Homocysteine Mini-Conference
Poznań 2019

Runge Collegium, Poznań University of Life Sciences

September 26, 2019

Welcome

Dear Conference Participants,

We are delighted to welcome you to the Homocysteine Mini-Conference Poznań 2019 held at the Poznań University of Life Sciences. The main goal of the conference is to bring together people working at academic and research institutions who share common scientific interests, facilitate exchange of their latest research findings, to discuss emerging ideas and potential collaborations.

The Homocysteine Mini-Conference Poznań 2019 received the honorary patronage of the Rector of the Poznań University of Life Science.

We hope that the conference will facilitate scientific exchange and discussion of recent developments in the field of homocysteine biology and pathophysiology for both advanced and beginning researchers. We are pleased to host 36 participants, including 12 speakers, and 13 poster presenters. The participants are affiliated with University "Luigi Vanvitelli", School of Medicine, Naples, Italy; Rutgers-New Jersey Medical School, Newark, USA; the Poznań University of Life Sciences, the Poznan University of Medical Sciences, the Institute of Bioorganic Chemistry PAS, the University of Lodz, the University of Bialystok, and the Medical University, Lublin.

The conference is generously sponsored by Animalab, A.P. Instruments, Luminex, Merck, Q4Lab, Sanlab, and Sarstedt.

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Scientific Program

8:00 – 9:00	Registration and setting up posters
9:00 – 10:50	**Session 1** Chair Hieronim Jakubowski
9:00 – 9:10	Welcome by Hieronim Jakubowski
9:10 – 9:50	**Diego Ingrosso**, University "Luigi Vanvitelli" – School of Medicine Derangements of sulfur amino acid and one carbon metabolisms in chronic kidney disease
9:50 – 10:10	**Joanna Perła-Kaján**, Poznań University of Life Sciences Homocysteine thiolactone and PON1 activity are associated with mortality and graft failure in renal transplant recipients
10:10 – 10:30	**Jerzy Bełtowski**, Medical University, Lublin Paraoxonase 1 phenotype and protein *N*-homocysteinylation in patients with rheumatoid arthritis
10:30 – 10:50	**Joanna Mikołajczyk-Stecyna**, Poznań University of Life Sciences Effect of maternal nonalcoholic fatty liver disease and dietary choline intake on body mass and body composition in rat offspring
10:50 – 11:20	Coffee break
11:20 – 13:00	**Session 2** Chair Diego Ingrosso
11:20 – 12:00	**Hieronim Jakubowski**, Rutgers University, New Jersey Medical School; Poznań University of Life Sciences Homocysteine Modification in Protein Structure/Function and Human Disease
12:00 – 12:20	**Olga Włoczkowska**, Poznań University of Life Sciences Anti-*N*-homocysteine-protein autoantibodies, brain atrophy, and mild cognitive impairment: the VITACOG trial
12:20 – 12:40	**Joanna Suszyńska-Zajczyk**, Poznań University of Life Sciences Hyperhomocysteinemia during pregnancy causes neurological deficiencies in musculature and brain function in neonatal mouse pups
12:40 – 13:00	**Łukasz Witucki**, Poznań University of Life Sciences Phf8-mediated epigenetic dysregulation of mTOR signaling/autophagy increases amyloid beta levels and accelerates neurodegeneration in hyperhomocysteinemic and bleomycin hydrolase-deficient mice
13:00 – 14:00	Lunch
14:00 – 14:40	Poster session
14:40– 16:00	**Session 3** Chair Jerzy Bełtowski
14:40 – 15:00	**Krzysztof Brzeziński**, University of Białystok Metal-cation regulation of enzyme dynamics might influence the activity of S-adenosyl-L-homocysteine hydrolase
15:00 – 15:20	**Justyna Piechocka**, University of Lódź Application of GC-MS technique for quantification of homocysteine thiolactone in human urine
15:20 – 15:40	**Anna Malinowska**, Poznań University of Life Sciences Is gut microbiota composition associated with homocysteine and folate serum concentrations?
15:40 – 16:00	**Damian Skrypnik**, Poznan University of Medical Sciences The effect of a 12-week supplementation of multistrain probiotic on homocysteine concentration in women with obesity
16:10 – 16:30	Closing ceremony, poster and oral presentation award

Derangements of sulfur amino acid and one carbon metabolisms in chronic kidney disease

Diego Ingrosso, MD, PhD
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Sulfur amino acid metabolism is characterized by a strict connection among methionine-homocysteine cycle, transsulfuration pathway and one carbon metabolism. Two related compounds, homocysteine and hydrogen sulfide, are both involved in vascular homeostasis and involved in transsulfuration, *i.e.* the major pathway for both homocysteine metabolism and hydrogen sulfide biosynthesis, respectively. Hyperhomocysteinemia is a powerful cardiovascular risk factor. Homocysteine toxicity on the vasculature is mainly indirect, being mediated by its derivatives, interfering with various molecular mechanisms, including protein covalent modifications and DNA methylation (1). Hydrogen sulfide, on the other hand has been recently assessed as the third volatile vasodilator, after nitric oxide and carbon monoxide.

Hydrogen sulfide has drawn the attention in cardiovascular medicine because of its protective effects on the vasculature. Its metabolic derangement and, hence, potential involvement in the pathogenesis of hypertension and vascular damage, particularly in chronic kidney failure, does occur through mechanisms partly related to inflammation (2, 3). On the other hand, kidney failure represents a worldwide growing health emergency and a condition centered on cardiovascular risk factors and their mechanisms of action. Association of both traditional and renal specific risk factors - also including hyperhomocysteinemia - affects this complex pathologic condition. Several differences in sulfur metabolism have been so far characterized in kidney disease vs healthy individuals. Major alterations involve homocysteine, *S*-adenosylhomocysteine and hydrogen sulfide, as well as a number of other sulfur derivatives, which display the typical behavior of retention compounds. Lanthionine also accumulates in circulation in uremia, to partial satisfaction of criteria for classification among uremic toxins (4, 5). The effects of lanthionine have been assessed in zebrafish, where it has been shown that lanthionine determines significant alterations of larval cardiovascular development, together with behavioral modifications, which are partly reversible upon glutathione treatment (6).
Present findings, as a whole, support (a) the interpretation that hyperhomocysteinemia, as such, is a red flag in a disrupted circuit; (b) derangements of various sulfur compounds, thus also including lanthionine, may trigger inflammatory response and contribute to elicit cardiovascular damage in uremia.
After almost three decades of studies, homocysteine still holds great promise of new exciting issues to unravel.

1. Ingrosso D, et al. Lancet (2003) 361:1693-9.
2. Jankowski J, et al. Semin Nephrol (2014) 34:135-50.
3. Perna AF, et al. J Cell Biochem (2013) 114:1536-48.
4. Perna AF, et al. Biochimie (2016) 126:97-107.
5. Perna AF, et al. Toxins (2017). 9, pii: E26.
6. Perna AF, et al. Int J Mol Sci; (2018) Apr 29;19(5). pii: E1323.

Homocysteine thiolactone and PON1 activity are associated with mortality and graft failure in renal transplant recipients

Joanna Perła-Kaján[1]*, Kamila Borowczyk[2], Josephine LC Anderson[3], Rafał Głowacki[2], Olga Włoczkowska[1], Małgorzata Kupc[1], Stephan JL Bakker[3,4], Hieronim Jakubowski[1,5].

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Background: Patients with renal disease have dysregulated homocysteine (Hcy) metabolism and suffer from cardiovascular complications and mortality. A toxic Hcy metabolite, Hcy-thiolactone (HTL), is generated in the human body by methionyl-tRNA synthetase, turned over in the circulation by paraoxonase 1 (PON1), and cleared by the kidney. HTL has the ability to modify proteins, which impairs their structure/ function and renders them autoimmunogenic [1]. Recent findings indicate that HTL is a predictor of acute myocardial infarction in coronary artery disease patients [2]. However, it is not known whether HTL levels are related to outcomes in renal transplant recipients (RTR).

