelial dysfunction in Sol-Eng' mice. However, these data do not rule out the possibility that sEng might contribute to alteration of endothelial function in combination with other risk factors related to cardiovascular disorders.

This work was supported by grants GAUK (300811/C and 115843/C), Charles University in Prague (SVV/2014/260064). The publication is co-financed by the European Social Fund and the state budget of the Czech Republic (Project No. CZ.1.07/2.3.00/30.0061).

EXTRACT FROM SILYBUM MARIANUM INCREASES SERUM HDL-CHOLESTEROL AND REDUCES ECTOPIC LIPID ACCUMULATION IN HEREDITARY HYPERTRIGLYCERIDEMIC RATS

O. Oliyarnyk1, H. Malinska1, V. Skop1, L. Kazdova1, R. Vecera2
1Center for Experimental Medicine, IKEM, Prague, Czech Republic
2Institute of Pharmacology, Palacky University, Olomouc, Czech Republic

Hepatoprotective effect of silymarin is well established, but its low absorption and rapid excretion limits its bioavailability. Using micronized extracts (ME) and phytosomes (PhE) formulation might be beneficial for increase of its metabolic activity. We investigated the effects of these compounds on disorders associated with metabolic syndrome in hereditary hypertriglyceridemic rats (HHTg). Adult male rats were fed a standard laboratory diet without (control group) or with supplementation of silymarin extracts (1 % SE); (1 % ME ) and (1.5 % PhE) for 3 weeks. Silymarin and its complexed forms decreased serum triacylglycerols (SE-48 %, ME-61 %, PhE-61 %), insulin and serum triacylglycerols (SE-48 %, ME-61 %, PhE-61 %), insulin and serum insulin sensitivity (SE-25 %, ME-43 %, PhE-50 %). We tested whether the sEng expression in aorta and heart in vivo in these conditions were different. sEng expression was found to be increased in aorta and heart in control group and decreased in treated groups. This indicates that sEng expression is regulated by silymarin and its complexed forms and may be involved in the improvement of metabolic parameters in hypertriglyceridemic rats.

Supported by MH CR-DRO (‘IKEM, IN 0002300’).

REMANN LIPOPROTEINS AS A RISK FACTOR FOR Atherosclerosis in menopausal transition

J. Piňha1, O. Auszký2, P. Štavěk2, R. Dembovská1, I. Králová-Lesná1, J. Kovář1, S. Adamková1, E. Babková1, T. Adamek1, M. Lejskova1, J. Mrázková1, R. Houdek1
1Laboratory for Atherosclerosis Research, Institute for Clinical and Experimental Medicine, 1First Internal Clinic of Thomayer Hospital, 2Institute for Postgraduate Medical Education, Prague, Czech Republic

In our previous studies we detected increased sensitivity for development and progression of atherosclerosis in menopausal transition. In the recent study we analyzed changes of lipid factors including calculated remann lipoproteins (RLP) and their dependence on reproductive status and smoking behavior – in longitudinal study. We analyzed lipid parameters after 6 years follow up. RLP were calculated as total minus LDL minus HDL cholesterol. Changes in time were evaluated by pair t-test. Differences in changes were evaluated by non-pair t-test (STATA). In women which were at the beginning of the study in menopausal transition and were non-smokers (n=44) RLP decreased (-0.125±0.42 mmol/l, p=0.056); in contrast in smoking women (n=31) RLP increased (+0.095±0.42 mmol/l, p=0.094). Differences in changes of RLP between non-smokers and smokers were statistically significant (p=0.015). These differences we detected neither in premenopausal non-smoking (n=80) and smoking (n=118) women nor in postmenopausal non-smoking (n=97) and smoking (n=100) women. We did not observe similar differences for other lipid parameters. In conclusion, remann lipoproteins could be accelerators of atherosclerosis in smoking women in menopausal transition.

Supported by grant NT 14008-3/2013 (Internal Grant Agency of the Ministry of Health of the Czech Republic).