Aim: Our objectives were to examine: 1) baseline urinary HTL as a risk marker of cardiovascular/all-cause mortality and graft failure in RTR patients; 2) determinants of baseline HTL; 3) anti-*N*-Hcy-protein autoantibody levels in RTR patients *vs*. kidney donors as controls; 4) the role of PON1 in mortality and graft failure in RTRs.

Methods: We used an HPLC-based assay to quantify HTL in urine from RTR patients after kidney transplantation (n=680, 53±13-year-old, 59% male), healthy kidney donors before (Pre-D, n=297, 54±11-year-old, 46% male) and after kidney donation (Post-D, n=191). The levels of anti-*N*-Hcy-protein IgGs were quantified by ELISA using *N*-Hcy-albumin as an antigen. Paraoxonase and arylesterase (PhAcase) activities of PON1 were assayed spectrophotometrically with paraoxone and phenyl acetate as substrates, respectively.

Results: Baseline urinary HTL excretion was significantly reduced (median (range): 4.1 (0.01-2814) vs. 18.5 (0.01-4666) vs. 28.65 (0.01-2459); mean±SD: 26.2±135.5 vs. 48.2±279.4 vs. 75.7±212.6, p= 0.0071) while titers of serum anti-*N*-Hcy-protein autoantibodies were significantly increased in RTRs relative to Pre-D and Post-D (0.17±0.19 vs. 0.11±0.12, p=0.000). HTL levels in RTR patients were significantly associated with plasma folate (r=-0.0828, p=0.035), log[vitamin B_6] (r=0.085, *p*=0.032), logNtProBNP (r=-0.105, *p*=0.008), urinary sulfate (r=0.111, *p*=0.005) and thiosulfate levels (r=0.156, p<0.000). During median 6.8-year follow-up, Kaplan-Mayer analysis showed significantly better survival in RTR patients with higher urinary HTL levels compared with those lower levels (p=0.0481). Cox regression analysis showed that urinary HTL was associated with cardiovascular (HR 0.81, 95% CI 0.65-1.00, *p*=0.05) and all-cause mortality (HR 0.85, 95% CI 0.74-0.97, p=0.02) in a model adjusted for gender, and urinary protein excretion. HTL was also associated with graft failure (HR 0.62, 95% CI 0.39-0.98, p=0.04) in a model adjusted for gender and glomerular filtration rate. Kaplan-Mayer analysis showed significantly lower mortality and better graft survival in RTR patients with higher PhAcase activity compared with those lower activity (p<0.0363).

Conclusions: These findings suggest that negative outcomes in RTR patients are due to impaired renal clearance of HTL. The accumulation of HTL in the blood and tissues increases protein modification/damage, which induces an autoimmune response [1], manifested as significantly elevated anti-*N*-Hcy-protein autoantibodies in RTR patients, which in turn could contribute to negative outcomes of kidney transplantation.

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Paraoxonase 1 phenotype and protein N-homocysteinylation in patients with rheumatoid arthritis

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Background: Rheumatoid arthritis (RA) is a chronic, systemic autoimmune disease characterized by symmetrical inflammatory polyarthritis of peripheral joints. RA affects approximately 1% of the adult population with females being more frequently affected than males. Patients with RA are characterized by high risk of cardiovascular diseases but the underlying mechanism is incompletely understood. Paraoxonase 1 (PON1) plays an important role in inhibiting atherogenesis by hydrolyzing the toxic metabolite of homocysteine (Hcy), Hcy thiolactone.
Aim: To assess PON1 phenotype (192R/Q polymorphism, enzyme activity toward both synthetic substrates and Hcy thiolactone and enzyme concentration) as well as the level of protein N-homocysteinylation in patients with RA in comparison to healthy control subjects.
Methods: The study was performed in 74 patients with rheumatoid arthritis (60 females, 14 males) and 70 sex- and age-matched control subjects. Patients were classified according to DAS-28ESR score. Serum PON1 activity was measured toward 2 synthetic substrates, paraoxon, phenyl acetate and Hcy thiolactone. 192R/Q polymorphism was examined by double-substrate method. PON1 protein concentration was measured by ELISA.
Results: There was no difference in the distribution of PON1 RR, RQ and QQ genotypes between control and RA groups. PON1 concentration was similar in both groups as well. However, PON1 activities toward paraoxon, phenyl acetate, and Hcy thiolactone were lower in RA patients than in control subjects. Total Hcy concentration did not differ between groups whereas protein-bound Hcy thiolactone was higher in RA patients. When RA patients were categorized into high-activity (DAS-28ESR>5.1) and low-activity (DAS-28ESR>5.1) subgroups, PON1 activities toward all 3 substrates were lower and protein-bound Hcy thiolactone was higher in the high-activity subgroup. In low-activity subgroup, PON1 activity toward synthetic substrates as well as protein-bound Hcy thiolactone did not differ from control although PON1 activity toward Hcy thiolactone was still lower than in control group. Plasma lipids as well as apolipoprotein A-I and A-II concentrations did not differ between groups.
Conclusions: Rheumatoid arthritis is associated with reduced PON1 activity which results in enhanced protein N-homocysteinylation despite normal total Hcy concentration. PON1 deficiency and protein N-homocysteinylation may contribute to accelerated atherosclerosis in RA patients. Decrease in PON1 activity and protein N-homocysteinylation correlate with disease activity.

Effect of maternal nonalcoholic fatty liver disease and dietary choline intake on body mass and body composition in rat offspring

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Background: Both maternal metabolic status and nutrition during pregnancy and lactation may have programming effects on the metabolism of offspring; the effect is more pronounced in male sex.

Aim: To examine the role of dietary choline supply during pregnancy and lactation in rat dams suffering from nonalcoholic fatty liver disease (NAFLD) on body mass and body composition of their progeny.

Methods: The research protocol was approved by the local ethics committee. The study groups included the offspring of: 1. healthy dams receiving choline during pregnancy and lactation (the control group); 2. NAFLD dams receiving choline during pregnancy and lactation (NN); 3. NAFLD dams receiving choline during pregnancy and a choline-deficient diet during lactation (ND); 4. NAFLD dams receiving a choline-deficient diet during pregnancy and a supply of choline during lactation (DN); and 5. NAFLD dams receiving a choline-deficient diet during both pregnancy and lactation (DD). Body mass was assessed in male rats from each group on day 3 (3d), day 24 (24d), and day 90 (90d); body composition was assessed on day 24 (24d) and day 90 (90d). The differences were analyzed by using Student's *t*-test. *P* values < 0.05 were taken as significant.

Results: Body mass was significantly lower in the male offspring of the DD and DN groups than in the control group. Differences were observed at all times (3d: $p = 0.0023$; 24d: $p < 0.0001$; 90d: $p < 0.0001$). Moreover, body mass was significantly higher in the control group than in any of the other groups.

We observed differences in the body fat percentage and body lean percentage between the DD and control groups (fat: 24d: $p = 0.0155$; 90d: $p = 0.0118$; and lean: 24d: $p = 0.044$; 90d: $p = 0.0062$), the DD and NN groups (fat: 24d: $p = 0.0029$; 90d: $p = 0.0337$; and lean:24d: $p = 0.0017$; 90d: $p = 0.0343$), and the DD and DN groups (fat: 24d: $p < 0.0001$; 90d: $p = 0.0338$; and lean: 24d: $p = 0.0278$; 90d: $p = < 0.0001$) measured at days 24 and 90 of life. The DD group had the lowest level of body fat and the highest level of body lean tissue of all the groups.

Conclusions: Maternal NAFLD and dietary choline status during pregnancy and lactation affect body mass and composition in rat male offspring.