STIMULATION OF INFLAMMATION INCREASES Atherosclerosis in experiment

R. Polk01, I. Králová Lesná1, P. Štavěk1, A. Králová1, H. Blyuss2
1Laboratory for Atherosclerosis Research, Centre for Experimental Medicine (IKEM), Prague, Czech Republic
2Mickiewicz University in Poznan, Poland

Interferon Gamma (IFN-G) substantially stimulates a “sterile” inflammation in adipose tissue and IFNG KO mice are resistant to this process and contrary they are more sensitive to insulin. We tried to analyzed a direct effect of IFN-G on atherosclerosis using the model of apoe KO mice. Two groups of apoe KO mice were injected intraperitoneal by 5 000 IU of IFN-G in 0.1 ml of physiological solution 5 times in week for a period 2 (n=5), resp. 6 (n=8) weeks. Control animals obtained the same volume of physiological solutions. All groups were fed a Western type diet with 0.1 % of cholesterol. The surface of atherosclerotic lesions in aorta was significantly higher (12.7±6.3 %) compared to controls (2.9±0.9 %, p=0.004) in animals injected for longer time. Differences after two weeks of IFN-G injection did not reach statistical significance (2.3±1.4 % vs. 1.2±0.7 %). Surprisingly injection of IFN-G for 6 weeks significantly decrease total cholesterol concentration (25.38±5.59 mmol/l in controls and 13.58±8.82 mmol/l in IFN-G group, p<0.01). In conclusion, IFN-G activates inflammation in the arterial wall and stimulates atherogenesis.

Supported by grant P305/13-130440S of Czech Science Foundation.

METFORMIN ATTENUATES MITOCHONDRIAL RESPIRATION AND ATP PRODUCTION IN STEATOTIC LIVER

E. Palenickova1,2, Z. Drahota1, M. Burian1, Z. Papackova1, M. Cahova1, L. Kazdova1
1Center for Experimental Medicine, Institute for Clinical and Experimental Medicine, Prague, Czech Republic
2Department of Cell Biology, Faculty of Science, Charles University, Prague, Czech Republic

Metformin (Mf) is drug widely used as the medication for T2D but precise mechanism of action remains elusive. It is supposed that its therapeutic effect may be derived from cellular energy charge reduction and consequent decrease in hepatic glucose output. We focused on Mf effect on energy metabolism in steatotic liver, particularly on: 1) Mf effect on isolated mitochondria respiration either in vitro to or after long-term administration in vivo; 2) in vivo ATP repletion after short-term partial ischemia and reperfusion. The Wistar rats fed chow or high-fat diet (HFD) for 13 weeks. Half of the HFD was enriched by Mf (HFD+Mf, 150 mg/kg b.w.). Mitochondrial respiration was detected as O2 uptake in liver homogenate. Liver ATP content was measured by 31P NMR. 2.5 mM Mf in vitro inhibited malate+palmitoylcarnitine (M+PC) oxidation by 33 %. Mf administered in vivo (final serum concentration 20 μM) reduced M+PC oxidation in isolated mitochondria by 24 % compared with Mf-un-treated rats. Mf lowered ATP repletion during reperfusion both in terms of ATP concentration and synthesis rate. Effect of Mf was additive to effect of HFD itself. In conclusion, we proved that Mf administered both in vivo and in vitro inhibits the mitochondrial respiratory chain. This effect involves both mitochondrial electron transfer and ATP production.
ENDOGLIN IS NOT CO-EXPRESSED WITH CELL ADHESION MOLECULES IN AORTA DURING ATHEROGENESIS IN APOE-DEFICIENT MICE

J. Rathouská1, K. Ježková1, I. NČÍRNT-14009/3.

1Department of Biological and Medical Sciences, Faculty of Pharmacy in Hradec Králové, Charles University in Prague, Czech Republic

Endoglin (TGF-β receptor III) affects vascular endothelium and atherosclerosis. Endoglin is involved in inflammation, in leukocyte adhesion and transmigration in vitro and in vivo but not in atherosclerotic vessels. We evaluate endoglin expression in 2 segments of aorta and assess its potential simultaneous expression with cell adhesion molecules in aortas of apoE-deficient mice. Ten-week-old fem. apoE-deficient mice on a C57BL/6J background (n=24) were randomly divided into 3 groups and were fed either chow diet (for another 2 and 4 months). Immunohistochemical staining of endoglin, VCAM-1 and P-selectin in aortic sinus and ascending aorta was performed. Endoglin expression was detected only in endothelial cells and varied during atherogenic process in aorta but not in aortic sinus, endoglin positivity was detected only in endothelium covering atherosclerotic lesions. Endoglin was not expressed with P-selectin and VCAM-1 in aortic endothelium in any tested group. In conclusion, this study shows that endothelial expression of endoglin is related to the atherogenic process predominantly in aorta outside the heart. Endoglin is not localized with cell adhesion molecules involved in atherosclerosis, suggesting it might not participate in leukocyte accumulation in aorta of apoE-deficient mice during atherogenesis.