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Homocysteine Modification in Protein Structure/Function and Human Disease*

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Background: Epidemiological studies established that elevated homocysteine (Hcy), an important intermediate in folate, vitamin B_{12}, and one carbon metabolism, is associated with poor health, including heart and brain diseases. Earlier studies show that patients with severe hyperhomocysteinemia (HHcy), first identified in the 1960s, exhibit neurological and cardiovascular abnormalities and premature death due to vascular complications. Although Hcy is considered to be a non-protein amino acid, studies over the past two decades have led to discoveries of protein-related Hcy metabolism and mechanisms by which Hcy can become a component of proteins. One such mechanism involves metabolic conversion of Hcy to Hcy-thiolactone (HTL), which then modifies protein lysine residues in a process called *N*-homocysteinylation.
Aim: Our objectives were: 1) examine urinary HTL, a chemically reactive metabolite generated by methionyl-tRNA synthetase and cleared by the kidney, as a risk predictor of acute myocardial infarction (AMI) in a randomized clinical trial; 2) evaluate effects of B vitamins supplementation on HTL during a follow-up; 3) elucidate mechanisms and identify pathological consequences of protein *N*-homocysteinylation.
Methods: We analyzed urine samples from the WENBIT trial participants (n=2049, 69.8% men) and used statistical models to examine a value of HTL as a predictor of AMI (n=183) during 4.7-year follow-up. We used protein chemistry and mass spectrometry methods, gene expression analysis (proteomics, transcryptomics), and HPLC-based assays to quantify Hcy-thiolactone, *N*-Hcy-protein and identify specific *N*-Hcy-Lys residues in proteins to study protein-related Hcy metabolism in human cell cultures, mouse models, and humans.
Results: Cox regression analysis showed that baseline urinary HTL/creatinine predicted AMI during follow-up (hazard ratio = 1.58, 95% confidence interval = 1.10–2.26, P = 0.012 for trend; adjusted for age, gender, tHcy). B vitamin/folate supplementation did not affect HTL/creatinine levels and its association with the AMI risk. Tissue culture studies showed that HTL induced pro-atherogenic changes in gene expression in human vascular endothelial cells and bioinformatic analyses showed that Hcy-thiolactone, but not Hcy, was strongly associated with AMI. Proteomic/mass spectrometry analyses identified two proteins important for function of the cardiovascular system, fibrinogen and collagen, whose functional lysine residues are *N*-homocysteinylated in HHcy: FGA α-K562Hcy, which impairs fibrin clot lysis in *CBS*$^{-/-}$ humans and Col1A1 reside K160Hcy, which prevents normal collagen crosslinking in *Cbs*$^{-/-}$ mice.
Conclusions: These findings support a hypothesis that metabolic conversion of Hcy to HTL and concomitant protein *N*-homocysteinylation underlie the involvement of HHcy in cardiovascular disease.
Support: National Science Center (2016/23/B/NZ5/00573, 2018/29/B/NZ4/00771) and American Heart Association (17GRNT32910002).

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Anti-*N*-homocysteine-protein autoantibodies, brain atrophy, and mild cognitive impairment: the VITACOG trial

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Background: Homocysteine (Hcy) is a risk factor for cognitive impairment and Alzheimer's disease (AD). Plasma concentrations of Hcy can be lowered by dietary administration of B vitamins. Homocysteine thiolactone (HTL) reacts with protein lysine residues, generating toxic *N*-Hcy-protein aggregates, which are autoimmunogenic. Anti-*N*-Hcy-protein autoantibodies are associated with increased risk of stroke. However, it is not known whether these autoantibodies are associated with mild cognitive impairment (MCI) and AD.

Aims: The main aim was to examine whether anti-*N*-Hcy-protein autoantibodies can predict brain atrophy and MCI in humans and to explore potential effect modification by B vitamins. The secondary aim was to study determinants of anti-*N*-Hcy-protein autoantibodies.

Methods: This was a double-blind, single center study, which included participants with MCI (n=196, 76.8-year-old, 60% female), randomly assigned to two groups, one treated with folic acid (0.8 mg/d), vitamin B_{12} (0.5 mg/d) and B_6 (20 mg/d) (n=97), the other with placebo (n=99) for 24 months. To quantify brain atrophy, a subset of participants (n=187) had cranial MRI scans at the start and the end of the study. Cognitive abilities were analyzed by standard tests. Serum anti-*N*-Hcy-protein autoantibodies were quantified by ELISA.

Results: Levels of anti-*N*-Hcy-protein autoantibodies were similar for men and women. B vitamin treatment did not affect levels of the autoantibodies. Baseline anti-*N*-Hcy-protein autoantibodies tended to be negatively correlated with the brain atrophy rate ($r = -0.136$, $p = 0.106$; $n = 141$). The correlation between anti-*N*-Hcy-protein autoantibodies and the brain atrophy rate was stronger at the end of the study in the placebo ($r = -0{,}289$, $p = 0{,}016$; $n = 70$), but not in the treatment group ($r = 0.016$, $p = 0.220$; $n = 71$). Cognitive testing showed that levels of anti-*N*-Hcy-protein autoantibodies were higher in those participants who scored better on semantic memory (Graded Naming Test, $p = 0.039$) and global cognition tests (Clinical Dementia Rating, CDR, $p = 0.010$; Mini mental score, $p = 0.032$). In contrast, although baseline anti-*N*-Hcy-protein autoantibodies were not associated with executive function (Clox delta $p = 0.700$), the autoantibodies at the end of study were associated with Clox delta regardless of the treatment (placebo group, $p = 0.032$; treatment group, $p = 0.049$). Lower levels of anti-*N*-Hcy-protein autoantibodies were associated with more severe MCI as shown by CDR. In multiple regression analysis anti-*N*-Hcy-protein autoantibodies were associated with hemoglobin ($\beta = 0.25$, $p = 0.0003$), Mini mental score ($\beta = 0.15$, $p = 0.029$) and coronary artery bypass graft ($\beta = -0.20$, $p = 0.003$).

Conclusions: Anti-*N*-Hcy-protein autoantibodies appear to protect from brain atrophy and the loss of cognitive function: higher level of autoantibodies were associated with reduced atrophy, better scores in cognitive testing, and less severe MCI.

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Hyperhomocysteinemia during pregnancy causes neurological deficiencies in musculature and brain function in neonatal mouse pups

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Background: Hyperhomocysteinemia (HHcy) is associated with neurological deficiencies including impairments in cognition and the neuromotor system. However, it is not known how exposure to HHcy during pregnancy affects these functions in neonatal pups.

Aim: Our objective was to test a hypothesis that dietary HHcy causes cognitive and neuromotor impairments in neonatal pups.

Methods: Wild type C57BL/6J mice (6-week-old, n=16) were divided into two groups: 1. HHcy-group (4 females and 4 males) that received 1% methionine in drinking water for 8 weeks, 2. Control group (4 females and 4 males) provided with plain water. At 3.5 months of age, mice in each group were mated in pairs and the dietary treatments were continued. The weight and survival rate of pups produced from those matings were determined. The motor function was examined between post natal day (PND) 6 and 30 by using the hind limb, surface righting, negative geotaxis, grip strength, and spontaneous activity tests. The short-term memory was assessed by studying spontaneous alternation behavior in the Y-maze test.

Results: High Met diet significantly elevated urinary tHcy in parental females (251±130 vs. 48.8±2.2 μM*, p*=0.047) and males (137±51 vs. 49.9±14.2 μM, *p*=0.046*)*. HHcy led to breeding impairments: slower breeding rate, in-utero fetal death, reduced pups' body weight, and increased mortality. Pups born from the HHcy parents exhibited significantly elevated urinary tHcy (1.26- and 1.85-fold at PND 6 and 30; *p*=0.028 *and p*=4E-05*,* respectively). Hind limb strength was reduced in HHcy (n=36) *vs.* control mice (n=45), demonstrated by a decrease in hanging score similar for both sexes (2.8±0.5 *vs.* 3.8±0.3, *p*=1E-19). Hind limb suspension time was significantly shorter in HHcy male, but not female, pups (27.9±10.6s (n=24) *vs.* 43.6±12.7s (n=16); *p*=6E-04). Male, but not female, HHcy pups manifested slower surface righting at PND 6 (25.2±17.8s *vs.* 7.4±4.9s; *p*=0.002), significantly weaker grip at PND13 (mean angle at fall 103±18º *vs.* 121±20º; *p*=0.007) and decreased spontaneous activity in a cylinder at PND 30 (mean number of rears 17.4±7.3 *vs.* 21.3±4.3; *p=0.038*) compared to control mice. HHcy females, but not males at PND 30 showed significantly decreased spontaneous alternation in Y-maze (49.4±9.6 *vs.* 59.7±9.4; *p*=0.007) and significantly higher number of doubles (1.88 *vs.* 0.47; *p*=3,5E-05) compared to control mice; however, there was no change in the animals' activity in the Y-maze test.