The study was supported by grant from The Grant Agency of Charles University in Prague number 1284214/C, grant SVV/2014/260064 and by the European Social Fund and the state budget of the Czech Republic, project no. CZ.1.07/2.3.00/30.0061.

EXPERIMENTAL MODELLING OF IRON-LOADED HUMAN THP-1 MONOCYTE CELL LINE

University Hospital Královské Vinohrady, Prague, Czech Republic

Elevation of the body iron stores is one of the non-classical risk factors of cardiovascular diseases. Macrophages play a crucial role in the underlying atherosclerosis and the iron metabolism. In order to investigate how macrophage iron status affects its phenotype and behavior, a suitable in vitro model of iron-overloaded cell is needed. Human monocytic THP-1 cells were grown in suspension and fed with iron (4-12 μM) in forms: a) transferrin, b) ferric-ammonium citrate (FAC) and c) hemin, for 24 and 24 hours. Lactate dehydrogenase release to the medium was employed as a marker of toxicity. Labile iron pool (LIP) was estimated in living cells according to Cabantchik, and expression of both heavy (H) and light (L) ferritin chains was assessed by Western immunoblotting. Only feeding with hemin was toxic to the cells. Elevated LIP was observed at 2 hours with all forms of iron, most markedly with hemin and FAC. Intracellular ferritin synthesis induction was observed at 24 hours, again most strongly after hemin and FAC loading. Whereas FAC did not change the proportion of H and L ferritin, hemin at 4-8 μM and transferrin induced preferentially the synthesis of L ferritin. In conclusion, our experimental model using THP-1 monocytes enables assessment of intracellular iron, both in H or L ferritin, and within the LIP in cultivated cells exposed to iron loading. We assume that iron-rich monocytes/macrophages significantly contribute to atherosclerosis progression and plaque destabilization.

Supported by the grant IGA MZ ČR NT 13671-4/2012.

Supported by “Long-term plan of development of organization 1011”.

FH HOMOZYGOATE WITHOUT CARDIOVASCULAR DISEASE AT THE AGE OF 46

L. Schwarzwäč, J. A. Hubáček, A. Felliňová1, L. Zlatohlávké, M. Prusiková, R. Čeřka, M. Vrablík
1CEM, Institute for Clinical and Experimental Medicine, Prague, Czech Republic; 2Centre for preventive cardiology, Third Department of Internal Medicine, First Faculty of Medicine, Charles University, Prague, Czech Republic

Familial hypercholesterolemia (FH) is an autosomal disease of lipid metabolism, caused mostly by the mutations within the LDL receptor. The prevalence of this disorder has been estimated to be 1:200, thus making FH the most common inherited metabolic disease. 135 Czech FH patients were screened by PCR-RFLP for the most common LDL receptor mutation c.1775G:A (Gly592Glu). Oligos 5´TTCACTCCATCTCAAGCACAAGTGTCGTCAT 5´AAAGTCTTGTACATACGTAGGTAAGCCAC and restriction enzyme TaqI were used. Mutation was confirmed by direct sequencing. One c.1775G:A homozygote, female born in 1973, was detected. Her plasma lipid levels when the diagnosis of FH was established (2004) were – TC 10.4, LDL-C 8.3, HDL-C 1.3 and TG 0.6; all in mmol/l. The subsequent treatment (statins+ezetimibe) lipid levels have been as follows; TC 4.5±0.7 and LDL-C 2.7±0.6; all in mmol/l. The carotid intimamedia thickness (cIMT) measurement (2007) showed no plaque with cIMT of 0.77 mm. The patient is free of symptomatic cardiovascular or cerebrovascular disease. In conclusion, we have detected an FH homozygote with well controlled dyslipidemia and no overt CVD at the age of 46. This case contradicts general belief the homozygous FH patients respond poorly to lipid lowering therapy. It also documents FH causing mutation itself does not cause accelerated atherosclerosis when plasma LDL cholesterol levels can be controlled.

Supported by the project (Ministry of Health, Czech Republic) for development of research organization 00023001 (IKEM, Prague, Czech Republic) and IGA NT 14186-3 (CPC, Third Dep't of Internal Medicine).

SELECTED COMPONENTS OF THE ANTIOXIDANT SYSTEM – SERUM LEVEL DEPENDING ON NATURE FOOD OR DIETARY SUPPLEMENTS EATING

H. Stritečká1, P. Hlubík2
1Faculty of Military Health Sciences, University of Defence, Hradec Králové, Czech Republic; 2Center for nutrition disorders, Hradec Králové, Czech Republic

Prevalence of dyslipoproteinaemia and cardiovascular diseases (CVD) is very high in The Czech Republic. Condition for satisfactory nutrition is sufficient intake of essential substances. When one of them is missing, it can lead to serious health damage; it can even endanger the life. At group of 171 volunteers (52 men, 19 women, age 34±7,9 years, 82 % non-smokers) were studied the effects of dietary polyphenols or supplements on cardiovascular risk factors. For evaluation up-to-date health condition volunteers recorded their eating habits by simple questionnaires, which were focused on the consumption of meat, fish, milk, eggs, vegetables, fruit, alcohol and dietary supplements. Volunteers who stated daily consummation of fruit and vegetables had two times higher β-carotene levels in serum than those who did not consume them at all. Vitamin C concentration was on an average 33 % higher and the atherogenous index was 20 % lower. It is clear that almost 20 % of examined volunteers regularly use preparations for antioxidant supplementation. The effect of regular supplementation was only seen in increased β-carotene and vitamin C levels. Retinol and α-tokopherol concentrations did not significantly change. In conclusion, diets rich in fruits and vegetables promote health and attenuate or delay the onset of CVD. Polyphenol extracts from fruits and herbs such as apples and green tea can reduce risks of heart diseases.

Supported by “Long-term plan of development of organization 1011”. 
CHANGE OF THE HDL CHOLESTEROL CONCENTRATION IN THE COURSE OF THE DIETARY – PHYSICAL INTERVENTION OF OVERWEIGHT OR OBSESE PERSONS

P. Suchanek, P. Stevaj, J. Mrázková, V. Lanská, J. A. Hubacek
CEM, Institute for Clinical and Experimental Medicine, Prague, Czech Republic

Obesity represents serious risk CVD factor. Effect of the dietary-physical intervention becomes positively evident in change of lipid parameters. There were selected 180 women in age extent 25-65 years, with BMI 25-29.9 kg/m² with abdominal obesity type. 65 women with BMI 25-29.9 kg/m², 115 of them had BMI over 29.9 kg/m². For reporting purpose we analysed blood draw results and anthropometric changes after first 14 days and after 28 days dietary – physical intervention. Lipid parameters were analysed enzymatically, physical activities was followed telemetrically. At the beginning and at after first 14 days and after 28 days there were followed anthropometric, lipid parameters, blood glucose level, CRP and NEMK concentration. Besides expected statically significant reduction at level p ≤ 0.001 at anthropometric parameters within both checks (14th and 28th day), there was found in both groups even significant decrease at level p≤0.001 of LDL concentration of set cholesterol. After 14th day HDL concentration decrease even more significant in a group of obese person than overweight persons (p<0.001 vs p=0.002). In conclusion, at the dietary-physical intervention beginning of overweight and obese persons there comes to significant concentration decrease of HDL cholesterol and such decrease is more noticeable with obese persons group.

Supported by Ministry of Health, Czech Republic – conceptual development of research organization („Institute for Clinical and Experimental Medicine – IKEM, IN: 00023001“).

OPPORTUNITIES OF METABOLIC SURGERY IN THE TREATMENT OF DYSLIPIDEMIA

P. Sucharda
Third Department of Internal Medicine, First Faculty of Medicine, Charles University, Prague, Czech Republic

The term of metabolic surgery is being historically connected with the treatment of extreme hypertriglyceridemias by partial small intestine resection. After quite a number of years it has been filled with much broader sense: „surgical change of an organ or an organ system aiming at health improvement through a biological effect“ . Such a biological effect might be long term remission of type 2 diabetes, which can be achieved in 4/5 of metabolic procedures (e.g. gastric bypass, bilipancreatic diversion). The bariatric/metabolic procedures reduce the all-cause mortality by up to 90 %. This impact is, to some extent, mediated by favourable changes of lipid levels, e.g. decrease of triglycerides accompanied by HDL cholesterol increase. Similar beneficial effect has been described also in a patient with familial lipoprotein lipase deficiency. Despite this, dyslipidemia is not a currently acknowledged indication for bariatric/metabolic surgery.