Conclusions: Dietary HHcy affects the cognition and the neuromotor system in neonatal pups in a sex-dependent manner. It impairs memory and cognition in female pups and weakens muscle strength in male pups.

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Phf8-mediated epigenetic dysregulation of mTOR signaling/autophagy increases amyloid beta levels and accelerates neurodegeneration in hyperhomocysteinemic and bleomycin hydrolase-deficient mice

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Background: Hyperhomocysteinemia (HHcy) is associated with cognitive impairment and neurodegeneration including Alzheimer`s disease (AD). The mechanistic target of rapamycin (mTOR) signaling and autophagy pathways and the Hcy-thiolactone-hydrolyzing enzyme bleomycin hydrolase (Blmh) are linked to AD, but underlying mechanisms are not fully understood.

Aim: Our objective was to test a hypothesis that HHcy causes amyloid beta (Aβ) accumulation and cognitive impairment *via* epigenetic effects on brain mTOR signaling and autophagy.

Methods: We generated a new AD model: *Blmh*$^{-/-}$5xFAD mice harboring a human transgen with mutations in the *APP* and *PSEN1* genes. HHcy was induced in 1-month-old mice by providing 1% methionine in drinking water for 4 and 12 months. Control mice received plain water. Neurological deficiencies were assessed by behavioral testing of 1-year-old mice. Brain Aβ levels, proteins of the mTOR/autophagy pathways, and the epigenetic regulators *Phf8* histone demethylase and Lys20 methylation in histone 4 (H4K20me1), were quantified by Western blotting.

Results: *Blmh*$^{+/+}$5xFAD and *Blmh*$^{-/-}$5xFAD fed with an HHcy high methionine diet for 1 year showed significantly impaired activity (beaker test), cognition (novel object recognition test), and locomotion (gait test) relative to *Blmh*$^{+/+}$5xFAD mice fed with control diet. *Blmh*$^{+/+}$5xFAD and *Blmh*$^{-/-}$5xFAD fed with an HHcy diet showed significantly increased accumulation of Aβ, phosphorylated forms of mTOR, decreased autophagy markers Beclin1, Atg5, Atg7 relative to *Blmh*$^{+/+}$5xFAD mice fed with control diet. In HHcy mice we observed significantly decreased level of the histone demethylase Phf8 and increased H4K20me1 which are involved in mTOR regulation. Similar changes in the Phf8/H4K20me1 and the mTOR/autophagy pathways were observed in 4-month-old *Blmh*$^{-/-}$5xFAD relative to *Blmh*$^{+/+}$5xFAD mice.

Conclusion: Epigenetic up-regulation of mTOR signaling and down-regulation of autophagy by HHcy, mediated by increased H4K20 methylation, causes Aβ accumulation and the cognitive and neuromotor impairments in mice. Similar changes in the Phf8/H4K20Me1->mTOR->autophagy pathway induced by Blmh deficiency suggest the involvement of Hcy-thiolactone in epigenetic dysregulation of mTOR/autophagy and neurological impairment.

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Metal-cation regulation of enzyme dynamics might influence the activity of *S*-adenosyl-L-homocysteine hydrolase

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SAM-dependent methylation generates equimolar amounts of *S*-adenosyl-L-homocysteine (SAH), which is a potent negative-feedback inhibitor of SAM-dependent methyltransferases; its intracellular accumulation suppresses the SAM-dependent processes. Therefore, its concentration has to be precisely controlled. In all organisms, this task is fulfilled by two distinct enzymes: 5'-methylthioadenosine/SAH nucleosidase (mtnN) and/or SAHase. The former enzyme hydrolyzes SAH to adenine and *S*-ribosyl-L-homocysteine, whereas SAHase catalyzes reversible breakdown of SAH to adenosine and L-homocysteine. Depending on the organism, the genome encodes both enzymes or only one of them.

Numerous genomes of eucaryotic and procaryotic organisms lack any copy of the *mtnN* gene, however, a single or muliple copies of the gene encoding SAHase are present in these organisms. In these organisms SAHase is the only enzyme involved in the regulation of SAM-dependent methylation reactions SAHases are typicaly homotetrameric proteins, where up to 500 amino acid residues subunit is folded into three domains: the substrate- and cofactor-binding domains, and a smaller C-terminal domain. Each subunit of the active enzyme binds one NAD^+ molecule in the cofactor-binding domain and one substrate/product molecule in the substrate-binding domain. The two principal domains are connected by a hinge element. During the hydrolytic cycle, the enzyme oscillates between two conformational states: open and closed.

The activity of SAHase can be influenced *via* regulation of the protein dynamics, i.e. oscillations between two conformational states during the catalytic cycle. The enzymatic activity strongly depends on the type of monovalent cation coordinated in the area of the hinge region, which strongly influences the transformation from the open to the closed state. The K^+ ion, but not other alkali cations, enables unique dynamic properties (domain movement) of the enzyme to ensure its maximum catalytic activity. The K^+ ion stabilizes the enzyme-substrate complex in the closed conformation for a time interval required to complete the catalytic cycle. Thus, SAHase activity can be influenced *via* regulation of the protein dynamics, which depends on the type of the coordinated ion. It was also shown that transition metal ions have a potent inhibitory effect on SAHase. They bind in a different site from the monovalent cation binding site, between two major domanins domains Their coordination switches the gate to a shut state and inhibits the enzyme by arresting the protein in its closed conformation.

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Application of GC-MS technique for quantification of homocysteine thiolactone in human urine

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Background: Investigation of the role of homocysteine thiolactone (HTL) in living organisms began in the 70's. Despite many years of research work, its physiological and pathological role in human body still remains unclear. So far, it has been recognized that urinary HTL can act as a risk predictor of acute myocardial infarction in patients with coronary artery disease independent from other established risk factors and plasma homocysteine [1]. Moreover, merely few methods enabling determination of HTL in human biofluid have been developed. Therefore, more robust and versatile platforms for comprehensive assessment of HTL content in various biological samples are still needed to facilitate quantification of the target analyte.
Aim: The main aim of our current studies has been focused on providing new powerful analytical tools for HTL determination. Since gas chromatography – mass spectrometry (GC-MS) is one of increasingly popular technique but shows a negligible extent of utility of HTL analytics, we have decided to extend its application.
Methods: Two new assays for the determination of urinary HTL in the form of its volatile isobutyl chloroformate [2] or trimethylsilyl derivative [3] by GC-MS have been elaborated for the first time. In each case sample preparation procedure involves three critical steps including isolation (liquid-liquid extraction), derivatization and lyophilization followed by dilution of the residue and chromatographic separation.
Results and conclusions: An attractive and high-throughput GC-MS assays were proven to be an excellent tools for the determination of HTL in human urine samples. Despite some limitations, elaborated methods could be considered as a valuable analytical tools useful for thoroughly studies of the physiological and pathological role of HTL in human body.

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Is gut microbiota composition associated with homocysteine and folate serum concentrations?

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Background: Gut microbiota may be a source of folates in humans, as it is produced by some species of gut bacteria—mainly *Bifidobacterium* and *Lactobacillus*. The composition of human gut microbiota depends on dietary intake and health status. Blood homocysteine concentration depends on age, sex, and diet. Folic acid supplementation lowers plasma homocysteine. We hypothesize that plasma homocysteine and folate concentration depend not only on these factors, but also on the potential of gut microbiota to produce folates, and that this potential depends on the diet of the host.
Aim: To assess the effects of diet, body composition, the potential of gut microbiota to produce folates, and gut microbiota composition on the concentration of plasma homocysteine and folate.
Methods: A group of 200 men and women, aged 31 to 50 years old, participated in the study. Their diet was assessed using three-day dietary records. Plasma folate was assessed using the ELISA method and plasma homocysteine was determined with the use of HPLC-UV method. To estimate the potential of gut microbiota to produce folate, cultures containing feces, L-glutamine, and 4-aminobenzoic acid (substrates for folate production) were incubated under anaerobic conditions for 24 hours. The concentration of folate before and after incubation were measured with the ELISA method. Bacterial DNA was isolated from the feces of the participants and microbiota composition was determined using metagenomic sequencing of the V3–V4 region of the 16S rRNA gene on the MiSeq Illumina platform.
Results: Plasma homocysteine (tHcy) concentration correlated positively with fat-free mass percentage ($r = 0.18$) and negatively with general diet quality ($r = -0.21$) and intake of vitamin B_1 ($r = -0.15$), B_2 ($r = -0.16$), B_6 ($r = -0.16$), vegetables ($r = -0.16$), and fruits ($r = -0.14$). Serum tHcy and serum folate did not differ between people having microbiota with higher and lower potential to produce folates (10.8 µM vs. 11.2 µM and 21.4 ng/ml vs. 20.7 ng/ml, respectively). The ability of gut microbiota to produce folates was not correlated with a relative abundance of *Bifidobacterium* genus, but correlated positively with the *Bacteroidetes* ($r = 0.27$) and negatively with the *Firmicutes* ($r = -0.28$) phyla. Abundance of these bacteria was not associated with serum tHcy and folate.
Conclusions: Diet and body composition is associated with plasma homocysteine concentration. Gut microbiota is not a determinant of serum folate or homocysteine in human.

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The effect of a 12-week supplementation of multistrain probiotic on homocysteine concentration in women with obesity

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Background: Increased blood homocysteine (Hcy) level is an independent risk factor for cardiovascular diseases. A promising intervention to lower serum Hcy concentration is supplementation with probiotic bacteria. It is supposed that probiotics may serve as an effective natural therapeutic solution for reducing cardiometabolic risk.

Aim: The aim of the trial was to investigate the effect of a 12-week supplementation of a multispecies probiotic preparation on Hcy concentration, oxidative stress, inflammation and lipid profile.

Methods: A population of 50 obese women (aged 45-70 years) underwent this randomized double-blind placebo-controlled study. Subjects were randomized into two groups taking either a multispecies probiotic supplement (n=25) or placebo (n=25) for 12 weeks. The supplement contained nine bacterial strains in an amount of $2.5x10^9$ CFU/g. Fasting blood samples were collected at baseline and at completion of 12-weeks long intervention.

Results: Comparing to the baseline values, subjects in probiotic group presented a significant decrease in Hcy ($p<0.0001$), TAS ($p=0.0076$), TNF-α ($p=0.0001$), total cholesterol (TC) ($p=0.0020$), LDL-cholesterol ($p=0.0149$) and triglyceride ($p=0.0140$) levels. Comparative analysis revealed, that the change (Δ) of Hcy ($p=0.0263$), TNF-α ($p=0.0002$), TC (0.0472) and TAS ($p=0.0032$) level were statistically significant in the probiotic group, compared to those shown in the placebo group. Comparative analysis of TNF-α concentration and TAS level at the completion of the intervention period revealed a statistically significant difference between groups ($p=0.0070$; $p=0.0017$ respectively).

Conclusions: The 12-week multispecies probiotic - Ecologic®BARRIER supplementation effectively reduced Hcy concentration, inflammation, oxidative stress, and improved lipid profile. By modification of these risk factors, probiotic bacteria used in this trial showed multidirectional effects and effective reduction of the cardiometabolic risk in obese women.

P 01

Dietary hyperhomocysteinemia and major urinary proteins expression in mice

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Background: Major urinary proteins (MUPs) are small pheromone binding proteins, excreted in urine at high concentrations, important for sexual signaling in mice. Previously we demonstrated that total urinary MUP levels were reduced ~2-fold in 8-week-old *Cbs*$^{-/-}$ mice *vs*. *Cbs*$^{+/-}$ controls, both females (2.3 ± 1.1 *vs*. 4.9 ± 1.5 mg protein/mM creatinine, p=0.056) and males (5.9 ± 2.0 *vs*. 12.0 ± 0.9 mg protein/mM creatinine, p<0.01). A male-specific MUP20 also called „darcin" was present in *Cbs*$^{-/-}$, but not in *Cbs*$^{+/-}$, female urine. This changes were also observed at protein and mRNA level in mouse livers.

Aim: The aim was to examine MUPs expression in C57BL/6J mice with dietary hyperhomocysteinemia (HHcy).

Methods: We analyzed MUP expression in livers and MUP concentration in urine of C57BL/6J mice which dietary HHcy. To induce HHcy mice were provided with 1% L-methionine in drinking water for 4-12 weeks starting at 4 weeks of age. Control mice drank water without methionine.

Results: We did not observe significant changes in MUP level in livers of 8-week-old HHcy mice compared with control animals, both female and male. However, MUP concentration in urine of HHcy females was significantly lower than in control females (1.47-fold ; p=0.01). MUP expression in livers of 16-week-old females was also lower than in controls (3.36-fold p<0.000) but there were no significant differences between these groups in MUP concentration in urine. Darcin was absent in urine and livers of females fed with a HHcy diet, both 8- and 16-week-old.

Conclusions: These findings suggest that dietary HHcy reduces MUP expression in livers of 16-week-old female, but not male, mice. However, HHcy affects MUP levels in urine of 8-week-old females but not in older mice.

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P 02

Homocysteine and homocysteine thiolactone dysregulate autophagy in murine neuroblastoma cells N2a-APP$_{Swe}$

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Background: Hyperhomocysteinemia (HHcy) and attenuated autophagy are associated with Alzheimer's disease (AD). HHcy inhibits autophagy which contributes to the accumulation of amyloid beta (Aβ) in brains of HHcy mice model (*Cbs*$^{-/-}$). Our preliminary studies show that HHcy upregulates mTOR pathway and inhibits autophagy in the mouse brain. Because homocysteine thiolactone (HTL) modifies proteins generating *N*-homocysteinylated proteins which tend to aggregate, we hypothesize that treatments with HTL will have a greater effect compared with homocysteine (Hcy) treatment on deregulation of autophagy.

Aim: Our aim was to compare effects of Hcy and HTL on autophagy in Aβ overproducing murine neuroblastoma cells N2a-APP$_{Swe}$.

Methods: N2a-APP$_{Swe}$ cells harbor the human amyloid precursor protein (APP) gene with K670N and M671L Swedish mutation. Cells were treated with Hcy or HTL (0.1 mM or 1 mM) for 24h. Cell protein extracts were analyzed by Western Blot. To measure mean autophagy intensity after 24h of Hcy or HTL treatment, cells were either starved for 4 hours to induce autophagy or kept under fed conditions, and then stained with the anti-LC3/Alexa Fluor®555 conjugated antibody. Signals were acquired using the Muse™ Cell Analyzer (Luminex).

Results: Treatment with Hcy and HTL significantly elevated levels of monomethylated lysine 20 in histone 4 (H4K20me1), mTOR protein, phosphorylated mTOR (S2448) as well as phosphorylated Ulk1 (S757). We also found significantly reduced levels of Atg 7, Atg 5 and Beclin-1 in Hcy- and HTL-treated cells. However, treatments with HTL appeared to caused more pronounced changes relative to Hcy in phospho-mTOR (1.7 ± 0.2 *vs*. 3.1 ± 0.5, $p<0.05$), H4K20me1 (1.9 ± 0.2 *vs*. 3.1 ± 0.4, $p<0.02$), and Beclin-1 (0.7 ± 0.1 *vs.* 0.3 ± 0.05, $p<0.02$). There was also a decline in autophagosome associated LC3-II protein level in starving cells treated with both Hcy and HTL.

Conclusions: The dysregulation of autophagy by Hcy and HTL may lead to accumulation of toxic aggregates which provides a mechanism explaining the neurodegeneration associated with HHcy.

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P 03

Analysis of risk factors of vascular diseases in migraine patients

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Background: Migraine is one of the most common neurological diseases. It is divided into two main clinical subtypes: migraine with aura (MA) and migraine without aura (MO). Numerous meta-analyses underline that MA doubles the risk of ischemic stroke. The association is stronger among younger adults, especially women and may be caused by a numerous factors, e.g. elevated homocysteine (Hcy) and matrix metalloproteinase-9 (MMP-9) concentration.