THE EFFECT OF METFORMIN ON HYPERTRIGLYCERIDEMIA-INDUCED DICARBONYL STRESS

V. Škopl, H. Malinská, J. Tmavská, L. Kazdová
Center for Experimental Medicine, Institute for Clinical and Experimental Medicine, Prague, Czech Republic

Biogenesis of reactive dicarbonyls leads to the production of advanced glycation end products and play a key role in the development of diabetic vascular complications. Metformin is the most widely prescribed glucose-lowering agent; however, its effect on dicarbonyls metabolism in tissues is not clarified. In our study we analyzed levels of individual reactive dicarbonyls – methylglyoxal (MG), glyoxal (GL) and 3-deoxyglucosone (3-DG) in relation to lipid disorders and after metformin administration (300 mg/kg b.w.) in non-obese hereditary hypertriglyceridemic (HHTg) rats. The concentrations of dicarbonyls were determined by HPLC-method with fluorescence detection. Compared with control Wistar rats, HHTg rats exhibited markedly increased serum level of GL and MG, increased levels of MG, GL and 3-DG in myocardium and increased level of GL and MG in kidney cortex. Metformin treatment was associated with significantly reduced level of all measured reactive dicarbonyls in the myocardium and reduced level of MG in serum. However, dicarbonyls concentrations in the kidney cortex were not affected by metformin. In conclusion, results indicate that hypertriglyceridemia is associated with increased dicarbonyl stress in serum and tissues. Beneficial effect of metformin therapy on reactive dicarbonyls in heart could contribute to its cardioprotective effects.

Supported by the grant GAČR P303/13-04420S.

NEW DIAGNOSTIC SCHEME FOR AUTOSOMAL DOMINANT HYPERCHOLESTEROLEMIA IN THE CZECH REPUBLIC

L. Tichy1,2, T. Freiberger1,2, P. Zapletalova1, O. Letocha2, L. Fajkusova1,2
1Department of Internal Medicine – Hematology and Oncology, University Hospital Brno, Brno, Czech Republic, 2Center of Molecular Medicine, Central European Institute of Technology, Masaryk, University, Brno, Czech Republic, Centre for Cardiovascular Surgery and Transplantation, St. Anne's University Hospital Brno, Brno, Czech Republic

Autosomal dominant hypercholesterolemia (ADH) is predominantly associated with mutations in the genes encoding the low-density lipoprotein receptor (LDLR), its ligands apolipoprotein B (APOB) and apolipoprotein E (APOE), and proprotein convertase subtilisin/kexin type 9 (PCSK9). Mutations were previously determined using exon by exon screening methods based on individual exon amplifications, restriction analysis, sequencing and MLPA. In 2014 we modified this scheme due to implementation of next-generation sequencing technology. The first step of our scheme is represented by MLPA. We use ADH MASTER assay (Multiplicom N.V., Belgium) for amplification the coding and promoter regions of the genes LDLR, PCSK9 and APOE, and part of the exon 26 (c.10200 c.11100) of APOB in MLPA-negative samples. New sequence variants were found in analyzed genes. Variants with unknown phenotype were identified in LDLR (p.(Glu266Lys)) and APOE (p.(Arg137Gly)) genes. Most probably pathological variant p.(His3570Tyr) in APOB gene was found in 2.8 % of probands suspected to have ADH. In conclusion, the presented results indicate that the ADH MASTER assay seems to be a suitable tool for identification of mutations in ADH patients and can be used for routine diagnostics.

This study was supported by the project NTI4186 of the Ministry of Health of the Czech Republic.