Aim of the study: The aim of the study was to analyze Hcy and MMP-9 plasma concentration, *MTHFR* C677T and *MMP9* C1562T polymorphisms, and clinical features of migraine.

Material and methods: The study included 80 female migraine patients (MA: 40, MO: 40) and 80 healthy women as controls. Mean age of participants was 35±12 years. The high resolution melting (HRM) analysis and Sanger sequencing were used for genotyping, high performance liquid chromatography with electrochemical detection (HPLC/EC) and ELISA for determining Hcy and MMP-9 plasma level, respectively.

Results: There were no significant differences in Hcy and MMP-9 concentration between MA, MO and controls. The TT *MTHFR* C677T genotype was more common in MA patients as compared to MO and controls. Moreover, in all groups the TT genotype of *MTHFR* C677T was associated with the higher Hcy level, while the CT *MMP9* C1562T with higher MMP-9 level as compare to CC genotypes. MA and MO patients with the TT *MTHFR* C677T genotype had longer and more frequent migraine attacks than patients with the CC genotype. Patients with longer migraine attacks had also higher MMP-9 concentration.

Conclusion: The TT *MTHFR* may be a risk factor for MA. Polymorphisms in *MTHFR* and *MMP9* genes are associated with the Hcy and MMP-9 concentrations.

P 04

Label-free proteomic analysis reveals changes in atherosclerosis signaling and red-ox homeostasis in the kidney of cystathionine β-synthase deficient mice

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Background: Homocysteine (Hcy) arises from the metabolism of methionine (Met). Cystathionine β-synthase (CBS) catalyzes the first step of the transsulfuration pathway that affords cysteine. Human CBS deficiency causes severe hyperhomocysteinemia (HHcy) and diverse clinical manifestations, including oxidative stress and pathologies in cardiovascular system. However, mechanisms underlying these are not fully understood.

Aim: We hypothesize that CBS deficiency induces changes in gene expression that impair kidney homeostasis and can lead to changes in red-ox homeostasis and thrombotic complications.

Methods: To identify the genes involved and gain an insight into functions of *Cbs* deficiency we analyzed the kidney proteome of $Cbs^{-/-}$ (n= 14) and $Cbs^{+/+}$ mice (n= 14). Using label-free relative quantitative mass spectrometry approach mouse kidney proteomes were analyzed. Proteins with a minimum of 2 identified peptides and *p* values <0.05 were considered as differentiating. Bioinformatic analyses were carried out using DAVID resources and Ingenuity Pathway Analysis software.

Results and Conclusions: We identified 87 kidney proteins whose expression was significantly altered as a result of the *Cbs* gene inactivation. 72 were up-regulated and 15 down-regulated. The most striking features were upregulation of heptaglobin (Hp) and down regulation of Acyl-coenzyme A synthetase (Acsm1), in the $Cbs^{-/-}$ mice. The GO analysis of the biological processes revealed that the differentially expressed proteins participated in diverse processes such as glutathione and lipid metabolism. Top canonical pathways affected by *Cbs* inactivation was atherosclerosis signaling (APOA4, APOE, CLU, COL18A1, COL1A1, COL1A2, SERPINA1) and glutathione redox reactions I (GPX3, GSTP1, PRDX6). Proteins affected by CBS deficiency were significantly enriched in 6 molecular pathways. The three top-scoring networks were: „Connective Tissue Disorders, Dermatological Diseases and Conditions, Developmental Disorde”(score=40), „Amino Acid Metabolism, Drug Metabolism, Protein Synthesis”(score=40) and „Cardiovascular Disease, Cellular Compromise, Inflammatory Response”(score=35). These findings suggest that CBS deficiency has an effect on kidney proteome. Deregulation of genes involved in red-ox reactions shows that CBS deficiency increases oxidative stress in kidneys and impairment of atherosclerosis signaling could explain at least in part pathologies in cardiovascular system observed in HHcy.

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P 05

Associations between serum folate concentration, body weight, body composition, and BMI

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A link has recently been found between one-carbon metabolism and body weight and body composition, suggesting that folate may account for body mass and for lipid metabolism.

The aim of this study was therefore to analyze whether serum folate concentration is associated with body weight, body mass index (BMI), percentage body fat, waist circumference, and hip circumference.

Four hundred subjects aged 20–40 were enrolled in Poznań, Poland between 2016 and 2018. Weight and height were measured using an electronic scale and a stadiometer, respectively. BMI was calculated as body weight in kilograms divided by height in meters squared. Fat mass and lean body mass were determined using whole-body air-displacement plethysmography (BodPod, Cosmed, Italy). Hip and waist circumferences were measured using nonelastic tape. Food intake was assessed using three-day food records. Serum folate concentrations were measured using an enzyme-linked immunosorbent assay method (ELISA), following the manufacturer's protocol. Folate intake was calculated based on food composition tables using the Diet 5.0 program (National Food and Nutrition Institute, Poland). To determine the associations between serum folate concentration and other variables, we used multiple regression with adjustments for age, sex, misreporting, energy, and folate intake.

The mean body weight was 78.57 ± 18.14 kg, the mean BMI was 25.96 ± 5.28 kg/m2, and the mean percentage fat was 29.20% ± 10.78%. The median folate concentration was 36.46 ng/ml. Folate serum levels were negatively associated with body weight ($p < 0.01$), BMI ($p < 0.05$), body fat percentage, and waist circumference ($p < 0.001$). There was no association between folate concentration and hip circumference.

Our findings showed that serum folate concentration is associated with body weight and body composition. Higher folate intake could have a protective effect against obesity, but further studies are necessary to investigate the underlying mechanism.

The authors declare that they have no conflict of interests.

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P 06

One-carbon metabolism in postmenopausal women with metabolic syndrome: A case-control study

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Background: Abnormal concentrations of one-carbon metabolism biomarkers are well-known risk factors for cardiovascular diseases. Moreover, changes in one-carbon metabolism may lead to the accumulation of fat in the liver. This is one of the possible mechanisms linking one-carbon metabolism with metabolic syndrome (MetS).
Aim: The aim of this study was to determine the relationship between one-carbon metabolism, lipid metabolism, body composition, and MetS in postmenopausal women.
Methods: A case-control study of one hundred and thirty-one postmenopausal women was performed. Body composition was determined by plethysmography. Serum lipid profile was measured using the colorimetric method. Betaine, choline, trimethylamine (TMA), and trimethylamine-N-oxide (TMAO) levels in plasma were performed using liquid chromatography/mass spectrometry with electrospray. The total homocysteine (tHcy) levels in plasma were measured using high-performance liquid chromatography. MTHFR genotype analysis was performed with the use of single-tube TaqMan SNP Genotyping Assay. Anthropometric and atherogenic indexes were calculated.
Results: There were no differences in the concentrations of tHcy, betaine, choline, TMA, and TMAO between women with and without MetS. Subjects with higher betaine levels (above the median value) had lower waist-to-height ratios (WHtR) and higher choline level. A lower level of TMA (below the median value) was associated with higher waist circumference, higher waist-to-hip (WHR), and higher WHtR, higher fat mass (FM), as well as with choline and lower fat-free mass (FFM). MTHFR genotype was not associated with tHcy, lipid profile, or anthropometric and atherogenic indices. Moreover, women with at least one minor allele had lower FM and higher FFM. TMA was negatively correlated with WHR in the group of women with MetS and positively correlated with low-density lipoproteins in women without MetS. Moreover, TMAO was positively correlated with body mass and atherogenic indices, and betaine was negatively correlated with body mass in women without MetS.
Conclusions: MetS is not associated with the level of one-carbon metabolites in this study group. Neither the MTHFR genotype nor the tHcy level in plasma were associated with the lipid profile or the anthropometric or atherogenic indices. One-carbon metabolites are associated with body composition in postmenopausal women with and without MetS.