EFFECT OF METFORMIN ON GLUCOSE AND FATTY ACID UTILIZATION IN BROWN ADIPOSE TISSUE

J. Tmavská1, V. Škopl1, H. Malinská1, L. Kazdová1
1Center for Experimental Medicine, Institute for Clinical and Experimental Medicine, Prague, Czech Republic

Metformin is the first-line drug for the treatment of type 2 diabetes and its antihyperglycemic properties are well-characterized. Metformin increase energy expenditure but the mechanism is still unclear. Brown adipose tissue is known for its high fuel utilization during thermogenesis. The aim of this study was investigate if metformin increase fuel utilization by BAT and ameliorate related parameters of metabolic syndrome (MS). Treatment of Wistar rats (n=6, age 8 months) with metformin (dose: 300 mg/kg/day) for 4 weeks significantly decreased body weight (~25 %, P<0.001), adiposity (~61 %, P<0.002), serum triglycerides (TAG) (~48 %, P<0.03) and ectopic accumulation of TAG in liver (~72 %, P<0.02), heart (~66 %, P<0.03) and diaphragm (~80 %, P<0.01). BAT activity was measured by lipolysis and palmitic acid (PA) or glucose oxidation and incorporation into interscapular BAT ex vivo. Metformin increased lipolysis (~57 %, P<0.01) PA oxidation (~45 %, P<0.03) and PA incorporation into BAT (~26 %, P<0.01). There were not significant differences in glucose oxidation among groups but incorporation of glucose into BAT was higher after metformin treatment (basal +61 %, P<0.004; insulin stimulated +60 %, P<0.01). In conclusion, results indicate that hypertriglyceridemia is associated with increased dicarbonyl stress in serum and tissues. Beneficial effect of metformin therapy on reactive dicarbonyls in heart could contribute to its cardioprotective effects.
indicate that metformin-induced increase of lipid and glucose utilization in BAT may participate in the improvement of MS disorders.

Supported by the grant GACR P303/13-04420S.

THE PROGNOSTIC VALUE OF CAROTID INTIMA-MEDIA THICKNESS IN CHILDREN

Z. Urbanová1, B. Grauová2

1Department of Pediatrics, 2Third Department of Internal Medicine, First Faculty of Medicine and General University Hospital, Charles University in Prague, Czech Republic

In adults, increased cIMT is associated with CAD and is predictive of future cardiovascular events. Cardiovascular risk factors have been associated with cIMT, including age, male sex, diabetes mellitus, cholesterol and smoking. Assessment of cIMT also has been used extensively in children and young adults with known risk factors for CAD as familial hypercholesterolemia, hypertension, obesity and diabetes mellitus. Despite the clear value of this tool in the assessment of cardiovascular risk in children and adolescents, its application has been restricted by a number of factors, including limited access to trained investigators and appropriate ultrasound equipment, variable protocols for data acquisition and analysis. Also some of the changes in cIMT that occur with age represent normal vascular adaptation. cIMT is one of the most widely used noninvasive measures of subclinical atherosclerosis in pediatrics, however, before cIMT can be applied in the clinical setting, many questions must be answered: What are normal cIMT values by age/sex? At what age are changes in cIMT with growth and large enough magnitude to be measurable? At what age or arterial size does cIMT become sufficiently reproducible? How do cIMT measures relate to anatomy of atherosclerosis/other measures of arterial structure? Are cIMT measures cost-effective? cIMT could help us to improve risk profiling of the highest-risk children for the most aggressive interventions and treatment.

EFFECTS OF HIGH SOLUBLE ENDOGLIN ON ENDO- THELIAL CELLS IN VITRO – PILOT STUDY

M. Varejčkova, P. Fikrova, I. Nemeckova, K. Jezkova, J. Rathouska, P. Nachtigal

Department of Biological and Medical Sciences, Faculty of Pharmacy in Hradec Králové, Charles University in Prague, Czech Republic

Endoglin (Eng) is an accessory type III receptor for the TGF-β superfamily cytokines. It is a membrane protein highly expressed in the vascular endothelium. In addition to membrane bound endoglin, a soluble form of endoglin (sEng) has been detected in various pathological conditions related to cardiovascular system. Since, the detailed relation between sEng and endothelial function/inflammation has not been uncovered yet, the aim of our pilot study was to evaluate possible effects of high levels of sEng on endothelial cells in vitro. Human endothelial vein cells (HUVEC) were obtained from Lonza from Clonetics™ Laboratories. Cells were cultured in gelatin-coated flasks in EGM-2 medium. HUVEC cells were serum starved for 3 h and then incubated for 16 h with recombinant human sEng (1 nM) in combination with starving conditions. Spectrum of selected markers of endothelial dysfunction was evaluated using Western blot analysis and immunofluorescence. Our results demonstrated no significant differences between treated and control cells in the expression of endoglin, endothelial NO-synthase, VEGF, VE-Cadherin, VCAM-1 and HO-1. In conclusion, this study shows that soluble endoglin did not directly affect endothelial cells with respect to inflammation and endothelial cells functions. Further studies are needed to evaluate whether soluble might affect endothelial cells in combination with cholesterol and inflammation.

This work was supported by grant GAUK number 1158413C and grant SVV/2014/260064. The publication is co-financed by the European Social Fund and the state budget of the Czech Republic, project no. CZ.1.07/2.3.00/30.0061.

IMPACT OF LIFE STYLE CHANGES IMPACT LEVELS ON PLASMA LIPIDS AND BODY IRON STORES IN PRAGUE SUBURBAN COMMUNITY – THE DUBEČ STUDY 20 YEARS AFTER

K. Vonašková1, P. Kraml1, J. Střížová1, J. Potočková1, V. Rejšek1, R. Poleđinec, M. Anděl2

1Second Internal Clinic, Third Faculty of Medicine, Charles University and University Hospital Královské Vinohrady, Prague, Czech Republic, 2Laboratory for Atherosclerosis Research, Institute for Clinical and Experimental Medicine, Prague, Czech Republic

In this retrospective study we compared the concentrations of plasma lipids and body iron stores in the subgroup of subjects participating in the preventive cardiology project Dubeč in 1992-1995 and in 2014. Since body iron stores reflect the consumption of heme iron in meat and meat products, we focused on possible association between plasma ferritin and lipid parameters, insulin resistance and anthropometric markers. 34 adult men were randomly selected for this sub- analysis. After the initial examination, all subjects were exposed to community intervention based on dietary education and increase of physical activity. Plasma concentration of ferritin, glucose, insulin, total cholesterol (C), HDL-C, LDL-C, triglycerides (TAG) were measured. Statistical evaluation was performed using the STATISTICA program. After 20 years, there were significantly lower concentrations of plasma ferritin (p<0.001), TAG (p<0.001) and LDL-C (p<0.001) when compared to the initial levels. An increase was observed in waist circumference (p<0.001), WHR (p<0.003), insulinemia (p<0.03) and HOMA IR (p<0.003). BMI remained unchanged. Initially, plasma ferritin correlated with insulinemia (p<0.05). In conclusion, 20 years after cardiovascular preventive intervention in Prague suburban community Dubeč was performed, we see a significant decrease in total and LDL-C accompanied by drop of ferritin. We believe that this reflects adoption of healthy life-style and lower dietary intake of heme iron and saturated fat in meat products.

Supported by program PRVOUK 031 3. LF UK.

THE OBESITY PARADOX IN STROKE SURVIVORS

P. Wohlhaler1,2,3, F. Lopez-Jimenez1, A. Kracjovicheva1, M. Jozišova1, O. Mayer1, J. Vaněk1, J. Filipovsky1, E. M. Llano1, R. Cífková1,2,4

1Center for Cardiovascular Prevention of the First Faculty of Medicine, Charles University and Thomayer Hospital, Prague, Czech Republic, 2International Clinical Research Center, St Ann’s University Hospital, Brno, Czech Republic, 3Department of Preventive Cardiology, Institute for Clinical and Experimental Medicine, Prague, Czech Republic, 4Department of Cardiovascular Diseases, Department of Medicine, Mayo Clinic Rochester, Rochester, MN, USA, 5Second Department of Internal Medicine, Charles University, Center for Hypertension, Pilsen, Czech Republic, 6University of Texas Southwestern Medical School, Dallas, TX, USA, 7Department of Cardiology and Angiology, First Faculty of Medicine, Charles University, Prague, Czech Republic