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Key words:
One-carbon metabolism, homocysteine, lipid metabolism, postmenopausal women, metabolic syndrome

P 07

Nutritional management of homocystinuria

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Background: Classic homocystinuria (HCU) is an inherited disorder of methionine metabolism caused by a deficiency of cystathionine beta synthase enzyme. The main symptoms of HCU include ectopia lentis or severe myopia, excessive height, scoliosis, long limbs, thromboembolism, and developmental delay. There are several gene variants which can lead to this phenotype, with CBS, ILE278THR being the most common one. The major aim of HCU treatment is to lower the plasma total homocysteine (tHcy) level into the normal range.

Aim: This poster provides information on nutritional management of homocystinuria (HCU).

Results and conclusions: There are two main nutritional strategies for lowering plasma tHcy levels used in pyridoxine-responsive and nonresponsive patients. In pyridoxine-responsive patients, it is recommended to use pyridoxine in pharmacological doses alongside folate supplementation; vitamin B_{12} deficiency should also be corrected. In patients not responsive to pyridoxine, biochemical targets can be achieved using a low-methionine diet that includes a methionine-free amino acid formula and betaine supplementation. Methionine occurs in all protein-containing foods, with the highest amounts in eggs, fish, meat, and dairy products. This poster summarizes effective dietary strategies in HCU treatment.

P 08

Hyperhomocysteinemia affects translation, protein folding and post-translational modifications, apoptosis, oxidative stress, and TORC1 signaling in *Saccharomyces cerevisiae*

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Background: Hyperhomocysteinemia (HHcy) inhibits growth and is cytotoxic to bacterial, yeast, and mammalian cells. In humans, HHcy a risk factor for heart and brain diseases. However, the mechanisms underlying HHcy toxicity are not fully understood. We hypothesize that the dysregulation of cellular proteostasis and modification of protein structures by homocysteinylation are involved in the toxicity of HHcy.

Aim: To identify proteins and biological pathways affected by HHcy by analyzing yeast cellular proteome and protein homocysteinylation.

Methods: Cultures of the yeast *S. cerevisiae* (BY4742, a lysine auxotroph derived from S288C) were treated with Hcy (1 - 10 mM, 3 h, 24 C). Cellular proteomes were analyzed using SILAC, iTRAQ, on a Q Exactive mass spectrometer. PANTHER and STRING resources were used for bioinformatic analyses. mRNA was quantified by RT-qPCR, protein levels and post-translational modifications by Western-blot.

Results: We identified 70 HHcy-responsive yeast proteins, of which 38 were up-regulated (1.06-3.58-fold) and 32 were down-regulated (0.21-0.88-fold). Up-regulated proteins are involved in amino acid biosynthesis (CYS4, SER1, HIS7, ARG4), vitamin B6 (PDX3) and red-ox metabolism (DUG1, GND1). Down-regulated proteins are involved in ribosome biogenesis and translation (RPP0, YEF3, TEF4, TEF1), protein folding (MSC82, FPR1, HSP60, HSC82, KAR2), apoptotic signaling (KAR2), oxidative stress response (TRX1,2), S-adenosylmethionine biosynthesis (SAM2), and sulfate assimilation (MET10). We also identified 25 *N*-Hcy-Lys (KHcy) residues in 20 yeast proteins involved in ribosome biogenesis/translation (RPL13B, RPS16B, RPL17A, RPS21A, YEF3), glycolysis, biosynthesis of secondary metabolites and amino acids (ENO2, YRO2, GPP1, GPP2, PDC1, DIT1, PDH1, IPP1, PGI1, INO1, TDH3, GPM1, YHL012W), oxidative stress (CSD1), DNA replication (POL32). mRNAs for ribosomal proteins RPS5, RPS6, RPS7A, RPS9B, and kinases SNF1 (phosphorylates KOG1) and YPK3 (phosphorylates RPS6), involved in TORC1 signaling were down-regulated by HHcy. In addition, HHcy inhibited phosphorylation of SNF1 and RPS6.

Conclusion: These findings suggest that protein *N*-homocysteinylation and dysregulation of cellular proteostasis affecting ribosomal proteins and TORC1 signaling are involved in the toxicity of HHcy in yeast. Homologous proteins are likely to be involved in HHcy toxicity in human and animal cells.

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P 09

The levels of homocysteine and miR-650 in plasma of Alzheimer's disease patients

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Background:

Alzheimer's disease (AD) is a progressive form of dementia, characterized by brain deposition of amyloid β (Aβ) and neurofibrillary tangles. AD is frequently accompanied by improper biothiols turnover, including homocysteine (Hcy). Hcy is associated with disturbed mitochondrial function, augmented oxidative stress and inordinate apoptosis of neurons in the central nervous system (CNS). Hcy affects also epigenetic mechanisms, such as DNA methylation and microRNA (miRNA) expression. MiRNAs are small, single-stranded molecules responsible for fine-tuning of protein production expressed from more than 70% of all human genes. One of miRNAs, miR-650 was recently suggested to play a role in the regulation of apoptosis in the CNS.

Aim:

The aim of the study was the analysis of the Hcy and miR-650 levels in plasma of AD patients and age-matched controls.

Methods:

Hcy concentrations were measured by HPLC/EC in 88 AD patients, 80 controls without (UC), 62 controls with (RC) positive family history of AD, while the level of miR-650 was assesed by RT-PCR.

Results: We showed that in nearly two thirds of AD patients the Hcy concentrations exceeded 15 µmol/L ($p<0.01$ *vs.* UC, $p<0.001$ *vs.* RC). The highest levels of Hcy were found in preclinical dementia (mild cognitive impairment) and in AD patients before introduction of pharmacotherapy.

Subsequently, we found that miR-650 levels correlated with the degree of cognitive impairment measured in MMSE scale ($R=+0.385$, $p=0.033$). In severe dementia, the reduction of median miR-650 level reached nearly 60%. What is more, only in healthy controls we observed that reduced miR-650 level correlated with increased Hcy concentration ($R=-0.384$, $p=0.012$).

Conclusions: It would seem that elevated Hcy plasma concentration and reduced level of miR-650 could constitute a useful predictive markers of AD pathological changes developing in the CNS.

P 10

Homocysteine concentrations, folate intake, and polymorphism of the *MTHFR* gene (rs1801133) in young Polish women

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BACKGROUND: The relation between folate and iron intake, gene polymorphisms, and homocysteine concentrations in Polish women is not well understood.

AIM: The aim of this study was to determine the intake of folate and iron, and to assess the effect of selected SNPs on biochemical parameters.

METHODS: This study was carried out with the approval of the local ethical committee (approval no. 917/16). We enrolled 189 Polish women aged 20-35 from 2016 to 2018. During recruitment, all women participated in medical consultations. Blood samples were taken after an overnight fast. Iron, unsaturated iron binding capacity (UIBC), and folate concentrations were assayed in serum using commercial kits (Folate III test, Roche Diagnostics, Germany, and DiaSys, Germany respectively) with a biochemical analyzer. Plasma homocysteine (Hcy) levels were determined using enzyme-cycling Hcy assay with commercial kits (Diazyme Homocysteine Assay, Diazyme Laboratories, USA) and Konelab 20i Analyzer (Thermo Electron, Vantaa, Finland). Genotyping of the *RFC* (rs1734762), *MTHFR* (rs1801133), *DHFR* rs70991108, *DMT* (rs224589), and *TFR2* (rs7385804) genes was performed using TaqMan probes on a Light Cycler 480 Instrument (Roche). Dietary intake was assessed using three-day food records. Statistical analysis was performed with Statistica using nonparametric tests ($p < 0.05$).