While obesity is a risk factor for stroke and achieving normal weight is advocated to decrease stroke risk, the risk associated with obesity and weight loss after stroke has not been well established. We analysed the association of obesity at the time of stroke admission and weight loss after stroke with total mortality. We analysed 736 consecutive patients (66±11 years, 58 % male) hospitalized for their first ischemic stroke. Body weight at admission and at the outpatient visit were used in the analysis. After multivariate adjustment, obesity at admission was associated with lower mortality risk as compared with normal weight (HR 0.50, p=0.03). At the last outpatient visit (median follow-up time 16 months), 21 % of patients had lost >3 kg of weight. Stroke severity, heart failure, transient ischemic attack and depression after stroke were independently associated with significant weight loss. Weight loss >3 kg was associated with increased mortality risk (HR 5.87, p<0.001) independently of other factors. Similar results were seen when weight loss was defined as losing over 3 % of baseline weight, (HR 4.97, p=0.004). Weight gain >5 % of the baseline weight tended to be associated with better survival when compared with no weight change (log-rank test p=0.07). In conclusion, normal weight at hospital admission and weight loss after ischemic stroke are independently
THE IMPACT OF PHYSICAL ACTIVITY AND DIETARY MEASURES ON COMPONENTS OF THE METABOLIC SYNDROME IN OBESE CHILDREN

L. Zlatohlávek1, J. A. Hubáček2, M. Vrablič1, H. Pejšová1, V. Lánská2, R. Češka1
1Third Department of Internal Medicine, General University Hospital and First Faculty of Medicine, Charles University, Prague, Czech Republic, 2Center for Experimental Medicine, Institute for Clinical and Experimental Medicine, Prague, Czech Republic

The aim of the study was to monitor the importance of laboratory, anthropometric and genetic determination of the presence of risk factors for atherosclerosis, obesity, dyslipidemia and components of the metabolic syndrome in obese children and the response to dietary and regimen interventions in obese children. 353 pediatric patients (46% boys) aged 8–16 years, who took part in a one-month lifestyle intervention program (comprising a reduction of energy intake and a supervised exercise program consisting of 5 units per day, 50 min each) were examined with obesity and dyslipidemia. Standard biochemical methods, including Lp-PLA2 were applied, anthropometric measurements as well as genetic analyzes were performed. During the reduction program of children, there was a statistically significant decrease in all anthropometric indicators of overweight (P<0.001) and in lipid parameters, as well as LpPLA2. Carriers of the FTO G genotype and/or MC4R CC genotype lost significantly more body weight in comparison to the non-carriers (P<0.0009 for BMI). In conclusion, child obesity is an important social issue. After regimen interventions, there is weight loss, as well as improvement in biochemical parameters. There are individuals with obesity predispositions, as well as individuals with a better response to regimen interventions.

Supported by grant: NT 14152-3/2013.

CHANGE IN METABOLIC SYNDROME PARAMETERS ACCORDING TO INITIATION OF PRANDIAL GLUCAGON-LIKE PEPTIDE-1 RECEPTOR AGONISTS (GLP-1RA) LXISENATIDE IN OBESE TYPE 2 DM PATIENT WITH HISTORY OF METFORMIN INDUCED PHOTOTOXIC DERMATITIS

P. Žák, P. Stastná
Second Internal Department of Masaryk University and University Hospital of Svatá Anna Brno, Czech Republic

Guidelines of ADA/EASD recommend initiation of metformin therapy immediately after T2DM diagnosis. If there is no chance to initiate this therapy according to history of metformin adverse events, there is a limited offer of therapy targeted to metabolic syndrome parameters. Incretin antiglutamides are new agents and include inhibitors of dipeptidyl peptidase-4 (DPP4) and GLP-1RA. Clinical studies have demonstrated lowering of postprandial glycemia, glycosylated Hb (HbA1c) and decrease in body weight following the therapy with prandial GLP-1 RA lixisenatide. We demonstrate change in metabolic syndrome parameters: insulin (hepatic) sensitivity ISI (HOMA) and whole-body insulin sensitivity ISI (composite) following initiation of GLP-1 RA lixisenatide in obese type 2 diabetes patient with history of metformin induced phototoxic dermatitis. After a 12-h overnight fast, subject received a 75-g OGTT at 7.00 A.M. Blood samples were taken at 0, 30, 60, 90, and 120 min for the measurement of plasma glucose, insulin concentrations and C-peptide. A composite measure of whole-body insulin sensitivity that encompasses both hepatic and peripheral tissues can be derived by combining the preceding ISI during the OGTT with that obtained during the basal state. The whole body insulin sensitivity has been calculated in Matsuda index rule. After 6 months of lixisenatide therapy we observed significantly improved insulin (hepatic) sensitivity ISI-HOMA, whole-body insulin sensitivity, decrease in body weight and HbA1c.

Supported by grant No. NT 14027-3/2013 from IGA MH CR.