RESULTS: Women were divided into two groups: a control group with no iron and folate deficiency (C, n = 87) and a second group with iron and or folic acid deficiency, or both (S, n = 113). Concentrations of the biochemical parameters were as follows: UIBC 236.21 vs. 307.07 µg/dl and folate 9.86 vs. 5.75 ng/ml. Hcy concentrations were lower in the C group than in the S group (8.72 vs. 10.09 µM/l, $p < 0.1$). Ferritin levels were higher in the C group than in S group, at 34.96 and 24.89 µg/l, respectively ($p < 0.005$). Only 20% of participants met their needs for folate. The intake of iron (11.63 and 11.13 mg/day, in the C and the S group, respectively) and folate (325.63 vs. 310.37 µg/day, in the C and the S groups, respectively) did not differ between groups. There were differences between the Hcy concentrations in the T allele carriers of the *MTHFR* rs1801133 and the CC homozygotes (10.27 vs. 8.79 µM/l, $p < 0.01$), but not in folate level. There were no differences between the genotype groups of *RFC* (rs1734762), *DHFR* (rs70991108), and *DMT* (rs224589). However, there were differences in iron concentration depending on *TFR2* genotype, with the A allele carriers having lower iron concentrations than the CC homozygotes (95.00 vs. 111.02 µg/dl, $p < 0.05$).

CONCLUSION: Women with iron and folic acid deficiencies had higher Hcy concentrations than those with no iron or folate deficiency, and this effect was unrelated to folate intake. The *MTHFR* polymorphism (rs1801133) is associated with Hcy concentrations in young Polish women.

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P 11

Serum Proteome Alterations in Cystathionine β-Synthase Deficiency and Ischemic Stroke Subtypes in Humans

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Background: Stroke induces brain injury by the thrombotic, embolic or hemorrhagic mechanisms involving large or small vessels. Cystathionine β-synthase deficiency (CBS), an inborn error of metabolism, is associated with vascular thromboembolism, the major cause of morbidity and mortality in affected patients. Because thromboembolism involves the brain vasculature in these patients, we hypothesize that CBS-deficiency and ischemic stroke have similar molecular phenotypes.

Aim: Our objective was to examine changes in serum proteomes of CBS deficient patients and ischemic stroke subtypes.

Methods: We used the label-free mass spectrometry for quantification of changes in serum proteomes in CBS-deficient patients and gender/age matched unaffected controls as well as in patients with cardioembolic, large-vessel, or lacunar stroke. Ingenuity Pathway Analysis resources were used for bioinformatics analysis.

Results: We identified 40 differentially expressed serum proteins in CBS-deficient patients. We also identified differentially expressed serum proteins in ischemic stroke patients, some of which were unique to a specific subtype: 10 of 32 for cardioembolic *vs*. large-vessel, 6 of 33 for cardioembolic *vs*.lacunar, and 6 of 23 for large-vessel *vs*. lacunar. There were significant overlaps between proteins affected by CBS deficiency *and* ischemic stroke subtypes, similar to protein overlaps between ischemic stroke subtypes. Top molecular pathways affected by CBS deficiency *and* ischemic stroke subtypes involved Acute Phase Response Signaling and Coagulation System. Similar molecular networks centering on NFκB were affected by CBS deficiency and stroke subtypes.

Conclusions: Proteomic signatures suggest common mechanisms between CBS-deficiency and ischemic stroke subtypes. Subtle proteomic differences between ischemic stroke subtypes involve unique sets of serum proteins that might be exploited in treatment/prognostication of outcomes.

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P 12

Identification of interactions between BLMH and GLOD4 in brains of 5xFAD mice and Alzheimer's disease patients

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Background: Bleomycin hydrolase (BLMH) is one of enzymes capable of degrading homocysteine thiolactone (HcyTL), a metabolite implicated in heart and brain diseases. Blmh activity is reduced in brains of Alzheimer's disease patients. *Blmh*$^{-/-}$ mice accumulate HcyTL in the brain and exhibit astrogliosis, indicative of undefined brain pathology. A new variant of glyoxalase domain protein 4 (Glod4) was identified in the brains of *Blmh*$^{-/-}$ mice. A polymorphism in the human GLOD4 gene is associated with familial Alzheimer's disease (AD).

Aim: To elucidate the mechanism underlying the involvement of GLOD4 in AD, we generated a new animal model of AD, the *Blmh*$^{-/-}$5xFAD mouse, which carries a human transgene harboring 5 mutations in the *APP* and *PSEN1* genes, which leads to early (at 3 months) accumulation of beta amyloid (Aβ). We collected brains from 5 months-old *Blmh*$^{+/+}$, *Blmh*$^{-/-}$, *Blmh*$^{+/+}$5xFAD, and *Blmh*$^{-/-}$5xFAD mice and examined the *Glod4* mRNA expression. We also examined the *GLOD4* gene expression in AD and age- and gender-matched control human brains.

Methods: Total RNA was purified by Trizol and a column-based method. RT-qPCR was used to quantify mouse *Glod4* and human *GLOD4* mRNAs, with mouse β-actin mRNA and human *B2M*, *XPNPE1*, or *GAPDH* mRNAs, respectively, as references. Mouse Glod4 and human GLOD4 protein was quantified in brain extracts by Western blotting.

Results: We found that *Glod4* mRNA expression was significantly down-regulated by the *Blmh*$^{-/-}$ genotype in 5xFAD female, but not male, mice: 0.76 *vs*. 1.28 in *Blmh*$^{-/-}$5xFAD *vs*. *Blmh*$^{+/+}$5xFAD, respectively, p=0.003. In contrast, however, *Glod4* mRNA expression was unaffected by the *Blmh*$^{-/-}$ genotype in female and male mice without the 5xFAD human transgene. We also found that *Glod4* mRNA expression was down-regulated by the human 5xFAD transgene itself: 0.94 *vs*. 1.74 (p=0.02) for male and 1.28 *vs*. 1.58 (p=0.29) for female *Blmh*$^{+/+}$5xFAD *vs*. *Blmh*$^{+/+}$ mice, respectively, and 0.84 *vs*. 1.92 (p=0.009) for male and 0.76 *vs*. 2.06 (p<0.000) for female *Blmh*$^{-/-}$5xFAD *vs*. *Blmh*$^{-/-}$ animals. Similar to the *Blmh*$^{-/-}$5xFAD mice, *GLOD4* mRNA was lower in AD human brains compared to healthy brains. GLOD4 protein levels were also lower in AD brains compared to controls (0.50 *vs*. 1.35, p=0.009)

Conclusions: These findings provide evidence for interactions between BLMH, GLOD4, and Aβ in the human and mouse brains and suggest that dysregulation of these interactions contributes to AD.

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P 13

Effect of betaine supplementation on homocysteine, cholesterol, and triacylglycerol levels

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Background: Betaine is a derivative of the amino acid glycine. It occurs naturally in many foods, but is also produced endogenously. Betaine participates in one-carbon metabolism, where it is a donor of methyl groups. It participates in the methylation of homocysteine to methionine, which leads to a decrease in its concentration in blood. Numerous studies have shown that increased levels of homocysteine in blood as well as total cholesterol (TC), LDL cholesterol, and triacylglycerol (TAG) are risk factors for cardiovascular events. Cardiovascular disease is the most common cause of death in the general population. There are about 175,000 deaths from cardiological causes in Poland every year, representing 46% of all deaths. Numerous studies have indicated how to deal with elevated homocysteine levels, and betaine supplementation may be one way. On the other hand, an increasing body of evidence suggests that betaine may have an adverse effect on lipid metabolism.

Aim: The aim of this study is to determine the impact of betaine supplementation on cardiovascular risk factors.

Methods: Two meta-analyses were performed. The meta-analysis of the effect of betaine supplementation on homocysteine level included five randomized controlled-trials (RCTs). The minimum betaine dose was 4 g/d and the duration of the studies varied from six to twenty-four weeks. The meta-analysis of the effect of betaine on blood lipids included data from six RCTs. The minimum betaine dose was 4 g/d and the duration of the intervention was at least four weeks. The endpoints were TC, TAG, LDL-C, and HDL cholesterol (HDL-C) concentrations in the blood.

Results: Betaine significantly decreased the homocysteine concentration by 1.23 µmol/L, but also increased TC by 0.34 mmol/L. Betaine supplementation had no effect on HDL-C, LDL-C, or TAG concentrations.

Conclusions: Betaine supplementation at doses of 4–6 g/d can affect the risk of cardiovascular diseases in two ways: it lower risk by decreasing the concentration of homocysteine, and it increases the risk by raising the TC concentration. However, there is insufficient data to draw a clear conclusions. There are no studies directly assessing the effect of betaine supplementation on cardiovascular disease risk.

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